Published online: 2021-12-23

Modulating Autoimmunity against LDL: Development of a Vaccine against Atherosclerosis

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Hamostaseologie 2021;41:447–457.

Abstract

Atherosclerosis is a chronic inflammatory disease of the arterial wall that leads to the build-up of occluding atherosclerotic plaques. Its clinical sequelae, myocardial infarction and stroke, represent the most frequent causes of death worldwide. Atherosclerosis is a multifactorial pathology that involves traditional risk factors and chronic low-grade inflammation in the atherosclerotic plaque and systemically. This process is accompanied by a strong autoimmune response that involves autoreactive T cells in lymph nodes and atherosclerotic plaques, as well as autoantibodies that recognize low-density lipoprotein (LDL) and its main protein component apolipoprotein B (ApoB). In the past 60 years, numerous preclinical observations have suggested that immunomodulatory vaccination with LDL, ApoB, or its peptides has the potential to specifically dampen autoimmunity, enhance tolerance to atherosclerosis-specific antigens, and protect from experimental atherosclerosis in mouse models. Here, we summarize and discuss mechanisms, challenges, and therapeutic opportunities of immunomodulatory vaccination and other strategies to enhance protective immunity in atherosclerosis.

Keywords
- atherosclerosis
- vaccination
- T cells
- antibodies
- autoimmunity

Zusammenfassung

Die Atherosklerose stellt eine chronisch entzündliche Erkrankung der Arterienwand dar, die zur Bildung von Gefäß-verengenden atherosklerotischen Plaques führt. Ihre klinischen Folgen, Herzinfarkt und Schlaganfall, repräsentieren die weltweit häufigsten Todesursachen. Der Erkrankung liegt ein multifaktorieller Krankheitsprozess zu Grunde, der traditionelle Risikofaktoren und eine chronische lokale und systemische Entzündungsreaktion umfasst. Die Entstehung der Atherosklerose wird von einer starken Autoimmunreaktion begleitet, an der autoreaktive T-Zellen in Lymphknoten und atherosklerotischen Plaques sowie Autoantikörper beteiligt sind, die gegen *low-density lipoprotein* (LDL) Cholesterin und Apolipoprotein B (ApoB) gerichtet sind.

Schlüsselwörter
- Atherosklerose
- Impfung
- T Zelle
- Antikörper
- Autoimmunität

received
July 5, 2021
accepted after revision
October 4, 2021

DOI https://doi.org/10.1055/a-1661-1908.
ISSN 0720-9355.

Georg Thieme Verlag KG, Rüdigerstraße 14, 70469 Stuttgart, Germany

Vielfältige präklinische Untersuchungen aus den vergangenen 60 Jahren konnten zeigen, dass eine immunmodulatorische Impfung mit LDL, ApoB und ApoB-Peptiden das Potenzial hat, die Autoimmunität in er atherosklerotischen Plaque abzuschwächen, eine Toleranz gegenüber Arteriosklerose-spezifischen Antigenen auszubauen und vor Atherosklerose in Mausmodellen zu schützen. In diesem Artikel diskutieren wir die Mechanismen, Herausforderungen und therapeutischen Möglichkeiten einer immunmodulatorischen Impfung und anderer Strategien, die zur einer Stärkung der protektiven Immunantwort in der Atherosklerose führen.

Introduction

Atherosclerosis is now recognized as a chronic inflammatory disease of middle- to large-size arteries that is characterized by the development of occluding plaques in the subendothelial intimal layer.[1] Its clinical complications, myocardial infarction (MI) and stroke, are the leading causes of death worldwide.[2] While originally perceived as a lipid-storage disease of the arterial wall with an excessive accumulation of low-density lipoprotein cholesterol (LDL-C),[3] it is now established that the progression of atherosclerotic plaques is driven by a chronic low-grade inflammatory and immune response encompassing inflammatory cells of myeloid origin and of the adaptive immune system.[4,5] Epidemiologic, preclinical, and interventional studies have demonstrated that in addition to the traditional risk factors smoking, hypertension, obesity, diabetes, and environmental stressors, LDL-C is the main culprit of atherosclerosis.[6,7] LDL-C continuously accumulates in the subintimal space of arteries, where it is oxidatively modified and taken up by tissue-resident macrophages, which become "foam cells" and secrete proinflammatory cytokines, such as interleukin (IL)-1β[8]. LDL-C-lowering strategies promote plaque regression, inhibit macrophage proliferation, and reduce cardiovascular mortality.[7,9] Besides the myeloid cellular response, LDL-C initiates an autoimmune response in atherosclerotic plaques with autoreactive $CD4^+$ T-helper cells and B-cell-derived autoantibodies that target LDL and its core protein, apolipoprotein B (ApoB).[5,10,11] The modulation of this autoimmune response with immunomodulatory vaccination strategies has been increasingly investigated in the last decades. Here, we present and discuss the development of a vaccine against atherosclerosis.

T-Cell Immunity in Atherosclerosis

T cells and B cells represent the adaptive limb of cellular and humoral immunity against pathogens, such as bacteria or viruses. B cells target pathogens by plasma cell-derived immunoglobulin G (IgG) antibodies, $CD8^+$ cytotoxic T cells neutralize infected cells by cytotoxic mechanisms, and $CD4^+$ T-helper cells (T_H) orchestrate the adaptive immunity by secreting cytokines that can either dampen or accelerate the immune response or can exhibit cytotoxic effects themselves.[12] The recognition of cognate antigens by B and T cells is facilitated by specific immunoreceptors on the cell surface, the B cell (BCR) and T-cell receptor (TCR). These have the ability to either bind complex antigens (BCR) or an antigen-derived peptide presented on major histocompatibility complex (MHC)-I ($CD8^+$) or MHC-II ($CD4^+$).[12] Besides B and T cells, other immune cell populations are relevant in atherosclerosis.[5] T cells are the largest leukocyte population in human atherosclerotic plaques, while B cells are found only in relevant quantities in the adventitia of the vessel wall.[13,14] The activation of $CD4^+$ T cells in the plaque requires antigen presentation on MHC-II by antigen-presenting cells (APCs), such as dendritic cells or plaque macrophages.[5,11,15] Antigen recognition, along with costimulatory signals from the APC, promotes the differentiation and activation of T_H cells.[16] As a result, naive T cells develop into effector T (T_{eff}) cells that express distinct intracellular transcription factors and cytokines. T_{eff} cells have different and partially contrasting roles in atherosclerotic disease.

A proportion of lesional $CD4^+$ T cells in humans are proatherogenic T_H1 cells that express the transcription factor T-bet and secrete interferon (IFN)-γ.[17] On the contrary, T regulatory (T_{reg}) cells are characterized by the expression of the transcription factor FoxP3 and IL-2 receptor (CD25). T_{reg} cells maintain *self*-tolerance by secreting the immunosuppressive cytokine IL-10, transforming growth factor (TGF)-β, and by direct contact inhibition of T_{eff} cells.[18,19] T_{reg} cells in the plaque induce an alternative activation pathway in macrophages, block pathogenic T_H1 immunity, resolve inflammation, and support plaque regression in the mouse.[20] Circulating T_{reg} numbers and plasma IL-10 levels are negatively correlated with human cardiovascular disease.[21,22] T_H17 cells express the transcription factor RORγT, produce IL-17, are central for mucosal immunity, and have been associated with autoimmune disease.[23] While some experimental studies in the mouse showed a proatherogenic role of T_H17 cells, others demonstrated atheroprotective properties, or no effect.[24] T-follicular helper cells (T_{FH}) express Bcl-6 and provide support for B cells for antibody isotype switching.[25] T_{FH} cell depletion protects from experimental atherosclerosis.[26] T_{FH} represents a direct link between humoral and cellular immunity. Antigen-specific $CD4^+$ T cells in the murine atherosclerotic plaque show mixed T_{reg}, T_H17, and T_H1 cells.[27] These seem to originate from initially immunosuppressive $FoxP3^+$ T_{reg} cells, which downregulate FoxP3 expression and become exT_{reg} cells.[26–30] Many $CD4^+$ T cells in the atherosclerotic plaque in the mouse express low levels of FoxP3 as well as IFN-γ and T-bet.[27,29,30] These mixed T_H cells seem to account for up to 50% of lesional T cells.[27]

Concept of Autoimmunity in Atherosclerosis

Autoimmunity is the abnormal response of immune cells against endogenous proteins or other structural components of the body. Protein autoantigens are recognized by $CD4^+$ T cells, which respond with proinflammatory cytokine secretion and activate other immune and stromal cell types. Autoantibodies bind to antigens, neutralize these, and build antibody–antigen complexes that may be proinflammatory. Ultimately, autoimmunity can lead to tissue damage, cell death, and organ dysfunction.[31] T_{reg} cells have the ability to protect from this pathogenic response by recognizing the same *self*-peptides and -antigens as pathogenic T-cell clones.[19] Secretion of immunosuppressive cytokines, such as IL-10, direct contact inhibition of T_{eff}, and removal of the $CD4^+$ T cell survival factor IL-2 are important T_{reg} effector functions. Whether atherosclerosis is a mere autoimmune disease has been a matter of discussion. Recent evidence has pointed out that autoreactivity needs to be considered as a pro-atherogenic stimulus in addition to myeloid-cell–driven inflammation and various other pathogenic mechanisms.[5] Several observations suggest the presence of a strong autoimmune response in atherosclerosis: Both $CD4^+$ and $CD8^+$ T cells accumulate in the atherosclerotic plaques in mice and humans. They show signs of chronic activation, have a predominant effector-memory phenotype, and express known T-cell activation markers, including CD69, CD38, granzyme, and others, suggestive of repetitive antigen exposure by cognate, either *self*- or foreign antigens.[11,14,32] $CD4^+$ T cells in human plaques show increased expression of programmed cell death protein 1 (PD-1) that is typically upregulated in chronically stimulated and exhausted T cells and prevents cell activation.[14] These findings suggest that a natural counterregulatory checkpoint, which protects from ongoing autoimmunity, exists in atherosclerosis.[33,34] Clinical checkpoint inhibition by PD-1 antibodies enhances the risk for autoimmunity in the heart. It is therefore plausible that a reversal of exhaustion may reactivate hibernating, autoreactive T cells in the plaque.[14,35] In the mouse plaque, T cells physically interact with tissue-resident APCs.[15] This process is antigen dependent, involves MHC-II-dependent antigen presentation, and drives the secretion of proinflammatory cytokines by T cells.[15] Antigen presentation and costimulation can be simulated in vitro in restimulation assays with plaque-derived T cells, APCs, and known *self*-peptides or more complex antigens in rodents and mice.[10,27,36] A proportion of T cells secretes IFN-γ[15] and proliferates when restimulated with LDL or peptides derived from ApoB in an MHC-II-dependent fashion.[10,27,36] ApoB-specific T_H cells have been detected in humans by MHC-II tetramers[37] and by restimulation with ApoB peptides in peripheral blood.[27] T-cell proliferation, as direct consequence of antigen presentation and costimulation, is observed in murine atherosclerotic plaques in histology and scRNAseq.[15,32,38] Sequencing of the TCR in mice and humans has validated that a relevant proportion of plaque T cells stems from a small number of unique, antigen-specific T cells.[27,36,39,40] Whether this restriction of TCR usage observed in bulk tissue analysis is caused by $CD8^+$ or $CD4^+$ T cells or both is currently unclear. In addition to a cellular response involving T cells, circulating autoantibodies that bind ApoB or LDL have been detected in patients with atherosclerosis.[41] Autoantibodies mostly target oxidation-specific epitopes of LDL.[42,43]

These findings suggest that at least a part of the pathogenic lymphocyte response in atherosclerosis is caused by autoreactivity against LDL/ApoB or other autoantigens. It was long believed that this autoimmune response would be solely pathogenic. However, initial findings with RAG-1-deficient mice, which lack mature B and T cells, have suggested that adaptive immune cells do either not affect atherosclerosis or only in the very initial stages of plaque development.[44,45] These studies with immunosuppressive mice remain highly controversial since mice with a severe combined immunodeficiency (*scid*) were partially protected from atherosclerosis.[46] An explanation sparked by more recent findings is that the autoreactive response in atherosclerosis is heterogeneous, subtype- and context-dependent, and can be pro- and anti-inflammatory (immune activating and immunosuppressive) at the same time, which may explain an overall neutral net effect in *Rag1*$^{-/-}$ mice.[5,11] Indeed, ApoB-reactive T helper cells in the early stages of atherosclerosis seem to be immunosuppressive.[27] Likewise, genetic abrogation of MHC-II-dependent antigen presentation aggravates atherosclerosis.[47] These findings argue for a partially protective autoimmune response in the early stages of disease—a concept denoted as "protective autoimmunity in atherosclerosis."[48] In the later stages of atherosclerosis, the protective phenotype is lost and gradually replaced by a more pathogenic phenotype.[11,27] Functionally heterogeneous subtypes are also observed in the B-cell compartment, where protective B1 cells secrete IgM antibodies that mostly protect from atherosclerosis, and conventional B2 cells that secrete IgG antibodies and likely accelerate vascular inflammation.[42] It is important to highlight that the immune system is likely more diverse than initially anticipated. Recent advances in single-cell technologies demonstrate several unexpected, partially overlapping, highly dynamic phenotypes, which are particularly evident within the population of autoreactive $CD4^+$ T helper cells.[11,49] These phenotypes dissociate from classical cellular identities, for example, T_H types of immunity, and may have opposing functional outcomes despite an apparent commitment to the same T_H type. For instance, IL-17 secretion is a hallmark of T_H17 cells but depending on concomitant cytokine and transcription factor expression, IFN-g/T-bet or IL-10/FoxP3, the factual (functional) identity can range from pathogenic T_H1-like to atheroprotective T_{reg}-like cells. It is therefore critical to identify the precise cellular targets for atheroprotective vaccination to selectively enhance the immunosuppressive limbs of ApoB autoimmunity.

Other (Auto-) Antigens in the Atherosclerotic Plaque

Beyond LDL-C and ApoB, two other groups of autoantigens have been discussed: heat shock proteins (HSPs) and β2-glycoprotein I (β2GPI).[50,51] HSPs are intracellular chaperones

that are required to protect against physical and chemical noxes.[52] Autoantibodies recognizing HSPs exist in humans and positively correlate with the presence of cardiovascular disease.[53] Vaccination with peptides or the entire protein complex HSP60/65 ameliorates experimental atherosclerosis in mice.[54] Whether HSPs represent autoantigens per se or caused by a molecular mimicry with HSP from foreign (bacterial) HSPs is a matter of discussion. Notably, it has been observed that bacterial HSP65 induces IgG antibodies that also bind human HSP. Human HSP shares immunodominant B cell epitopes with its bacterial counterpart.[55] A similar mechanism has been proposed to explain potentially beneficial effects of vaccination against *Streptococcus pneumoniae* that shares epitopes with oxidatively modified LDL in humans.[43,56] β2GPI is a regulator of the coagulation and complement system and the target of anticardiolipin antibodies in the antiphospholipid syndrome.[57,58] β2GPI is located in the plasma, but has also been detected in human atherosclerotic lesions.[59] Whether vaccination using β2GPI protects from atherosclerosis in mice is controversial.[50,60,61]

The clinical correlation of infectious disease and atherosclerosis has also sparked the idea that a proportion of T cells in the plaque may recognize foreign antigens, that is, peptides from bacteria or viruses. Indeed, clinical observations have suggested that varicella zoster virus (VZV) and influenza virus infection increases the risk for MI and stroke.[62] Influenza vaccination ameliorates the outcome of patients with clinically relevant atherosclerosis and is therefore recommended for patients with heart disease.[63,64] Several other viruses, including human cytomegalovirus, herpes simplex virus, Epstein–Barr virus, VZV, and influenza virus, have either been detected in arterial tissue or are suspected to promote inflammation of the vasculature.[62] These associations, however, could be explained by indirect effects, for instance, the induction of local tissue injury, thrombotic pathways, or a systemic inflammatory response[65,66] as observed in SARS-CoV-2 infection, which promotes inflammation and dysfunction of the vasculature and enhances thrombogenicity.[67,68] SARS-CoV-2 viral particles have been detected in endothelial cells[69] and the myocardium.[70] Whether SARS-CoV-2-specific T cells are enriched in atherosclerotic plaques is currently not known.

Immunomodulatory Vaccination with LDL-C/ApoB

Up to now, vaccination with LDL and peptides from ApoB has been exclusively tested in rodents. In 1959, Gero et al were the first to observe a decrease of atherosclerotic lesion formation following a vaccination of rabbits with LDL.[71] In the last decades, numerous studies in rodents have validated that vaccination with native LDL, oxidized LDL, or peptides from ApoB induces an atheroprotective T-cell response.[50,72–80] The main protein immunogen in LDL is ApoB. So far, nine distinct ApoB peptides have been validated in peptide vaccination studies: p3, p6, p18, p101, p102, p103, p210, p265, and p295.[27,37,81–84] In these studies, peptide vaccination was mostly tested in a preventive setting to inhibit de novo atherosclerosis in ApoE- or LDLR-deficient mice. Peptides were largely delivered subcutaneous as emulsion of peptides with complete Freund's adjuvant (CFA) as prime and subsequent boost injections intraperitoneally with incomplete Freund's adjuvant (IFA).[50] In some reports, peptides were linked to keyhole limpet hemocyanin (KLH)[84] or the B subunit of cholera toxin subunit B (CTB).[83] Different routes, such as intranasal delivery, have been described as well.[83] Vaccination with ApoB peptides has been demonstrated to induce antigen-specific T cells, T-cell proliferation, and cytokine secretion.[81] ApoB-specific $CD4^+$ T cells have been found in the spleen and in the peritoneal cavity, which is likely related to tested routes of vaccination. Induction of IL-10 secreting T_{reg} cells in the atherosclerotic aorta and spleen has been proposed as the main protective mechanism.[82,83,85,86] T_{reg}-independent IL-10 secretion has also been observed[81,87] as well as an inhibition of T_H1 immunity.[86] In conclusion of the reported mechanisms (extensively reviewed in the article by Nettersheim et al[50]), it is believed that preexisting ApoB-specific T_{reg} cells or IL-10 secreting non-T_{reg} cells (T_R1 cells) selectively expand after vaccination with ApoB, either at the site of injection or in draining lymph nodes. Cytokines secreted by ApoB-specific T cells may circulate and act on atherosclerotic plaques in a remote fashion (►Fig. 1). The direct migration of T cells into atherosclerotic lesions has been extensively studied[89] but has not yet been demonstrated for vaccination-induced T cells. The function of ApoB-specific T cells seems to be highly flexible. For instance, vaccination with p6 was atheroprotective in *Apoe*$^{-/-}$ mice after a prime in CFA and four subsequent boosts in IFA. In this study, mice received an atherogenic diet for 13 weeks 1 week after the prime vaccination.[81] In *Apoe*$^{-/-}$ mice pre-fed with an atherogenic diet for 5 weeks, one prime and one boost in CFA dramatically exacerbated atherosclerotic lesion size within the next 5 weeks.[90] A potential explanation for this discrepancy is that vaccination may only be capable of expanding the existing pool of antigen-specific T cells with a certain phenotype but not of reversing phenotypes. As mentioned earlier, ApoB-specific T cells undergo a phenotypic transformation from a T_{reg}-like T_H17 cell with a protective phenotype in healthy animals into a pathogenic T_H1-like phenotype with proinflammatory cytokine secretion in established disease.[27] Therefore, atheroprotective peptide vaccination may only be effective in the absence or in early stages of atherosclerotic disease when ApoB-specific T_{reg} cells dominate but not in later stages after the phenotypic switch when T_H1-like pathogenic cells outnumber protective T_{reg} cells. It remains, however, to be tested whether vaccination in established disease preferentially expands pathogenic T-cell clones.

Vaccination to Neutralize Atherosclerosis-Relevant Proteins, Apolipoproteins, and Cells

As mentioned earlier, vaccination is historically designed to remove target proteins, such as toxins, or other harmful noxes during infection. Therefore, vaccination against infectious agents needs to be capable of inducing high-affinity B-cell-derived IgGs that bind to immunodominant

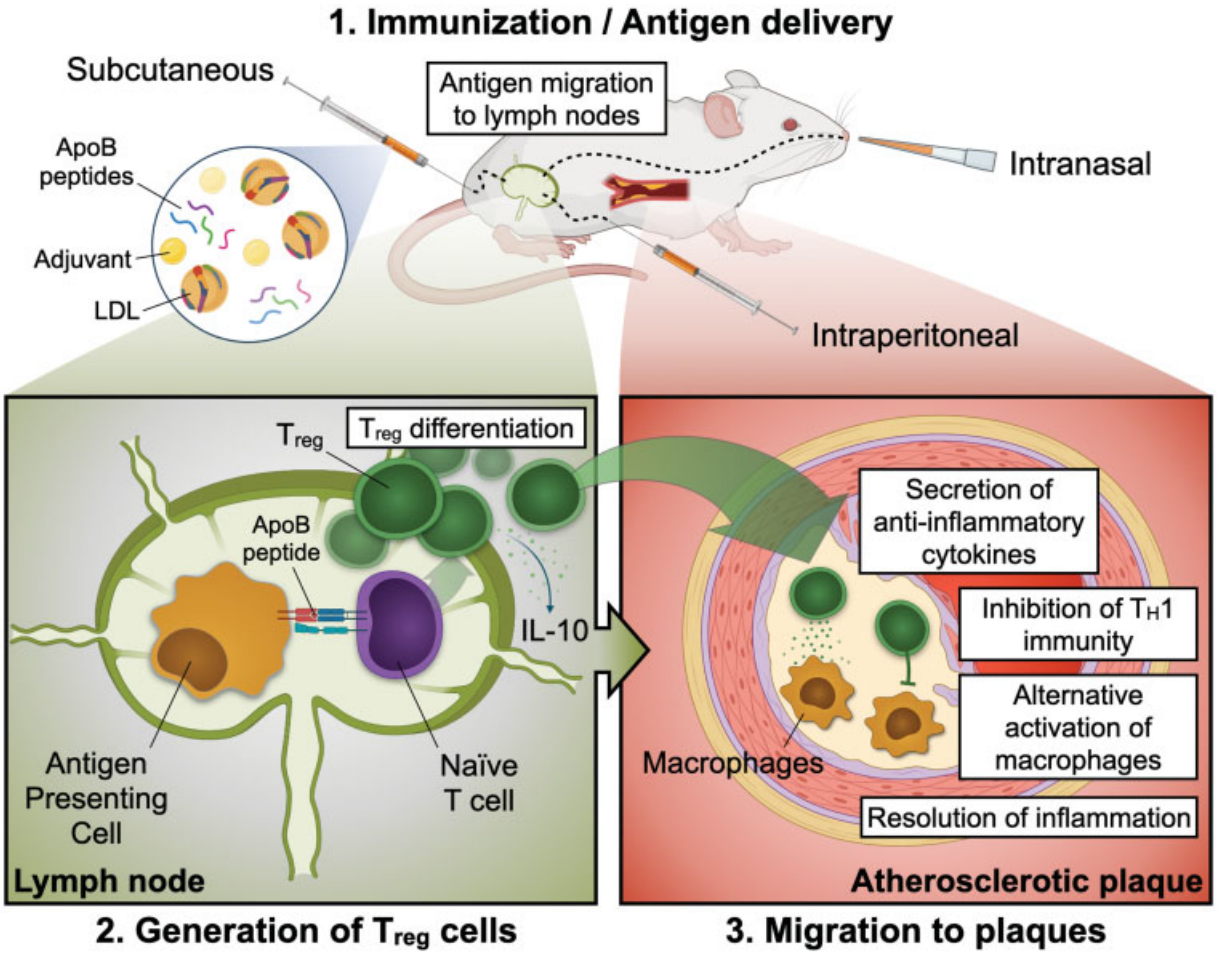

Fig. 1 **Proposed mechanism of immunomodulatory LDL/ApoB vaccination in mice.** A combination of autoantigens (native LDL, oxidized LDL, ApoB peptides) and the adjuvant (mostly CFA or IFA) is injected subcutaneous or in the peritoneal cavity. Intranasal application of an ApoB–cholera toxin fusion protein has also been described (1). Antigens are transported to locally draining lymph nodes, taken up by antigen-presenting cells (APCs), processed, and peptides from the antigen are presented to naive T cells. T cells are activated, and proliferate and differentiate into IL-10 secreting regulatory T cells (T_{reg}) or IL-10 secreting non-T_{reg} (T_R1) (2). LDL/ApoB-specific T cells redistribute to other lymph nodes or directly migrate into atherosclerotic plaques (3). Vaccination-induced T cells secrete the immunosuppressive cytokine IL-10, which suppresses cellular proliferation and activation of effector T cells. In addition, T_{reg} cells inhibit pathogenic T_H1 pathways in the plaque, induce alternative-activation pathways in macrophages, and support resolution of inflammation. These local and systemic effects retard atherosclerotic lesion progression. Created with Biorender.com.

epitopes and neutralize the antigen through various mechanisms. In addition, vaccination-induced IgGs serve as biomarkers of vaccination efficacy and indicate the need for booster immunization. Antigen-specific T cells may support rapid recall responses even in the absence of long-lasting IgG titers, as recently demonstrated for SARS-CoV-2 vaccines.[91] It is important to note that the generation of neutralizing IgG antibodies requires a proinflammatory response with T_H1 polarized $CD4^+$ T cells. This principle fundamentally differs from an immunomodulatory immunization, which aims to induce a protective response with immunosuppressive T_{reg} cells. Whether and how antibodies support this response is currently unknown. Yet, the induction of neutralizing IgG antibodies may be helpful to inhibit some important propagators of atherogenesis. For instance, a recent study has highlighted that naturally occurring antibodies to ALDH4A1, a mitochondrial dehydrogenase, can delay atherosclerosis progression.[92] Vaccination strategies may therefore also be designed to remove endogenous proteins with a pathogenic potential through an endogenous clearance by IgG antibodies as a substitution for repetitive exogenous IgG injections.

Neutralizing Interleukin-12

Neutralizing interleukin-12 (IL-12) is a T_H1-inducing cytokine, which is secreted by macrophages and other APCs. Blocking IL-12 protects from atherosclerosis, likely by preventing proinflammatory and pathogenic $CD4^+$ T cell clones and pathogenic T_H1 polarization.[93] $LDLR^{-/-}$ mice immunized with a complex of IL-12 and adjuvant developed anti-IL-12 antibodies that neutralized IL-12 downstream signaling and IFN-γ secretion in T cells, resulting in smaller atherosclerotic lesions with a plaque phenotype resembling plaque stability in humans.[94] Essentially, these results confirm findings from initial studies that made use of exogenous antibody injections. The main limitation of blocking IL-12 is the broad and unspecific inhibition of T_H1 responses that are vital for host defense and may therefore induce relevant immunosuppression in a clinical setting.

Vaccination as an Alternative Strategy to Lower LDL-C

LDL-C is considered the main culprit of atherosclerosis.[5] LDL-C lowering correlates with an improvement of cardiovascular outcomes in an almost linear fashion and current clinical guidelines recommend individual LDL-C target ranges based on the individual risk for cardiovascular disease and the presence of clinically relevant atherosclerotic cardiovascular disease (ASCVD).[95] Currently, LDL-C lowering is mainly achieved by an inhibition of HMG-CoA reductase by statins, and an inhibition of cholesterol uptake in the intestine.[95] An alternative strategy to lower LDL-C is the inhibition of proprotein convertase subtilisin/kexin type 9 (PCSK9), which degrades the receptor for LDL and results in a diminished peripheral uptake of LDL-C. PCSK9 expression correlates with cardiovascular events and individuals with a loss-of-function mutation are greatly protected in genome-wide associations studies.[96] Clinically, humanized, monoclonal antibodies against PCSK9 are now recommended in cardiovascular patients with LDL-C levels above the individual target range.[95] Neutralizing antibodies against PCSK9 can also be induced in mice and primates by an immunization with virus-like particles that display PCSK9-derived peptides,[97] albeit these antibodies did not seem to reduce LDL-C in preclinical studies. Another strategy is the inhibition of cholesteryl ester transfer protein (CETP), which transfers cholesterol from high-density lipoproteins (HDLs) to LDL and very-low density lipoproteins (VLDL). LDL and VLDL propagate atherosclerosis. Immunization with a fusion protein of CETP and tetanus toxin resulted in neutralizing CETP antibodies in mice.[98,99]

Targeting Atherosclerosis-Relevant T Cells by Blocking Specific TCRs

While immunomodulatory approaches are designed to specifically induce immunosuppressive T-cell clones (e.g., T_{reg} cells), it is also reasonable to discuss the therapeutic removal of pathogenic T-cell clones. This strategy requires to be most specific for ApoB-specific T cells. This technical challenge could be theoretically achieved by MHC-II tetramer-based strategies.[100] Hermansson et al have proposed a less specific but effective strategy to remove T cells expressing the predominant TCR variable (V) β segments by neutralizing monoclonal antibodies. It is important to emphasize that T cells selected on broad criteria, such as TCR β segment usage, contain other specificities beyond LDL/ApoB. In their study, LDL-specific T-cell clones mostly expressed the TCRBV31 segment. The V segment is required for TCR–MHC-II interaction. Vaccination of atherosclerosis-prone mice with a TCRBV31 peptide induced blocking antibodies against TCRBV31 and protected from atherosclerosis.[36] It may be possible that this strategy either blocks TCR–MHC-II interactions or eliminates pathogenic T-cell clones.[36]

Translational Strategies

Vaccination to protect against bacterial and viral infection is the most successful intervention of medical history.[101] The concept of immunomodulatory vaccination to induce a preferable immune phenotype during autoimmune disease has been mainly demonstrated in mouse models of diabetes and multiple sclerosis.[102] Atheroprotective vaccination has remained at a preclinical level and only a few, recent studies have interrogated the existence of ApoB-specific T cells in humans.[27,37] Several questions, which are fundamental to understand human immunity against ApoB/LDL-C, need to be addressed in the future to develop a vaccine against human atherosclerosis.

Human Self-Peptides from ApoB

As highlighted earlier, the induction of an immune response—either during presentation of the natural occurring antigenic peptides or of the peptide vaccine—requires a high affinity of the peptide and MHC-II. In contrast to inbred laboratory mice, which only express one variant of MHC-II ($I\text{-}A^b$ in the C57BL/6 mouse), humans express a high number of MHC-II variants with eight different class II allotypes, which results in a total of approximately 10,000 different HLA allelic forms that all bear different affinities to potential peptides. The identification of suitable peptides is therefore limited by several aspects: first, the MHC-II alleles must be determined by sequencing in every individual before vaccination. Second, every MHC-II variant would potentially require its own tested antigenic peptide in the vaccine, even if recent screening strategies have identified several peptide sequences with moderate to high affinities across several MHC-II variants.[27] In addition, every peptide is likely to bind to several MHC-II alleles with different affinities. It is important to note that is has not been entirely resolved whether low- versus high-affinity peptides may promote divergent T-cell phenotypes with pro- and anti-inflammatory T-cell clones simultaneously.[103]

Adjuvants and Routes of Administration

Immunologic adjuvants are substances that are added to the antigens to enhance the effectiveness of the immune reaction during vaccination.[104] Traditional adjuvants make use of evolutionary conserved motifs, pathogen-associated molecular patterns, for example, in lipopolysaccharides that augment the immune response by binding to pattern-recognition receptors.[105] Frequently used adjuvants in rodents are CFA that contains heat-inactivated *Mycobacterium tuberculosis* in an emulsion with mineral oil and IFA that does not contain *M. tuberculosis* for booster injections.[106] The sequence of CFA-IFA as prime and boost injection is atheroprotective,[107] while a single vaccination with CFA and the same antigen seems to be pro-atherogenic.[90] CFA, however, induces several side effects and is therefore obsolete in clinical practice.[108] In contrast, many clinically used adjuvants make use of aluminum salts (alum) or CTB. One important consideration is that adjuvants alone may have the ability to modulate atherosclerosis, as shown for IFA and alum. Both seem to induce an atheroprotective immune response even in the absence of a specific antigen.[87,109,110] Of the clinically used adjuvants, only Addavax, a squalene oil-based nano-emulsion, has an efficacy similar to that of CFA/IFA in a head-to-head comparison in the mouse testing ApoB vaccination in de novo atherosclerosis.[86] Currently, Addavax is frequently used in flu vaccines.

In rodents, booster injections are often made in the peritoneal cavity—a strategy that is not practical in humans. Alternative routes of antigen delivery in rodents with partially divergent pro- or anti-atherogenic effects include nasal and oral application[111,112] as well as intra- or subcutaneous injection.[113,114]

Identification of Patients for LDL/ApoB Vaccination

Vaccination of atherosclerosis-prone *Ldlr*$^{-/-}$ and *Apoe*$^{-/-}$ mice with LDL/ApoB preparations has exclusively been tested in preventative settings, where vaccination was highly efficient in preventing de novo atherosclerosis. Whether atheroprotective vaccination has the potential to dampen or even reverse established atherosclerosis remains to be tested. Particularly in light of the constantly transforming phenotype of ApoB-specific T cells in the course of disease, a vaccine should be capable of reversing the phenotype of late-stage T_H1-primed and pathogenic T cells[27] back to an immunosuppressive T_{reg}-like phenotype or to generate new T_{reg} cells. In addition, patients susceptible to vaccination need to be identified in clinical practice. It has been increasingly appreciated that different risk scenarios of atherosclerosis exist. Even under optimal lipid-lowering therapies, a residual inflammatory risk persists.[115] While this risk may be lowered by the addition of anti-inflammatory therapies, a considerable high rate of event remains, giving rise to the speculation that a fraction of this excessive risk, which is currently not addressable by medical therapy, relates to immune activation. Besides immunoglobulins that recognize LDL/ApoB, the quantification of ApoB-specific T cells, such as by recently introduced re-stimulation[27] or by tetramers,[37] may help identify the suitable patient for vaccination.

DNA and mRNA Vaccination

Antigens are traditionally delivered as recombinant proteins, peptides, attenuated pathogens, and more complex formulations. DNA vaccines refer to the delivery of naked DNA or DNA packed into plasmids that encodes for the antigen. DNA vaccines have been tested in animal models of autoimmunity, including experimental autoimmune encephalomyelitis (EAE) and rheumatoid arthritis.[116–118] In atherosclerosis, DNA vaccination against vascular endothelial growth factor 2 (VEGF-2) induced $CD8^+$ cytotoxic T cells, which neutralized VEGF-2 expressing endothelial cells and protected from atherosclerosis.[119] VEGF-2 is known to be expressed by stressed endothelium. mRNA vaccines contain the messenger RNA (mRNA) that codes for antigen. mRNA vaccines have recently been successfully developed against SARS-CoV-2 and promise low-cost manufacturing, validated safety profiles, rapid developments, and high potency.[120] mRNA vaccines against LDL/ApoB have not yet been developed. One challenge in DNA and mRNA vaccines remains their ability to be expressed in target dendritic cells and to prevent simultaneous activation of dendritic cells (DCs). Recently, it was shown that this limitation may be circumvented with a nanoparticle-formulated 1 methylpseudouridine-modified messenger RNA that induced a strong T_{reg} response and prevented EAE in mice.[121]

Immunotherapy with Immunoglobulins

IgG antibodies against LDL and ApoB are detectable in untreated mice and humans, correlate with atherosclerotic disease, and rise after vaccination with LDL/ApoB.[37,42,51,72–74,76,107,122,123] Whether they exhibit a biological activity is uncertain. The generation of antibodies against peptides of ApoB in the inner core of LDL particles, however, renders it unlikely that these may physically interact with intact LDL particles. Instead, they may serve as biomarkers of vaccination efficacy. In addition, preclinical evidence has suggested both pro- and anti-atherogenic outcomes after passive vaccination with some IgG antibodies.[113,124–126] On the contrary, most IgM antibodies are naturally occurring and target oxidation-specific epitopes with a convincing anti-atherogenic function.[42] Immunotherapy with injectable immunoglobulins has been tested only in humans with an IgG1 antibody targeting the oxidation-specific epitope p45 of ApoB (anti-oxLDL, MLDL1278A). This antibody was effective in mouse atherosclerosis[125,126] and in primates.[127] In the multicenter, randomized phase-II GLACIER trial (Goal of Oxidized LDL and Activated Macrophage Inhibition by Exposure to a Recombinant Antibody), MLDL1278A was tested for its efficacy to reduce arterial wall inflammation quantified by positron emission tomography (PET) imaging with ^{18}F-fluorodeoxyglucose (FDG). MLDL1278A failed to reach its primary endpoint, but many methodological weaknesses render the interpretation of GLACIER results difficult.[128] In conclusion, it is still under debate whether IgG immunotherapy works in humans.

TCR/CAR T-Cell Immunotherapy

T cells expressing a chimeric antigen receptor (CAR T cells) or a transgenic T cell receptor have recently emerged in cancer immunology. CAR T cells are constructed to express an engineered antigen receptor, often a single-chain variable fragment (scFv) from antibodies that specifically recognizes a specific antigen on cancer cells. $CD8^+$ cytotoxic T cells engineered to express a CAR can therefore be guided to neutralize cancer cells after an adoptive transfer. Similarly, TCR T cell immunotherapy bases on T cells expressing a transgenic TCR with a certain specificity.[129] CAR T cell therapy has been approved for leukemia and lymphoma in humans.[130] Recently, it was shown that cardiac fibrosis can be prevented by redirecting $CD8^+$ T cells to attack cardiac fibroblasts in mice.[131] Because ApoB-reactive T_{reg} cells prevent murine atherosclerosis after an adoptive transfer,[27] it is plausible to speculate that the infusion of engineered T_{reg} cells with an ApoB-reactive TCR (TCR immunotherapy) or a CAR recognizing plaque-specific proteins (CAR T-cell immunotherapy) would be effective in treating atherosclerosis in future. However, the identification of atherosclerosis-specific ligands for CAR and HLA-adapted ApoB-specific TCRs in humans remains a future challenge.

Conclusion

The recent introduction of novel LDL-lowering strategies (PCSK9), new anti-inflammatory treatments (IL-1β blockade by monoclonal antibodies), and novel therapies to address the

excessive risk in patients with diabetes mellitus (SGLT-2 inhibition, GLP-1 agonism) has greatly improved medical strategies in atherosclerosis and established a novel path for precision medicine in cardiology. In addition to excessive inflammatory or metabolic risk, solid evidence from numerous preclinical and clinical studies has identified a substantial role for an immune-related pathogenesis of atherosclerosis with a humoral and cellular response against autoantigens. LDL and ApoB have been in the center of investigations in the last decades, but the exact immunodominant epitopes and potential other antigens are still unknown. "Vaccination against atherosclerosis" has emerged as a novel type of immunomodulation that holds the promise of causality, minimal side-effects, and cost-effectiveness. Several major concepts have emerged as vaccination-based therapies: (1) passive immunization with exogenous IgG antibodies that make use of naturally occurring immunoglobulins. Albeit this type of immunotherapy has advanced into the clinical stage, early clinical results were negative; (2) vaccines that are designed to neutralize a pro-atherogenic protein by endogenously produced IgG antibodies, such as PCSK9, CETP, or ALDH4A1. These therapies, however, offer no substantial advantage over existing or future biologicals that are administered by injection; (3) tolerogenic vaccination to weaken the existing pathogenic immune response against the naturally occurring autoantigens LDL and ApoB. While this path is the most existing one and offers a truly preventative vaccination strategy, the conceptual framework, antigens, doses, routes, and vaccination pipelines will have to be clarified in future. Even if this type of vaccination will not succeed, current advances in understanding the adaptive immune response in atherosclerosis have already opened several new avenues. These strategies focus on promoting the pool of T_{reg} cells with injectable IL-2 [132] or on an exogenous propagation of antigen-specific T_{reg} cells for TCR- or CAR-T-cell immunotherapy.

Funding

This project has received funding from the European Research Council (ERC) under the European Union's Horizon 2020 research and innovation program (grant agreement no. 853425). T.M. and D.W. are members of the SFB1425, funded by the Deutsche Forschungsgemeinschaft (DFG, German Research Foundation).

Conflict of Interest

The authors declare no potential conflict of interest.

D.W., T.M., and T.A. report support for the present manuscript (e.g., funding, provision of study materials, medical writing, and article processing charges) from the European Research Council (ERC) for European Union's Horizon 2020 research and innovation program (grant agreement no. 853425) and from Deutsche Forschungsgemeinschaft for SFB1425 (D.W. and T.M. are members).

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