Immunomodulation of atherosclerosis with a vaccine

Prediman K Shah*, Kuang-Yuh Chyu, Gunilla N Fredrikson and Jan Nilsson

SUMMARY

Experimental observations have established that the innate and adaptive immune mechanisms both have roles in the modulation of atherosclerosis. The complex function that the immune system has in the pathophysiology of atherosclerosis is highlighted by the fact that both proatherogenic and atheroprotective effects of immune activation can be demonstrated. An immune response to the protein and lipid components of oxidized LDL cholesterol has been observed in experimental models, and immunization with these antigens has generally reduced atherosclerosis. The findings suggest the tantalizing possibility that an atheroprotective vaccine can be developed. Our laboratories have identified several antigenic epitopes in the human apolipoprotein B100 component of LDL cholesterol. Active immunization with some of these epitopes has reduced atherosclerosis in hyperlipidemic mice. We believe, therefore, that a vaccine based on apolipoprotein B100-related peptide could have a role in reducing atherosclerosis. In this review, we discuss the possible immunologic mechanisms by which vaccines against atherosclerosis might work and the ways in which such treatment might be most effectively administered.

KEYWORDS atheroprotective, atherosclerosis, immune response, proatherogenic, vaccination

REVIEW CRITERIA

A search for original articles focusing on atherosclerosis was performed in MEDLINE and PubMed. We searched for articles published between 1965 and 2005, with the search terms "vaccination", "immunization", "atherosclerosis" and "immunity". All papers identified were English-language, full-text papers. We also searched the reference lists of identified articles for further papers.

PK Shah is the Director and K-Y Chyu is a Cardiologist in the Division of Cardiology and Atherosclerosis Research Center, Cedars-Sinai Medical Center, Los Angeles, CA, USA. GN Fredrikson is an Assistant Professor and J Nilsson is a Professor of Medicine in the Department of Clinical Sciences, Malmö University Hospital, Sweden.

Correspondence
*Cardiology Division, Cedars Sinai Medical Center, Suite 5531, 8700 Beverly Boulevard, Los Angeles, CA 90048, USA
shahp@cshs.org

Received 29 April 2005 **Accepted** 8 August 2005
www.nature.com/clinicalpractice
doi:10.1038/ncpcardio0372

INTRODUCTION

In the past few years, considerable evidence has accumulated in favor of the paradigm that atherosclerosis is a chronic disease in which inflammatory and immune responses contribute to the initiation, progression and destabilization of atherosclerotic lesions.[1,2] The innate and adaptive immune responses have both been shown to modulate atherosclerosis.[3,4] Like many other biologic systems, it has become apparent that activation of the immune system has two juxtaposed roles in atherosclerosis, with evidence of both proatherogenic and atheroprotective effects.[3–5] Selective activation of the atheroprotective pathway of the immune response might offer a potentially novel therapeutic or preventive approach against this prevalent disease. In this paper we discuss the possible immunologic mechanisms by which vaccines against atherosclerosis might work and the ways in which such treatment might most effectively be administered.

INNATE IMMUNITY AND ATHEROSCLEROSIS

The innate immune response is orchestrated by macrophages and dendritic cells with a rapid, blunt and somewhat nonspecific response against many extrinsic, mostly infectious, threats, although various quasi-infectious and endogenous noninfectious stimuli are relevant.[4,6] Exogenous infectious triggers interact with TOLL-LIKE RECEPTORS (TLRs), which act as pattern-recognition receptors, leading ultimately to the transcription of several genes involved in acute inflammation and the eventual release of inflammatory cytokines that characterize the acute innate immune response (Figure 1).[6] In this regard, it is interesting to note that TLR4 and other members of the TLR family are expressed by macrophages and endothelial cells in murine and human atherosclerotic lesions.[7,8] Genetic interference in innate immune signaling, through disruption of TLR4 or myeloid differentiation

GLOSSARY

TOLL-LIKE RECEPTORS
A family of transmembrane receptors that specifically discriminate between self-antigens and microbial nonself-antigens by recognizing conserved molecular patterns

PHOSPHORYLCHOLINE HEAD GROUP
Part of the phosphatidylcholine molecule, which is the predominant phospholipid in all cell membranes and of circulating blood lipoproteins

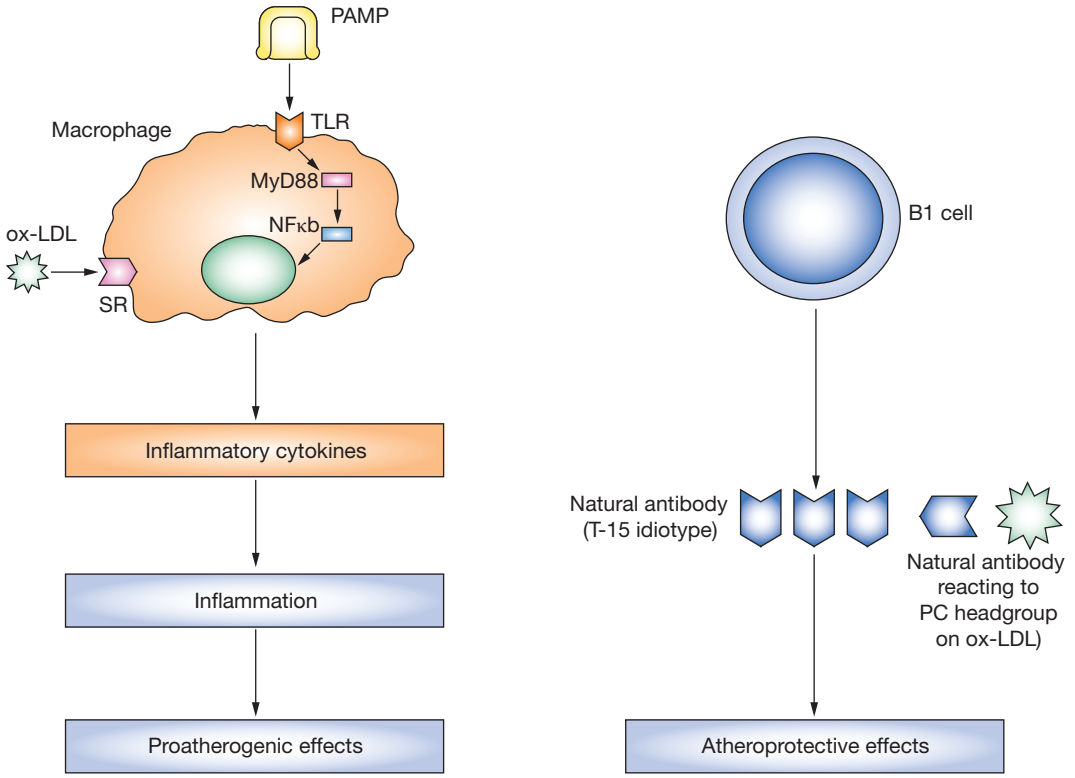

Figure 1 A schematic representation of the potential juxtaposed roles of the innate immune response in atherosclerosis. The proatherogenic component of this system is related to mediation of inflammatory signaling by Toll-like receptors and myeloid differentiation factor 88, a downstream adaptor molecule. The atheroprotective component is mediated by B1-cell-derived natural antibody to phosphorylcholine head group, which cross-reacts with phosphorylcholine on oxidized LDL cholesterol. MyD88, myeloid differentiation factor 88; NKκB, nuclear factor κ B; Ox-LDL, oxidized LDL cholesterol; PAMP, pathogen-associated molecular patterns; PC, phosphorylcholine; SR, scavenger receptor; TLR, Toll-like receptor.

factor 88, a downstream adaptor molecule involved in TLR4 signaling, reduces atherosclerosis, plaque inflammation and circulating inflammatory proteins in mice independent of changes in circulating cholesterol levels.[9,10] Multiple cell types express these genes and, therefore, experiments with mice deficient in the genes encoding TLR4 and myeloid differentiation factor 88 cannot reveal the precise cell types involved in the associated phenotype. Nevertheless, these data demonstrate a proatherogenic role for the innate immune response that is mediated by TLR4 and myeloid differentiation factor 88.

On the other hand, a naturally occurring, humoral, innate immune response without previous antigen exposure might have an atheroprotective role. This type of immune response involves natural antibodies, a class of predominantly IgM antibodies that are produced by self-renewing B1 lymphocytes without T-cell or thymic involvement.[11–13] These natural antibodies recognize the PHOSPHORYLCHOLINE HEAD GROUP present in the phospholipid moiety of oxidized LDL, apoptotic cells, and the cell wall of pneumococcus.[11,12] These natural antibodies to phosphorylcholine, which can also be stimulated by pneumococcal vaccination, reduce the uptake of oxidized LDL by macrophages and attenuate murine atherosclerosis.[12] Binder *et al.*[14] have demonstrated that immunization of mice with malondialdehyde-modified LDL, which does not expose the phosphorylcholine head group, led to a significant increase in natural antibodies to phosphorylcholine. This effect was attributable to an increase in interleukin (IL)-5, a T-helper (T_H)2 cytokine; therefore, a novel link was seen between the adaptive immune response and innate immunity. Neto *et al.*[15] developed a monoclonal antibody to

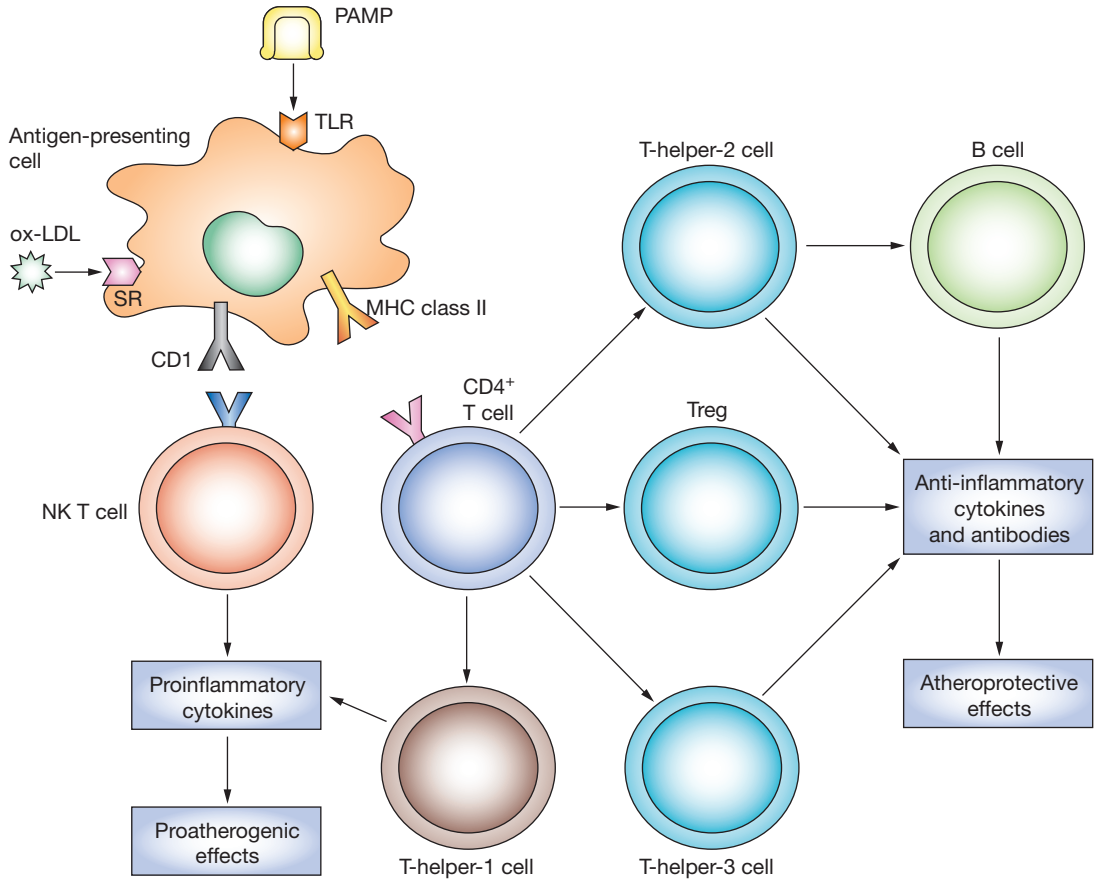

Figure 2 A schematic representation of the potential juxtaposed roles of the adaptive immune response to specific antigens. The proatherogenic component results from T-helper-1 cell and natural killer T-cell activation triggered by the presentation of antigens by the major histocompatibility complex class II or CD1 molecules. The atheroprotective component is mediated by the secretion of anti-inflammatory cytokines (interleukin-10 and transforming growth factor-β), mediated by T-helper-2, T-helper-3, and regulatory T cells, and antibody response mediated by B cells. MHC class II, major histocompatibility complex class II; NK T cell, natural killer T cell; Ox-LDL, oxidized LDL cholesterol; PAMP, pathogen-associated molecular patterns; SR, scavenger receptor; TLR, Toll-like receptor; Treg, regulatory T cells.

GLOSSARY

COMPLEMENT 3
A β-globulin component in the alternative complement cascade; its proteolytic product C3b binds to the surface of the microbe without a role for antibody

phosphorylcholine and showed that passive immunization of hyperlipidemic mice with this antibody reduces accelerated vein-graft atherosclerosis, which further supports an atheroprotective role for humoral immune response to phosphorylcholine.

Complement and acute-phase reactants are other components of the innate immune system that have been linked to atherosclerosis. Genetic deficiency of COMPLEMENT 3 reduced atherosclerosis in LDL-receptor mice and apolipoprotein $E^{-/-}$ plus $LDL\text{-receptor}^{-/-}$ mice.[16,17] The precise mechanism by which deficiency of complement 3 improves atherosclerosis remains unclear, although local vascular effects and dyslipidemia have been implicated.[16,17]

ADAPTIVE IMMUNITY AND ATHEROSCLEROSIS

Unlike the innate reaction, the adaptive immune response to an antigen is specific, taking days or weeks to be fully orchestrated. Stochastic rearrangement takes place during the development of immunoblasts, which generates a large number of T-cell and B-cell receptors and immunoglobulins, which can recognize specific foreign antigens. Adaptive immune response occurs when a specific antigen, processed and presented by antigen-presenting cells, is recognized by the immune system, leading to proliferation of T and B cells. Following uptake by the scavenger receptors on macrophages, and possibly dendritic cells, oxidized LDL is processed and epitopes derived from the LDL particle are presented by major

histocompatibility complex class II proteins for recognition by specific $CD4^+$ T cells. When T cells encounter their specific antigens on a major histocompatibility complex class II molecule, an adaptive immune response is activated, including clonal proliferation of the T cell and production of cytokines, and subsequent activation of B cells to produce immunoglobulins (Figure 2).

Several subtypes of $CD4^+$ cells have been identified, including T_H1 cells, which secrete proinflammatory cytokines such as interferon-γ, and T_H2 cells, which produce IL-4, IL-5 and IL-10.[3,4] In general, T_H1 cells and their cytokines promote macrophage activation, inflammation, and atherosclerosis, whereas T_H2 cells and their cytokines mediate antibody production, allergic reactions, and generally have anti-inflammatory and antiatherogenic effects.[18–20] During the early stages of plaque development in apolipoprotein $E^{-/-}$ mice, circulating IgG acting on oxidized LDL is mainly of the IgG_{2a} subtype, which is characteristic of a T_H1 immune response. At more advanced stages of the disease, however, a shift towards expression of T_H2-specific IgG has been noted in severely hypercholesterolemic animals. This finding suggests that the immune system is adapting to evolve into a less-proinflammatory and less-proatherogenic response.[18–20]

Reduction of atherosclerosis in hyperlipidemic apolipoprotein $E^{-/-}$ mice with a lack of functional T and B cells, and reversal of this phenotype effect on reconstitution with functional $CD4^+$ T cells, suggests proatherogenic effects of adaptive immunity.[21] Other $CD4^+$ T-cell subsets have been defined that might modulate the adaptive immune response. Regulatory T cells constitutively express CD25 and inhibit the activation of other T cells by producing immune inhibitory cytokines, such as IL-10 (regulatory T1 cells) or transforming growth factor-β, also produced by T_H3 cells, which can favorably influence inflammation and atherosclerosis in murine models.[22–25] Activation of yet another subset of $CD4^+$ T cells, which expresses natural killer 1.1 receptors found on natural killer cells and recognize lipid antigens presented by the class-I-like molecule CD1, worsens early atherosclerosis in apolipoprotein $E^{-/-}$ mice.[26]

AUTOIMMUNE RESPONSE IN ATHEROSCLEROSIS

Findings from several experimental and clinical studies have demonstrated that autoantibodies to autoantigens, such as oxidized LDL, heat-shock protein 60, and β-2 glycoprotein, are present in atherosclerosis.[27–30] Furthermore, T cells reactive to oxidized LDL have been identified within the atherosclerotic lesions,[31] and the cytokines of both T_H1 and T_H2 systems have been shown to modulate atherosclerosis.[3,4] Evidence reported so far suggests that activation of an adaptive immune response to heat-shock protein 60 and β-2 glycoprotein is proatherogenic, whereas the adaptive immune response to oxidized LDL might be proatherogenic or atheroprotective.[3–5,32–34] Palinski *et al.*[35] first demonstrated atheroprotective effects of immunization with malondialdehyde-modified LDL in Watanabe rabbits. The following year, further studies demonstrated atheroprotective effects of immunization with native and *ex vivo* oxidized homologous LDL in cholesterol-fed rabbits.[36,37] These observations of atheroprotective effects of immunization with oxidized LDL have also been demonstrated in hypercholesterolemic mice.[38–40] The available experimental evidence thus suggests that, while adaptive immunity in hypercholesterolemic animals is predominantly proatherogenic, active immunization with oxidized LDL inhibits atherosclerosis. This atheroprotective effect is possibly achieved by shifting the endogenous immune response against oxidized LDL from a proinflammatory T_H1 response towards an anti-inflammatory T_H2 or T_H3 response. The autoimmunity of any antigen is, however, difficult to prove and more work is needed to further elucidate the roles of the autoantigens identified so far. Work is also needed to identify other autoantigens that might have a role.

THE CONCEPT OF A VACCINE FOR ATHEROSCLEROSIS

Although vaccines have eradicated many serious infectious diseases, vaccination has also been tested against noninfectious chronic inflammatory or autoimmune diseases, such as Alzheimer's disease and cancer, with variable results. Immunization with synthetic amyloid β peptides in murine models of Alzheimer's disease have resulted in decreased amyloid deposits with concomitant beneficial neuropathologic and behavioral changes.[41] Subsequent human trials have shown beneficial effects of immunization with amyloid β peptide on cognitive functions, but one phase II trial was discontinued shortly after its initiation when approximately 5% of

the treated patients developed cerebral inflammation.[41] The mechanism responsible for these adverse events is not fully understood, but might have involved the use of a proinflammatory T_H1-inducing adjuvant in the clinical trial, as opposed to the anti-inflammatory T_H2-favoring adjuvants that were used in the animal studies and which had produced more-favorable results.[41]

Oxidized LDL is believed to have a key role in atherosclerosis because it causes intimal inflammation and foam-cell formation. Oxidation of polyunsaturated fatty acids in phospholipids and cholesteryl esters generates breakdown products such as malondialdehyde and 4-hydroxynonenal, which form covalent adducts with amino acids containing free amino groups in apolipoprotein B100, the main protein component of LDL. Oxidation of LDL also leads to degradation of apolipoprotein B100 into numerous peptide fragments.[42] It is thought that these modifications make oxidized LDL a target for the immune system. Several clinical studies have shown the presence of circulating autoantibodies to oxidized LDL, in healthy individuals and patients with cardiovascular disease; however, the relationship between the antibody titer and cardiovascular disease has been inconsistent.[28,43–45]

Some antigenic sequences that are normally absent or concealed become available after the oxidation of LDL; these have been termed neoepitopes.[46] The structure of apolipoprotein B100 component of LDL was studied to identify potential antigenic epitopes that could be responsible for the atheroprotective effects of immunization with oxidized LDL,[47] and involved the synthesis of 302 peptide sequences, spanning the entire human apolipoprotein B100 molecule, each 20 amino acids long with an overlap of five amino acids.[48] Of these potential antigenic epitopes, 102 have been found to be associated with an antibody response in pooled human serum.[49] Some of the peptide sequences, but not others, provoke an atheroprotective response in hypercholesterolemic mice when incorporated into a vaccine formulation with alum as an adjuvant; a 40–70% reduction in aortic atherosclerosis and a reduction in plaque inflammation was observed.[48,49] Furthermore, studies have shown that such atheroprotection can be passively transferred to nonimmunized mice through adoptive transfer of splenocytes from immunized mice.[49] Passive immunization, using a monoclonal antibody to one of the atheroprotective peptide epitopes, has also been shown to reduce atherosclerosis in hyperlipidemic mice.[50] These observations have raised the tantalizing possibility that an atheroprotective vaccination strategy based on certain specific apolipoprotein-B100-related peptide epitopes is feasible.

Most of the experimental studies of immunization with oxidized LDL and peptide antigens specific for oxidized LDL have demonstrated the efficacy of this approach in the prevention of early atherosclerotic lesions when used before significant lesion development; whether immunization is beneficial after lesions have already formed remains to be established. Although prevention of atherosclerosis by immunization at an early age remains an exciting, potential long-term goal, investigation of whether immunization slows plaque progression, induces stabilization of existing plaques or both is also warranted. Observations from experimental studies have shown that immunization favorably changes the composition of established plaques, indicated by decreased plaque inflammation and increased collagen content,[40,48,49] but further studies addressing this important question are required.

For clinical testing of a vaccination strategy based on apolipoprotein-B100-related peptides, a number of critical issues will need to be addressed: identification of the most effective antigen or antigens responsible for activation of atheroprotective immune responses, selection of the most suitable immune adjuvant and the optimum route of administration, and assessment of the durability and safety of response. The immune response to an antigen is modulated by the type of antigen, its route of administration, and the choice of the adjuvant, which can influence antigen delivery and presentation, induction of immunomodulatory cytokines, and effects on antigen-presenting cells. Gels containing aluminium salt, the only adjuvants approved for general use in US-licensed vaccines, primarily function by forming a complex with antigens and retaining them at the site of injection. FREUND'S COMPLETE ADJUVANT, which contains substantial amounts of heat-shock protein, usually induces a strong T_H1 or delayed-type hypersensitivity and antibody responses. In comparison, FREUND'S INCOMPLETE ADJUVANT induces antibody production but promotes

GLOSSARY

FREUND'S COMPLETE ADJUVANT
An emulsion of water, mineral oil, and antigen to which micro-organisms—frequently heat-killed mycobacteria—are added for vaccination

FREUND'S INCOMPLETE ADJUVANT
An emulsion of water, mineral oil, and antigen for vaccination, without the addition of micro-organisms

GLOSSARY

UNMETHYLATED CPG MOTIFS
Regions of genomic DNA containing the cytosine–guanine dinucleotide, in which cytosine remains unmethylated, especially in prokaryotic DNA

delayed-type hypersensitivity reactions to a lesser extent than the complete adjuvant.[5] DNA sequences containing UNMETHYLATED CPG MOTIFS activate antigen-presenting dendritic cells through TLR ligation, leading to enhanced immune responses to the antigen.

Several new types of adjuvant have been tested in preclinical and clinical trials, including liposomes, immunostimulatory complexes, and biodegradable polymer microspheres.[5] Of these adjuvants, some might become relevant for testing in immunomodulation of atherosclerosis because they mimic the pattern through which, for example, oxidized LDL antigens are presented. Other adjuvant approaches include coadministration of cytokines, such as IL-12 and interferon-γ, and genetic synthesis of fusion proteins containing peptide sequences for both antigen and costimulatory molecules.[5] Understanding the mechanisms through which each antigen contributes to the disease might be necessary in finding the optimum combination of antigen and vehicle of administration. Accumulating evidence suggests that activation of immunity in atherosclerosis primarily involves proinflammatory T_H1 cells that promote disease development. Accordingly, adjuvants that favor a shift towards an anti-inflammatory T_H2 response, such as alum and Freund's incomplete adjuvant, might be more effective than adjuvants favoring T_H1 responses.[5]

Immune tolerance induction as an antiatherogenic strategy

Autoimmune response to heat-shock protein 60 and β-2 glycoprotein has been shown to accelerate atherosclerosis in animal models.[25–30] Mucosal (nasal or oral) administration of autoantigens reduces organ-specific inflammation and severity of several autoimmune diseases in animals through induction of a response by regulatory T cells, leading to suppression of the T_H1-type response and secretion of anti-inflammatory cytokines IL-4, IL-10, and transforming growth factor-β, or leading to clonal deletion or anergy at high doses.[51,52] Induction of tolerance to heat-shock protein 65, a mycobacterial heat-shock protein homologous to human heat-shock protein 60, and β-2 glycoprotein by exposure of the oral mucosa to the antigen before the development of atherosclerosis has been shown to reduce early-stage atherosclerosis in animal models.[53,54] Similar results were also reported with nasal administration of heat-shock protein 65 in LDL receptor$^{-/-}$ mice.[55] These proof-of-concept studies highlight the possibility that induction of immune tolerance through activation of a mucosal immune response could emerge as yet another novel immunomodulating therapy for atherosclerosis, and this strategy merits further evaluation.

A VACCINE TO RAISE HDL CHOLESTEROL LEVELS

The beneficial effects of high HDL cholesterol levels against atherosclerosis are well known. Cholesterol ester transfer protein is a key enzyme involved in HDL metabolism. Inhibition of this enzyme results in marked elevation of HDL, with a reduction in atherosclerosis in hyperlipidemic rabbits.[56] Experimental studies have shown the feasibility and atheroprotective efficacy of raising HDL levels by inhibition of cholesterol ester transfer protein through the induction of neutralizing antibodies by active immunization or by directly blocking antibodies to this enzyme.[56] Clinical testing of such a vaccine strategy is ongoing.[56]

Effects of the influenza vaccine

Influenza promotes arterial and plaque inflammation in experimental models, and triggering of acute myocardial infarction by upper respiratory infection has been suggested in humans. It has been suggested that influenza increases the risk of cardiovascular disease by triggering destabilization of existing vulnerable plaques.[57] The findings from two case–control studies have suggested that influenza vaccination is associated with a markedly reduced short-term risk of myocardial infarction.[57] Nichol *et al.*[58] reported that the incidence of hospitalization for cardiac disease in a community-dwelling cohort of 288,238 people, who were aged 65 years or older, was 19% lower in those who received influenza vaccination than in those who did not. In a randomized pilot trial of 301 patients with existing coronary disease, Jackson *et al.*[57] observed a significant reduction in cardiovascular death 6 months after influenza vaccination. These researchers were, however, unable to identify a protective effect of vaccination in a younger cohort of survivors of a first myocardial infarction.[57] Thus, the role of influenza vaccination in prevention of cardiovascular disease remains to be fully established.

CONCLUSIONS AND PERSPECTIVES

Modulation of immune responses involved in atherosclerosis using vaccines and passive immunization with antibodies, particularly to apolipoprotein-B100-related peptide, potentially represent novel approaches to the management of atherosclerotic cardiovascular disease. Experimental proof-of-concept studies have provided encouraging data, but continued investigation of this promising new approach is warranted to ensure safety and durability of benefits before clinical testing is considered. Even though understanding of the complex role of the immune system in atherosclerosis remains incomplete, there is cautious optimism for the potential of vaccination as a complementary approach to existing and partially effective antiatherogenic strategies in atherosclerosis. It is hoped that a vaccination strategy will one day become part of the routine childhood vaccination program.

References

1 Ross R (1999) Atherosclerosis—an inflammatory disease. *N Engl J Med* **340:** 115–126
2 Shah PK (2003) Mechanisms of plaque vulnerability and rupture. *J Am Coll Cardiol* **41 (4 Suppl S):** S15S–S22S
3 Binder CJ *et al*. (2002) Innate and acquired immunity in atherogenesis. *Nat Med* **8:** 1218–1226
4 Hansson GK *et al*. (2002) Innate and adaptive immunity in the pathogenesis of atherosclerosis. *Circ Res* **91:** 281–291
5 Nilsson J *et al*. (2005) Immunomodulation of atherosclerosis: implications for vaccine development. *Arterioscler Thromb Vasc Biol* **25:** 18–28
6 Gordon S (2002) Pattern recognition receptors: doubling up for the innate immune response. *Cell* **111:** 927–930
7 Xu XH *et al.* (2001) Toll-like receptor-4 is expressed by macrophages in murine and human lipid-rich atherosclerotic plaques and upregulated by oxidized LDL. *Circulation* **104:** 3103–3108
8 Edfeldt K *et al*. (2002) Expression of toll-like receptors in human atherosclerotic lesions: a possible pathway for plaque activation. *Circulation* **105:** 1158–1161
9 Michelsen KS *et al*. (2004) Lack of TLR4 or myeloid differentiation factor 88 reduces atherosclerosis and alters plaque phenotype in mice deficient in apolipoprotein E. *Proc Natl Acad Sci USA* **101:** 10679–10684
10 Bjorkbacka H *et al*. (2004) Reduced atherosclerosis in MyD88-null mice links elevated serum cholesterol levels to activation of innate immunity signaling pathways. *Nat Med* **10:** 416–421
11 Shaw PX *et al*. (2000) Natural antibodies with the T15 idiotype may act in atherosclerosis, apoptotic clearance, and protective immunity. *J Clin Invest* **105:** 1731–1740
12 Binder CJ *et al*. (2003) Pneumococcal vaccination decreases atherosclerotic lesion formation: molecular mimicry between *Streptococcus pneumoniae* and oxidized LDL. *Nat Med* **9:** 736–743
13 Binder CJ *et al*. (2005) The role of natural antibodies in atherogenesis. *J Lipid Res* **46:** 1353–1363
14 Binder CJ *et al*. (2004) IL-5 links adaptive and natural immunity specific for epitopes of oxidized LDL and protects from atherosclerosis. *J Clin Invest* **114:** 427–437
15 Neto JRF *et al*. (2004) Passive immunization with monoclonal (IgM) antibody to phosphorylcholine, an oxidized LDL related neoantigen , reduces accelerated atherosclerosis in a vein-graft model in apo E null mice [abstract]. *Circulation* **110:** III–52
16 Buono C *et al*. (2002) Influence of C3 deficiency on atherosclerosis. *Circulation* **105:** 3025–3031
17 Persson L *et al*. (2004) Lack of complement factor C3, but not factor B, increases hyperlipidemia and atherosclerosis in apolipoprotein E–/– Low-density Lipoprotein receptor–/– mice. *Arterioscler Thromb Vasc Biol* **24:** 1062–1067
18 Constant SL and Bottomly K (1997) Induction of Th1 and Th2 $CD4^+$ T cell responses: the alternative approaches. *Annu Rev Immunol* **15:** 297–322
19 Frostegard J *et al*. (1999) Cytokine expression in advanced human atherosclerotic plaques: dominance of pro-inflammatory (Th1) and macrophage-stimulating cytokines. *Atherosclerosis* **145:** 33–43
20 Zhou X *et al*. (1998) Hypercholesterolemia is associated with a T helper (Th) 1/Th2 switch of the autoimmune response in atherosclerotic apo E-knockout mice. *J Clin Invest* **101:** 1717–1725
21 Zhou X *et al*. (2000) Transfer of $CD4^+$ T cells aggravates atherosclerosis in immunodeficient apolipoprotein E knockout mice. *Circulation* **102:** 2919–2922
22 Sakaguchi S (2000) Regulatory T cells: key controllers of immunologic self-tolerance. *Cell* **101:** 455–458
23 Mallat Z *et al*. (2003) Induction of a regulatory T cell type 1 response reduces the development of atherosclerosis in apolipoprotein E-knockout mice. *Circulation* **108:** 1232–1237
24 Pinderski Oslund LJ *et al*. (1999) Interleukin-10 blocks atherosclerotic events in vitro and *in vivo*. *Arterioscler Thromb Vasc Biol* **19:** 2847–2853
25 Robertson AK *et al*. (2003) Disruption of TGF-β signaling in T cells accelerates atherosclerosis. *J Clin Invest* **112:** 1342–1350
26 Tupin E *et al*. (2004) CD1d-dependent activation of NKT cells aggravates atherosclerosis. *J Exp Med* **199:** 417–422
27 Frostegard J (2002) Autoimmunity, oxidized LDL and cardiovascular disease. *Autoimmun Rev* **1:** 233–237
28 Tornvall P *et al*. (2003) Autoantibodies against modified low-density lipoproteins in coronary artery disease. *Atherosclerosis* **167:** 347–353
29 Perschinka H *et al*. (2003) Cross-reactive B-cell epitopes of microbial and human heat shock protein 60/65 in atherosclerosis. *Arterioscler Thromb Vasc Biol* **23:** 1060–1065
30 Xu Q *et al*. (1993) Association of serum antibodies to heat-shock protein 65 with carotid atherosclerosis. *Lancet* **341:** 255–259
31 Stemme S *et al*. (1995) T lymphocytes from human atherosclerotic plaques recognize oxidized low density lipoprotein. *Proc Natl Acad Sci USA* **92:** 3893–3897
32 Afek A *et al*. (2000). Immunization of low-density lipoprotein receptor deficient (LDL-RD) mice with heat shock protein 65 (HSP-65) promotes early atherosclerosis. *J Autoimmun* **14:** 115–121
33 Xu Q *et al*. (1992). Induction of arteriosclerosis in normocholesterolemic rabbits by immunization with heat shock protein 65. *Arterioscler Thromb* **12:** 789–799
34 George J *et al*. (1998) Induction of early atherosclerosis in LDL-receptor deficient mice immunized with β_2 glycoprotein I. *Circulation* **11:** 1108–1115
35 Palinski W *et al*. (1995) Immunization of low density lipoprotein (LDL) receptor-deficient rabbits with homologous malondialdehyde-modified LDL reduces atherogenesis. *Proc Natl Acad Sci USA* **92:** 821–825

Acknowledgments

We gratefully acknowledge the contributions of many colleagues in the Atherosclerosis Research Center at Cedars Sinai Medical Center and at the University of Lund to this work. We thank the Eisner Foundation, the Heart Fund, United Hostesses, and the Entertainment Industry Foundation for support. We used hybridoma technology provided by Dr Patricia Gearhart at the NIH in our work on this topic.

Competing interests

PK Shah and J Nilsson declared competing interests; go to the article online for details. The other authors declared they have no competing interests.

36 Ameli S *et al*. (1996) Effect of immunization with homologous LDL on early atherosclerosis in hypercholesterolemic rabbits. *Arterioscler Thromb Vasc Biol* **16:** 1074–1079
37 Nilsson J *et al*. (1997) Immunization with homologous oxidized low density lipoprotein reduces neointimal formation after balloon in hypercholesterolemic rabbits. *J Am Coll Cardiol* **30:** 1886–1891
38 Freigang S *et al*. (1998) 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* **18:** 1972–1982
39 Zhou X *et al*. (2001) LDL immunization induces T-cell-dependent antibody formation and protection against atherosclerosis. *Arterioscler Thromb Vasc Biol* **21:** 108–114
40 Chyu KY *et al*. (2004) Timing affects the efficacy of LDL immunization on atherosclerotic lesions in apo E (–/–) mice. *Atherosclerosis* **176:** 27–35
41 Monsonego A and Weiner HL (2003) Immunotherapeutic approaches to Alzheimer's disease. *Science* **302:** 834–838
42 Fong LG *et al*. (1987) Non enzymatic oxidative cleavage of peptide bonds in apoprotein B-100. *J Lipid Res* **12:** 1466–1477
43 Hulthe J *et al*. (2001). Antibodies to oxidized LDL in relation to intima-media thickness in carotid and femoral arteries in 58-year-old subjectively clinically healthy men. *Arterioscler Thromb Vasc Biol* **21:** 101–107
44 Fukumoto M *et al*. (2000) Antibodies against oxidized LDL and carotid artery intima-media thickness in a healthy population. *Arterioscler Thromb Vasc Biol* **20:** 703–707
45 Hulthe J *et al*. (2001) Antibodies to oxidized LDL in relation to carotid atherosclerosis, cell adhesion molecules, and phospholipase A_2. *Arterioscler Thromb Vasc Biol* **21:** 269–274
46 Palinski W and Witztum JL (2000) Immune responses to oxidative neoepitopes on LDL and phospholipids modulate the development of atherosclerosis. *J Intern Med* **247:** 371–380
47 Fredrikson GN *et al*. (2003) Identification of immune responses against aldehyde-modified peptide sequences in ApoB associated with cardiovascular disease. *Arterioscler Thromb Vasc Biol* **23:** 872–878
48 Fredrikson GN *et al*. (2003) inhibition of atherosclerosis in ApoE-null mice by immunization with ApoB-100 peptide sequences. *Arterioscler Thromb Vasc Biol* **23:** 879–884
49 Reyes OS *et al*. (2002) Immunization with a novel human Apo B100 related peptide reduces atherosclerosis and inflammation in Apo E null mice. *J Am Coll Cardiol* **39 (Suppl A):** S240A
50 Schipou A *et al*. (2004) Recombinant human antibodies against aldehyde-modified apolipoprotein B-100 peptide sequences inhibit atherosclerosis. *Circulation* **110:** 2047–2052
51 Chen Y *et al*. (1995) Peripheral deletion of antigen-reactive T cells in oral tolerance. *Nature* **376:** 177–180
52 Miller A *et al*. (1991) Antigen-driven bystander suppression following oral administration of antigens. *J Exp Med* **174:** 791–798
53 Harats D *et al*. (2002) Oral tolerance with heat shock protein 65 attenuates *Mycobacterium tuberculosis*-induced and high-fat-diet-driven atherosclerotic lesions. *J Am Coll Cardiol* **40:** 1333–1338
54 George J *et al*. (2004) Suppression of early atherosclerosis in LDL-receptor deficient mice by oral tolerance with β_2-glycoprotein I. *Cardiovasc Res* **62:** 603–609
55 Maron R *et al*. (2002) Mucosal administration of heat shock protein-65 decreases atherosclerosis and inflammation in aortic arch of low-density lipoprotein receptor-deficient mice. *Circulation* **106:** 1708–1715
56 Davidson MH *et al*. (2003) The safety and immunogenicity of a CETP vaccine in healthy adults. *Atherosclerosis* **169:** 113–120
57 Madjid M *et al*. (2003) Influenza and cardiovascular disease: a new opportunity for prevention and the need for further studies. *Circulation* **108:** 2730–2736
58 Nichol KL *et al*. (2003) Influenza vaccination and reduction in hospitalizations for cardiac disease and stroke among the elderly. *N Engl J Med* **348:** 1322–1332