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Review Article

Vaccination against atherosclerosis: An overview

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ABSTRACT

Atherosclerosis, an inflammatory disorder involving innate and adaptive immune responses with both atheroprotective and proatherogenic roles, is a life wasting and economic demanding disorder that continues to be the leading cause of morbidity and mortality worldwide. Thus, the need for a long-lasting and highly effective treatment has made researchers to find new strategies. Many efforts made thus far to reduce the burden of the disease have been toward the modification of cardiovascular risk factors.

Vaccination against atherosclerosis has been investigated as a promising strategy to overcome the disorder. Several kinds of vaccination methods have been investigated mostly in mice, with promising results in the attenuation of atherosclerosis, inflammation, and lipid concentration. The most conflicting part of this strategy is finding appropriate antigens and adjuvants. Some antigens have been used, including OxLDL, apoB100, CETP, PCSK9, HSP60, MHC-II-derived peptides, and interleukins. The DNA-based vaccination method has opened a new window in this field. There is an increasing necessity for developing an effective, economical, long-lasting, accessible, and convenient vaccination method. There are large gaps in evidence for the selection of proper human sampling to test the vaccines, route of delivery, safety, strength, scheduling, and side effects, all of which must be considered in clinical trials in the future.

1. Background

Atherosclerosis is a chronic inflammatory condition in which arterial walls are stiffened by the atherosclerotic plaque formation.[1,2]

Atherosclerosis, as a multifactorial, noncommunicable disease (NCD), has always been a major and leading cause of death worldwide. Cardiovascular disease (CVD) including ischemic heart disease and cerebrovascular disease, the two major causes of disability, is estimated to be the cause of 24% of NCD-related disease-adjusted life years (DALYs) worldwide.[3]

In addition, CVDs impose economic loss in all countries, with more significant effect in low and middle-income countries. Actually, CVDs affect working-age individuals in low and middle-income countries as compared to those in high-income countries.[4]

To date, most of the attempts to reduce the burden of atherosclerosis have been made toward the regulation of different modifiable risk factors. Despite considerable endeavors regarding the reduction of the load of CVDs, the noticeable morbidity, mortality, and loss of resources still exist, which promote investigators to search for new methods to overcome it.

Both innate and adaptive immune responses are involved in the process of atherosclerosis as a chronic inflammatory, autoimmune-like disease, which is initiated by lipid accumulation and inflammatory processes.[5–7,196]

Vaccination, which is a principal method of prevention to create a desired immune response against antigens, has been used for several bacterial and viral diseases for many years. However, this technique has also been used as a preventive method to avert some autoimmune diseases in humans.[8–10]

During the recent years, vaccines have been developed for NCDs such as cancer,[11] atherosclerosis,[12] hypertension,[13] Alzheimer's disease,[14] and diabetes[15] and successfully applied in some autoimmune diseases.[16,17]

However, with regard to atherosclerosis, this method is not as simple and easy as it looks at the first glance. Atherosclerosis is a multifactorial, chronic, and complex process that begins in childhood and progresses with time. Vaccination can be a tempting, an alternative, and, probably, a promising and exciting approach to reduce the load of the disease.

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One of the first studies in this regard was conducted by Palinski and his colleagues in 1995. Malondialdehyde (MDA)-modified lysine is an epitope of oxidized LDL (OxLDL), and a low level of circulating autoantibodies are generated against which OxLDL in some species. Immune complexes between these autoantibodies and OxLDL are present in the lesions. Immunization with MDA-LDL generated high titers of antibodies with a similar specificity as that of natural autoantibodies. Immunized animals showed a significant reduction in atherosclerosis compared with control animals. Thus, the results of this study suggested that the immune system played an important role in the process of atherosclerosis, and consequently, a new window was opened in this area.[18]

Another study by Ameli and his fellow researchers in 1996 showed that immunization with LDL and OxLDL reduced atherosclerotic lesions in the proximal aorta of rabbits by 74% and 48%, respectively.[19]

During years, thereafter, several studies have been conducted with hope to determine the best immunization method against atherosclerosis. Despite substantial efforts made in this field, vaccination against atherosclerosis is still in its infancy. It appears that the most complicated step in this zone is the finding a proper atherogenesis-related antigen.

1.1. Pathogenesis of atherosclerosis

1.1.1. Endothelium and early phase of atherosclerosis

During the past 25 years, it has been apparent that endothelium is not just a simple layer of cells that just operate as a barrier but a multifunctional, dynamic, and complex organ whose healthy status is essential to impede the free passage of molecules and cells to the underlying interstitial layer of the arterial walls.[20]

A healthy endothelium is able to produce a wide variety of factors in response to physical and chemical signals that regulate vascular tone, cellular adhesions, thrombus resistance, smooth muscle cell proliferation, and vessel wall inflammation.[20,21] In fact, a healthy endothelium does not normally attach white blood cells. Endothelial function could be negatively affected by a variety of stimuli such as OxLDL, free radicals produced by smoking, hypertension, diabetes, genetic factors, elevated homocysteine levels, and infections.[22] Among different markers, nitric oxide (NO), a particular mediator that causes endothelium-dependent vasodilatation having anti-inflammatory and antithrombotic properties, is the earliest and most significant factor whose function is altered during the early phase of the process of atherosclerosis.[23]

NO synthesis is modulated by asymmetric dimethylarginine (ADMA), an endogenous inhibitor of nitric oxide synthase (NOS), which catalyzes the formation of NO from L-arginine; therefore, it is an atherosclerosis risk factor.[198]

Increased expression of the receptor of endothelin 1 (ET-1), a vasoconstrictor substance that is in strict balance with NO,[24] has been detected in human atherosclerotic plaques.[25]

In atherosclerosis-promoting circumstances, endothelial cells begin to express adhesion molecules such as P-selectin, E-selectin, intercellular adhesion molecules (ICAMs), and vascular cell adhesion molecule (VCAMs), which act as receptors for integrins and glycoconjugates present on the monocytes and T-cells.[26] Leukocytes decelerate through interaction with P-selectin and E-selectin. The interface between VCAM-1 and ICAM-1 with very late antigen-4 (VLA-4) and lymphocyte function-associated antigen-1 (LFA-1), respectively, leads to a stronger adhesion of leukocytes.[27–29]

In the next step, leukocytes migrate through the inter-endothelial junction into the sub-endothelial space, a process called diapedesis. This process is facilitated by several adhesion molecules such as platelet/endothelial adhesion molecule-1 (PEAM-1) and junctional adhesion molecule-1 (JAM-1), some chemokines and interleukins secreted by the activated endothelial cells. Among chemokines, the two most significant chemoattractant for leukocytes are monocyte chemoattractant protein-1 (MCP-1), which is responsible for the migration of monocytes into the intima, and T cell chemoattractant, which supports the penetration of lymphocytes into the intima.[30]

Once monocytes enter inside the intima, they are converted to macrophages and express scavenger receptors with the mediation of macrophage colony-stimulating factor (M-CSF), which is produced by activated endothelial cells and smooth muscle cells (SMCs).[30,31] This factor facilitates phagocytosis of modified lipoproteins as well as multiplication and differentiation of monocytes into macrophage foam cells. All the processes explained above lead to the formation of the earliest feature of atherosclerosis, named fatty streak, which remains asymptomatic and might be reversible at this stage. This lesion could progress into more complex lesions during the following years in the presence of cardiovascular risk factors.[32]

1.1.2. Progression of atherosclerosis

Within the fatty streak, activated T cells produce some factors such as tumor necrosis factor (TNF)-β, γ-interferon (INF- γ), fibrogenic mediators, and growth factors promoting the migration and proliferation of SMCs and the production of a dense extracellular matrix around them, which is the characteristic of an advanced atherosclerotic lesion. On the other hand, medial SMCs express specialized enzymes that degrade the elastin and collagen in response to inflammatory stimulation easing the penetration of the SMCs through the internal elastic laminae and their passage to the subintimal area.[33]

The process of inflammation will continue by secretion of some factors by SMCs.[34] A vicious circle would be initiated by three components of the lesion (macrophage lipid, T-lymphocytes, and fibromuscular components) in which cell migration to the subintimal space, cell proliferation, and overproduction of fibrous tissue result in intimal thickening, intermediate lesions, and reorganization of the atheroma. These components secret proinflammatory mediators that mediate adhesion of leukocytes to the endothelium, continuation of inflammation, and progression of atherosclerosis.[35]

An advanced atherosclerotic lesion is characterized by the necrotic core, which contains apoptotic foam cells, cell debris, lipids, and necrotic tissues. The necrotic core is highly immunogenic and leads to recruitment of more inflammatory cells to the intima. The necrotic core is covered by a fibrous cap formed by SMCs and extracellular matrix proteins under the influence of the cytokines and growth factors from T cells and macrophages, which protects the lesion from blood flow[1] [See Fig. 1].

Progression of an atherosclerotic plaque will restrict the blood flow that would be compensated by an outward remodeling.[36]

Gradually, the outward remodeling will severely limit blood flow, leading to ischemia of distal tissues and finally revealing symptoms of angina.[37,38]

However, the role of inflammation is not limited in the stable phase of atherosclerotic plaque. It plays a key role in destabilization; rupture of the fibrous cap; thrombus formation of the plaque; and, ultimately, the occurrence of myocardial infarction (MI), fatal coronary thrombosis, and stroke.[38]

Activated T-cells may stimulate matrix metalloproteinase production by macrophages in the lesion, which leads to degradation of the collagen of the fibrous cap, resulting in plaque rupture.[39] Additionally, INF-ϒ produced by T lymphocytes might be able to halt collagen production by the SMCs, limiting the capacity of SMCs to restore the collagen that reinforces the cap.[40]

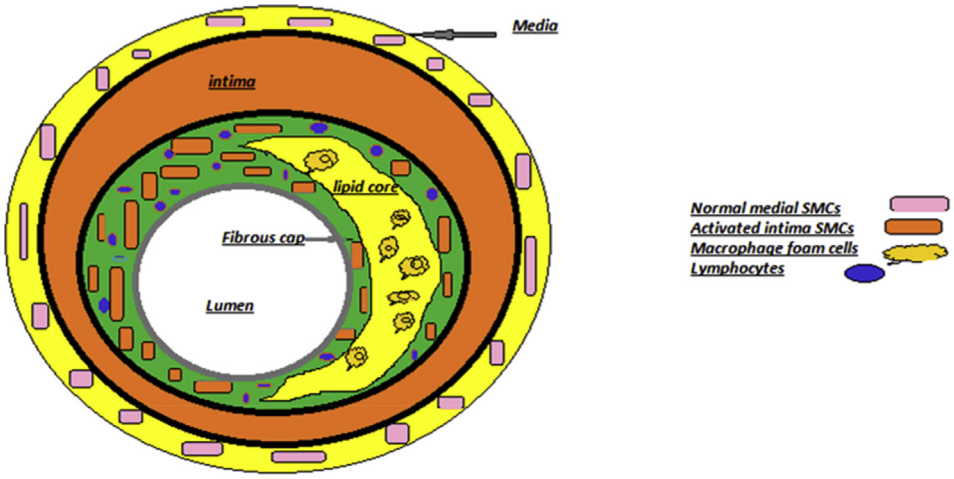

Fig. 1. Anatomy of atherosclerotic plaque. SMCs: Smooth Muscle Cells.

2. Role of Immune System in Atherosclerosis

2.1. Innate immune system

Innate immune response is the first line of protection for the host against pathogens, which is characterized by fast and blunt responses. It includes important immune cells such as neutrophils, mast cells, macrophages, and natural killer (NK) cells. Among these cells, neutrophils are probably involved in the later phase of atherosclerosis and have been found in the ruptured atherosclerotic plaques.[41]

Mast cells are activated in the sites of plaque rupture and are degranulated and exocytosed, resulting in the destabilization of the atherosclerotic plaque.[42,43]

In contrast, NK cells have been shown to play a role in the early phase of atherosclerosis; however, the precise role of these immune cells in the process of atherosclerosis has not been well understood.[32,44,45]

Macrophages are the key inflammatory cells of the innate immune system involved in the formation of atherosclerosis and send immune signals to the adaptive immune system.[46]

2.2. Adaptive immune system

Adaptive immune cells have been demonstrated to have a significant role in the process of atherosclerosis in some studies, in which nonfunctional adaptive immune cells in mice result in decreased lesion formation.[47]

In contrast to the innate immune system, the adaptive immune system is identified by a slower response and exerts a high specificity for its target. Several kinds of immune cells are involved in the adaptive immune system, such as dendritic cells (DCs), T cells, B cells, and macrophages. Most T cells present in the atherosclerotic lesions are CD3+, CD4+, and TCR αβ+.[48,49] CD8+ T cells are found in atherosclerotic lesions, but their role has not been well established. When T cells recognize a specific antigen, which is presented by an antigen-presenting cell (APC), an adaptive immune response against that specific antigen is initiated. All cells involved in the adaptive immune response demonstrate versatile effects, which augment and regulate the innate and adaptive immune cells.[32]

Some T cells within the atherosclerotic lesion might have a key role in producing interleukin 17 (IL-17), which is used in the process of vaccination. Among the population of T cells, T helper (Th)1, Th2, Th17, and regulatory T cells (Tregs) are important and related to vaccine production.[32] Polarizing cytokines such as IL-12 and IL-18 differentiate T cells into Th1 cells.[50,51] It has been demonstrated that Th1 cells are mainly found in atherosclerotic lesions and stimulate atherosclerosis by the production of INF-ϒ (a characteristic of Th1 cells) and TNF-α.[52,53] INF-ϒ stimulates the activation of macrophages and endothelial cells, which results in the production of more adhesion molecules, proinflammatory cytokines, and chemokines and thus leads to more T cell recruitment. INF-ϒ also promotes of protease production and inhibits collagen production, leading to plaque instability.[52]

The proinflammatory role of Th1 cells was demonstrated by Buono et al who observed a 75% reduction in lesion size in LDLr- and IFN-ϒ-deficient mice.[54]

On the contrary, Th2 cells produce the so-called "anti-atherosclerotic cytokines" such as IL-3, IL-4, IL-5, IL-9, IL-10, and IL-13,[7] which have inhibitory effects on Th1 cells.

Tregs, which have been recently recognized, exert their anti-inflammatory effects by recognition of specific autoantigens. They are a group of cells essential in maintaining immune homeostasis and preventing autoimmunity.[55,56]

Some studies suggest that Tregs are important in the prevention of Th1-mediated autoimmune diseases such as multiple sclerosis,[57] diabetes mellitus,[58] and atherosclerosis.[59]

Tregs are characterized by the expression of both CD4 and CD25 and subdivided into adaptive or inducible and natural Tregs. Adaptive Tregs develop from immature T cells in the periphery and can produce IL-10 and transforming growth factor (TGF)-β. While natural Tregs originate from the thymus as $CD4^{+}CD25^{+}$ cells and exert their suppressive function on foam cell formation in atherosclerotic lesions, they express Foxp3 as well, a member of the Forkhead/winged-helix family of transcription factors, which is essential for the development of Tregs.[60–63]

It has been shown that oral administration of atherosclerosis-related antigens (HSP60 and OxLDL) increases the number of Foxp3-expressing Tregs in several organs, which leads to a decrease in the development of atherosclerotic lesions in $LDLr^{-/-}$mice.[64,65] T. Van. Es et al established the protective role of $Foxp3^{+}$ Tregs in atherosclerosis and demonstrated that vaccination against $Foxp3^{+}$ Tregs aggravates atherosclerotic lesion formation.[32]

Th-17 cells, the newly recognized T cells, are proposed to have an important role in the process of atherosclerosis. The increase in Th-17 cells in patients with acute coronary syndrome has been described by Cheng X et al.[66] The most important interleukin produced by these cells is IL-17, which exerts various biological

effects on different cell types in conjunction with atherosclerosis, such as endothelial cells, vascular SMCs, and macrophages,[67–69] resulting in the production of pro-inflammatory cytokines, chemokines, and matrix metalloproteinase.[69,70]

However, according to the existing data, the role of IL-17 in CVD may vary in relation to the cell type producing IL-17 and the cytokine profile of the local microenvironment where IL-17 functions. Enhanced IL-17 production associated with increased IL-10 and reduced IFN-γ will most probably limit lesion development and promote plaque stability. On the contrary, the production of IL-17 and IFN-γ will most likely promote lesion development and instability.[71]

B cells, an important group of immune cells involved in the adaptive immune system, are fundamental in the production of antibodies against specific antigens.[72] A specific subpopulation of B cells, B1 cells, has been shown to have atheroprotective effects by producing natural immunoglobulin M (IgM) class antibodies.[73–75] On the other hand, depletion of B cells aggravates atherosclerosis.[76]

B2 cells and immunoglobulin G (IgG) antibodies have proatherogenic roles based on the most available data. However, the function of antibodies is not solely related to their isotypes but is affected by their specificities.[77]

DCs are the most potent APCs of the immune system and the key players in the regulation of the adaptive immune system.[78] In general, DCs have functionally been ascribed to play the main role in initiating antigen-specific adaptive immune responses and maintaining tolerance to self-antigens, whereas macrophages excel in phagocytotic processes.[79]

In fact, a low number of DCs are found in the intima of healthy but susceptible arteries to atherosclerosis and in the intima of vessels with the progression of atherosclerosis.[44,80]

Briefly, both innate and adaptive immune systems have significant roles in all stages of atherosclerosis; as the innate immune system kicks off atherosclerosis and the adaptive immune system continues the growth and the progression of the lesion.[32]

3. Vaccination

Despite all primary and secondary prevention strategies and treatment, the burden of ischemic cardiovascular conditions continues to increase to become a leading cause of morbidity, loss of useful life years, and mortality worldwide.[81] For this issue, researchers are trying to find alternative solutions.

One of these alternative solutions is vaccination. As atherosclerosis is an inflammatory disorder wherein the immune system has an active role in the initiation, progression, and complication of it, it would be attractive to consider specific strategies to target immune or inflammatory components as a novel approach against inflammation and atherosclerosis.[82]

An important difference between atherosclerosis and other chronic inflammatory diseases such as psoriasis and rheumatoid arthritis is that atherosclerosis develops at early ages where inflammation dominates the very beginning of the disease.[83]

One specific strategy targeting the immune components is modulation of the athero-promoting adaptive immune response. Given that immune activation during the process of atherogenesis is an unavoidable host response, modification of this response may be beneficial in atherosclerosis.[82]

A successful immunomodulatory therapeutic strategy must have at least one of the following points: 1) stop the growth of the atherosclerotic plaque; 2) total or partial regression of the plaque; 3) stabilize the plaque; and 4) diminish the inflammatory process associated with atherosclerotic plaque formation.[83]

Several approaches have been investigated to consider the possibility of inhibiting atherosclerosis by active immunization or directly providing antibodies against proteins related to the development of the disease.[84] The emerging strategies by which immune system modulation occurs make this concept that the development of a vaccine in the prevention and/or treatment of atherosclerosis would be a realistic approach.[2,85]

With regard to vaccination against atherosclerosis, there are two essential aspects:

1) Selective suppression of pro-atherogenic immune responses
2) Selective activation of anti-atherogenic immune responses[83]

Many trials conducting vaccination for atherosclerosis concentrate on a) the presence of pre-existing immune responses that are part of the pathologic process, such as immune responses against OxLDL epitopes and HSP 60 to empower them and b) the targets like the cholesteryl ester transfer protein (CETP) and TNF-α to produce neutralizing antibodies that inhibit the consequence of the preferred antigens[83] [See Table 2].

Because the identification of key antigens responsible for the activation of immune responses related to atherosclerosis is a prerequirement for any immunization therapy, it is challenging to find the suitable adjuvant and the most appropriate administration route.

3.1. Adjuvants

Adjuvants have been used in human vaccines for more than 90 years. They are substances added to vaccines to boost the immunogenicity of highly purified antigens that have inadequate immunostimulatory capacities. This unfairly neglected part of immunization has been tagged by Charles Janeway as “the dirty little secret of immunology”.[86]

Adjuvants elicit immune responses through one or more of the following mechanisms:

1) Sustained release of the antigen at the site of injection
2) Upregulation of cytokines and chemokines
3) Cellular recruitment at the site of injection
4) Increase in antigen uptake and presentation to APCs
5) Activation and maturation of APCs.[87]
6) Activation of inflammasomes, which are innate immune system receptors/sensors that regulate the activation of caspase-1 and induce inflammation in response to infectious microorganisms and molecules derived from host proteins.[88]

Commonly used adjuvants are Freund's adjuvant, which holds mineral oil and heat-killed mycobacterium and considerable amounts of HSPs, promoting adaptive immune response,[89] and aluminum salt adjuvant, which chiefly operates by complexion and preservation of antigens at the site of injection.[89]

Table 1
Most common practical antigens used in vaccination against atherosclerosis

Lipid-related antigens	Non-lipid-related antigens
OX-LDL and apoB100	MHC-II-derived peptides
Cholesteryl ester transfer protein (CETP)	Heat shock proteins
Proprotein convertase subtilisin/kexin type 9 (PCSK9)	*Streptococcus pneumoniae*
	Interleukins
	Vascular endothelial growth factor receptor 2 (VEGFR2)
	Fibronectin
	β2-Glycoprotein I
	C5a receptor

Table 2
A summary of some important studies of active immunization against atherosclerosis

Antigen	Animal	Route of administration	Adjuvant	Site of evaluation for atherosclerosis	Effect	Reference
Mixture of p143 and p210	ApoE$^{-/-}$ mice	NA	Aluminum (Pierce	descending aorta	Reduced	92
MDA-modified p45 or p74	ApoE$^{-/-}$ mice	NA	Aluminum (Pierce)	descending aorta	Reduced	93
p2	ApoE$^{-/-}$ mice	SQ followed by intraperitoneal	Aluminum (Pierce)	aorta	Reduced	94
p210	LDL-R$^{(-/-)}$/human apoB-100 transgenic mice	NA	Aluminum (Pierce)	descending aorta	Reduced	95
P210	APOE-/mice	Intranasal	CTB (p210-CTB fusion protein)	Aortic sinus	Reduced	96
p210	ApoE$^{-/-}$ mice	SQ	Alum (Pierce)	descending aorta	Reduced	97
Mixture of p210, MDA-p210, and p240 or p210 only	ApoE$^{-/-}$ mice	Continuous SQ	No adjuvant	aortic sinus	Reduced	98
p210	ApoE$^{-/-}$ mice	SQ	Aluminum (Pierce)	aorta	Reduced	99
ApoB$_{3501-3516}$ or ApoB$_{978-993}$	APOE-/mice	SQ followed by intraperitoneal	Freund's complete followed by incomplete adjuvant	aorta and aortic sinus	Reduced	100
Cuox-LDL or AGE-LDL	LDL-R$^{-/-}$ and APOE-/ mice	SQ	Aluminum (Pierce)	aorta and aortic sinus	Reduced	101
Cuox-LDL	ApoE$^{-/-}$ mice	Intranasal	None	aorta and aortic sinus	Reduced	102
Cuox-LDL	LDL-R$^{-/-}$ mice	IV delivery of oxLDL-pulsed dendritic cells	Dendritic cells	carotid artery	Reduced	103
MDA-LDL	ApoE$^{-/-}$ or apoE/CD4 double-knockout mice	SQ	Freund's complete followed by incomplete adjuvant		Reduced	104
Native LDL	ApoE$^{-/-}$ mice	SQ	IL-12	aortic sinus	Reduced	105
Plaque homogenate or MDA-LDL	ApoE$^{-/-}$ mice	Foot pad injection	Freund's complete followed by incomplete adjuvant	aortic sinus	Reduced	106
MDA-LDL	ApoE$^{-/-}$ mice	SQ	Freund's complete followed by incomplete adjuvant	aortic sinus	Reduced	107
Fc-CETP$_6$	NZW rabbits on a high-fat diet	SQ	complete and incomplete Freund's adjuvants	aorta	Reduced	108
HSP65-CETPC	Rabbit on a high-cholesterol diet	NA	Aluminum	aorta	Reduced	109
plasmid pCR-X8-HBc-CETP[a]	Rabbits on a high-cholesterol diet	IM	NA	aorta	Reduced	110
HCPTC	NZW rabbits on a high-cholesterol diet	Intranasal	NA	aorta	Reduced	111
CETP	Human	Injection[c]	Aluminum	NA	NE (Just for CETP function and anti-CETP antibody)	112
PCSK9-peptide	Wild-type (wt), LDLr−/− mice	SQ	NA	NA	NE (significantly reduced LDL)	113
PCSK9Qβ-003[b]	Balb/c mice and LDLR$^{+/-}$ mice	NA	NA	NA	NE (LDL, TC decreased and LDLR upregulated)	114
peptide (AFFITOPE®) with mimicry pattern of N-terminal epitope of PCSK9	APOE*3 Leiden.CETP mice on a western-type diet	SQ	NA	aorta	Reduced	115
PCSK9 displaying VLPs	Balb/c mice and Macaque	NA	incomplete Freund's adjuvant (mice) and Alhydrogel or no adjuvant (Macaque)	NA	NE (decreased TC, free cholesterol, phospholipids, and TGs)	150
Ep1.B (ApoE$_{239-252}$)[d]	Non-obese diabetic (NOD) wild-type mice	Intraperitoneal OR footpad injection	Incomplete Freund's adjuvant	NA	NE (induction of plasmacytoid dendritic cells (pDCs) promoted T_{reg} cell generation)	152
Ep1.B	ApoE null mice and Sprague Dawley rat	IV	NA	aorta and carotid artery	Reduced	151
Hsp65	NZW rabbits	Intranasal	NA	aorta	Reduced	161
IL-1α-C-Qβ[e]	APOE−/− on a western-type diet	Injection	NA	Aorta (Root and descending parts)	Reduced	169
IL12	LDLR−/− mice on a western-type diet	IM	oil-in-water emulsion adjuvant	Carotid artery	Reduced	51
IL15	LDLR−/− mice on a western-type diet	Oral	NA	carotid artery	Reduced	170
IL17	LDLr−/− mice	IM		Carotid artery	Reduced	32

Table 2 (*continued*)

Antigen	Animal	Route of administration	Adjuvant	Site of evaluation for atherosclerosis	Effect	Reference
VEGFR2 (DNA vaccine)	APOE$^{-/-}$ and mice/ LDLr−/−	Oral	NA	Carotid artery	Reduced (both initiation in LDLr−/− and progression in APOE−/− mice)	179
MDA-modified fibronectin	APOE$^{-/-}$ mice	Injection	Aluminum	Aorta and subvalvular area	Reduced	180
β2-glycoprotein I; IL-2; IL-10	LDLR$^{-/-}$ mice on a high-fat diet	IV	NA	Thoracic aorta	Reduced in β2-glycoprotein I group and ts protective effect enhanced with IL2, IL10, or both	187
C5a receptor	LDlr (tm1Her) Apob (tm2Sgy) mice[6] on a high-fat diet	Injection	Aluminum	Aorta sinus and descending aorta	Reduced	192

CTB, cholera toxin B; Cuox, copper oxidized; IL, interleukin; LDL, low-density lipoprotein; LDL-R, LDL receptor; MDA, malondialdehyde; NA, not applicable; NE, not evaluated; NZW, New Zealand White; oxLDL, oxidized LDL; SQ, subcutaneously; CETP, cholesteryl ester transfer protein; FC-CETP, fragment-crystalizable-CETP; HSP, heat shock protein; plasmid pCR-X8-HBc-CETP; HCPTC, Hsp65-CETP-PADRE-TT-CETP; PADRE, pan-HLA-DR-binding T-helper epitope; TT, tetanus toxin; PCSK9, Proprotein Convertase Subtilisin Kexin 9; PCSK9Qβ-003; VLPs, virus-like particles; Ep1.B; IL, interleukin IL-1α-C-Qβ; VEGFR2, vascular endothelial growth factor receptor2; DNA, deoxyribonucleic acid; C5a receptor, complement 5a receptor; LDlr (tm1Her)Apob(tm2Sgy).

[a] pCR-X8-HBc-CETP encoding a B-cell epitope of cholesteryl ester transfer protein (CETP) C-terminal fragment (CETPC) displayed by a Hepatitis B virus core (HBc) particle.

[b] A Qβ bacteriophage VLP-peptide-designated PCSK9Qβ-003 vaccine that elicits strong antibody responses against PCSK9.

[c] "Injection" is used whenever there is no specified type of injection in the main article.

[d] C-terminal ApoE-derived peptide, Ep1.B ($ApoE_{239-252}$).

[e] Full-length, native IL-1α chemically conjugated to virus-like particles derived from the bacteriophage Qβ.

[6] Mice homozygous for the LDLr (tm1Her) have elevated serum cholesterol level of 200-400 mg/dl and >2000 mg/dl when fed a high fat diet.

In fact, the outcome of vaccination is determined by the adjuvant along with the target.[90]

Although the finding of a suitable adjuvant might not be more important than the finding of a suitable antigen, it definitely has equal importance. Adjuvants favoring a shift toward an anti-inflammatory Th2 response, such as aluminum and Freund's incomplete adjuvant (which lacks mycobacterium), may be more preferable than adjuvants favoring Th1 responses.[89] A perfect vaccine must aim at restoring the self-tolerance to autoantigens like LDL and HSPs, reducing the inflammation, and balancing the pro- and antiatherogenic immune response[91] [See Table 2].

3.2. Finding suitable antigens

The most conflicting and challenging step in immunization against atherosclerosis has always been finding suitable antigens to induce sufficient protection against atherosclerosis [See Table 1].

3.2.1. Lipid-related antigens

3.2.1.1. OxLDL and apoB-100-derived peptides. AS LDL and other apoB100 containing lipoproteins are the principal offenders with the strongest causative link with atherothrombosis, antigens derived from lipoproteins have always been at the forefront of vaccine development.

LDL, in its native state, does not activate an immune response. LDL is a complex particle composed of a high-molecular-weight protein, apolipoprotein B-100 (ApoB100), neutral and polar lipids, and lipophilic antioxidants.[116]

OxLDL plays a crucial role in the development of atherosclerosis. Modification of LDL into its oxidized form occurs by several different mechanisms. One clinically relevant pathway is through myeloperoxidase (MPO) and its oxidant product hypochlorite (HOCl).[117] MPO seems to predict well in the vulnerable population and to correlate well with the severity of the disease.[118–120] In mice and humans, increased titers of autoantibodies against HOCl-OxLDL have been reported during atherogenesis.[121–124]

Anti-OxLDL antibodies are present in both healthy individuals and in patients with atherosclerosis.[125,126] Use of OxLDL immunization in animals has shown a positive correlation between high titers of anti-OxLDL antibodies and the extent of protection against atherosclerosis.[18,19,72] In one study, the high titers of anti-OxLDL antibody were inversely associated with the intima-media thickness of the carotid arteries in a healthy population, with no clinical signs of atherosclerosis.[127] These studies support the defensive role of anti-LDL antibodies in atherosclerosis, which seem to be native antibodies that deactivate OxLDL.[127,128]

In addition, the transfer of B cells from atherosclerotic apolipoprotein (Apo) E-knockout mice (ApoE−/−) to young, ApoE−/− mice protected the latter from developing advanced disease.[126] Passive administration of recombinant human antibodies against aldehyde-modified apolipoprotein B-100 peptide sequences was detected to hinder atherosclerosis in ApoE−/− mice.[129] These antibodies were found to modulate the development of fatty streaks as well as their progression to atherosclerotic plaque.[130]

Actually, a positive correlation between serum OxLDL concentration and the severity of coronary artery disease (CAD), on the one hand, and acute coronary syndrome, on the other hand, has been demonstrated in several studies.[23,35,119]

It seems that antibodies to lipoproteins have both pro-atherogenic and protective roles against atherosclerosis. In the human body, a natural IgM antibody recognizing the epitopes in OxLDL and phosphorylcholine (PC) headgroups on the surface of apoptotic cells inhibits the uptake of OxLDL and apoptotic cells by macrophages, as passive immunization with a natural IgM antibody directed to HOCl-OxLDL reduced atherosclerotic development in mice.[121–124]

On the other hand, the generation of IgG antibodies and its predominance over IgM antibodies favors the formation of IgG-containing immune complexes with proinflammatory properties. Immune complexes formed with modified LDL and IgG antibodies have been reported to have stronger pro-atherogenic and proinflammatory properties than the modified LDL itself.[131–134]

Indeed, IgG1 and IgG3 antibodies have capabilities to activate the complement system and to interact with Fcγ receptors in phagocytic cells, and thus, they have been identified as proinflammatory antibodies.[18] However, there are few studies showing

negative or no correlation between anti-LDL antibodies and atherosclerosis.[135,136]

Anti-OxLDL antibodies can induce adaptive immune response, leading to inflammation. Different subclasses of anti-OxLDL antibodies with a range of pathogenic effects are reported in humans, which are the striking candidates to investigate immune modulation.[137]

Two major subclasses of OxLDL antigens have been used for immunization against atherosclerosis and have been considered to be atheroprotective: specific MDA-modified peptide sequences in apoB-100 and oxidized phospholipids containing a PC head group, present either as isolated lipid or as covalently bound to an apoB-100 peptide sequence.[19,64,92,138]

In the case of active immunization, Fredrickson and his colleagues, in collaboration with Dr. Jan Nilsson's laboratory at Lund University in Sweden, made a broad effort to spot potential antigenic epitopes within apoB-100, the major protein component of LDL, and other atherogenic lipoproteins, which could then be used as antigens to achieve atheroprotective effects through immunization. They screened the entire 4,536-amino acid human apoB-100 protein and designed a library of 302 peptides spanning the entire apoB-100 sequence. They selected 102 peptides based on the humoral immune response detected in pooled human plasma as potential candidates for the next round of screening.[92] Later studies disclosed that use of immunoreactive peptides including p2, p143, and p210 in vaccine formulation resulted in a 40%-70% decrease in atherosclerosis and reduction in plaque inflammation in hypercholesterolemic mice.[94,184] Later on, P210 had been more frequently used as a prototype antigen in vaccine formulations owing to its steady atheroprotective effects.[95,96,99]

In one study, immunization with the p210 vaccine resulted in a significant amelioration of aortic atherosclerosis compared with the control group in a murine model of atherosclerosis. In this study, CD8+ T-cells were activated by p210 vaccine, and it was demonstrated that adoptively transferring CD8+ T-cells from p210-immunized mice into nonimmunized mice reiterated the atheroprotective effect of active immunization, confirming that CD8+ T-cells mediate the atheroprotective effect of the p210 vaccine.[99] However, the mechanism of atheroprotection seen in these studies remains unexplained.[139]

3.2.1.2. CETP. CETP is a hydrophobic glycoprotein secreted in the liver and circulates in plasma, bound mainly to high density lipoprotein (HDL). CETP augments the rate of reverse cholesterol transfer (RCT), a process by which cholesterol is removed from tissues and delivered to the liver for excretion from the body. In fact, it endorses the redistribution of cholesteryl esters, triglyceride (TG), and, to a lesser extent, phospholipids between plasma lipoproteins. The pickup of lipids from lipoprotein particles and their drop off in other lipoproteins by CETP maintains an equilibration of lipids between lipoprotein fractions. From this point of view, CETP seems to be an atheroprotective factor.[140,141] On the other hand, CETP-mediated transfer of cholesteryl esters from HDL could result in a reduction in the cholesterol content, the apoA-I content, and the size of HDL particles. Consequently, CETP redistributes cholesteryl esters from the non-atherogenic HDL to the potentially atherogenic VLDL/LDL, implying that it may also be a pro-atherogenic factor.[141]

Given this controversy, the results of animal and human studies in which CETP inhibitors are used as treatment for atherosclerosis showed conflicting results as well.

For instance, immunization of rabbits against CETP-induced neutralizing antibodies obviously increased HDL-cholestero (HDL-C) levels, which was linked to reduced atherosclerosis.[111]

In another study, nasal immunization of rabbits with a vaccine targeting both CETP and HSP65 has also been identified to reduce aortic atherosclerosis.[109,110,142]

Phase I human trial shows neither consistent induction of CETP antibody nor significant changes in CETP function or HDL levels with CETP immunization.[112]

The ILLUSTRATE (Investigation of Lipid Level Management Using Coronary Ultrasound to Assess Reduction of Atherosclerosis) study was conducted for a global analysis of patients taking torcetrapib, the first small-molecule inhibitor of CETP, showed that the majority of them demonstrated no regressions of coronary atherosclerosis.[143]

However, in a post hoc analysis, a significant regression of coronary atherosclerosis was detected in patients in the highest HDL-C quartile.[144]

Additionally, torcetrapib was tested in the ILLUMINATE (Investigation of Lipid Level Management to Understand its Impact in Atherosclerotic Events), a large-scale phase 3 end point trial. Male and female patients between the ages of 45 and 75 years with a history of CVD (n = 15,067) were randomly assigned to receive atorvastatin plus torcetrapib or atorvastatin plus placebo. The study was interrupted earlier than anticipated because of an excess of deaths and cardiovascular events in the group receiving torcetrapib. CVD, cancer, and infection were the main causes of death.[140]

Because of the undesired adverse effect of torcetrapib in the ILLUMINATE trial, it raised the suspicion that there are some unnoticed aspects regarding the CETP function. Later studies found novel functions of CETP, such as protection against lipoprotein oxidation, anti-inflammatory activity, and anti-adipogenic actions.[145]

3.2.1.3. PCSK9. The proprotein convertase subtilisin/kexin type 9 (PCSK9) is primarily secreted in the liver and binds to the LDL receptor, thereby prohibiting it from recycling to the cell surface to take up more LDL particles and leading to increase in the circulating level of LDL-C.[146] It has been suggested that there is a strong correlation between PCSK9 levels and future cardiovascular risk.[197] During the recent years, PCSK9 has emerged as a promising therapeutic target for the treatment of hypercholesterolemia and atherosclerosis.

In normal circumstances, the LDL/LDL-R complex is endocytosed by endosomes. The affinity of LDL for LDL-R is decreased by the acidic pH of endosome, hence aiding in recycling of LDL-R back to the plasma membrane. PCSK9 binding inhibits this rearrangement and locks the LDL-R, thereby preventing its recycling, and hence, it is directed to lysosomes for degradation.[147,148]

Immunization against PCSK9 has been developed by passive immunization through administration of PCSK9 monoclonal antibodies, which results in reducing LDL cholesterol concentration. Alicrumab was approved by the FDA in July 2015 for adult patients with heterozygous familial hypercholesterolemia or in patients with clinically significant atherosclerotic CVD requiring additional LDL lowering after being on a diet control and maximally tolerated statin therapy. Evolocumab (Repatha) was also approved in August 2015 for use in adult patients with heterozygous familial hypercholesterolemia, homozygous familial hypercholesterolemia, or clinical atherosclerotic CVD requiring additional lowering of LDL-C after being on a controlled diet and maximally tolerated statin therapy. However, due to their short half-lives in vivo, they need to be injected intermittently, and this demands a high cost.[149]

Given the need for frequent administrations of anti-PCSK9 monoclonal antibodies at high doses, which may be associated with side effects, poor drug adherence, and high cost, active immunization against PCSK9 might be a promising approach to overcome the drawback of the passive immunization.

Some studies have shown that PCSK9 peptide-based vaccines have been able to elicit a PCSK9-specific antibody with a significant reduction of LDL-C.[113,150]

C. Landlinger and his colleagues investigated the effect of immunization of APOE*3Leiden.CETP mice on atherosclerosis with AT04A.[115] This peptide (AFFITOPE®) was previously designed to mimic the N-terminal epitope of the mature human and homolog mouse PCSK9 protein (153aa-692aa) and formulate into the AT04A vaccine.[113] Mice were immunized five times subcutaneously either with AT04A or with a control vaccine in biweekly intervals. The results showed a persistent and strong immune response induction and plasma PCSk9 reduction. Additionally, lesion area and number of lesions were significantly reduced in vaccinated mice compared with those of controls. Moreover, the necrotic core content of lesions, vascular inflammation, inflammatory biomarkers, LDL-C, and HDL-C all reduced in vaccinated mice.[115] It appears that the AT04A anti-PCSK9 vaccine would be an ideal therapeutic agent to accomplish the requirements for long-term LDL-C management because of its constant effectiveness and cost-effective application, along with anti-inflammatory effects. AT04A is currently being tested in a phase I clinical trial.[115]

3.2.2. Non-lipid-related antigens

3.2.2.1. MHC-II-derived peptides. Another kind of antigen derived from non-apoB100 proteins are peptides eluted from murine class II MHC molecules. Apolipoprotein E (ApoE) is a lipid transport protein with a vast range of functions in cellular responses to tissue injury, immune regulation, and cell growth. The isoforms of ApoE and ApoE deficiency are associated intimately with accelerated plaque growth. ApoE carries out its protective function through vascular alterations by the N-terminus of the protein that contributes to arterial protection, while the function of the C-terminus is only partially defined. Among peptides eluted, Ep1.B (239–252) was demonstrated to reduce early atherosclerosis when administered intravenously.[111] In fact, EP1.B (239-252) is the 14-amino acid C-terminal ApoE peptide that is a fragment of a naturally processed peptide (236-252) of murine ApoE. Ep1.B injection reduced neointimal hyperplasia after vascular surgery in rats and mice. When given during early plaque progression in ApoE-deficient mice, Ep1.B injections also prevented plaque growth. Treatment with Ep1.B, however, did not reduce established plaque growth in older mice.[151] Later, S. M. Bellemore and his colleagues demonstrated that Ep1.B treatment induced plasmacytoid DC (pDC). They showed that Ep1.B-induced pDCs promote the generation of Treg cells that stimulate the peripheral tolerance in adaptive immunity and potentially contribute its anti-atherogenic activity.[152]

3.2.2.2. HSPs. HSPs are a kind of proteins expressed at a high level in response to stress such as low level of oxygen or pH alteration. In these circumstances, HSPs repair denatured proteins or prevent them to be denatured and increase the cell's viability under stressful stimuli. HSPs are also reported to be linked to atherosclerosis.[152–156]

HSP antigen is marked on the surface of endothelial cells, mononuclear cells, and vascular SMCs in human atherosclerotic plaques.[157] Circulating antibodies against HSPs have been identified in patients with atherosclerosis, and HSP60-specific T cells were observed within the atherosclerotic plaques.[158,159] Studies conducting immunization with HSP65 have reported conflicting results, in part due to different adjuvants and different routes of administration.[160]

G. Foteinos et al showed that IV injection of anti–HSP antibodies derived from the blood of patients with coronary heart disease significantly increased atherosclerotic lesions in aorta after 8 weeks in the apolipoprotein E–deficient mice.[159]

On the other hand, mucosal administration of HSP-based vaccines attains a downmodulation of immune responses to specific antigens. For instance, intranasal vaccinations using either plasmid DNA encoding HSP65, whole protein HSP65, or both in phosphate-buffered saline (PBS) in rabbits induced HSP65 IgG responses, increased serum IL-10, reduced IFN-γ, and reduced atherosclerosis along with decreased cholesterol levels.[161] However, the effects of these three forms of HSP65 were different (the strongest for the HSP65 protein) and complicated, and thus, the exact mechanisms of action have remained to be established. Nonetheless, intranasal administration of the Hsp65 protein alone is an advisable way to interfere the development of atherosclerosis compared with the other two immunization methods.[161]

3.2.2.3. S. pneumoniae. It has been suggested that there is a molecular mimicry between *Streptococcus pneumoniae* (*S. pneumoniae*) and OxLDL. For the first time, C. J. Binder and his colleagues incidentally found that many autoantibodies to OxLDL derived from "naive" atherosclerotic mice share complete genetic and structural characteristics with antibodies from the classic anti-PC B-cell clone, T15, which have protective effects against common infectious pathogens including pneumococci. They immunized $LDLR^{-/-}$ mice with *S. pneumoniae*, thereby resulting in induction of the high circulating levels of OxLDL-specific T15 IgM-secreting B cells mainly in the spleen, which were cross-reactive with pneumococcal determinants. It was observed that pneumococcal immunization reduced the extent of atherosclerosis. The plasma of these mice had a superior capacity to block the binding of OxLDL to macrophages.[74,162,163]

Conversely, when it comes to reducing myocardial infarction or stroke as the endpoints, observational cohort studies in humans did not show consistent protective effects of pneumococcal vaccines.[164–167] However, in a systematic review and meta-analysis of cohort studies, it was observed that pneumococcal vaccination was associated with decreased risk of cardiovascular events and mortality. This benefit was higher in older age and the subjects with a high cardiovascular risk. The protective effect of vaccine decreased as the time elapsed from vaccination.[168]

3.2.2.4. Interleukins. IL-1α is a powerful proinflammatory cytokine that has been linked to the development of atherosclerosis. Vaccination against IL-1α was investigated by Tissot AC et al. They immunized ApoE-deficient mice with IL-1α vaccine and observed that vaccination resulted in a consistent and robust reduction in plaque load both at the root of the aorta, where atherosclerosis develops early due to disturbed flow patterns, and in the descending aorta, where lesions may develop later. Neutralization of IL-1α targets the key processes in atherogenesis by reduction in expression of cellular adhesion molecules and recruitment of monocytes and possibly other leukocytes to the plaque.[169]

IL-12 has been identified as a key inducer of Th1 cells, which is thought to contribute to the development of atherosclerosis. A. Hauer and his colleagues demonstrated that IL-12 vaccination resulted in the induction of anti-IL-12 antibodies, which functionally blocks the action of IL-12. IL-12 vaccination of LDLr (−/−) mice resulted in a significant reduction in atherosclerosis (68.5%) and improved plaque stability by increasing SMC and collagen content in the neointima.[51]

IL-15 is a pro-inflammatory cytokine involved in inflammatory diseases and expressed in atherosclerotic plaques. Van Es T et al demonstrated the upregulation of IL-15 within spleen and blood by hypercholesterolemia induced by Western type of diet in LDL-r-mice. They immunized mice by administration of live attenuated *Salmonella typhimurium* bacteria transformed with a eukaryotic expression vector encoding IL-15. They observed the killing of over-

expressing IL-15 cells and a 75% reduction in atherosclerotic lesion size as well.[170]

Another interleukin that is highly indicated to be involved in the process of atherosclerosis is IL-17. IL-17 exhibits pleiotropic effects on atheroma-associated cell types and induces the secretion of pro-inflammatory cytokines and chemokines.[32] The role of IL-17 in atherosclerosis was determined by the expression of IL-17 in atherosclerosis-prone mice, fed a Western type of diet. A significant 2-fold increase in the IL-17 gene expression in the spleen was observed three weeks after the start of the Western type of diet and continued to steadily increase to more than 3-fold at week 6 of feeding a Western type of diet. Subsequently, the expression of IL-17 rapidly decreased and declined to basal levels at 12 weeks of using a Western type of diet, indicating specifically the induction of IL-17 during the initiation of atherosclerosis. By vaccination of male LDLr−/− hypercholesterolemic mice against IL-17, a significant reduction of approximately 90.2% in plaque size was observed in carotid arteries, 59% in the aortic valve region, 87.5% reduction in the intima/media ratio, and 79.6% reduction in the intima/lumen ratio. Furthermore, the transplantation of IL-17-deficient bone marrow into LDLr−/− mice inhibited atherosclerosis by 50%.[32]

IL-27 is an interleukin consisting of two subunits (P28 and EBI3) with a contradictory but not fully recognized activity. It was first described as a proinflammatory cytokine with Th1-inducing activity. Nevertheless, following effort has demonstrated that mice deficient in IL-27 receptor (IL27Rα) showed aggravated inflammatory responses to a variety of challenges, suggesting that IL-27 has important immunoregulatory functions *in vivo*.[171–173] Furthermore, Goldberg et al suggested that vaccination against the P28 subunit of IL-27 resulted in suppressing experimental autoimmune encephalomyelitis and adjuvant-induced arthritis.[174,175]

IL-27 is related to the IL-12, IL-23, and IL-6 family, which are associated with atherosclerosis. IL-27 is a heterodimeric cytokine composed of Epstein-Barr virus-induced gene 3 (EBI3) and p28.[32] Thomas Van et al examined the expression of the two subunits of IL-27 in LDLr−/− mice, which received a western type of diet. They observed that the subunits of IL-27 were upregulated in early phases of atherosclerosis.

Then, they used DNA vaccine against the p28 subunit to block the function of IL-27 in mice. Vaccination against the P28 subunit of IL-27 showed a significant aggravation of atherosclerosis. This effect might provide an explanation for the antiatherogenic properties of the P28 subunit of IL-27, which could be exerted through the balance between Th17 cells and Treg cells. Vaccination against P28 altered this balance in favor of Th17 cells, causing atherosclerosis aggravation. However, vaccination against IL-27 did not show significant changes in plaque composition and collagen content.[32]

3.2.2.5. Oral DNA vaccination. The base of oral DNA vaccination is the target of some cell surface proteins considered to contribute to atherosclerosis. In this strategy, an expression plasmid that encodes the antigen transfers the genetic material from the carrier to host phagocytes in the gastrointestinal tract. The phagocytes then express the antigen de novo in the cytosol and present it on MHC molecules.[176]

Vascular endothelial growth factor receptor 2 (VEGFR2), which is not expressed on healthy arteries and veins, is strongly expressed both on endothelial cells during angiogenesis and on the luminal endothelium of human atherosclerotic vessels.[177] The interaction between VEGF and VEGFR2 is a key factor to pathologic angiogenesis of plaque stimulating, endothelial cell migration, and proliferation.[178]

One of the studies using the oral DNA vaccination method was performed by A.D. Hauer and his colleagues, who employed the live attenuated bacterium *S. typhimurium* containing a VEGFR2-encoding plasmid, resulting in the induction of a cytotoxic CD8+ T cell response against VEGFR2 over-expressing cells in atherosclerosis-prone mice. They showed that vaccination against VEGFR2 significantly reduced the initiation of atherosclerosis by 77% and reduced the progression of pre-existing atherosclerotic lesions in apoE$^{-/-}$ mice by 66%.[179]

3.2.2.6. Fibronectin. LDL retention in the arterial wall by extracellular matrix such as fibronectin is an early step in the development of atherosclerotic lesions. In this process, the interaction of the apoB-100 protein in LDL with extracellular matrix proteoglycans plays a key role.[180] Furthermore, mice expressing LDL-binding defective proteoglycan develop less atherosclerosis.[181,182] Extracellular matrix proteins such as collagen, laminin, and fibronectin have been shown to bind lipoproteins.[183,184] The oxidation of LDL is associated with aldehyde modification of surrounding extracellular matrix proteins such as fibronectin. MDA–fibronectin is present in human atherosclerotic plaques, and autoantibodies against MDA–fibronectin can also be detected in human plasma. In a prospective case–control study, it was found that antibodies against MDA-modified fibronectin were associated with a lower risk for cardiovascular events.[185] To investigate the functional role of these antibodies in atherosclerosis, Dunér P et al immunized apoE$^{-/-}$ mice with MDA-modified fibronectin. They showed that immunization with fibronectin formulated with aluminum as the adjuvant significantly reduced atherosclerosis in apoE−/− mice and was associated with increased Th2-type antibody production and Tregs.[180]

3.2.2.7. β2-Glycoprotein I. β2-Glycoprotein I (β2-GPI) is a highly glycosylated plasma protein that eagerly binds negatively charged surfaces and substances.[186] It serves as a target of antiphospholipid antibodies in patients with a procoagulant state and with associated immune disorders. It has also been shown to bind apoptotic cells, serving as a transporter regulator of cellular traffic and has also been implied in atherogenesis.[187]

Anti-β2-GPI has an increasing effect on the in vitro uptake of OxLDL by macrophages, which exacerbates atherosclerosis.[188]

Further, former studies have demonstrated the presence of β2-GPI within atherosclerotic lesions that stimulates an autoimmune response enhancing fatty streak formation in LDLr−/− mice that were previously transferred with β2-GPI-reactive lymphocytes.[186]

In a study conducted by J. De Haro et al, LDLR$^{-/-}$ mice were maintained on a high-fat diet for 8 weeks after vaccination against β2-GPI. Thoracic aorta thickening was significantly different between the immunized and control groups. The thickness of aortic wall in the vaccinated group was significantly less than the aortic wall thickness of the control group.[187]

Interestingly, the protective effect of immunization against β2-GPI on atherosclerosis was enhanced by the downregulation of the cellular and humoral autoimmune response provoked by a single injection of IL-2 and IL-10.[187]

3.2.2.8. C5a receptor. The anaphylatoxin C5a, generated by the activation of the innate immunity complement component C5, is a potent protein fragment. It has proinflammatory characteristics when it binds to the C5a receptor present in immune-inflammatory cells, including monocytes, macrophages, neutrophils, and T cells.[189]

Among the innate immune components, C5, C5a, and C5aR are abundant and proposed to play critical roles in atherogenesis.[190,191]

In a study to evaluate the effect of vaccination with C5aR in mice, X. Lu et al found that C5a or C5aR is expressed in the lesion sites of aorta sinus. Lesion size was significantly smaller in immunized mice. They also found that immunization with C5aR peptides

apparently reduced lesion development despite that the peptides had no effect on the expression of both C5a and C5aR, suggesting that immunization with C5aR peptides may affect the function of inflammatory cells rather than the expression of C5aR and C5a. They noted that in immunized mice, the C5a cannot effectively bind to C5aR expressed on inflammatory cells present in the lesions indicating a possible mechanism that C5aR antibody produced by immunization may occupy the site, which was for C5a binding on the surface of $C5aR^+$ cells in lesions, therefore blocking C5a binding to C5aR.[192]

3.2.2.9. Plant-based vaccination. The concept of utilizing transgenic plants to produce and deliver subunit vaccines was introduced by Dr. Arntzen and his colleagues and confirmed that this idea can overwhelm the constraints in traditional vaccine production.[193]

The plant-based vaccine has some advantages over the traditional vaccines. It does not require complex storage, and its production is cost-effective and easy to scale up for large production. Moreover, it is able to provide a needleless, convenient, and easy route of administration. Plants also have the ability to produce complex recombinant proteins in the correct form (folding and post-translational modification) and with the desired biological function.[193]

Plant-based vaccine production mainly involves the integration of transgene into the plant cells. The genes could be delivered by direct and indirect methods. The direct method simply means the direct introduction of DNA or RNA into the plant cells, whereas indirect gene delivery involves the employment of plant bacteria, particularly *Agrobacterium* species and plant viruses, which naturally infect the plant cells and are able to integrate the gene of interest into the plant genome.[193]

The plants commonly used as bioreactors are tobacco, potato, tomato, corn, and rice. To date, there are many transgenic plants that have been used to produce four different types of vaccines: bacterial vaccines, viral vaccines, parasite vaccines, and immunocontraceptive vaccines.[194]

Plant-based vaccine could also be a possible vaccination strategy for atherosclerosis, which might provide a new window in this field. The consideration of plant-based vaccines emanates from the strong feeling to the necessity for development of low-cost vaccination strategies as a main concern to reach robust platforms for large-scale vaccination programs, especially in developing countries.[195]

Plant-based vaccines could structure a raised area with a significant potential to influence the field of vaccination against atherosclerosis and could create a new tendency in the development of new vaccination models and eventually novel vaccines.[195]

4. Conclusion

The burden of atherosclerotic CVDs has made researchers to investigate more effective and long-lasting treatment strategies in this regard. Most of the efforts respecting immunization against atherosclerosis have been directed toward the empowering atheroprotective immunity and weakening the proatherogenic immunity. Despite the very appreciable efforts made in the field, vaccination against atherosclerosis has not still achieved a place in the prevention or treatment of atherosclerosis. Most of the studies have been performed in animals and very few studies conducted in humans. It might be the time to conduct some research studies in humans along with continuing research in animals. Whether the vaccination against atherosclerosis could reduce the size of atherosclerotic plaques in human has remained to be established. There is also a necessity to conduct studies to evaluate the effectiveness of vaccination on the prevention of the formation of atherosclerotic plaques at the very early stages. In addition, important challenges such as choice of formulation and route of delivery, vaccine safety and stability, schedule and durability of immunization, proper determination and monitoring of efficacy endpoints in clinical studies, potential side effects of immunization such as undesirable immune activation, and proper selection of population for testing, all need to be addressed in early safety trials.[160] However, there is a long distance to reach the ideal vaccination strategy, which could be utilized from early years of life to protect humans against atherosclerosis. It will not be achieved unless the investigators make inexhaustible attempts and funding organizations, governments and universities provide financial support to research.

Conflict of interest

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