

# 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.

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## 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.<sup>1,2</sup> The innate and adaptive immune responses have both been shown to modulate atherosclerosis.<sup>3,4</sup> 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.<sup>3-5</sup> 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.<sup>4,6</sup> 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).<sup>6</sup> 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.<sup>7,8</sup> Genetic interference in innate immune signaling, through disruption of TLR4 or myeloid differentiation

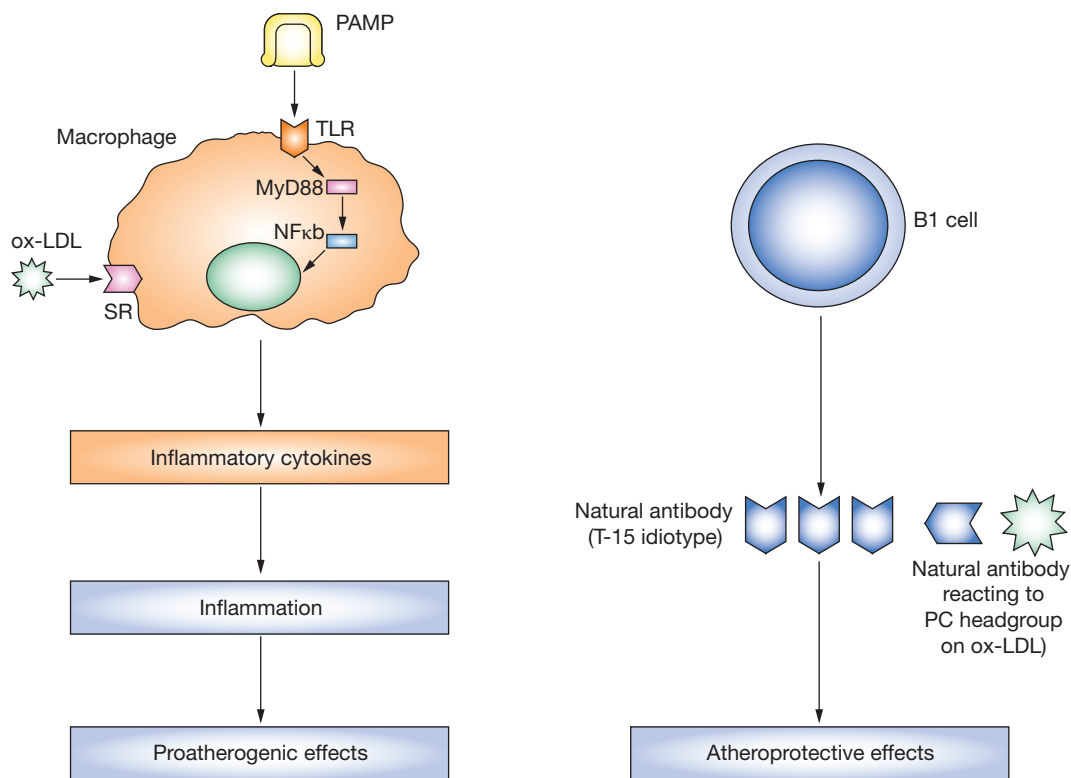
**GLOSSARY**

**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

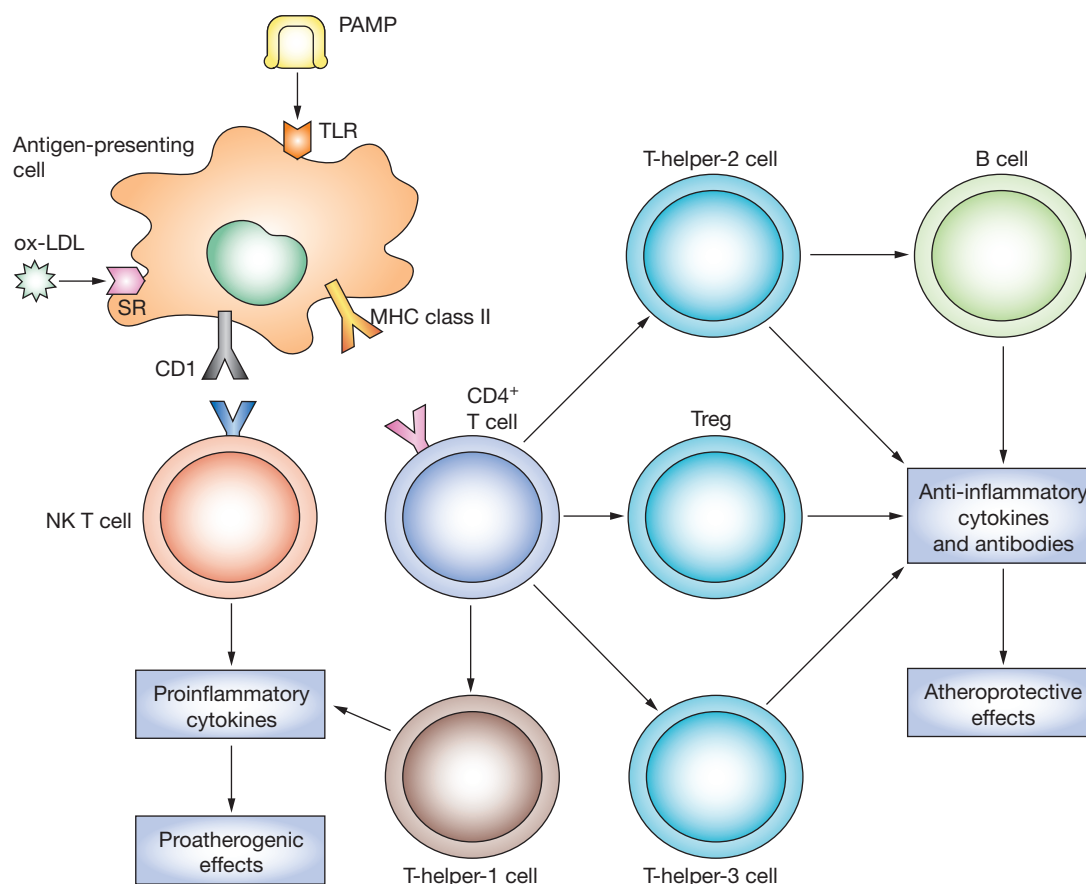


**Figure 1** A schematic representation of the potential juxtaposed roles of the innate immune response in atherosclerosis. The proatherogenic component of this system is related to mediation of inflammatory signaling by Toll-like receptors and myeloid differentiation factor 88, a downstream adaptor 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.<sup>9,10</sup> 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.<sup>11–13</sup> These natural antibodies recognize the PHOSPHORYLCHOLINE HEAD GROUP present in the phospholipid moiety of oxidized LDL, apoptotic cells, and the cell wall of pneumococcus.<sup>11,12</sup> 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.<sup>12</sup> Binder *et al.*<sup>14</sup> 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<sub>H</sub>)<sub>2</sub> cytokine; therefore, a novel link was seen between the adaptive immune response and innate immunity. Neto *et al.*<sup>15</sup> 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- $\beta$ ), 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.

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<sup>-/-</sup> plus LDL-receptor<sup>-/-</sup> mice.<sup>16,17</sup> The precise mechanism by which deficiency of complement 3 improves atherosclerosis remains unclear, although local vascular effects and dyslipidemia have been implicated.<sup>16,17</sup>

## 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

## GLOSSARY

### COMPLEMENT 3

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

histocompatibility complex class II proteins for recognition by specific CD4<sup>+</sup> 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<sup>+</sup> cells have been identified, including T<sub>H</sub>1 cells, which secrete proinflammatory cytokines such as interferon- $\gamma$ , and T<sub>H</sub>2 cells, which produce IL-4, IL-5 and IL-10.<sup>3,4</sup> In general, T<sub>H</sub>1 cells and their cytokines promote macrophage activation, inflammation, and atherosclerosis, whereas T<sub>H</sub>2 cells and their cytokines mediate antibody production, allergic reactions, and generally have anti-inflammatory and antiatherogenic effects.<sup>18–20</sup> During the early stages of plaque development in apolipoprotein E<sup>-/-</sup> mice, circulating IgG acting on oxidized LDL is mainly of the IgG<sub>2a</sub> subtype, which is characteristic of a T<sub>H</sub>1 immune response. At more advanced stages of the disease, however, a shift towards expression of T<sub>H</sub>2-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.<sup>18–20</sup>

Reduction of atherosclerosis in hyperlipidemic apolipoprotein E<sup>-/-</sup> mice with a lack of functional T and B cells, and reversal of this phenotype effect on reconstitution with functional CD4<sup>+</sup> T cells, suggests proatherogenic effects of adaptive immunity.<sup>21</sup> Other CD4<sup>+</sup> 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- $\beta$ , also produced by T<sub>H</sub>3 cells, which can favorably influence inflammation and atherosclerosis in murine models.<sup>22–25</sup> Activation of yet another subset of CD4<sup>+</sup> 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<sup>-/-</sup> mice.<sup>26</sup>

#### **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  $\beta$ -2 glycoprotein, are present in atherosclerosis.<sup>27–30</sup> Furthermore, T cells reactive to oxidized LDL have been identified within the atherosclerotic lesions,<sup>31</sup> and the cytokines of both T<sub>H</sub>1 and T<sub>H</sub>2 systems have been shown to modulate atherosclerosis.<sup>3,4</sup> Evidence reported so far suggests that activation of an adaptive immune response to heat-shock protein 60 and  $\beta$ -2 glycoprotein is proatherogenic, whereas the adaptive immune response to oxidized LDL might be proatherogenic or atheroprotective.<sup>3–5,32–34</sup> Palinski *et al.*<sup>35</sup> 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.<sup>36,37</sup> These observations of atheroprotective effects of immunization with oxidized LDL have also been demonstrated in hypercholesterolemic mice.<sup>38–40</sup> 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<sub>H</sub>1 response towards an anti-inflammatory T<sub>H</sub>2 or T<sub>H</sub>3 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  $\beta$  peptides in murine models of Alzheimer's disease have resulted in decreased amyloid deposits with concomitant beneficial neuropathologic and behavioral changes.<sup>41</sup> Subsequent human trials have shown beneficial effects of immunization with amyloid  $\beta$  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.<sup>41</sup> The mechanism responsible for these adverse events is not fully understood, but might have involved the use of a proinflammatory T<sub>H</sub>1-inducing adjuvant in the clinical trial, as opposed to the anti-inflammatory T<sub>H</sub>2-favoring adjuvants that were used in the animal studies and which had produced more-favorable results.<sup>41</sup>

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.<sup>42</sup> 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.<sup>28,43–45</sup>

Some antigenic sequences that are normally absent or concealed become available after the oxidation of LDL; these have been termed neopeptides.<sup>46</sup> 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,<sup>47</sup> 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.<sup>48</sup> Of these potential antigenic epitopes, 102 have been found to be associated with an antibody response in pooled human serum.<sup>49</sup> 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.<sup>48,49</sup> Furthermore, studies have shown that such atheroprotection can be passively transferred to nonimmunized mice through adoptive transfer of splenocytes from immunized mice.<sup>49</sup> Passive immunization,

using a monoclonal antibody to one of the atheroprotective peptide epitopes, has also been shown to reduce atherosclerosis in hyperlipidemic mice.<sup>50</sup> 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,<sup>40,48,49</sup> 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<sub>H</sub>1 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.<sup>5</sup> 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.<sup>5</sup> 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- $\gamma$ , and genetic synthesis of fusion proteins containing peptide sequences for both antigen and costimulatory molecules.<sup>5</sup> 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<sub>H</sub>1 cells that promote disease development. Accordingly, adjuvants that favor a shift towards an anti-inflammatory T<sub>H</sub>2 response, such as alum and Freund's incomplete adjuvant, might be more effective than adjuvants favoring T<sub>H</sub>1 responses.<sup>5</sup>

**Immune tolerance induction as an antiatherogenic strategy**

Autoimmune response to heat-shock protein 60 and  $\beta$ -2 glycoprotein has been shown to accelerate atherosclerosis in animal models.<sup>25–30</sup> 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<sub>H</sub>1-type response and secretion of anti-inflammatory cytokines IL-4, IL-10, and transforming growth factor- $\beta$ , or leading to clonal deletion or anergy at high doses.<sup>51,52</sup> Induction of tolerance to heat-shock protein 65, a mycobacterial heat-shock protein homologous to human heat-shock protein 60, and  $\beta$ -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.<sup>53,54</sup> Similar

results were also reported with nasal administration of heat-shock protein 65 in LDL receptor<sup>-/-</sup> mice.<sup>55</sup> 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.<sup>56</sup> 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.<sup>56</sup> Clinical testing of such a vaccine strategy is ongoing.<sup>56</sup>

**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.<sup>57</sup> The findings from two case–control studies have suggested that influenza vaccination is associated with a markedly reduced short-term risk of myocardial infarction.<sup>57</sup> Nichol *et al.*<sup>58</sup> 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.*<sup>57</sup> 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.<sup>57</sup> 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.

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**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.

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