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

Published in:
Atherosclerosis

DOI:
10.1016/j.atherosclerosis.2012.10.074

2013

Link to publication

Citation for published version (APA):

General rights
Copyright and moral rights for the publications made accessible in the public portal are retained by the authors and/or other copyright owners and it is a condition of accessing publications that users recognise and abide by the legal requirements associated with these rights.

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

Take down policy
If you believe that this document breaches copyright please contact us providing details, and we will remove access to the work immediately and investigate your claim.
Emerging biomarkers and intervention targets for immune-modulation of atherosclerosis – a review of the experimental evidence

Harry Björkbacka, Gunilla Nordin Fredrikson and Jan Nilsson

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

Word count (including abstract, manuscript, references and figure legends): 7727

Figures: 2

Keywords: biomarkers, immune modulation, regulatory T cells, monocytes, inflammation, autoimmunity

Corresponding author:

Harry Björkbacka
Experimental Cardiovascular Research
CRC 91:12, Lund University
Skåne University Hospital Malmö
Jan Waldenströms gata 35
SE-205 02 Malmö
Sweden
Phone: +46 (0)40 391205
Fax: +46 (0)40 391212
E-mail: harry.bjorkbacka@med.lu.se
ABSTRACT

The role of inflammation in atherosclerosis and plaque vulnerability is well recognized. However, it is only during recent years it has become evident that this inflammation is modulated by immune responses against plaque antigens such as oxidized LDL. Interestingly, both protective and pathogenic immune responses exist and experimental data from animal studies suggest that modulation of these immune responses represent a promising new target for treatment of cardiovascular disease. It has been proposed that during early stages of the disease, autoimmune responses against plaque antigens are controlled by regulatory T cells that inhibit the activity of auto-reactive Th1 effector T cells by release of anti-inflammatory cytokines such as IL-10 and TGF-β. As the disease progresses this control is gradually lost and immune responses towards plaque antigens switch towards activation of Th1 effector T cells and release of pro-inflammatory cytokines such as interferon-γ, TNF-α and IL-1β. Several novel immune-modulatory therapies that promote or mimic tolerogenic immune responses against plaque antigens have demonstrated athero-protective effects in experimental models and a first generation of such immune-modulatory therapies are now in early or about to enter into clinical testing. A challenge in the clinical development of these therapies is that our knowledge of the role of the immune system in atherosclerosis largely rests on data from animal models of the disease. It is therefore critical that more attention is given to the characterization and evaluation of immune biomarkers for cardiovascular risk.
Current therapies for prevention of cardiovascular disease rest almost exclusively on risk factor intervention. This approach has proven very successful and experience from randomized clinical trials has shown that up to 40% of cardiovascular events can be prevented by optimal medical risk factor intervention (1). However, in spite of these encouraging results the majority of treated patients still receive insufficient protection. It is likely that to further improve the efficacy of cardiovascular prevention new treatments directly targeting the disease process in the arterial wall need to be developed. Such treatments will need to specifically target atherosclerotic plaque inflammation. In this review we will discuss evidence suggesting that this can be achieved by modulating immune responses against plaque antigens as well as the need of developing validated biomarkers that can be used to measure such immune responses.

The concept of the immune system as an attractive target for future cardiovascular therapies is primarily based on experimental studies demonstrating that inhibition of inflammatory mediators and induction of specific immune responses can reduce atherosclerosis burden (2-5). The proposed pleiotropic and anti-inflammatory effects of statins and the usefulness of the inflammatory marker high-sensitivity C-reactive protein (hsCRP) in risk prediction in humans reinforce this notion (6, 7). On the other hand, the failures of non-steroidal anti-inflammatory drugs (NSAIDs) and COX-2 specific inhibitors have taught us that general anti-inflammatory treatment may not be a viable option for the treatment of atherosclerosis and the targeting of more specific immune responses will be needed (8, 9). Therefore, CRP, predominantly secreted by the liver and adipose tissue in response to inflammatory stress, is a relatively crude marker for the evaluation of specific immune responses in the vascular wall. It has become increasingly clear that to truly understand the role of inflammation in atherosclerosis CRP will be insufficient
and novel biomarkers assessing the complex role of the immune system in the disease need to be developed. There is today convincing evidence for presence of immune responses against plaque antigens in atherosclerosis and it has been proposed that disease progression occurs as a result of a loss of immune tolerance against these antigens in the plaque (10). However, atherosclerosis is probably not an autoimmune disease in the classical sense but rather a state of local immune dysfunction resulting in an imbalance between naturally occurring autoimmunity and the regulatory immune cells that should control this autoimmunity. The circumstance that women, which generally are more prone to develop autoimmune diseases, suffer from cardiovascular disease at an older age than men also suggest that atherosclerosis is not a true autoimmune disease.

_Atherosclerosis and the immune system_

An impressive list, encompassing almost all aspects of the immune system, can be compiled when trying to summarize immune mechanisms that contribute to atherosclerosis development in mice (Fig. 1). Depletion of monocytes, the precursors of macrophage foam cells in plaques, can to a large extent abrogate atherosclerosis development in mice (11, 12). Interestingly, this effect is more prominent at early stages of the disease while depleting monocyte/macrophages from advanced lesions does not alter plaque size or composition (13). Dendritic cells have been implicated in the formation of the earliest detectable plaques (14, 15). Neutrophils are found in increased numbers in hypercholesterolemic mice and neutrophil depletion effectively reduces atherosclerosis if neutrophil-depleting antibodies are administered during early atherosclerosis development (16, 17). A concept has been put forth where neutrophils pave the way for monocyte infiltration during early atherosclerosis development (18). Platelets also assist in the
recruitment of immune cells to the atherosclerotic plaque by facilitating interactions between endothelial cells and monocytes, neutrophils, dendritic cells and T cells (19). T cell responses have generally been regarded as pro-atherogenic, although protective T cell responses also exist. In this context it is interesting to note that cardiovascular complications are more common in human immunodeficiency virus–infected individuals than in age-matched uninfected individuals and that antiretroviral therapy, which increases T cell counts, reduces the cardiovascular risk in treated subjects (20).

**T cells modulate inflammation in atherosclerosis**

Initial studies in severely immune-deficient mice provided inconclusive and partly contradictory findings regarding the role of the immune system in atherosclerosis (21-23). It was later revealed that this was explained by the fact that both protective and atherogenic immune responses exist (24). There is now convincing evidence that type 1 T helper (Th1) cells promote disease and deletion of the transcription factor T-bet, which is required for Th1 lineage commitment, reduces atherosclerosis (25, 26). The role of type 2 T helper (Th2) cells in atherosclerosis is unclear as several Th2 cytokines have been assigned protective roles, whereas deletion of IL-4, the proteotypic cytokine for Th2 cells, reduces atherosclerosis development in some studies (27-29). The role of Th17 in atherosclerosis is also debated as conflicting reports exist (30-32). In contrast to CD4\(^+\) T helper cells, CD8\(^+\) cytolytic T lymphocytes have not been extensively studied, even though CD8\(^+\) T cells are abundant in atherosclerotic plaques and they have been shown to be activated in hypercholesterolemic Apoe\(^{-/-}\) mice (33). Activation of CD8\(^+\) T cells has also been associated with increased plaque formation in these mice (34). In apparent contrast with these observations, atherosclerosis protection achieved by immunization with apolipoprotein B (apoB)
peptide has been suggested to involve CD8⁺ T cells (35). On the other hand, Tap1-deficiency that leads to severely diminished CD8⁺ T cell populations does not alter atherosclerosis development in Apoe⁻/⁻ mice (36). Regulatory T cells expressing the transcription factor FoxP3, on the other hand, are clearly limiting atherosclerosis development (37). The development and activation of natural killer T (NK T) cells, a subset that expresses surface markers characteristic of both natural killer cells and conventional T cells, depends on the interaction of their T cell receptor with lipids and glycolipids presented on CD1d, a MHC-class I-type molecule. Deletion of CD1d, which also eliminates NK T cells, reduces atherosclerosis development, whereas administration of the exogenous activator αGalCer augments atherosclerosis (38, 39). The role of different immune cells in atherosclerosis is summarized in the figure 1.

**Emerging therapies promoting regulatory T cell responses**

The atherosclerosis quenching properties of regulatory T cells have attracted much attention in recent years and have spurred the development of therapies that inhibit atherosclerosis in mice by promoting regulatory T cells. Regulatory T cells prevent autoimmunity by controlling the activity of potentially auto-reactive T cells that have escaped deletion in the thymus. These natural regulatory T cells are characterized by expression of CD25 and the transcription factor FoxP3, which is considered the master regulator of the regulatory T cell transcription program. A wealth of data supports a protective role for regulatory T cells in atherosclerosis. Mice lacking the co-stimulatory molecules CD80/86, CD28 or ICOS have reduced numbers of regulatory T cells and consequently develop atherosclerosis more readily (37, 40). Furthermore, depletion of regulatory T cells with an anti-CD25 antibody or by immunizations targeting FoxP3 also significantly increases the formation of atherosclerotic plaques (37, 41). Regulatory T cells
generated in the periphery are characterized by expression of IL-10 (Tr1 cells) or TGF-β (Th3 cells). Adoptive transfer of a clone of ovalbumin-specific Tr1 cells together with its cognate antigen inhibits plaque development in mice and inhibition of Th3 cells through deletion of the receptor for TGF-β on T cells enhance disease progression (42, 43). Thus, inhibition of atherosclerosis has been associated with induction of several types of regulatory T cells including natural regulatory T cells in response to anti-CD3 and anti-CD45RB treatment (44, 45), Th3 cells through oral immunization with oxidized LDL (46) and Tr1 cell trough nasal immunization with an apoB peptide fused with the cholera toxin B subunit (47).

Both experimental and clinical evidence support the existence of autoimmune responses against LDL modified by oxidation (10). As much as 10 % of the T cells in atherosclerotic plaques are oxidized LDL-specific (48). Interestingly, the existence of T effector cells auto-reactive for native apoB has recently also been reported and neutralization of these cells by immunization against their specific T cell receptor was shown to result in decreased atherosclerosis (49). The existence of T cells that are auto-reactive for native or modified lipoproteins is not unexpected because the immune system is forced to allow the generation of some cells with limited auto-reactivity to avoid narrowing the capacity for immunological diversity required for an effective host defense. Such auto-reactive T effector cells will normally be controlled by regulatory T cells with similar antigen specificity. However, it has been proposed that the balance between plaque antigen specific T effector cells and regulatory T cells is shifted in atherosclerosis allowing plaque inflammation to progress (10). Accordingly, several novel strategies to promote peripheral tolerance against self-antigens in mice by modulating regulatory T cells have emerged and constitute an attractive novel approach to treat atherosclerosis. Particularly, the possibility to
induce antigen-specific tolerance has advantages over more general anti-inflammatory therapies that also may compromise defence against invading pathogens. Autoimmunity against self-antigens, such as oxidized LDL and heat shock proteins (HSPs), play an important role in the development of atherosclerosis and immunization with HSPs or apoB peptides are associated with a decrease in atherosclerosis development (50, 51). Atherosclerosis protection achieved by immunization with apoB peptide and Alum adjuvant has been credited to regulatory T cell dependent mechanisms (52, 53). Similarly, oral administration of oxidized LDL or HSP60 is associated with increased number of regulatory T cells and reduced atherosclerosis (46, 54).

Mucosal delivery of apoB peptides has also been shown to reduce atherosclerosis and to be associated with an increase in Tr1-type regulatory T cells (47). Furthermore, subcutaneous infusion of low doses of apoB peptide has been shown to inhibit atherosclerosis development and was found to promote antigen-specific regulatory T cells (55). Dendritic cells can also be used to confer tolerance to self-antigens. Intravenous administration of dendritic cells pulsed with the complete apoB protein and IL-10 reduces atherosclerosis by a mechanism involving activation of regulatory T cells (56). Taken together, these data imply that immunizations with self-antigens boosts existing regulatory T cell immunity and that breaking the tolerance to self-antigens may be an important contributor to atherosclerosis development (49, 57). The first of these therapies are now close to entering clinical testing, emphasizing the need for a better understanding of the role of regulatory T cells in cardiovascular disease in humans. Based on the experimental evidence both oxidized LDL and more well defined LDL antigens, such as different apoB peptides, represent possible components of future atherosclerosis vaccines for human use (Fig. 2). However, apoB peptides have the advantage of synthetic manufacturing under controlled conditions and a lower risk for contaminations.
Clinical studies of regulatory T cells and cardiovascular disease

Despite strong experimental evidence supporting a protective role of regulatory T cells in atherosclerosis our understanding of their clinical significance remains limited. Decreased levels of circulating regulatory T cells have been reported in patients with acute coronary syndrome (58-61), but prospective studies analyzing the association of regulatory T cells with cardiovascular risk have been lacking. Recently, however, Wigren and coworkers (62) reported that low baseline levels of regulatory T cells, defined as CD4⁺FoxP3⁺ T cells, was associated with an increased risk for development of acute myocardial infarction during a 15-year follow-up of 700 subjects taking part in the cardiovascular sub-study of the Malmö Diet and Cancer study. The hazard ratio for suffering a coronary event in the lowest tertile of CD4⁺FoxP3⁺ T cells was 1.9 compared to the highest tertile and this increase in risk was independent of other cardiovascular risk factors. It was also reported that low levels of CD4⁺FoxP3⁺ T cells was associated with an increased release of pro-inflammatory cytokines from activated peripheral blood mononuclear leukocytes. Although these observations are encouraging because they provide the first prospective evidence for a role of regulatory T cells in coronary artery disease they need to be interpreted with some caution due to the technical difficulties in defining human regulatory T cells. In mice regulatory T cells are easily identified based on expression of the surface markers CD4 and CD25 which characterizes a largely homogenous regulatory population of cells also expressing the transcription factor FoxP3 that is required for regulatory T cell development and function. However, the use of CD4 and CD25 is inadequate in humans because conventional CD4⁺ T cells also express CD25 in response to activation. One approach has been to identify CD4⁺CD25high cells as these have been shown to have regulatory properties (63), but
the limitation with this strategy is that it only identifies CD45RO$^+$ memory regulatory T cells and not CD45RA$^+$ naïve regulatory T cells (64). To overcome this problem the combination of CD25$^{\text{high}}$ and CD127$^{\text{low}}$ has been used which identifies a relatively pure population of FoxP3 expressing cells in humans (65), but this still fails to discriminate between regulatory T cells and conventional T cells that in response to activation up-regulate CD25 and down-regulate CD127 (64). Accordingly, it is much more complex to study the association between regulatory T cells and disease in humans than in mice. The recent observation that regulatory T cells may differentiate into pro-inflammatory Th17 cells further contributes to this challenge (66).

**B cells and antibodies have multifaceted roles**

Initial studies suggested that B cells have an overall protective role in atherosclerosis as B-cell deficient mice display increased atherosclerosis compared to control mice and transfer of B cells to atherosclerotic splenectomized mice reduces atherosclerosis (67, 68). However, more recently the protective role of B cells in atherosclerosis has been challenged as B cell depletion with anti-CD20 antibodies decreases atherosclerosis (69, 70). These discrepancies may in part be due to differential effects of B cell subsets. Depletion of B2 B cells ameliorates atherosclerosis, whereas B1a cells are protective presumably through the secretion of natural IgM antibodies that bind to oxidized LDL and apoptotic cells (70-73). Natural IgM may limit inflammation by facilitating the removal of damaged cells and lipoproteins, and high levels of these autoantibodies have been associated with a lower cardiovascular risk in several clinical studies (74). The observation that immunization with phosphorylcholine, the target for natural IgM, reduces atherosclerosis suggests that these antibodies represent an interesting novel intervention goal (75).
Oxidized LDL is also targeted by IgG autoantibodies. The role of these antibodies in the development of atherosclerosis has been controversial because their associations with atherosclerosis severity and cardiovascular risk have been inconsistent in clinical studies (76). This inconsistency may in part be due to difficulties in standardizing the oxidized LDL antigen used in the antibody ELISAs because studies based on defined apo B peptide antigens have provided more consistent findings. Interestingly, high levels of IgG autoantibodies against apoB peptides have generally been associated with less severe atherosclerosis and a lower cardiovascular risk (77, 78) suggesting that they may have a protective role. This notion has received support from experimental studies demonstrating that treatment of hypercholesterolemic mice with a recombinant human IgG antibody recognizing the MDA-modified 661-680 amino acid sequence of human apo B inhibits the development of atherosclerosis and potentiates plaque regression induced by cholesterol lowering (79, 80). The possible athero-protective effect of these antibodies in humans is presently being investigated in the GLACIER trial (Goal of oxidised Ldl and ACtivated macrophage Inhibition by Exposure to a Recombinant antibody, www.clinicaltrials.gov).

**Therapeutic opportunities to target chemokine receptors and monocytes**

In mice, monocytes are divided into a Ly-6C\textsuperscript{high}CX3CR1\textsuperscript{low}CCR2\textsuperscript{+} subset that is actively recruited into inflamed tissue and a Ly-6C\textsuperscript{low}CX3CR1\textsuperscript{high}CCR2\textsuperscript{−} subset that home into non-inflamed tissues (81). The Ly-6C\textsuperscript{high} monocytes are increased in blood during hypercholesterolemia and are preferentially recruited to atherosclerotic plaques by the use of CCR2 and CX3CR1 receptors (82, 83). In contrast, Ly-6C\textsuperscript{low} monocytes have been shown to enter plaques less frequently than Ly-6C\textsuperscript{high} monocytes, but they are, on the other hand, more
prone to develop into plaque resident cells expressing CD11c (83). Despite a high expression of CX3CR1, the Ly-6C\textsuperscript{low} monocytes depend mainly on CCR5 to enter plaques (83). Interestingly, some of the same receptors that regulate plaque entry may also contribute to atherosclerosis by altering the homeostasis of monocyte subsets. The mobilization of classical Ly-6C\textsuperscript{high} monocytes from bone marrow is severely impaired in the absence of CCR2, resulting in reduced numbers of this monocyte subset in blood, which could contribute to reduced atherosclerosis (84). Absence of CX3CR1 affect survival of Ly-6C\textsuperscript{low} monocytes, as CX3CR1 seems to mediate growth factor like signals, resulting in reduced Ly-6C\textsuperscript{low} monocyte levels (85). Notably, when the three chemokine pathways CCR2, CX3CR1 and CCR5 are simultaneously targeted, atherosclerosis in mice is almost completely abrogated (11). During atherosclerosis development in mice, the spleen supplements the hematopoietic function of the bone marrow by producing Ly-6C\textsuperscript{high} monocytes that readily accumulate in the aorta (86). Interestingly, Dutta et al. has recently shown that the accumulation of Ly-6C\textsuperscript{high} monocytes is accelerated in the aorta of mice subjected to a myocardial infarction by coronary ligation (87). These data indicate that myocardial infarction might be followed by a period of increased plaque growth and vulnerability fuelled by increased monocyte inflammation, which may explain the high risk of recurring events in acute coronary syndrome patients, if monocyte behaviour is similar in humans.

In humans at least three monocyte subsets can be defined by their expression of CD14 and CD16 (88). The classical (CD14\textsuperscript{++}CD16\textsuperscript{−}) monocytes express CCR2, whereas non-classical (CD14\textsuperscript{+}CD16\textsuperscript{++}) monocytes and intermediate (CD14\textsuperscript{++}CD16\textsuperscript{+}) monocytes express higher levels of CX3CR1 than the classical monocytes. Recently, Berg and co-workers reported that increased levels of classical CD14\textsuperscript{++}CD16\textsuperscript{−} monocytes at baseline were associated with an increased risk
for suffering cardiovascular events during a 15-year follow-up of 700 subjects from the Malmö Diet and Cancer Study population cohort (89). The hazard ratio for suffering a cardiovascular event in the highest tertile of classical monocytes was 1.66 compared to the lowest tertile even after adjustment for common risk factors. The classical monocytes did not, however, associate with the extent of atherosclerosis, measured as intima-media thickness (IMT), at baseline. In contrast, the percentage of monocytes expressing CD16 was negatively associated to the extent of carotid atherosclerosis at baseline. This association was independent of other common risk factors. These data might indicate that different monocyte subsets have different biological functions in cardiovascular disease in humans. CD14++CD16– classical monocytes might cause inflammation that weakens the fibrous cap covering plaques and thus be associated with increased risk of clinical events, whereas CD16-expressing monocytes might play a role in determining the size of the plaque, perhaps even having a protective, or reparative, rather than plaque promoting function.

Despite strong evidence in mice, Berg et al. found no association between CX3CR1 and CCR2 expression on monocytes and cardiovascular risk (89). CCR5 expression on non-classical CD14+CD16++ monocytes was, however, negatively associated to carotid IMT. Although the association of chemokine receptor expression on monocytes to cardiovascular risk is poorly understood, the potential to treat human disease by targeting chemokine receptors is emphasized by the fact that polymorphisms in the CX3CR1 gene have been found to protect against atherosclerosis (90-92). Evidence also indicate that a naturally occurring frame-shift mutation in the human CCR5 gene is associated with lower carotid intima-media thickness in the common carotid artery and reduced cardiovascular disease risk (93). Also, in mice, pharmacological
intervention of several chemokine receptors has been proven to reduce atherosclerosis (94). In addition, it has been speculated that several approved chemokine receptor antagonists, such as CCR5 antagonists approved for HIV therapy, could also limit plaque development (95). At present, clinical studies targeting atherosclerosis with these compounds have, however, not been initiated.

**Anti-inflammatory drugs and atherosclerosis**

The fact that atherosclerosis is an inflammatory disease taken together with observations that inflammatory biomarkers such as CRP predicts risk for cardiovascular events (96) suggests the possibility of using anti-inflammatory drugs in prevention and treatment of the disease. However, in conflict with this notion the use of non-steroidal anti-inflammatory drugs (NSAID) has been found to be associated with increased cardiovascular risk (8, 9). Statins, on the other hand, have been shown to have an anti-inflammatory effect both systemically (97) as well as in atherosclerotic plaques (98) which may explain part of their protective action. Novel approaches to target inflammation in atherosclerosis includes the Cardiovascular Inflammation Reduction Trial (CIRT) in which 7000 stable coronary artery disease patients with persistent elevation of CRP are allocated to either placebo or low-dose methotrexate (99) and the Cankinumab Anti-inflammatory Thrombosis Outcomes Study (CANTOS) in which 17200 stable post-myocardial infarction patients with persistent elevation of CRP are allocated to either placebo or three different doses of the IL-1β neutralizing antibody Cankinumab (100). The lipoprotein phospholipase A2 (Lp-PLA2) inhibitor darapladib represent another novel and interesting possibility to specifically inhibit plaque inflammation. Oxidized phospholipids in LDL are hydrolysed by Lp-PLA2 to generate pro-inflammatory lysophosphatidylcholines (lysoPCs) (101)
and recent studies have revealed very strong association between the levels of lysoPC and pro-inflammatory cytokines in human atherosclerotic plaques (102). Plasma levels of Lp-PLA2 and its activity have also been shown to be independent predictors of cardiovascular risk in several epidemiological studies (103). Darapladib is currently being studied in two large phase III trials: STABILITY (Stabilization of Atherosclerotic Plaque by Initiation of Darapladib Therapy Trial), involving 15,828 patients with coronary heart disease (103) and SOLID-TIMI 52 (the Stabilization of Plaques Using Darapladib - Thrombolysis in Myocardial Infarction 52 Trial) which is estimated to include 11,500 patients with acute coronary syndromes.

**Challenges facing the translation of experimental immune-modulation of atherosclerosis into clinical therapy**

Current therapies focusing on risk factor reduction such as lipid-lowering treatment have been shown to reduce the risk of ischemic cardiovascular events by up to 40% in randomized clinical trials (1). However, it is difficult to achieve additional risk reduction by treating risk factors alone leaving the majority of treated subjects without sufficient protection emphasizing the need for development of novel therapies directly targeting the atherosclerotic disease process in the plaque. Such therapies should preferentially act through specific inhibition of plaque inflammation. Down-regulation of pro-inflammatory autoimmune response against plaque antigens represents a potential approach to achieve this. As discussed above several tolerogenic plaque-antigen vaccines have been shown to be effective in animal models of atherosclerosis. However, the challenge of translating these results into clinically effective therapies should not be underestimated. Most of the available knowledge of the role of immunity in atherosclerosis is based on studies performed in mice and our understanding of the importance of these disease
mechanisms in humans remains limited. There is an urgent need for studies identifying and validating immune biomarkers for cardiovascular risk in man as well as to develop biomarkers that can be used to monitor the effect of atherosclerosis vaccines in clinical trials. Such biomarkers are likely to include plaque antigen-specific autoantibodies, antibodies against vaccine antigens and detailed characterization of circulating immune cells believed to be involved in the disease process. To obtain sufficient sensitivity these biomarkers also needs to be antigen specific. Although the key antigens in human atherosclerosis need to be more firmly established it will be relatively straight-forward to develop and standardize assays for determination of autoantibodies against these antigens. The same will most likely be true for vaccine antigens. However, the experience from animal studies suggests that the mechanism of action of athero-protective immune therapies involves modulation of cellular immunity that may be equally or more important than modulation of humoral immunity. Characterization of different T cells, such as Th1 and regulatory T cells using flow cytometry is relatively simple in the young genetically identical mice with limited exposure to pathogens used in experimental atherosclerosis studies. However, this is considerably more difficult in a clinical setting where the different T cell populations are much more heterogeneous. Such analysis should preferably also be done in an antigen-specific way, e.g. it should be possible to restrict the analysis to T cells specific for a certain atherosclerosis or vaccine antigens. Possible approaches to achieve this include ex vivo antigen challenge and the use of synthetic tetramer HLA-antigen constructs that binds only to T cells specific for that antigen. Important immune targets for monitoring disease and the efficacy of immune-modulatory therapies are summarized in Figure 2.
Another important limitation of the animal studies is that they with few exceptions have demonstrated effect of vaccines on early development of atherosclerosis rather than on the more clinically relevant advanced plaques. It is not unlikely that strategies for immune-modulatory therapy targeting advanced established plaques need to be different from those aiming to prevent early stages of disease. In this context it is encouraging to note that Herbin et al. (55) were able to completely halt the progression of advanced atherosclerosis by subcutaneous infusion of apo B peptides.

The clinical development of an immune-modulatory therapy for atherosclerosis also faces several regulatory challenges. If shown to be effective this type therapy could potentially be widely used even in subjects without clinically manifest disease. Accordingly safety standards must be very strict. The potential side effects include general immune suppression increasing the risk for infections as well as activation of immune process with adverse effects on the atherosclerotic disease process. Possible adverse side effects of an immune-modulatory therapy are likely to be closely related to its mode of action. Accordingly, it is essential to characterize the exact mechanisms through which such a therapy works not only in experimental animals but also in humans.

**Conclusions and perspectives**

Studies in mice have assigned atherosclerosis promoting or protective roles to different immune cells (2-5). For instance, increased numbers of monocytes and type 1 T helper (Th1) cells are thought to promote disease, whereas regulatory T cells have a protective role (5). Such generalized roles, however, have not been assigned for many human immune cells. In fact,
surprisingly few clinical studies have investigated the role of different immune cell populations in cardiovascular disease and most studies compare patients suffering acute clinical events to patients with stable or no disease. Prospective studies are needed to appropriately estimate the cardiovascular risk posed by various immune cells. Epidemiological studies relating immune cell populations in blood to the extent of atherosclerosis will also clarify which immune mechanisms are at play in human atherosclerosis. Moreover, immune cell populations could be valuable biomarkers when monitoring the efficacy of future therapies targeting the immune system in humans. Several strategies involving modulation of existing or induced specific immune responses have been devised to reduce atherosclerosis in mice (26, 104). However, our limited understanding of the role of the immune system in human atherosclerosis can impede the application of experimental therapies in mice to humans. Thus, clinical studies of the immune system and its association to cardiovascular events and atherosclerosis in humans are needed to design therapies directed against human atherosclerosis and its clinical manifestations.

Experimental studies have identified both active (vaccines) and passive (antibodies) immunization as possible immune-based therapies for atherosclerosis. It remains to be demonstrated which one (if any) of these approaches is most effective in humans. It could be argued that passive immunization may have a more rapid effect and that the risk of serious adverse immune-related side effects is more limited. Vaccines, on the other hand, could potentially activate several different protective pathways and also be more cost-effective.
REFERENCES


FIGURE LEGEND

**Figure 1:** The role of different immune cell populations in experimental atherosclerosis in mice and in prediction of cardiovascular disease risk in humans. Red arrows indicate a role in facilitating atherosclerosis development in mice or positive association in humans. Blue arrows indicate an inhibition of atherosclerosis development in mice or negative association in humans.

**Figure 2:** Possible immune targets for treatment of atherosclerosis in humans and immune targets important for monitoring disease and efficacy of immune-modulatory therapies.
Figure 1
Figure 2

Therapeutic targets

- Oxidized LDL
- Oxidation generated adducts
- ApoB
- ApoB-peptide
- Recombinant anti-apoB-peptide antibody
- HSP60
- Chemokine receptors

- Oral immunization
- Immunization
- Immunization with antigen-pulsed DCs
- Subcutaneous immunization
- Mucosal immunization
- Subcutaneous infusion
- Passive immunization
- Oral immunization

Targets for monitoring disease

- Regulatory T cell mediated tolerance
- Regulatory T cells
- Antigen specific T cells
- TCR
- MHC:Ag
- Treg
- Tr1
- Tr3
- IL-10
- TGFβ
- Antibody response
- Antigen specific auto-antibodies

Hans Björkbacka