Restoring Immune Tolerance in Atherosclerosis: Role of Regulatory Immune Response in Atheroprotection

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Abstract: Cardiovascular diseases remain the most significant cause of global mortality despite advances in medicine and new drug development. Atherosclerotic lesions start developing in childhood, progress over decades and manifest as coronary artery disease, stroke or peripheral arterial disease later in life. Chronic inflammation in the arterial wall mediated by altered immune response is vital during the development of atherosclerosis. Antigen specific immune therapy is an elegant approach to target disease related antigens. Immune tolerance to atherogenic self antigens has gained importance as an efficient therapy to control atherosclerosis in recent years. This review discusses the recent development and our understanding of immune tolerance in atherosclerosis and the role of regulatory immune response in protection against the disease.

Keywords: Atherosclerosis, Autoimmunity, Immune tolerance, Regulatory T cells.

INTRODUCTION

Cardiovascular diseases (CVD), including stroke and myocardial infarction are the major cause of global death and disability. The underlying cause of CVD is atherosclerosis, which is a multi-factorial, chronic inflammatory disease initiated by lipid accumulation [1]. Studies in the last two decades suggest that inflammation and immune response play an important role in the pathogenesis of the disease [2, 3]. The immune cells secrete pro inflammatory cytokines, proteases, and pro-thrombotic factors and play a crucial role in the formation and complication of atherosclerotic plaque [1, 4-6]. Accumulation of inflammatory cells and soluble mediators are characteristic of an unstable plaque which is prone to rupture and subsequent thrombosis [7]. Current therapies are targeted towards controlling the risk factors and are estimated to prevent only 30% of clinical events, suggesting an urgent need for newer therapeutic strategies [8]. Over the past decades chronic inflammation mediated by pathogenic T-cell response to endogenous antigens such as modified lipoproteins, β2 glycoprotein and heat shock protein (HSP), as well as exogenous antigens from pathogens including *Chlamydia pneumonia* and cytomegalovirus, have been implicated in the initiation of immune response during atherogenesis [9]. Thus antigen-specific immune modulation is an attractive therapeutic approach to treat atherosclerosis without affecting the normal immune function of the host. Immunotherapy for atherosclerosis is directed towards inducing tolerance to self-antigens by increasing the number of antigen-specific regulatory T cells (Tregs), which can suppress the pro-atherogenic immune response [10, 11]. These treatments for atherosclerosis have shown promising preclinical results, but have not yet entered clinical trials [12]. The major challenge in the treatment of cardiovascular disease is stabilization of vulnerable plaque. The rupture of these plaques results in coronary thrombosis, the main cause of unstable angina, acute myocardial infarction, and sudden cardiac death. It is now clear that biologic factors within the plaque play a critical role in plaque rupture rather than the plaque size or structure [13]. Although immune modulation has been studied extensively for preventing plaque formation very few studies report plaque stabilization or prevention of plaque rupture. The role of immune response in the disease process has been reviewed extensively and is this review will focus on the strategies for immune tolerance based therapy for atherosclerosis, and the role of regulatory immune response in reducing the burden of cardiovascular diseases.

IMMUNE SYSTEM AND INFLAMMATION IN ATHEROSCLEROSIS

Atherosclerosis was considered to be lipid mediated disease for a long time. Extensive research on the pathophysiology of the disease has resulted in a paradigm shift in our understanding of the disease development and its complication. Atherosclerosis is now accepted as a chronic inflammatory disease with the involvement of both innate and adaptive immune response in its development and progression [14-16]. Atherosclerotic plaques appear in the arterial intima as

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asymmetric focal thickening typically in the arterial curves and branching points, which experience higher shear stress due to disturbed blood flow [12]. It is characterized by the accumulation of lipids, macrophages and T cells in the sub endothelial region of the arteries leading to narrowing of the lumen. Early lesions contain fatty streaks composed of foam cells, lipid deposits and T lymphocytes (Figure **1**). As the lesion grows, other immune cells like dendritic cells mast cells macrophages accumulate which produce pro inflammatory mediators including interleukins (IL)-1, IL-2, IL-6, IL-8, IL-12, IL-10, tumor-necrosis factor, interferon-γ, and acute-phase reactants, such as serum amyloid A, fibrinogen and C-reactive protein [1, 16]. The infiltration of smooth muscle cells forms a fibrous cap around the lesion. Over a period of time apoptotic and necrotic cells along with cholesterol crystals and cell debris accumulate in the lesion forming a necrotic core. Enzymes produced by the macrophages in the lesion lead to thinning of the fibrous cap thus exposing the thrombogenic material in the core. This causes platelet aggregation, coagulation and formation of a thrombus, which manifests as severe ischemia, myocardial infarction and stroke requiring immediate medical attention. Recent evidence indicates hyperlipidaemia and myocardial injury induce innate immune response by activating toll like receptors (TLR) through pathogen-associated molecular patterns (PAMPs) [17, 18]. Engagement of TLR on immune and resident vascular cells generates pro-inflammatory cytokine release, lipid uptake, foam cell formation and activates cells of the adaptive immune system. Specific immune mechanisms may cause plaque instability and modulate platelet function and thrombus formation [19, 20]. Recent studies of atherosclerotic plaques from patients with acute coronary syndrome (ACS) as well as studies in animal models show the presence of activated macrophages, T cells and dendritic cells at sites prone to plaque rupture [21, 22].

INITIATION OF VASCULAR INFLAMMATION

Retention of cholesterol in the sub endothelial region of the vessel has been reported to be the central pathogenic event initiating atherosclerotic lesion

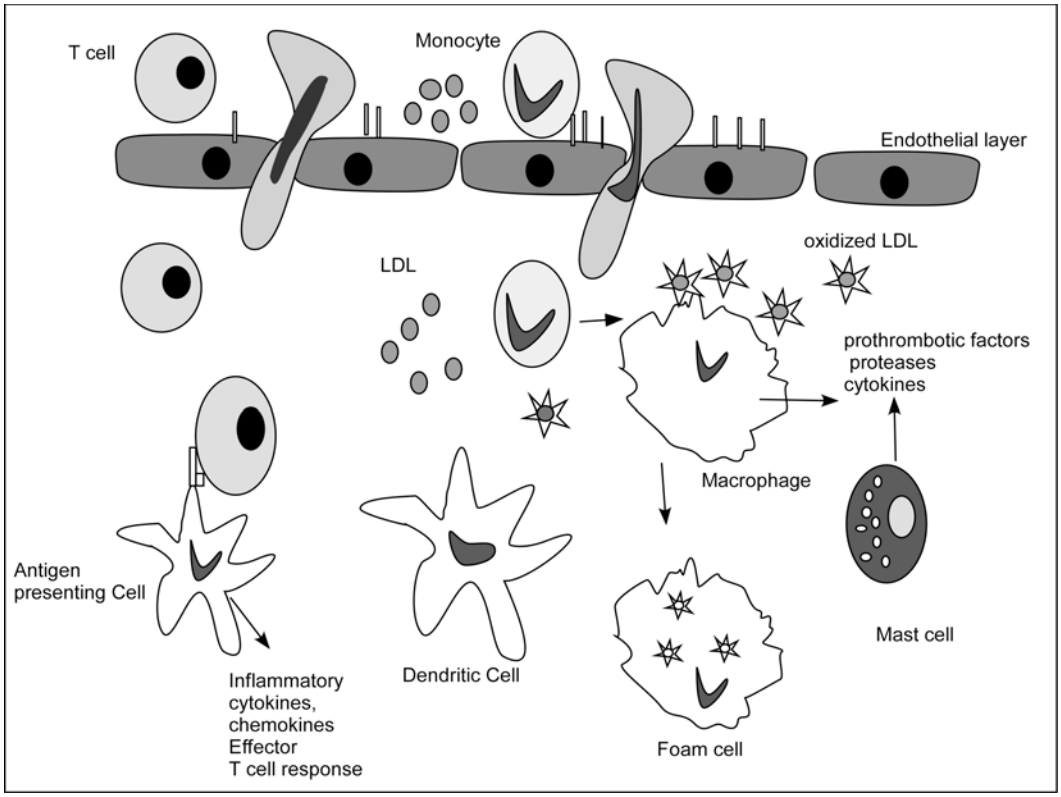

Figure 1: Immune cells in the early atherosclerotic plaque.

Atherosclerosis builds up in the intima, the innermost region of the artery. Several types of cells of the immune response are present throughout the atheroma including macrophages, T cells, mast cells and DCs. These cells secrete pro atherogenic inflammatory molecules which can recruit other immune cells to infiltrate into the plaque. As the plaque grows, necrotic cells and cholesterol crystals accumulate.

formation by several epidemiological studies [23]. A raise in plasma low density lipoprotein (LDL) levels results in increased accumulation of lipids in the arterial intima [24]. The interaction of positively charged apolipoprotein B (Apo B) carrying the LDL molecules with the negatively charged proteoglycans leads to retention of Apo B linked lipoproteins in the vessel wall [25]. These sequestered lipoproteins are susceptible to enzymatic cleavage, modification by oxidation, and aggregation [26]. Oxidized LDL induces the formation of foam cells and fatty streaks in the vessel wall which is the hallmark of initiation of atherosclerosis [27]. Immune response to modified lipoproteins drives the pathogenic evolution of the plaque by releasing pro inflammatory mediators leading to a chronic inflammatory reaction.

Apart from this, various classical risk factors of CVD can also induce vascular inflammation. The disturbed blood flow at arterial branches changes the hemodynamic conditions at these points, inducing endothelial stress. High arterial blood pressure, uncontrolled diabetes, smoking and microbial infection can also cause endothelial stress and damage leading to altered gene expression [28]. Activation of endothelial cells by any of these factors, increases the expression of heat shock proteins, adhesion molecules on the endothelial cell surface thus creating a pro inflammatory environment for the recruitment of monocytes, dendritic cells (DC) and lymphocytes into the intimal region [29]. Activated endothelial cells secrete macrophage colony stimulating factor which help in monocyte differentiation to macrophages. These macrophages engulf the oxidized lipid molecules to become foam cells that are characteristic of the atherosclerotic lesion. Activated T cells found in atherosclerotic lesion produce pro-atherogenic mediators and contribute to its growth and complications [9]. Vascular dendritic cells are found to be present within the tertiary lymphoid structures in the aortic adventitia of normal atherosclerotic vessels in children and animals and are thought to promote immune tolerance to auto antigens by regulating the T cell activation [12, 28]. However in the atherosclerotic lesion DCs are often seen as clustered with T cells suggesting their role in adaptive immune response and inflammation [30]. B cells and mast wells are fewer in the lesion but are found in the adventitial region of the atherosclerotic artery. Together these findings highlight the importance of innate and adaptive immunity and inflammation in the development and progression of the disease

ATHEROSCLEROSIS AS AN AUTOIMMUNE DISEASE

Autoimmune conditions, including rheumatoid arthritis, systemic lupus erythematosus and antiphospholipid syndrome, are characterized by enhanced atherosclerosis and consequently higher rates of cardiovascular mortality [31]. The autoimmune mechanisms in atherosclerosis are schematically represented Figure **2**. As discussed earlier, the activation of endothelium acts as a trigger for the development of atherosclerosis. Almost a decade back it was observed that auto immune response to cell surface antigens can also damage the endothelium similar to the action seen by other risk factors such as, such as hyperlipidaemia, smoking and disturbed blood flow [32]. The autoimmune concept of atherosclerosis emerges from the role of heat shock proteins (HSP) in the initiation and development of atherosclerosis. Adaptive immune response to bacterial HSP60 is generated in humans and animals in response to infection or vaccination protocols. Immune response to bacterial HSP60 may cross react with self protein due to the similarity between microbial and human HSP. The anti HSP60 immune response does not cause any damage under normal physiological conditions due to immune tolerance to autologous protein [14, 28]. HSP60 is normally a cytoplasmic protein but under stress it is expressed on the cell surface and can recognize the anti HSP60 antibodies [33]. Almost all the classical risk factors associated with CVD are known to induce the cell surface expression of HSP60 [28]. The cross reactive antibodies recognize stress induced cell surface expressed HSP60 and initiate a cascade of events leading to the development of atherosclerotic lesion [34, 35]. Several sero-epidemiological studies have shown that HSP60 auto antibodies are associated with the progression and severity of atherosclerosis [36-38]. Molecular mimicry between cytomegalovirus early proteins and HSP60 was demonstrated by Bason *et al.*, who showed that these cross reactive antibodies cause apoptosis of endothelial cells, a primary event in the pathogenesis of atherosclerosis [39]. Enhanced expression of HSP within the lesion has been reported in humans, rabbits and ApoE-/- mice [40] and HSP60-specific Th1 cells are found in lesions, which produce inflammatory cytokines like IFN-γ, IL12 and TNF-α [41]. Recently it was reported that HSP60-reactive T-cells can initiate atherosclerosis by recognizing atherogenic HSP60 epitopes in the intima [42]. The role of HSP60 in immune inflammatory reactions has been reviewed extensively by Gruntman *et al.* [14].

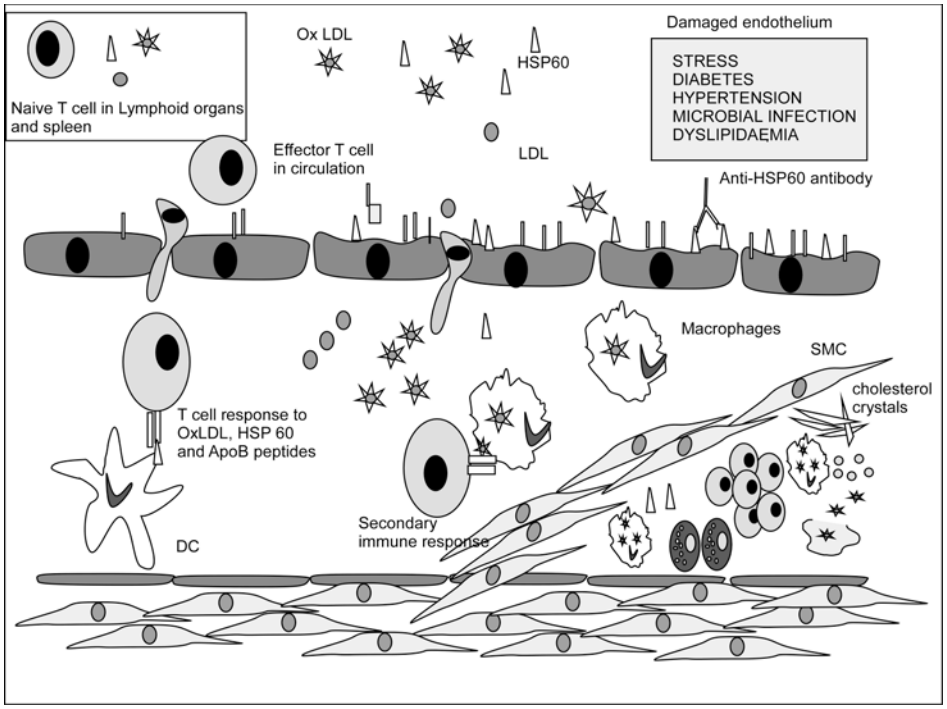

Figure 2: Autoimmune response in atherosclerosis.

Classical risk factors for cardiovascular diseases cause endothelial activation and higher expression of HSP60 and adhesion molecules on the endothelial cell surface. Hyperlipidemia increases the circulating concentration of LDL and its oxidation providing ideal condition for naïve T cell response to modified self antigens in the secondary lymphoid organs and spleen. Microbial infection can trigger autoimmune reaction by molecular mimicry. Cross-reactive antibodies to HSP60 act as stress factors and promote intimal infiltration by mononuclear cells. Primed T cells migrate into the intima of the arteries where they encounter the self antigens again thus eliciting a secondary immune response and release of pro inflammatory cytokines. As the lesion develops, macrophages and vascular smooth muscle cells migrate into the intima and form a fibrous cap around the lesion. As the lesion becomes more complex a necrotic core is formed which consists of apoptotic cells, cell debris, and cholesterol crystals, along with a fibrous cap that is infiltrated by activated T cells, macrophages, and mast cells that continue to release proinflammatory mediators. HSP, heat shock protein; oxLDL, oxidized LDL; SMC, smooth-muscle cell; DC, dendritic cell.

Apart from HSP60, antibodies to other self antigens such as oxidized LDL (ox-LDL), β2-glycoprotein-I (β2GPI), have also been demonstrated in atherosclerosis [43, 44]. The expression of β2GPI is observed in the within the sub-endothelial regions and in the intima–media layers at the border of human atherosclerotic plaques, supporting the assumption that β2GPI can serve as an autoantigen that promotes lesion progression [45]. Other pro-atherogenic properties of β2GPI include induction of monocyte adherence to endothelial cells, and acceleration of the influx of Ox-LDL into macrophages [46].

Oxidation of LDL degrades the apolipoprotein B (ApoB) into fragments resulting in the generation of new antigenic epitopes [47]. Some antigenic sequences that are normally masked become available after the oxidation of LDL and constitute a class of immunodominant antigens [48]. Although LDL is a self protein and the immune system develops a tolerance to this protein, oxidation exposes novel epitopes which can break the immune tolerance. Ox-LDL is reported to be present in sera of patients with coronary syndrome [49, 50] and also accumulates in atherosclerotic plaques [51]. Plasma ox-LDL levels are known to increase before the progression of atherosclerosis, suggesting its pathogenic role at the early stages of the disease [52]. Progression of the lesion is associated with reduction in plasma levels of ox- LDL and its accumulation in the lesion [53]. Increased levels of ox-LDL degradation products have also been observed in the urine of atherosclerosis patients compared to controls [54]. Ox-LDL-reactive T cells can be localized in plaques, lymph nodes, and in the plasma of

atherosclerosis patients and experimental animals [55]. Presence of auto-antibodies to ox-LDL has been reported in human and animal models of atherosclerosis [56, 57]. The prototypic and best-characterized antibody against ox-LDL is identical to T15, a natural antibody known to recognize phosphorylcholine expressed as a capsular epitopes on *Streptococcus pneumonia* [58]. Apart from this, cholesterol crystals, which are found in the necrotic cores, can activate inflammasomes in humans and in mouse, leading to interleukin-1β secretion [59, 60]. These results suggest that crystalline cholesterol can act as an independent signal to promote inflammation.

Other categories of self antigens may be expressed by apoptotic cells in the plaque. Recent studies have shown that failure to clear apoptotic cells has an impact on regulatory T cell function accelerates atherosclerosis suggesting a synergy between autoimmune diseases and atherosclerosis [61, 62].

IMMUNE TOLERANCE IN ATHEROSCLEROSIS

It is now well accepted that chronic inflammation mediated by a pathogenic immune response to endogenous antigens are responsible for atherogenesis [9]. Infections may also contribute to atherosclerosis through different mechanisms including direct effects on vascular cells, circulating cytokines and inflammatory mediators, activation of toll like receptors (TLR) as well as initiation of autoimmune reactions. The immune system generates regulatory T cells, which actively suppress immune activation and maintain immune homeostasis [63, 64]. An imbalance between pathogenic T cells producing proatherogenic mediators and Tregs with immunosuppressive properties is well established during the development of disease [15, 65, 66]. Thus, antigen-specific immune modulation is emerging as an attractive therapeutic option to control atherosclerosis [11, 67]. Immunotherapy is directed toward inducing tolerance to self-antigens which is mediated by either protective antibodies or by antigen-specific regulatory T cells [10, 11, 68-71]. An ideal immune therapy is aimed at restoring the self tolerance to auto antigens like LDL and heat shock proteins, reducing the inflammation, and balancing the pro and anti atherogenic immune response [65].

Over the last few years, considerable efforts have been made to develop an immune therapy using epitopes from lipoproteins and heat shock proteins [11, 72-75]. Table **1** summarizes the different approaches of immune modulation in atherosclerosis. Normally T cells reacting to self antigens get eliminated in the thymus by negative selection, leading to central tolerance [16]. Peripheral tolerance plays a role in maintaining an immune homeostasis to these self antigens under normal circumstances. The mucosal (intranasal or oral) route of administration is an effective method of inducing antigen-specific peripheral tolerance [76]. Oral administration of antigen leads to systemic unresponsiveness and represents a powerful tool for treating autoimmune and inflammatory diseases. Repeated administration of a low dose of antigen induces $CD4^+CD25^+$ $Foxp3^+$ Tregs and those secreting transforming growth factor β (TGF-β) or interleukin (IL)-10 [77]. $CD4+CD25^{+/high}$Foxp3+ regulatory T cells are a subpopulation of T cells, specialized in the suppression of pathogenic response from the immune system against self or foreign antigens. The anti inflammatory activity of Tregs is mediated by the cytokines TGF-β and IL10 [78]. Dendritic cells (DCs) play a major role in the development of antigen specific immune regulation to orally administered antigens [79]. Antigen-induced, tolerogenic $CD11c^+$, $CD11b^+$ DCs are shown to increase in payer's patches and confer protection against experimental arthritis by inducing regulatory T cells in mice [80]. Recent studies have shown that mucosal DCs expressing CD103 were able to induce $Foxp3^+$ Tregs in the presence of TGF-β [81].

Immunization against oxidized LDL has been shown to reduce atherosclerosis by a number of studies [68, 74, 82-88]. Immunization of hypercholesterolemic rabbits and $LDLr^{-/-}$ mice with both MDA-LDL and Cu-oxLDL were found to generate high titers of antibodies and inhibit atherosclerosis development by 40-70%, suggesting an induction of atheroprotective immune response [11, 57, 82, 89]. Induction of oral tolerance to Cu-oxLDL and MDA-LDL mediated by Treg cells and TGF-β were also reported to attenuate the initiation and progression of atherosclerosis in LDLr-/- mice [74]. Adoptive transfer of splenocytes from immunized mice as well as monoclonal antibody against ApoB peptide was found passively transfer protection [86]. Treatment with human recombinant IgG1 antibodies against the same epitope ameliorated the existing atherosclerotic lesions in ApoB-/- and LDLr-/- mice. The study also demonstrated reduction in macrophage MCP1 release leading to reduced inflammatory plaques and increased reverse cholesterol transport as a possible mechanism of protection [87]. Recently intranasal immunization with ApoB100 peptides was found to induce protective immune response mediated by antigen specific Treg cells. Intranasal immunization of Mycobacterium

Table 1: Comparison of Different Approaches of Immune Modulation in Atherosclerosis

S. No.	Mice strain	Antigen	Route	Reduction in lesion size	Reference
1	ApoB48/LDLr-/-	Recombinant multivalent antigen	Oral	47% in aortic root	Mundkur *et al.* *Int. J Cardiology* 2014
2	ApoB48/LDLr-/-	Recombinant multivalent antigen	SC	60% in aortic root	Lu *et al.* *Atherosclerosis* 2012
3	ApoB48/LDLr-/-	ApoB + HSP60 peptides	Oral	40% in aortic root	Mundkur *et al.*, *Plos One* 2013
4	ApoB48/LDLr-/-	ApoB + HSP60 peptides	SC	40% in aortic root	Lu *et al.* *Atherosclerosis* 2012
5	ApoE(-/-)	ApoB-100 fusion protein	Intranasal	35% in aortic root	Klingenberg *et al.*, *ATVB*, May 2010
6	ApoE-/-	Anti- CD3 antibodies	Oral	50% in aortic root	Naoto Sasaki, *Circulation* 2009
7	LDLr (-/-)	CD99-DNA vac	Oral	38% in Aortic root 69%, carotid artery	Van Wanrooij, *Cardiovasc. Res.*, 2008
8	LDLr (-/-) human ApoB 100 transgenic mice	ApoB peptides P45 and p210 + carrier catonized BSA with alum	IP	59-66 % in Descending aorta	G. N. Fredrikson, *J. Intern. Med.* 2008
9	New Zealand white rabbits	CETP-Chitosan Nano particles	Intranasal	57.9% in aortic sinus	Xiying Yuan, *Vaccine* 2008
10	LDLr (-/-)	VEGF receptor 2 (Flk1)-DNA vac	Oral	~ 10%	Petrovan, *ATVB*, 2007
11	ApoE-/-	S typhimurium DNA vaccine with murine VEGRF	Oral	25% in brachiocephalic artery	Arnaud D. Hauer, *Arterioscler. Thromb. ATVB*, 2007
12	LDLr (-/-)	HSP 60	Oral	80% in carotid artery and 27% in aortic root	G.H.M. van Puijvelde, *ATVB*, 2007
13	*Apobec-1_/_/LDLR_/_*	Antibody to modified LDL passive therapy	IP	50% in descending aorta	Alexandru Schiopu *et al.*- Nillson and PD Shah *J. Am. Coll. Cardiol.* 2007
14	ApoE-/-	Phosphoryl choline-KLH	IP	40% in aortic root	Giuseppina Caligiuri *et al.* 2007
15	LDLr (-/-)	Ox LDL	Oral	70% in carotid artery	G.H.M. van Puijvelde *Circulation* 2006
16	LDLr (-/-)	IL12	Intramuscular	68.5 % in carotid artery	Arnaud D. Hauer *Circulation* 2005
17	LDLr (-/-)	β2 glycoprotein 1	Oral	45% in aorta	Jacob George *et al.*, *Cardiovascular Research*, (2004)
18	LDLr (-/-)	Pneumococcal	Subcutaneous	En face 32%, aortic root 22%	Binder, *Nat. Med.*, 2003
19	LDLr (-/-)	Hsp65	Mucosal	~30% in aorta	Maron *et al.,* *Circulation*, 2002,

HSP65 in LDLr-/- mice was found to increase IL10 expression with significant reduction in the size of plaques as well as the number of T cells and macrophages in the plaque [73]. Induction of oral tolerance to HSP60 and a peptide derived from this protein (AA 253-268) were found to increase the number of regulatory T cells with increased production of IL10 and TGFβ resulting in a decrease in plaque size [75] opening a new strategy for the treatment of atherosclerosis.

STRATEGY FOR IMMUNE THERAPY FOR ATHEROSCLEROSIS

The understanding of the atherosclerosis pathology has evolved from being a lipid mediated disease to a complex inflammatory disease with an autoimmune etiology. In different autoimmune diseases, vaccination has proved to be an effective strategy to attenuate disease pathology, [90, 91]. However, the complication in atherosclerosis is that it is a multifactorial disease with several atherogenic antigens. Each molecule has

a distinct role to play in the initiation and progression of the disease. Can a multifaceted disease like atherosclerosis be treated by inducing immunological tolerance to a single peptide?

To address the multi-factorial, infection-mediated pathogenesis of atherosclerosis, we hypothesized that a multivalent vaccine containing epitopes from self antigens like, HSP60 and ApoB and infectious pathogens would give better protection. To test our hypothesis, we initially carried out efficacy studies with synthetic peptides and their combinations. Peptides were injected by subcutaneous route by rapid immunization method and our results showed that that combination of two peptides from ApoB 100 and HSP60 could reduce early atherosclerosis by 41.34 % in comparison with either ApoB peptide (14.66%) or hHSP60 peptide (22.15%) [92]. Mucosal tolerance to combination of ApoB and HSP60 peptides induced significantly higher level of protection compared to individual peptides further confirming our earlier observations. We also observed that tolerance to two different proteins induce atheroprotection by diverse mechanisms. While HSP60 tolerance resulted in increase in Treg cells and anti inflammatory cytokine secretion ApoB tolerance was effective in reducing the lipid deposition in the lesion [93].

Although we and other groups have shown promising results with ApoB100 and HSP60 peptides, translation of peptide-based vaccines from the preclinical phase to clinical trials has often been hampered by several issues. Peptides are known to have restricted immunogenicity due to low bioavailability. Free peptides often lack sufficient immunogenicity to evoke a regulatory immune response which is the key for mucosal tolerance based vaccines. To overcome these disadvantages of peptides, we have expressed multiple peptides in a single protein scaffold to generate a recombinant vaccine for atherosclerosis. Our preliminary results show up to 60 % reduction in early atherosclerosis in animal models [94]. We also observed that oral administration with multiantigenic construct induces atheroprotective immune tolerance to individual peptides in mice. Further evaluation of this molecule is in progress.

Atherosclerosis starts at an early age in humans and progresses slowly. Vaccination would be a successful approach to treat the disease provided that it has the ability to work at various stages of the disease progression. The most urgent unmet clinical need being responsible for 90% of deaths following an acute heart attack is related to rupture of an unstable plaque. Currently no treatments are available to prevent plaque rupture and stabilize the vulnerable plaque. Our studies show that immune tolerance to peptides can reduce the expression of plaque vulnerability markers suggesting that this approach can be developed to stabilize plaques [95]. From a clinical perspective, a vaccine will always be used as an adjunctive therapy along with lifestyle changes. We have observed that tolerance to combination of self peptides with diet control can prevent the progression of a established plaque in mice model [95]. Thus an effective strategy for immune therapy for atherosclerosis will be the one that can stabilize the plaque in addition to reducing plaque development.

MECHANISM OF PROTECTION

The immunotherapy of atherosclerosis is perused with an understanding that restoring the immune tolerance to self antigens will reduce inflammation and prevent the development of the disease [75, 96-98]. Antigen specific regulatory T cells have been shown to reduce vascular inflammation and prevent disease progression [66, 75, 98-100]. Recent reports have demonstrated an atheroprotective role of natural Treg (nTreg) cells expressing CD25 and the transcription factor Foxp3, which controls the expression of genes associated with regulatory function including cytotoxic T-lymphocyte antigen 4 (CTLA-4) and glucocorticoid-induced tumor necrosis factor receptor [66, 99]. Besides nTreg-adaptive regulatory cells, Tr1 and Th3 cells secreting IL-10 and TGF-β, respectively, have also been implicated in protection against atherogenesis [74, 96, 97]. Activation of regulatory cells and protective antibodies has been reported to be the key mediator of protection induced by vaccine.

Atherosclerotic mice immunized with ApoB peptides showed a marked reduction in atherosclerosis with a shift in the Th1/Th2 balance towards the more favorable Th2 response as measured by significant increases in Th2-specific IgG1 antibodies. Passive therapy with IgG1 antibodies against MDA-modified ApoB100 resulted in significant reductions in atherosclerosis, further corroborating these results [86, 87]. Later it was observed that immunization with ApoB100 peptides could also reduce the development of atherosclerosis in absence of altered antibody levels, suggesting other mechanisms were involved in protection [101]. The importance of Treg in atheroprotection was demonstrated when it was

observed that oral tolerance to oxidized low-density lipoprotein (LDL) in $LDL^{-/-}$ mice was associated with an increase in the number of $Foxp3^{+}$ cells in the spleen and lymph nodes, and increased TGF-β production [74]. Intranasal administration with ApoB100 p210 conjugated to cholera toxin B (CTB) was found to activate Treg cells and markedly reduce atherosclerosis in $ApoE^{-/-}$ mice [72]. We also observed an expansion of Treg cells with antigen-specific suppressor properties for both antigenic peptides following oral treatment with ApoB and HSP60 peptide [95]. Depletion of Treg cells completely abolished the atheroprotective effects of low subcutaneous doses of ApoB100 p210 vaccine demonstrating a direct evidence for the atheroprotective effects of Tregs [98]. Depletion of Tregs was reported to increase plasma cholesterol and an atherogenic lipoprotein profile with increased levels of VLDL and cholesterol-rich particles in the circulation. It was hypothesized that $FoxP3^{+}$ Tregs inhibit atherosclerosis by modulating lipoprotein metabolism in ApoE-/- mice [102]. In another study, myocardial infarction was induced in Foxp3DTR mice which allowed the depletion of Treg cells. The results showed an increase in infarct size with impaired resolution of inflammation and accumulation of neutrophils and Ly6C high monocytes in Treg depleted mice suggesting an important role played by Treg in atheroprotection [103].

TGF-β is a potent anti-atherogenic cytokine secreted by Treg cells and a key molecule contributing to peripheral tolerance [104]. Recent reports suggest that TGF-β–producing Th3 cells play a crucial role in inducing and maintaining peripheral tolerance by helping the differentiation of antigen-specific Foxp3+ cells in the periphery [105]. TGF-β can suppress the recruitment of macrophages into the lesion, uptake of oxidized LDL, formation of foam cells, and can activate Treg cells, phagocytosis, and collagen biosynthesis, thus resolving inflammation [106, 107].

Gut associated lymphoid organs are exposed to innumerable antigens and tolerance induction is a default immune response in the gut. Intestinal mucosa contains macrophages and DCs which play a key role in antigen uptake and regulation of mucosal immune response [108]. These tolerogenic DCs with non-inflammatory phenotype can activate Tregs, induce gut-homing receptors on responding cells and are

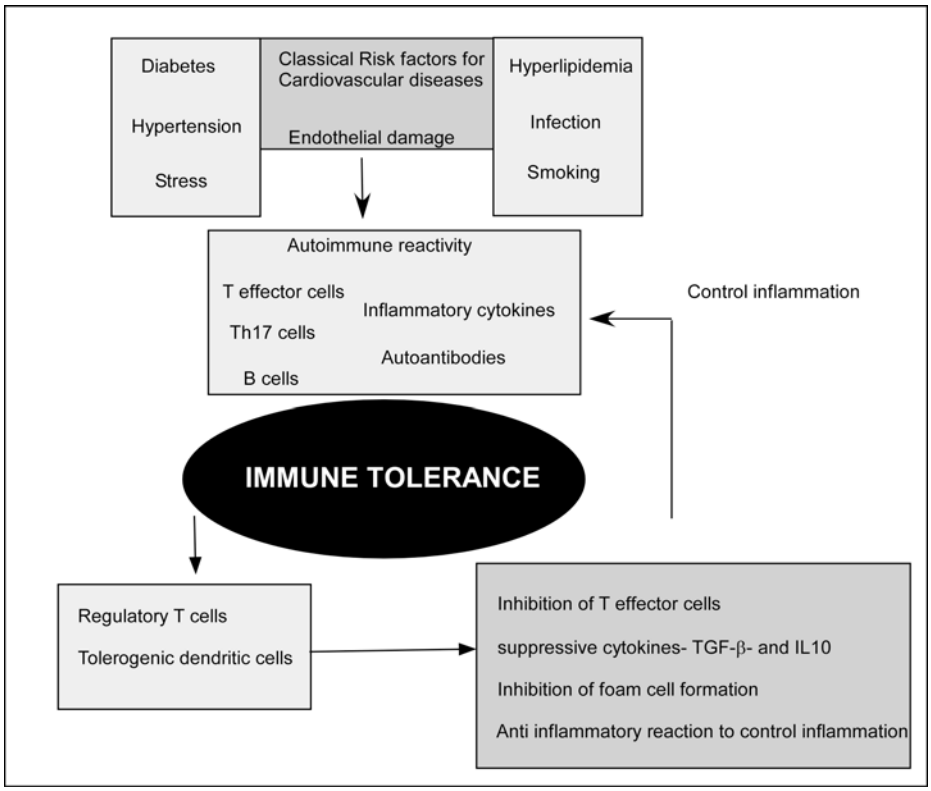

Figure 3: Regulatory immune respone in Atherosclerosis.

Restoration of tolerance induces regulatory T cells and tolerogenic dendritic cells which in turn can activate Tregs. The Tregs can inhibit the proliferation of effector T cell and also secrete anti inflammatory cytokines, TGF-β and Il10 which have atheroprotective properties.

known to migrate from intestine to induce peripheral tolerance. A distinct set of $CD11c^+$ DCs expressing α E integrin ($CD103^+$) and co expressing (CD11b) have been shown to induce the development of Tregs [80, 81, 100]. Recently Steinman and colleagues reported that classical DCs expressing CD103 were atheroprotective [109]. Another recent study by Tabas *et al.* has shown a dominant role for mature $CD11c^+$ DCs in the development of regulatory T cells through MYd88 signaling, which in turn suppresses the monocyte inflammatory response [110]. Our recent study also suggests a protective role for the $CD11c^+$ cells expressing CD103 in atherosclerosis [111]. Figure **3** summarizes the role of regulatory immune response in protection against atherosclerosis.

CONCLUSIONS

Although CVD is a major global health problem, current treatments only aim at reducing the risk factors and do not target the disease process in the artery wall. Recent investigations have revealed a complex interplay of immune response in atherosclerosis.

Since the adaptive arm of immune response is involved in both protection and inflammation, approaches to simultaneously inhibit pathogenic response and stimulate regulate protective response have proven to be highly effective in controlling the disease. Although immune tolerance to atherogenic proteins has shown effective protection in animal models, translation of this approach to human disease will have to face several hurdles. One possible disadvantage with activation of Tregs to reduce inflammation is that it may result in a general immunosuppression. Establishing preclinical safety of the vaccine will be very important aspect before it is approved for clinical trials. Second aspect will be a development of efficient delivery system for inducing tolerance. Other important issue is to clearly define the target population and determine if the vaccine will be given preventive or only to patients with established CVD. The clinical trial design will mainly be based on these points and should thus be resolved before moving into the clinical phase. Characterizing the immune pathways in patients with coronary atherosclerosis to establish alterations of immune functions can contribute to translation of animal studies to clinical trials. Although the road ahead has plenty of obstacles it will not be a long time before we can actually have an immune tolerance based therapy to control the global mortality due to atherosclerosis.

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CONFLICT OF INTEREST

None.

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