Immunization for Atherosclerosis

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Current Atherosclerosis Reports 2007, **9:**104–109
Current Medicine Group LLC ISSN 1523-3804

This review summarizes experimental findings that highlight the complex roles of the immune system in atherogenesis. Immune activation can have either proatherogenic or atheroprotective effects. Immune-modulation therapy via an active or passive immunization strategy aims to exploit the atheroprotective aspects of the immune system to modulate atherosclerosis. Several experimental studies have demonstrated that such an approach is feasible and effective, raising the tantalizing possibility that an atheroprotective vaccine can be developed for clinical testing. Several potential immunogens have been identified and tested for their atheroprotective efficacy with variable results. Although several questions such as choice of optimal antigens, choice of most effective adjuvants, the optimal route of administration, durability of effects, and safety remain to be answered, we believe that a vaccine-based approach to manage atherosclerotic cardiovascular disease is a potentially viable paradigm.

Introduction

Atherosclerotic cardiovascular disease continues to be the leading cause of morbidity and mortality in much of the Western world despite many advances in management. These observations highlight the need for new therapies targeting this highly prevalent disease. This review summarizes what we currently know about the complex and conflicting role of the immune system in atherogenesis and how such knowledge may be used to formulate novel immunomodulation therapies for atherosclerotic vascular disease.

Immunity and Atherosclerosis

Atherosclerosis is a complex, chronic inflammatory disease of the arterial walls as evidenced by the presence of inflammatory cells, activated immune cells, various molecules, and cytokines to recruit immune cells, complements, and immunoglobulins in the lesions, all of which highlight the involvement of the immune system [1,2]. The immune system can be largely divided into distinct yet overlapping innate and adaptive immunity, but both immunities modulate atherosclerosis. Innate immunity reacts quickly and recognizes common pathogen-associated microbial patterns, such as lipopolysaccharides in gram-negative bacteria and unmethylated CpG DNA motif [3]. It includes macrophages, natural killer (NK) cells, and mast cells and uses complements, various cytokines or chemokines, and cell-mediated cytotoxicity as its effector mechanism. Adaptive immunity reacts more slowly, recognizes more specific antigenic epitopes, its cellular components include T and B cells, and it uses antibodies, cytotoxic T cells, antibody-dependent cell-mediated cytotoxicity, cytokines, or chemokines as its effector mechanisms [3].

Different components of innate or adaptive immunity have been studied using gene knockout or bone marrow transplant strategies to determine their involvement in atherogenesis. Table 1 summarizes several such important examples and a few specific examples are worthy of further discussion. Toll-like receptors (TLR) are a group of pattern recognition receptors of innate immunity. Exogenous infectious triggers interact with TLRs, leading to transcription of several acute inflammatory genes and the eventual release of inflammatory cytokines that characterize the acute innate immune response [4]. Macrophages and endothelial cells in murine and human atherosclerotic lesions express TLR4 and other members of the TLR family [5,6]. Interference in innate immune signaling through genetic disruption of TLR4 or its downstream signaling adaptor molecule (myeloid differentiation factor 88) reduces atherosclerosis, plaque inflammation, and circulating inflammatory proteins in mice [7•,8•]. On the other hand, a naturally occurring IgM antibody with the T15 idiotype, produced by self-renewing B1 cells without T cell or thymic involvement, might have an atheroprotective role [9,10•]. These natural antibodies recognize the phosphorylcholine head group present in the phospholipid moiety of oxidized low-density lipoprotein (LDL), apoptotic cells, and the cell wall of pneumococcus, and they reduce the uptake of oxidized LDL by macrophages and attenuate murine atherosclerosis [9,11]. Our laboratory

Table 1. Effect of different immune components on atherogenesis

Model	Immune component	Effect on atherosclerosis	Study
ApoE/RAG-1	T and B cell deficiency	Decrease; no effect if mice on high-fat diet	Dansky et al. [13]
ApoE/RAG-2	T and B cell deficiency	No effect	Daugherty et al. [15]
LDLR/RAG-1	T and B cell deficiency	Delayed	Song et al. [14]
ApoE/SCID	T and B cell deficiency	Decrease	Zhou et al. [68]
ApoE/CD1d	Natural killer T cell deficiency	Decrease	Tupin et al. [23]
ApoE/CD1d	Natural killer T cell deficiency	Decrease	Major et al. [69]
LDLR/complement 3	Defect in classical and alternative pathways	Increase	Edfeldt et al. [6]
ApoE/complement 5	Defect in terminal complement complex	No effect	Patel et al. [70]
ApoE/Myd-88 or ApoE/TLR4	Defect in innate immunity	Decrease	Michelsen et al. [7•]
ApoE/Myd-88	Defect in innate immunity	Decrease	Bjorkbacka et al. [8•]
Splenectomy in ApoE-/- mice	Defect in adaptive immunity	Increase	Caligiuri et al. [17]
B cell deficiency in LDLR-/- mice	Defect in adaptive immunity	Increase	Major et al. [16]

Apo—apolipoprotein; LDLR—low-density lipoprotein receptor.

has recently demonstrated that passive immunization of hypercholesterolemic mice with this antibody, developed by hybridoma technology, reduces accelerated vein-graft atherosclerosis [12], which further supports an atheroprotective role for innate humoral immune response to the phosphorylcholine head group.

Although the models of total T cell and B cell deficiency in the setting of hypercholesterolemia provided conflicting reports on their roles in atherogenesis [13–15], other experimental approaches have provided a somewhat clearer view regarding the role of B or T cells in atherosclerosis. Using a bone marrow transplantation strategy, Major et al. [16] demonstrated that B cell deficiency in LDL receptor (LDLR) -/- mice was associated with a reduced level of anti–oxidized LDL antibody; these mice developed a 30% to 40% increase in the lesion area in the proximal and distal aortas. Caligiuri et al. [17] reported that splenectomy aggravated atherosclerotic lesions in apolipoprotein (apo) E-/- mice and such an increase could be ameliorated by adoptive transfer of B cells from donor mice. However, adoptive transfer of naive CD4+ T cells or CD4+ T cells from malondialdehyde (MDA)-LDL immunized donors into hypercholesterolemic immune-deficient mice resulted in aggravation of atherosclerosis, supporting the notion that CD4+ T-cell–mediated immunity is proatherogenic [18,19•]. A subset of CD4+ T cells that constitutively express CD25 produces immune inhibitory cytokines, such as IL-10 (regulatory T1 cells) or transforming growth factor-β, and inhibits the activation of other T cells and favorably influences inflammation and atherosclerosis in murine models [20–22]. Activation of yet another subset of CD4+ T cells, which express NK 1.1 receptors found on NK cells and recognize lipid antigens presented by the class I-like molecule CD1, worsens early atherosclerosis in apoE-/- mice [23]. Unlike CD4+ T cells, the role of CD8+ T cells in atherogenesis is currently unclear.

Through these studies, it is apparent that immune system activation could be either atherogenic or atheroprotective. Given these observations, many investigators have been interested in exploiting the concept of immune modulation as a potential therapy for atherosclerosis.

Immune Modulation Against Atherosclerosis

There are two major ways to modulate immune responses—via immune suppressive therapy or through immunization (active or passive). In experimental animals, immune suppressive agents have variable effects on atherosclerosis. For example, cyclosporin A was reported to have the opposite effect on atherosclerosis in hypercholesterolemic rabbits in two different studies [24,25]. Although mycophenolate mofetil reduces experimental atherosclerosis [26], FK506 has an opposite effect [27]. Additionally, immune suppressive therapy results in many undesirable side effects and is not likely to have major value for routine clinical use as a therapy for atherosclerotic vascular disease.

Vaccines have successfully reduced death from infectious disease and resulted in the global eradication of diseases such as smallpox and poliomyelitis. Vaccines are effective, specific, relatively inexpensive, and generally well tolerated. In recent years, the concept of vaccination has also been extended to treat or prevent noninfectious chronic inflammatory or autoimmune diseases such as Alzheimer's disease and cancer with variable results. The observation that immunization with lipoprotein could reduce atherosclerosis can be traced back to 1950s [28]; however, it

was not until the past two decades that scientists began to understand how immunization affects atherogenesis. Several molecules have been identified as potential candidate immunogens in atherosclerosis; some are proatherogenic whereas others are atheroprotective (Table 2).

Historically, native LDL or modified LDL was used as an immunogen in immunization studies to reduce atherosclerosis [29–32]. LDL is not an ideal immunogen because it is a large, heterogeneous molecule containing apolipoprotein, cholesteryl ester, triglyceride, and phospholipids. Which components are responsible for immunization-mediated atheroprotection is not known. Although oxidation of LDL leads to degradation of apoB-100 into numerous peptide fragments that could serve as targets for recognition by the immune system [33,34], the exact nature of these peptides is not known. Use of whole LDL as an immunogen for clinical testing is also not practical because of the requirement of its isolation in large quantities and the accompanied safety issues. Therefore, to exploit the potential atheroprotective effects of LDL immunization, it would be useful to be able to identify the protective antigenic epitopes in LDL.

Our laboratories have been studying the structure of the apoB-100 component of LDL to identify potential antigenic epitopes that could mediate the atheroprotective effects of immunization with oxidized LDL [35]. We have generated a library of 302 peptide sequences spanning the entire structure of the human apoB-100 molecule, each one being 20 amino acids in length with an overlap of 5 amino acids [36]. Among these peptides, we have identified 102 peptides that are associated with an antibody response in pooled human serum [35]. Some of the peptide sequences, but not others, when incorporated into a vaccine formulation with alum as an adjuvant, provoke an atheroprotective response in hypercholesterolemic mice, resulting in a 40% to 70% reduction in aortic atherosclerosis along with a reduction in plaque inflammation [36,37]. Such atheroprotection could be passively transferred to nonimmunized mice through adoptive transfer of splenocytes from immunized mice [37]. Passive immunization using a monoclonal antibody to one of the atheroprotective peptide epitopes was also shown to reduce atherosclerosis in hyperlipidemic mice [38]. These studies clearly identified apoB-100–related specific peptide epitopes that are able to trigger atheroprotective immunity and provided evidence that such vaccination strategy is feasible.

A different way to identify possible immunogens is through isolation of relevant antibodies against LDL. Horkko et al. [39] and Friedman et al. [40] successfully generated a panel of B cell hybridomas from naive (nonimmunized) apoE-/- mice. A number of clones were selected that generated antibodies with specific binding to epitopes in oxidized LDL. All clones were found to secrete IgM binding either to MDA-LDL (presumably aldehyde-modified apoB-100 peptide sequences) or to copper-oxidized LDL. Subsequent studies showed that all antibodies binding to copper-oxidized LDL recognized oxidized phospholipids [39,40]. The antibodies recognized epitopes both in the lipid moiety of oxidized LDL and in the delipidated apoB-100, suggesting that the antigen can exist as a free lipid as well as an adduct to apoB-100. One of the IgM antibodies is of the T15 idiotype, recognizes phosphorylcholine headgroups on the surface of apoptotic cells, and inhibits uptake of oxidized LDL and apoptotic cells in macrophages [9,39,41]. Antiphosphorylcholine antibodies of the T15 idiotype also protect against infection from *Streptococcus pneumoniae* [42]. Binder et al. [11] studied the functional role of antiphospholipid antibodies in atherosclerosis by immunizing LDLR knockout mice with *Streptococcus pneumoniae*. This treatment resulted in induction of high levels of oxidized LDL-specific IgM and a modest reduction of atherosclerosis. We subsequently used another clone of the antiphosphorylcholine IgM antibody of T15 idiotype and demonstrated its atherosclerosis-reducing effect in accelerated atherosclerosis in a murine vein-graft model via passive immunization [12]. Currently, we are testing its efficacy in reducing spontaneous atherosclerosis.

Table 2. List of immunogens that have been used in immunization studies

Atheropromotion via active immunization
Heat shock protein 65 [60–62]
β2-glycoprotein I [63]
Atheroprotection via active immunization
Native low-density lipoprotein [31,64]
Malondialdehyde–low-density lipoprotein [65,66]
Apolipoprotein B-100 peptides [36,37]
Phosphorylcholine head group on oxidized phospholipid [11]
Cholesteryl ester transfer protein [49,67]

Immunization using immunogens unrelated to LDL may also have an atheroprotective effect (Table 2). The beneficial effects of high high-density lipoprotein (HDL) levels against atherosclerosis are well established [43–46]. Cholesteryl ester transfer protein (CETP) is a key enzyme involved in HDL metabolism. Inducing neutralizing antibodies against CETP via immunization results in marked elevation of HDL with concomitant reduction in atherosclerosis in hyperlipidemic rabbits [47–49]. Clinical testing of such a vaccine strategy is ongoing [50]. Interestingly, two CETP inhibitors have been developed and are now undergoing clinical trials to assess their clinical benefits.

Recently, influenza virus infection has been shown to induce vascular inflammation and a prothrombotic state in animal models; it has also been linked to increased cardiovascular events in human subjects [51,52]. Several studies have observed that influenza vaccination is associated with a reduced incidence of various cardiovascular events such as myocardial infarc-

tion, out-of-hospital cardiac arrest, and stroke [53]. A randomized controlled trial of influenza vaccination, the FLU Vaccination Acute Coronary Syndromes (FLUVACS) study [54,55], provided the strongest evidence to support the use of influenza vaccination to reduce cardiovascular mortality in patients with atherosclerotic coronary disease. Based on these data, both the American Heart Association and the American College of Cardiology recently released a Science Advisory, widely endorsed by several professional associations, to include influenza vaccination as a part of secondary prevention measures for patients with cardiovascular disease [56]. However, only the inactivated form of vaccine should be administered to patients [56].

Unresolved Questions in Immunization Strategy for Atherosclerosis Management

In addition to the choice of immunogens, for clinical testing of a vaccination strategy based on apo-B100–related peptides a number of critical issues will need to be addressed: 1) the timing of immunization (childhood vs adulthood or both); 2) the most suitable immune adjuvant and the optimum route of administration; 3) vaccine safety and efficacy including durability of effect; and 4) appropriate methods to document an atheroprotective benefit.

The timing of the vaccination is an important issue to consider if a widespread vaccine strategy is to be implemented. Most of the experimental studies of immunization with oxidized LDL or peptide antigens specific for oxidized LDL have demonstrated the prevention of early atherosclerotic lesions when used before a significant lesion develops; whether immunization used after lesions have already formed remains to be established. Although prevention of atherosclerosis by immunization at an early age remains a potential, long-term goal, whether immunization slows plaque progression and/or induces stabilization of existing plaques is also worthy of investigation as a secondary prevention strategy. Our experimental studies have shown that immunization favorably changes the composition of established plaques, indicated by decreased plaque inflammation and increased collagen content [31]. Studies to further address this question in detail are required.

The immune response to an antigen is determined not only by the type of antigen (protein, lipid, or carbohydrate antigen) but also by its route of administration (subcutaneous vs intramuscular vs mucosal) and the choice of the adjuvant, which can influence antigen delivery, presentation, and induction of immunomodulatory cytokines. Commonly known adjuvants include aluminum salt and Freund's complete and incomplete adjuvant. Gels containing aluminum salt are the only adjuvant approved for use in vaccines in the United States. Several new types of adjuvants are now being tested in preclinical and clinical trials, including liposomes, immunostimulatory complexes, DNA sequences containing unmethylated CpG motifs, and biodegradable polymer microspheres. Understanding the mechanisms through which each antigen contributes to the disease may be necessary to find the optimum combination of antigen, adjuvant, and vehicle of administration.

For a vaccine to be adopted universally, it needs to be effective, safe, and conceptually acceptable to the general public. Several recent examples highlighted the importance of this issue. The concern of an increase in the incidence of autism with the measles-mumps-rubella (MMR) vaccine did raise issues around the MMR vaccination program, although no evidence suggests there is a link between autism and the MMR vaccine [57,58]. Immunization against amyloid β-peptide reduced amyloid peptide–induced neurotoxicity in mouse models of Alzheimer's disease; however, such a clinical trial was deemed to be unsafe and had to be terminated prematurely due to excessive occurrence of meningoencephalitis [59].

How to assess the clinical efficacy of immunization is another difficult question to answer. Atherosclerosis is a chronic disease and it is likely to take a long-term effort for any treatment to affect its hard endpoints such as death or myocardial infarction. Hence, the initial clinical trials to evaluate immunization efficacy would need to rely on the assessment of surrogate endpoints such as atherosclerotic plaque burden or composition using noninvasive and/or invasive imaging modalities. If this initial "proof-of-concept" human trial is successful, long-term trials involving hard endpoints will be required. The optimal duration of follow-up also needs to be defined.

Conclusions

Taken together, it is apparent that modulation of immune responses related to atherogenesis by active or passive immunization is a novel and promising approach to the prevention and treatment of atherosclerotic cardiovascular disease. Even though our understanding of the complex role of the immune system in atherosclerosis is incomplete and many questions remain unanswered, experimental studies have provided encouraging data. Continued investigation of this promising approach is warranted to ensure safety and durability of benefits before clinical testing is considered. We are cautiously optimistic that there is a future for vaccination for atherosclerosis as a complementary approach to the existing antiatherogenic strategies. It is our sincere hope and dream that someday an atherosclerosis vaccination strategy will become part of the routine childhood (and/or adulthood) vaccination program so that atherosclerosis and its complications can be markedly reduced.

Acknowledgment

Dr. Nilsson can be contacted at the Department of Clinical Sciences at the University of Lund in Sweden. The authors acknowledge the support of the HeartFoundation, Eisner Foundation, United Hostesses, Skirball Foundation, and Mr. Sam Spaulding.

References and Recommended Reading

Papers of particular interest, published recently, have been highlighted as:

- • Of importance
- •• Of major importance

1. Ross R: **Atherosclerosis—an inflammatory disease.** *N Engl J Med* 1999, **340:**115–126.
2. Hansson GK: **Immune mechanisms in atherosclerosis.** *Arterioscler Thromb Vasc Biol* 2001, **21:**1876–1890.
3. Abbas AK, Lichtman AH: *Cellular and Molecular Immunology*. Philadelphia: WB Saunders; 2003.
4. Gordon S. **Pattern recognition receptors: doubling up for the innate immune response.** *Cell* 2002, **111:**927–930.
5. Xu XH, Shah PK, Faure E, et al.: **Toll-like receptor-4 is expressed by macrophages in murine and human lipid-rich atherosclerotic plaques and upregulated by oxidized LDL.** *Circulation* 2001, **104:**3103–3108.
6. Edfeldt K, Swedenborg J, Hansson GK, Yan ZQ: **Expression of toll-like receptors in human atherosclerotic lesions: a possible pathway for plaque activation.** *Circulation* 2002, **105:**1158–1161.
7.• Michelsen KS, Wong MH, Shah PK, et al.: **Lack of Toll-like receptor 4 or myeloid differentiation factor 88 reduces atherosclerosis and alters plaque phenotype in mice deficient in apolipoprotein E.** *Proc Natl Acad Sci U S A* 2004, **101:**10679–10684.

This article provided experimental evidence supporting the role of TLR in atherogenesis.

8.• Bjorkbacka H, Kunjathoor VV, Moore KJ, et al.: **Reduced atherosclerosis in MyD88-null mice links elevated serum cholesterol levels to activation of innate immunity signaling pathways.** *Nat Med* 2004, **10:**416–421.

This article provided experimental evidence supporting the role of TLR in atherogenesis.

9. Shaw PX, Horkko S, Chang MK, et al.: **Natural antibodies with the T15 idiotype may act in atherosclerosis, apoptotic clearance, and protective immunity.** *J Clin Invest* 2000, **105:**1731–1740.
10.• Binder CJ, Shaw PX, Chang MK, et al.: **The role of natural antibodies in atherogenesis.** *J Lipid Res* 2005, **46:**1353–1363.

This review summarizes the experimental evidence supporting the role of IgM natural antibody in atherogenesis.

11. Binder CJ, Horkko S, Dewan A, et al.: **Pneumococcal vaccination decreases atherosclerotic lesion formation: molecular mimicry between Streptococcus pneumoniae and oxidized LDL.** *Nat Med* 2003, **9:**736–743.
12. Faria-Neto JR, Chyu KY, Li XJ, et al.: **Passive immunization with monoclonal IgM antibodies against phosphorylcholine reduces accelerated vein graft atherosclerosis in apolipoprotein E-null mice.** *Atherosclerosis* 2006, **189:**83–90.
13. Dansky HM, Charlton SA, Harper MM, Smith JD: **T and B lymphocytes play a minor role in atherosclerotic plaque formation in the apolipoprotein E-deficient mouse.** *Proc Natl Acad Sci U S A* 1997, **94:**4642–4646.
14. Song L, Leung C, Schindler C: **Lymphocytes are important in early atherosclerosis.** *J Clin Invest* 2001, **108:**251–259.
15. Daugherty A, Pure E, Delfel-Butteiger D, et al.: **The effects of total lymphocyte deficiency on the extent of atherosclerosis in apolipoprotein E-/- mice.** *J Clin Invest* 1997, **100:**1575–1580.
16. Major AS, Fazio S, Linton MF: **B-lymphocyte deficiency increases atherosclerosis in LDL receptor-null mice.** *Arterioscler Thromb Vasc Biol* 2002, **22:**1892–1898.
17. Caligiuri G, Nicoletti A, Poirier B, Hansson GK: **Protective immunity against atherosclerosis carried by B cells of hypercholesterolemic mice.** *J Clin Invest* 2002, **109:**745–753.
18. Zhou X, Nicoletti A, Elhage R, Hansson GK: **Transfer of CD4(+) T cells aggravates atherosclerosis in immunodeficient apolipoprotein E knockout mice.** *Circulation* 2000, **102:**2919–2922.
19.• Zhou X, Robertson AK, Hjerpe C, Hansson GK: **Adoptive transfer of CD4+ T cells reactive to modified low-density lipoprotein aggravates atherosclerosis.** *Arterioscler Thromb Vasc Biol* 2006, **26:**864–870.

This is an important observation indicating the proatherogenic role of CD4+ T cells.

20. Mallat Z, Gojova A, Brun V, et al.: **Induction of a regulatory T cell type 1 response reduces the development of atherosclerosis in apolipoprotein E-knockout mice.** *Circulation* 2003, **108:**1232–1237.
21. Pinderski OL, Hedrick CC, Olvera T, et al.: **Interleukin-10 blocks atherosclerotic events in vitro and in vivo.** *Arterioscler Thromb Vasc Biol* 1999, **19:**2847–2853.
22. Robertson AK, Rudling M, Zhou X, et al.: **Disruption of TGF-beta signaling in T cells accelerates atherosclerosis.** *J Clin Invest* 2003, **112:**1342–1350.
23. Tupin E, Nicoletti A, Elhage R, et al.: **CD1d-dependent Activation of NKT Cells Aggravates Atherosclerosis.** *J Exp Med* 2004, **199:**417–422.
24. Roselaar SE, Schonfeld G, Daugherty A: **Enhanced development of atherosclerosis in cholesterol-fed rabbits by suppression of cell-mediated immunity.** *J Clin Invest* 1995, **96:**1389–1394.
25. Drew AF, Tipping PG: **Cyclosporine treatment reduces early atherosclerosis in the cholesterol-fed rabbit.** *Atherosclerosis* 1995, **116:**181–189.
26. Greenstein SM, Sun S, Calderon TM, et al.: **Mycophenolate mofetil treatment reduces atherosclerosis in the cholesterol-fed rabbit.** *J Surg Res* 2000, **91:**123–129.
27. Matsumoto T, Saito E, Watanabe H, et al.: **Influence of FK506 on experimental atherosclerosis in cholesterol-fed rabbits.** *Atherosclerosis* 1998, **139:**95–106.
28. Gero S, Gergely J, Jakab L, et al.: **Inhibition of cholesterol atherosclerosis by immunisation with beta-lipoprotein.** *Lancet* 1959, **2:**6–7.
29. 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.
30. Freigang S, Horkko S, Miller E, et al.: **Immunization of LDL receptor-deficient mice with homologous malondialdehyde-modified and native LDL reduces progression of atherosclerosis by mechanisms other than induction of high titers of antibodies to oxidative neoepitopes.** *Arterioscler Thromb Vasc Biol* 1998, **18:**1972–1982.
31. Chyu KY, Reyes OS, Zhao X, et al.: **Timing affects the efficacy of LDL immunization on atherosclerotic lesions in apo E (-/-) mice.** *Atherosclerosis* 2004, **176:**27–35.
32. 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.
33. Palinski W, Witztum JL: **Immune responses to oxidative neoepitopes on LDL and phospholipids modulate the development of atherosclerosis.** *J Intern Med* 2000, **247:**371–380.
34. Horkko S, Binder CJ, Shaw PX, et al.: **Immunological responses to oxidized LDL.** *Free Radic Biol Med* 2000, **28:**1771–1779.
35. Fredrikson GN, Hedblad B, Berglund G, et al.: **Identification of immune responses against aldehyde-modified peptide sequences in apoB associated with cardiovascular disease.** *Arterioscler Thromb Vasc Biol* 2003, **23:**872–878.

36. 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.
37. Chyu KY, Zhao X, Reyes OS, et al.: **Immunization using an Apo B-100 related epitope reduces atherosclerosis and plaque inflammation in hypercholesterolemic apo E (-/-) mice.** *Biochem Biophys Res Commun* 2005, **338:**1982–1989.
38. 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.
39. Horkko S, Bird DA, Miller E, et al.: **Monoclonal autoantibodies specific for oxidized phospholipids or oxidized phospholipid-protein adducts inhibit macrophage uptake of oxidized low-density lipoproteins.** *J Clin Invest* 1999, **103:**117–128.
40. Friedman P, Horkko S, Steinberg D, et al.: **Correlation of antiphospholipid antibody recognition with the structure of synthetic oxidized phospholipids. Importance of Schiff base formation and aldol concentration.** *J Biol Chem* 2002, **277:**7010–7020.
41. Chang MK, Bergmark C, Laurila A, et al.: **Monoclonal antibodies against oxidized low-density lipoprotein bind to apoptotic cells and inhibit their phagocytosis by elicited macrophages: evidence that oxidation-specific epitopes mediate macrophage recognition.** *Proc Natl Acad Sci U S A* 1999, **96:**6353–6358.
42. Briles DE, Forman C, Hudak S, Claflin JL: **Anti-phosphorylcholine antibodies of the T15 idiotype are optimally protective against Streptococcus pneumoniae.** *J Exp Med* 1982, **156:**1177–1185.
43. Shah PK: **Focus on HDL: a new treatment paradigm for athero-thrombotic vascular disease.** *Expert Opin Investig Drugs* 2000, **9:**2139–2146.
44. Shah PK, Kaul S, Nilsson J, Cercek B: **Exploiting the vascular protective effects of high-density lipoprotein and its apolipoproteins: an idea whose time for testing is coming, part I.** *Circulation* 2001, **104:**2376–2383.
45. Shah PK, Kaul S, Nilsson J, Cercek B: **Exploiting the vascular protective effects of high-density lipoprotein and its apolipoproteins: an idea whose time for testing is coming, part II.** *Circulation* 2001, **104:**2498–2502.
46. Price MJ, Shah PK: **New strategies in managing and preventing atherosclerosis: focus on HDL.** *Rev Cardiovasc Med* 2002, **3:**129–137.
47. Rittershaus CW, Miller DP, Thomas LJ, et al.: **Vaccine-induced antibodies inhibit CETP activity in vivo and reduce aortic lesions in a rabbit model of atherosclerosis.** *Arterioscler Thromb Vasc Biol* 2000, **20:**2106–2112.
48. Gaofu Q, Jun L, Xin Y, et al.: **Vaccinating rabbits with a cholesteryl ester transfer protein (CETP) B-Cell epitope carried by heat shock protein-65 (HSP65) for inducing anti-CETP antibodies and reducing aortic lesions in vivo.** *J Cardiovasc Pharmacol* 2005, **45:**591–598.
49. Mao D, Kai G, Gaofu Q, et al.: **Intramuscular immunization with a DNA vaccine encoding a 26-amino acid CETP epitope displayed by HBc protein and containing CpG DNA inhibits atherosclerosis in a rabbit model of atherosclerosis.** *Vaccine* 2006, **24:**4942–4950.
50. Davidson MH, Maki K, Umporowicz D, et al.: **The safety and immunogenicity of a CETP vaccine in healthy adults.** *Atherosclerosis* 2003, **169:**113–120.
51. Naghavi M, Wyde P, Litovsky S, et al.: **Influenza infection exerts prominent inflammatory and thrombotic effects on the atherosclerotic plaques of apolipoprotein E-deficient mice.** *Circulation* 2003, **107:**762–768.
52. Madjid M, Awan I, Ali M, et al.: **Influenza and atherosclerosis: vaccination for cardiovascular disease prevention.** *Expert Opin Biol Ther* 2005, **5:**91–96.
53. Madjid M, Naghavi M, Litovsky S, Casscells SW: **Influenza and cardiovascular disease: a new opportunity for prevention and the need for further studies.** *Circulation* 2003, **108:**2730–2736.
54. Gurfinkel EP, de la Fuente RL, Mendiz O, Mautner B: **Influenza vaccine pilot study in acute coronary syndromes and planned percutaneous coronary interventions: the FLU Vaccination Acute Coronary Syndromes (FLUVACS) Study.** *Circulation* 2002, **105:**2143–2147.
55. Gurfinkel EP, Leon DL, Mendiz O, Mautner B: **Flu vaccination in acute coronary syndromes and planned percutaneous coronary interventions (FLUVACS) Study.** *Eur Heart J* 2004, **25:**25–31.
56. Davis MM, Taubert K, Benin AL, et al.: **Influenza vaccination as secondary prevention for cardiovascular disease. a science advisory from the American Heart Association/American College of Cardiology.** *Circulation* 2006, **114:**1549–1553.
57. Taylor B, Lingam R, Simmons A, et al.: **Autism and MMR vaccination in North London; no causal relationship.** *Mol Psychiatry* 2002, **7(Suppl 2):**S7–S8.
58. Elliman DA, Bedford HE: **Measles, mumps and rubella vaccine, autism and inflammatory bowel disease: advising concerned parents.** *Paediatr Drugs* 2002, **4:**631–635.
59. Orgogozo JM, Gilman S, Dartigues JF, et al.: **Subacute meningoencephalitis in a subset of patients with AD after Abeta42 immunization.** *Neurology* 2003, **61:**46–54.
60. Afek A, George J, Gilburd B, et al.: **Immunization of low-density lipoprotein receptor deficient (LDL-RD) mice with heat shock protein 65 (HSP-65) promotes early atherosclerosis.** *J Autoimmunity* 2000, **14:**115–121.
61. George J, Afek A, Gilburd B, et al.: **Cellular and humoral immune responses to heat shock protein 65 are both involved in promoting fatty-streak formation in LDL-receptor deficient mice.** *J Am Coll Cardiol* 2001, **38:**900–905.
62. George J, Shoenfeld Y, Afek A, et al.: **Enhanced fatty streak formation in C57BL/6J mice by immunization with heat shock protein-65.** *Arterioscler Thromb Vasc Biol* 1999, **19:**505–510.
63. George J, Afek A, Gilburd B, et al.: **Induction of early atherosclerosis in LDL-receptor-deficient mice immunized with beta2-glycoprotein I.** *Circulation* 1998, **98:**1108–1115.
64. 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.
65. 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.
66. Freigang S, Horkko S, Miller E, et al.: **Immunization of LDL receptor-deficient mice with homologous malondialdehyde-modified and native LDL reduces progression of atherosclerosis by mechanisms other than induction of high titers of antibodies to oxidative neoepitopes.** *Arterioscler Thromb Vasc Biol* 1998, **18:**1972–1982.
67. Gaofu Q, Jun L, Xin Y, et al.: **Vaccinating rabbits with a cholesteryl ester transfer protein (CETP) B-cell epitope carried by heat shock protein-65 (HSP65) for inducing anti-CETP antibodies and reducing aortic lesions in vivo.** *J Cardiovasc Pharmacol* 2005, **45:**591–598.
68. Zhou X, Nicoletti A, Elhage R, Hansson GK: **Transfer of CD4(+) T cells aggravates atherosclerosis in immunodeficient apolipoprotein E knockout mice.** *Circulation* 2000, **102:**2919–2922.
69. Major AS, Wilson MT, McCaleb JL, et al.: **Quantitative and qualitative differences in proatherogenic NKT cells in apolipoprotein E-deficient mice.** *Atheroscler Thromb Vasc Biol* 2004, **24:**2351–2357.
70. Patel S, Thelander EM, Hernandez M, et al.: **ApoE(-/-) mice develop atherosclerosis in the absence of complement component C5.** *Biochem Biophys Res Commun* 2001, **286:**164–170.