



LUND UNIVERSITY

Apolipoprotein B100 autoimmunity and atherosclerosis - disease mechanisms and therapeutic potential.

Nilsson, Jan; Björkbacka, Harry; Nordin Fredrikson, Gunilla

Published in:
Current Opinion in Lipidology

DOI:
[10.1097/MOL.0b013e328356ec7c](https://doi.org/10.1097/MOL.0b013e328356ec7c)

2012

[Link to publication](#)

Citation for published version (APA):
Nilsson, J., Björkbacka, H., & Nordin Fredrikson, G. (2012). Apolipoprotein B100 autoimmunity and atherosclerosis - disease mechanisms and therapeutic potential. *Current Opinion in Lipidology*, 23(5), 422-428. <https://doi.org/10.1097/MOL.0b013e328356ec7c>

Total number of authors:
3

General rights

Unless other specific re-use rights are stated the following general rights apply:
Copyright and moral rights for the publications made accessible in the public portal are retained by the authors and/or other copyright owners and it is a condition of accessing publications that users recognise and abide by the legal requirements associated with these rights.

- Users may download and print one copy of any publication from the public portal for the purpose of private study or research.
- You may not further distribute the material or use it for any profit-making activity or commercial gain
- You may freely distribute the URL identifying the publication in the public portal

Read more about Creative commons licenses: <https://creativecommons.org/licenses/>

Take down policy

If you believe that this document breaches copyright please contact us providing details, and we will remove access to the work immediately and investigate your claim.

LUND UNIVERSITY

PO Box 117
221 00 Lund
+46 46-222 00 00

Apolipoprotein B100 autoimmunity and atherosclerosis – disease mechanisms and therapeutic potential

Jan Nilsson, Harry Björkbacka, Gunilla Nordin Fredrikson

Department of Clinical Sciences Malmö, Skåne University Hospital, Lund University,
Sweden

Number of words excluding references: 3298

Figures: 1

Address for correspondence:

Jan Nilsson, Experimental Cardiovascular Research, Building 91;12, Jan Waldenströms gata
35 , SE-205 02 Malmö, Sweden

Phone: 46 40 391230, Fax: 46 40 391203,

Email: jan.nilsson@med.lu.se

Field Code Changed

Abstract

Purpose of review

Adaptive immune responses have been shown to play an important role in the atherosclerotic disease process and both pathogenic and protective immunity has been identified.

Apolipoprotein (apo) B100 appears to be a key antigen and novel therapies modulating immune responses against apo B100 have shown promising results in experimental models.

This review will discuss recent developments in the mechanistic understanding of apo B100 autoimmunity and approaches taken to use this knowledge for development of novel therapies.

Recent findings

It has recently been shown that not only apo B100 modified by oxidation but also non-modified apo B100 is targeted by autoimmune responses. This implies that a corresponding set of regulatory T cells with the same antigen specificity must exist and that these cells under normal circumstances are able to prevent autoimmunity against LDL. Recent studies also suggest that the athero-protective effect of apo B100 peptide immunization acts by re-enforcing the activity of such cells.

Summary

These novel findings suggest that aggravation of plaque inflammation may occur as result of a local loss of tolerance against LDL in the plaque due to insufficient activity of regulatory T cells. Restoration of lost tolerance represents an interesting novel approach for treatment of cardiovascular disease.

Key words: Autoimmunity, atherosclerosis, LDL, vaccine

Introduction

Atherosclerosis is characterized by a chronic inflammation affecting large- and mid-sized arteries and it has been established that this inflammation primarily is caused by an accumulation and oxidative modification of low density lipoprotein (LDL) in the artery wall [1]. The pro-inflammatory properties of oxidized LDL have been attributed to the generation of lysophospholipids and lipid peroxides as well as to general cytotoxicity. More recently it has also been found that this inflammation is modulated by immune responses against oxidized LDL antigens suggesting the involvement of autoimmunity in the atherosclerotic disease process [2, 3]. Interesting novel observations suggest that these autoimmune responses may not only target modified self-antigens in oxidized LDL but also un-modified apolipoprotein B (apoB)100 [4]**. There are several possible reasons for the existence of autoimmune responses against apo B100 and other antigens in oxidized LDL. The immune system is forced to allow a certain presence of autoreactivity in order to avoid limiting the immunological diversity required to mount effective immune attacks against all different forms of foreign antigens. Many bacterial proteins are for example very similar to proteins present in human cells and strictly deleting immune responses against such human proteins would compromise the defense against infections. The autoreactive T effector cells that escape deletion in the thymus are normally controlled by immune-suppressive regulatory T cells (Tregs) with corresponding self-antigen specificity preserving immunological tolerance. However, presentation of self-antigens in an inflammatory environment, such as an atherosclerotic plaque, favors a shift towards generation of pro-inflammatory Th1 cells and may result in loss of tolerance. It is also possible that the modifications that take place in response to oxidation are so significant that some antigens in the LDL particle are no longer recognized as self by the immune system. Interestingly, there is also evidence that some of the autoimmune responses against LDL antigens are protective and may facilitate oxidized LDL

Field Code Changed

Field Code Changed

Field Code Changed

Field Code Changed

removal as well as to limit oxidized LDL-induced inflammation in a site-specific manner [2].

Field Code Changed

This has generated interest in the possibility of developing immune-modulatory therapies specifically targeting and enforcing athero-protective immune responses [5]. This review will focus on immune responses against apo B100 and how these can be targeted by novel intervention strategies.

Field Code Changed

Oxidized LDL and atherosclerotic plaque inflammation

LDL particles that accumulate in the artery wall may become oxidized within a few hours resulting in activation of the transcription factor NF- κ B that subsequently initiates the expression of pro-inflammatory cytokines and adhesion molecules [6]. Several mechanisms through which oxidized LDL can activate inflammation have been identified. Accumulating evidence indicate a role for the toll-like receptors (TLR) 2 and 4, that are key regulators of innate immune responses, in recognition of oxidized LDL [7, 8]. TLR4 has also been shown to mediate uptake of LDL in macrophages [9, 10]. Consistently, deletion of TLR2, TLR4 or MyD88, a key signal transduction protein of TLR 2 and 4, markedly reduces lesion development in hypercholesterolemic mice suggesting that activation of this pathway has an important role in atherosclerosis [11]. Lysophosphatidylcholine (lysoPC) and its metabolite lysophosphatidic acid (LPA) have been proposed to mediate the inflammatory effect of oxidized LDL. LysoPC is formed when oxidized phospholipids in LDL are hydrolysed by lipoprotein-phospholipase A2 (Lp-PLA2). Both lysoPC and LPA activate inflammatory responses in cultured vascular cells and recent studies have shown strong associations between the human atherosclerotic plaque contents of lysoPC, LPA and pro-inflammatory cytokines [12]. The importance of these lipid mediators is further supported by observations that treatment with an inhibitor of Lp-PLA2 reduces the development of coronary

Field Code Changed

Field Code Changed

Field Code Changed

Field Code Changed

Field Code Changed

Field Code Changed

Field Code Changed

atherosclerosis in experimental animals [13] and halts the progression of plaque necrotic core expansion in man [14]. Ketelhuth and co-workers recently identified a sequence in apo B100 that when presented as a fragment to human mononuclear leukocytes activated the expression of IL-6, IL-8 and CCL2 [15]**. This apo B100 fragment was referred to as the apo B100 danger associated signal 1 (ApoBDS-1). It was shown to be present in a biologically active form in human atherosclerotic plaques and to function through activation of calcium signalling and mitogen-activated protein kinase indicating involvement of a specific receptor.

Field Code Changed

Field Code Changed

Field Code Changed

Adaptive immune responses against oxidized LDL antigens

The first indication of the existence of autoimmune response against oxidized LDL came from studies identifying the presence of oxidized LDL-specific antibodies in human serum [16].

Field Code Changed

This notion of oxidized LDL autoimmunity was subsequently supported by findings of oxidized LDL-specific autoantibodies and T cells in atherosclerotic plaques [17, 18]. Early studies aiming to characterize the functional role of these immune responses unexpectedly

Field Code Changed

Field Code Changed

showed that immunization with oxidized LDL resulted in a partial protection against atherosclerosis development rather than the anticipated aggravation of disease [19, 20]. This outcome was initially difficult to explain as other studies carried out at the same time

Field Code Changed

Field Code Changed

appeared to confirm the original hypothesis that adaptive immune responses activated by hypercholesterolemia were pro-atherogenic [21]. However, as these studies continued to

Field Code Changed

evolve it became evident that the role of the immune system in atherosclerosis was much more complex than originally anticipated. Deletion of cytokines and transcription factors

required for CD4⁺ Th1 effector cell function was consistently found to be associated with

reduced development of atherosclerosis whereas studies on Tregs uniformly pointed to a

protective role of the latter cells [3]. The role of CD4⁺ Th2 effector T cells appears to be more

Field Code Changed

multifaceted. There is evidence of a protective role of the Th2 cytokine IL-5 through stimulation of B1 cells to secrete so called natural IgM antibodies, some of which are binding to phospholipids on oxidized LDL and the plasma membrane of apoptotic cells. IL-33, a potent inducer of Th2 T cell responses, has also been shown to reduce atherosclerosis [22]. In contrast, there is experimental data suggesting a pro-atherogenic role of the Th2 signature cytokine IL-4 [3].

Field Code Changed

Field Code Changed

Targeting cellular and humoral immune responses against apo B and oxidized LDL to inhibit atherosclerosis

The observation of an athero-protective effect of oxidized LDL immunization taken together with the increased understanding of the role of different T cell subsets in atherosclerosis has stimulated efforts to develop novel immune-modulatory therapies for treatment and prevention of cardiovascular disease (figure). We were able to identify several native and malondialdehyde (MDA)-modified peptide sequences in apo B100 that were recognized by autoantibodies in human plasma and showed that several of these peptides could inhibit the development of atherosclerosis when used to immunize *Apoe*^{-/-} mice [23, 24]. The athero-protective effect of a prototype vaccine based on the MDA-modified apo B100 peptide p45 (amino acids 661-680) was shown to be associated with generation of MDA-p45 specific IgG [25]. To investigate the role of these antibodies recombinant human IgG with the same specificity were generated. Treatment of *Apoe*^{-/-} mice with these antibodies was found to inhibit the development of atherosclerosis, reduce inflammation of remaining plaques and to facilitate plaque regression when applied in combination with lipid lowering [26, 27]. The mechanisms involved in the athero-protective effect of these antibodies remains to be fully characterized but appear to involve facilitation of oxidized LDL removal as well as

Field Code Changed

Field Code Changed

Field Code Changed

Field Code Changed

Field Code Changed

neutralization of the pro-inflammatory effects of oxidized LDL. The possible athero-protective effect of these antibodies in humans is presently being investigated in the GLACIER trial (Goal of oxidised Ldl and ACTivated macrophage Inhibition by Exposure to a Recombinant antibody, www.clinicaltrials.gov).

Field Code Changed

Although the studies described above point to an important role of antibodies in mediating the effect of immunization with apo B100 peptides other studies have shown that protection can occur also in the absence of an antibody response [28]. Recent studies by Wigren et al [29, 30]* have suggested that this protection involves activation of Tregs and that it may be enhanced by the use of aluminum-containing adjuvants. The possibility that the protective effect of apo B100 peptide immunization is mediated by activation of Tregs is in line with the documented athero-protective effect of these cells [31] as well as with studies demonstrating that mucosal delivery of oxidized LDL is associated with inhibition of atherosclerosis [32]. Mucosal delivery of antigens is a well-established approach to induce tolerance and studies by van Puijvelde et al [32] have shown that oral administration of oxidized LDL results in generation of oxidized LDL-specific Tregs. Taking advantage of this knowledge Klingenberg and coworkers [33] developed an intranasal vaccine based on recombinant fusion protein containing the native apo B sequence 3136-3155 (p210) and the B-subunit of cholera toxin (CTB). CTB promotes uptake of antigens via the nasal and oral mucosa and CTB-antigen conjugates have evolved as a promising therapeutic strategy to induce tolerance in a variety of autoimmune diseases. Immunization with p210-CTB for 12 weeks caused a reduction in aortic lesion size of *Apoe*^{-/-} mice. The reduction of lesion size was accompanied by induction of regulatory T cells that markedly suppressed effector T cells re-challenged with apo B100 and increased numbers of interleukin (IL)-10⁺CD4⁺ T cells. Another approach to activate apo B100 tolerance was applied by Herbin *et al.* [34]** that used subcutaneously implanted

Field Code Changed

Field Code Changed

Field Code Changed

Field Code Changed

Field Code Changed

Field Code Changed

Field Code Changed

Field Code Changed

osmotic mini-pumps to continuously administer low doses of apo B100 peptides in absence of adjuvant during a 2-week period. This treatment lead to a reduction of lesion development in young *ApoE*^{-/-} mice and abrogated atherosclerosis progression in older *ApoE*^{-/-} mice. The protection conferred by apo B100 peptide administration was associated with activation of an apo B100-specific Treg response and could be cancelled by depletion of Tregs.

It is interesting to note that the different types of apo B100 peptide vaccines that have been shown to be athero-protective almost exclusively contain native peptides rather than peptides modified by aldehydes or other oxidation products suggesting that the target antigen could be present in un-modified rather than in oxidized LDL. A possible explanation to this apparent paradox was recently provided by Hermansson and co-workers [4]** that identified the existence of apo B100 reactive CD4⁺ T cells. This observation came out of studies in which human apo B100 transgenic mice were immunized with oxidized LDL and T cell hybridomas subsequently generated by fusing cells from draining lymph nodes with thymoma cells. Unexpectedly, it was found that several of the generated hybridomas were specific for apo B100, whereas none reacted to oxidized LDL. The apo B-reactive T cell hybridomas were all characterized by expression of the T cell receptor variable β chain TRB31. Immunization of the mice with a TRB31-derived peptide blocked T cell recognition of apo B100 and inhibited the development of atherosclerosis. It remains to be fully clarified if these apo B100 autoreactive T cells target completely normal LDL particles or only particles with minor modifications. These observations still have considerable implications as they suggest that the existence of apo B100-reactive T cells may not be associated to atherosclerosis pathology in itself. If this notion is correct atherosclerosis would instead develop when Tregs with the corresponding antigen-specificity lose control over these cells. It is an interesting possibility

Field Code Changed

that apo B100 peptide vaccines work by targeting the balance between apo B100-reactive T effector and Tregs.

Most studies have focused on CD4⁺ cells as being the most important T cells in atherosclerosis. However, Chyu et al [35]* recently reported that adoptive transfer of CD8⁺, but not CD4⁺ T cells from mice immunized with apo B100 p210 vaccine reduced atherosclerosis in recipient of *ApoE*^{-/-} mice. They also presented evidence that this effect was mediated through CD8⁺ T cell cytotoxicity of dendritic cells. Studies carried out in mice lacking the ability to present antigens to CD8⁺ T cells through MHC class I did not demonstrate any effect on atherosclerosis [36], but this does not exclude that both pro- and anti-atherogenic sub-sets of CD8⁺ T cells may exist. Accordingly, it is possible that both CD4⁺ and CD8⁺ T cells are involved in mediating the protective effect of apo B100 peptide immunization.

There has also been attempts to further enhance the efficacy of apo B peptide vaccines by including other antigens in the vaccine and Lu *et al.* [37] have reported that immunization with the apo B100 p45 peptide in combination with a peptide derived from HSP60 is more effective than immunization with the apo B100 p45 peptide alone.

Dendritic cell therapy

Dendritic and other antigen presenting cells play a key role in determining the nature of T cell activation. Dendritic cells that express co-stimulatory molecules, such as CD80 and CD86, and secrete IL-12 activates differentiation and clonal expansion of pro-inflammatory effector T cells, while dendritic cells that interacts with co-inhibitory molecules, such as CTLA4 and PD1 on T cells, in presence of IL-10 induce Tregs [38]. The expression of co-stimulatory

Field Code Changed

Field Code Changed

Field Code Changed

Field Code Changed

molecules and IL-12 is typically induced by activation of TLR receptors. Hjerpe *et al.* [39] reported that dendritic cells that have been isolated, pulsed with MDA-LDL in presence of the TLR4 activator LPS and subsequently transferred into apo E^{-/-} mice aggravate atherosclerosis. However, using an almost identical design Habets *et al.* [40] found that injection of dendritic cells pulsed with oxidized LDL inhibited the development of atherosclerosis in *Ldlr*^{-/-} mice. The reason for these contradictory observations remains to be clarified but both findings underline the possibility of using *ex vivo* modulation of dendritic cell function as a therapeutic approach for prevention and treatment of atherosclerosis. This possibility was further investigated in a study by Hermansson *et al.* [41]** who showed that injection of human apo B100 transgenic mice with dendritic cells pulsed with apo B100 in the presence of IL-10 inhibits the development of atherosclerosis as compared with injection of dendritic cells pulsed with Apo B100 alone.

Field Code Changed

Field Code Changed

Field Code Changed

Biomarkers of apo B100 and oxidized LDL autoimmunity

The most commonly used approach to study presence of autoimmune responses against oxidized LDL has been to determine circulating levels of autoantibodies against copper-oxidized or MDA-modified LDL. The presence of different organ-specific autoantibodies is a diagnostic criterion in many autoimmune diseases. This is not the case for oxidized LDL autoantibodies and cardiovascular disease, suggesting that atherosclerosis is not an autoimmune disease in the traditional sense. Instead autoantibodies against oxidized LDL can be detected in almost all individuals and the levels are often highest at an early age before the onset of clinical disease [42]. A large number of studies have been carried out to determine the association between autoantibodies against oxidized LDL and cardiovascular disease.

Field Code Changed

However, the results of these studies have not been consistent with some reports

demonstrating inverse associations between the level of circulating autoantibodies against oxidized LDL and the severity of atherosclerosis while others have shown the opposite association [43]. The reason for these inconsistencies remains to be fully clarified but may involve difficulties in standardizing the LDL antigens used in the antibody assays as LDL is extremely sensitive to modifications *ex vivo*. Notably, many of these studies also determined antibody levels against native LDL but only used these measures for background subtraction purposes. One approach to overcome the standardization problem of oxidized LDL antibody assays has been to determine autoantibodies against specific native or MDA-modified apo B100 peptide sequences. These studies have primarily used the p45 (amino acids 661-680) and p210 (amino acids 3136-3155) apo B100 peptides used in the development of immune-modulatory therapies discussed above. Inverse associations have been found between the level of autoantibodies against these apo B100 peptides and the severity of atherosclerosis and subjects that later suffered from acute myocardial infarction has been reported to have lower apo B peptide autoantibodies levels than controls matched for age and gender [44-47].

Field Code Changed

Field Code Changed

Conclusions

It has become well established that immune responses against atherosclerotic plaque antigens play an important role in the disease process and that both pathogenic and protective immune responses exist. It was originally assumed that modified self-antigens, such as oxidized LDL, were the main targets for these immune responses but recent research implies that immune responses against native apo B100 may be of equal importance. The identification and cloning of the T cell receptor of apo B100 autoreactive CD4⁺ cells represents a particularly important breakthrough in this respect and suggest that there exists a naturally occurring autoimmunity against LDL. As a consequence, this implies that a corresponding set of Tregs with the same

antigen specificity must exist and that these cells under normal circumstances are able to prevent autoimmunity against LDL. Recent studies also suggest that the athero-protective effect of apo B100 peptide immunization acts by re-enforcing the activity of such cells. Taken together, these novel findings suggest that aggravation of plaque inflammation and disease progression may occur as result of a local loss of tolerance against LDL in the plaque due to insufficient recruitment or activation of Tregs. If this is true, immune-modulatory therapy enhancing LDL tolerance represents an interesting novel approach for prevention and treatment of cardiovascular disease.

Key points

- Immune responses against atherosclerotic plaque antigens play an important role in the disease process and both pathogenic and protective immunity has been identified.
- It was originally assumed that modified self-antigens generated as a result of LDL oxidation were the most important auto-antigens in atherosclerosis but recent findings demonstrate that T cells that react with un-modified apolipoprotein B100 also exist.
- These apo B100 auto-reactive T cells should normally be controlled by regulatory T cells with corresponding antigen specificity, but this control appears to be lost in atherosclerotic lesions.
- Novel therapies enforcing apo B100 tolerance have shown promising results in experimental models.

Acknowledgements

Disclosures

Jan Nilsson is signed as co-inventor on patents describing the use of immune-modulatory therapy for atherosclerosis and has assigned patent-rights to Forskarpatent, Sweden.

Forskarpatent has licensed patent-rights to Cardiovox, US and Bioinvent, Sweden. Jan Nilsson has received research support from Cardiovox and Bioinvent.

Figure legend

Apo B100 autoimmunity and novel therapeutic strategies modulating apo B100

autoimmune responses. Immunization with apo B100 peptides inhibits the development of atherosclerosis in mice. Modified and unmodified apo B100 peptides are recognized by autoantibodies in human plasma and treatment of mice with these antibodies can inhibit the development of atherosclerosis. Adoptive transfer of dendritic cells pulsed with apo B100 in the presence of IL-10 inhibits the development of atherosclerosis in mice. Also, adoptive transfer of CD8⁺ T cells from mice immunized with apo B100 p210 vaccine reduced atherosclerosis in recipient mice. The existence of apo B100 reactive CD4⁺ T cells raises the possibility that Tregs with the corresponding antigen-specificity exist to control these apo B-reactive T cells. When the balance between apo B100-reactive T effector and Tregs is shifted in favour of the effector T cells, a local loss of tolerance against LDL in the plaque could aggravate atherosclerosis. Restoration of lost tolerance represents an interesting novel approach for treatment of cardiovascular disease.

ApoBDS-1 = apo B100 danger associated signal 1.

References

- [1] Hansson GK. Inflammation, atherosclerosis, and coronary artery disease. *N Engl J Med* 2005; 352:1685-1695.
- [2] Nilsson J, Hansson GK. Autoimmunity in atherosclerosis: a protective response losing control? *J Intern Med* 2008; 263:464-478.
- [3] Hansson GK, Hermansson A. The immune system in atherosclerosis. *Nature immunology* 2011; 12:204-212.
- [4] Hermansson A, Ketelhuth DF, Strodthoff D *et al.* Inhibition of T cell response to native low-density lipoprotein reduces atherosclerosis. *J Exp Med* 2010; 207:1081-1093.** This study identifies the presence of apo B 100 autoreactive T cells and demonstrates that deletion of these cells by immunization against their T cell receptor reduces lesion development. It remains to be fully clarified if these autoreactive T cells are specific for completely native apo B 100 or apo B with minor modifications.
- [5] Shah PK, Chyu KY, Nilsson J. Immunotherapy for atherosclerosis: an emerging paradigm. *Rev Cardiovasc Med* 2004; 5:194-203.
- [6] Calara F, Dimayuga P, Nieman A *et al.* An animal model to study local oxidation of LDL and its biological effects in the arterial wall. *Arterioscl Thromb Vasc Biol* 1998; 18:884-893.
- [7] Seimon TA, Nadolski MJ, Liao X *et al.* Atherogenic lipids and lipoproteins trigger CD36-TLR2-dependent apoptosis in macrophages undergoing endoplasmic reticulum stress. *Cell metabolism* 2010; 12:467-482.
- [8] Miller YI, Viriyakosol S, Worrall DS *et al.* Toll-like receptor 4-dependent and -independent cytokine secretion induced by minimally oxidized low-density lipoprotein in macrophages. *Arterioscler Thromb Vasc Biol* 2005; 25:1213-1219.
- [9] Choi SH, Harkewicz R, Lee JH *et al.* Lipoprotein accumulation in macrophages via toll-like receptor-4-dependent fluid phase uptake. *Circ Res* 2009; 104:1355-1363.
- [10] Levin MC, Jirholt P, Wramstedt A *et al.* Rip2 deficiency leads to increased atherosclerosis despite decreased inflammation. *Circ Res* 2011; 109:1210-1218.
- [11] Bjorkbacka H. Multiple roles of Toll-like receptor signaling in atherosclerosis. *Curr Opin Lipidol* 2006; 17:527-533.
- [12] Goncalves I, Edsfeldt A, Ko NY *et al.* Evidence Supporting a Key Role of Lp-PLA2-Generated Lysophosphatidylcholine in Human Atherosclerotic Plaque Inflammation. *Arterioscler Thromb Vasc Biol* 2012 ;32(6):1505-12.
- [13] Wilensky RL, Shi Y, Mohler ER, 3rd *et al.* Inhibition of lipoprotein-associated phospholipase A2 reduces complex coronary atherosclerotic plaque development. *Nat Med* 2008; 14:1059-1066.
- [14] Serruys PW, Garcia-Garcia HM, Buszman P *et al.* Effects of the direct lipoprotein-associated phospholipase A(2) inhibitor darapladib on human coronary atherosclerotic plaque. *Circulation* 2008; 118:1172-1182.
- [15] Ketelhuth DF, Rios FJ, Wang Y *et al.* Identification of a danger-associated peptide from apolipoprotein B100 (ApoBDS-1) that triggers innate proatherogenic responses. *Circulation* 2011; 124:2433-2443, 2431-2437. ** The pro-inflammatory properties of oxidized LDL have previously been attributed to the generation of reactive aldehydes and phospholipids. This study identifies a sequence in apo B100 that when presented as a fragment to human mononuclear leukocytes activated the expression of several chemokines and cytokines.
- [16] Palinski W, Rosenfeld ME, Yla-Herttuala S *et al.* Low density lipoprotein undergoes oxidative modification in vivo. *Proc Natl Acad Sci U S A* 1989; 86:1372-1376.
- [17] Yla-Herttuala S, Palinski W, Butler SW *et al.* Rabbit and human atherosclerotic lesions contain IgG that recognizes epitopes of oxidized LDL. *Arterioscler Thromb* 1994; 14:32-40.
- [18] Stemme S, Faber B, Holm J *et al.* T lymphocytes from human atherosclerotic plaques recognize oxidized low density lipoprotein. *Proc Natl Acad Sci U S A* 1995; 92:3893-3897.

- [19] Palinski W, Miller E, Witztum JL. Immunization of low density lipoprotein (LDL) receptor-deficient rabbits with homologous malondialdehyde-modified LDL reduces atherogenesis. *Proc Natl Acad Sci U S A* 1995; 92:821-825.
- [20] Ameli S, Hultgardh-Nilsson A, Regnstrom J *et al.* Effect of immunization with homologous LDL and oxidized LDL on early atherosclerosis in hypercholesterolemic rabbits. *Arterioscler Thromb Vasc Biol* 1996; 16:1074-1079.
- [21] Binder CJ, Chang MK, Shaw PX *et al.* Innate and acquired immunity in atherogenesis. *Nat Med* 2002; 8:1218-1226.
- [22] Miller AM, Xu D, Asquith DL *et al.* IL-33 reduces the development of atherosclerosis. *J Exp Med* 2008; 205:339-346.
- [23] Fredrikson GN, Hedblad B, Berglund G *et al.* Identification of immune responses against aldehyde-modified peptide sequences in apo B-100 associated with cardiovascular disease. *Arterioscler Thromb Vasc Biol* 2003; 23:872-878.
- [24] Fredrikson GN, Soderberg I, Lindholm M *et al.* Inhibition of Atherosclerosis in ApoE-Null Mice by Immunization with ApoB-100 Peptide Sequences. *Arterioscler Thromb Vasc Biol* 2003; 23:879-884.
- [25] Fredrikson GN, Andersson L, Soderberg I *et al.* Atheroprotective immunization with MDA-modified apo B-100 peptide sequences is associated with activation of Th2 specific antibody expression. *Autoimmunity* 2005; 38:171-179.
- [26] Schiopu A, Bengtsson J, Soderberg I *et al.* Recombinant human antibodies against aldehyde-modified apolipoprotein B-100 peptide sequences inhibit atherosclerosis. *Circulation* 2004; 110:2047-2052.
- [27] Schiopu A, Frendeus B, Jansson B *et al.* Recombinant antibodies to an oxidized low-density lipoprotein epitope induce rapid regression of atherosclerosis in apobec-1(-/-)/low-density lipoprotein receptor(-/-) mice. *J Am Coll Cardiol* 2007; 50:2313-2318.
- [28] Fredrikson GN, Bjorkbacka H, Soderberg I *et al.* Treatment with apo B peptide vaccines inhibits atherosclerosis in human apo B-100 transgenic mice without inducing an increase in peptide-specific antibodies. *J Intern Med* 2008; 264:563-570.
- [29] Wigren M, Kolbus D, Duner P *et al.* Evidence for a role of regulatory T cells in mediating the atheroprotective effect of apolipoprotein B peptide vaccine. *J Intern Med* 2011; 269:546-556. * Prototype vaccines based on apo B 100 peptides have previously been shown to reduce development of atherosclerosis. This study demonstrates that immunization with the apo B 100 peptide p210 is associated generation of regulatory T cells and that deletion of regulatory T cells removes the protective effect of immunization.
- [30] Wigren M, Bengtsson D, Duner P *et al.* Atheroprotective effects of Alum are associated with capture of oxidized LDL antigens and activation of regulatory T cells. *Circ Res* 2009; 104:e62-70.
- [31] Mallat Z, Ait-Oufella H, Tedgui A. Regulatory T-cell immunity in atherosclerosis. *Trends Cardiovasc Med* 2007; 17:113-118.
- [32] van Puijvelde GH, Hauer AD, de Vos P *et al.* Induction of oral tolerance to oxidized low-density lipoprotein ameliorates atherosclerosis. *Circulation* 2006; 114:1968-1976.
- [33] Klingenberg R, Lebens M, Hermansson A *et al.* Intranasal immunization with an apolipoprotein B-100 fusion protein induces antigen-specific regulatory T cells and reduces atherosclerosis. *Arterioscler Thromb Vasc Biol* 2010; 30:946-952.
- [34] Herbin O, Ait-Oufella H, Yu W *et al.* Regulatory T-cell response to apolipoprotein B100-derived peptides reduces the development and progression of atherosclerosis in mice. *Arterioscler Thromb Vasc Biol* 2012; 32:605-612. **This study add further support to the notion that regulatory T cells are involved in the athero-protective effect of immunization with apo B 100 peptides. It is demonstrated that subcutaneous slow release of apo B 100 peptides inhibits early stages of atherosclerosis development and halts plaque progression in advanced disease. The protective effect was cancelled following depletion of regulatory T cells.
- [35] Chyu KY, Zhao X, Dimayuga PC *et al.* CD8 T Cells Mediate the Athero-Protective Effect of Immunization with an ApoB-100 Peptide. *PLoS ONE* 2012; 7:e30780. ** Previous studies have

attributed the the protective effect of immunization with apo B 100 peptiudes to CD4⁺ regulatory T cells. This study provide evidence that also CD8⁺ T may be involved in mediating this protection.

[36] Kolbus D, Ljungcrantz I, Soderberg I *et al.* TAP1-Deficiency Does Not Alter Atherosclerosis Development in Apoe(-/-) Mice. *PLoS ONE* 2012; 7:e33932.

[37] Lu X, Chen D, Endresz V *et al.* Immunization with a combination of ApoB and HSP60 epitopes significantly reduces early atherosclerotic lesion in Apobtm2SgyLdlrtm1Her/J mice. *Atherosclerosis* 2010; 212:472-480.

[38] Gotsman I, Sharpe AH, Lichtman AH. T-cell costimulation and coinhibition in atherosclerosis. *Circ Res* 2008; 103:1220-1231.

[39] Hjerpe C, Johansson D, Hermansson A *et al.* Dendritic cells pulsed with malondialdehyde modified low density lipoprotein aggravate atherosclerosis in Apoe(-/-) mice. *Atherosclerosis* 2010; 209:436-441.

[40] Habets KL, van Puijvelde GH, van Duivenvoorde LM *et al.* Vaccination using oxidized low-density lipoprotein-pulsed dendritic cells reduces atherosclerosis in LDL receptor-deficient mice. *Cardiovasc Res* 2010; 85:622-630.

[41] Hermansson A, Johansson DK, Ketelhuth DF *et al.* Immunotherapy with tolerogenic apolipoprotein B-100-loaded dendritic cells attenuates atherosclerosis in hypercholesterolemic mice. *Circulation* 2011; 123:1083-1091. ** This interesting study showed that injection of human apo B100 transgenic mice with dendritic cells pulsed with apo B100 in the presence of IL-10 inhibits the development of atherosclerosis as compared with injection of dendritic cells pulsed with Apo B100 alone.

[42] Tinahones FJ, Gomez-Zumaquero JM, Garrido-Sanchez L *et al.* Influence of age and sex on levels of anti-oxidized LDL antibodies and anti-LDL immune complexes in the general population. *J Lipid Res* 2005; 46:452-457.

[43] Hulthe J. Antibodies to oxidized LDL in atherosclerosis development--clinical and animal studies. *Clin. Chim. Acta* 2004; 348:1-8.

[44] Fredrikson GN, Schiopu A, Berglund G *et al.* Autoantibody against the amino acid sequence 661-680 in apo B-100 is associated with decreased carotid stenosis and cardiovascular events. *Atherosclerosis* 2007; 194:e188-192.

[45] Sjogren P, Fredrikson GN, Samnegard A *et al.* High plasma concentrations of autoantibodies against native peptide 210 of apoB-100 are related to less coronary atherosclerosis and lower risk of myocardial infarction. *Eur Heart J* 2008; 29:2218-2226.

[46] Engelbertsen D, Anand DV, Fredrikson GN *et al.* High levels of IgM against methylglyoxal-modified apolipoprotein B100 are associated with less coronary artery calcification in patients with type 2 diabetes. *J Intern Med* 2012; 271:82-89.

[47] Fredrikson GN, Anand DV, Hopkins D *et al.* Associations between autoantibodies against apolipoprotein B-100 peptides and vascular complications in patients with type 2 diabetes. *Diabetologia* 2009; 52:1426-1433.

