



LUND UNIVERSITY

Emerging biomarkers and intervention targets for immune-modulation of atherosclerosis - A review of the experimental evidence.

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

Published in:
Atherosclerosis

DOI:
[10.1016/j.atherosclerosis.2012.10.074](https://doi.org/10.1016/j.atherosclerosis.2012.10.074)

2013

[Link to publication](#)

Citation for published version (APA):

Björkbacka, H., Nordin Fredrikson, G., & Nilsson, J. (2013). Emerging biomarkers and intervention targets for immune-modulation of atherosclerosis - A review of the experimental evidence. *Atherosclerosis*, 227(1), 9-17. <https://doi.org/10.1016/j.atherosclerosis.2012.10.074>

Total number of authors:
3

General rights

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

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

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

Take down policy

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

LUND UNIVERSITY

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

**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-1 β . 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 prototypic 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 α GalCer 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 through 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⁺CD25^{high} 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^{high} and CD127^{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^{high}CX3CR1^{low}CCR2⁺ subset that is actively recruited into inflamed tissue and a Ly-6C^{low}CX3CR1^{high}CCR2⁻ subset that home into non-inflamed tissues (81). The Ly-6C^{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^{low} monocytes have been shown to enter plaques less frequently than Ly-6C^{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^{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^{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^{low} monocytes, as CX3CR1 seems to mediate growth factor like signals, resulting in reduced Ly-6C^{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^{high} monocytes that readily accumulate in the aorta (86). Interestingly, Dutta *et al.* has recently shown that the accumulation of Ly-6C^{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⁺⁺CD16⁻) monocytes express CCR2, whereas *non-classical* (CD14⁺CD16⁺⁺) monocytes and *intermediate* (CD14⁺⁺CD16⁺) monocytes express higher levels of CX3CR1 than the classical monocytes. Recently, Berg and co-workers reported that increased levels of classical CD14⁺⁺CD16⁻ 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-1 β 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 need 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

1. Baigent C, Keech A, Kearney PM, Blackwell L, Buck G, Pollicino C, et al. Efficacy and safety of cholesterol-lowering treatment: prospective meta-analysis of data from 90,056 participants in 14 randomised trials of statins. *Lancet*. 2005;366(9493):1267-78.
2. Hansson GK. Inflammation, atherosclerosis, and coronary artery disease. *N Engl J Med*. 2005;352(16):1685-95.
3. Hansson GK, Libby P. The immune response in atherosclerosis: a double-edged sword. *Nat Rev Immunol*. 2006;6(7):508-19.
4. Hansson GK, Robertson A-KL, Naucler CS. Inflammation and Atherosclerosis. *Annu Rev Pathol Mech Dis*. 2006;1:297-329.
5. Hansson GK, Hermansson A. The immune system in atherosclerosis. *Nat Immunol*. 2011;12(3):204-12.
6. Vaughan CJ, Murphy MB, Buckley BM. Statins do more than just lower cholesterol. *Lancet*. 1996;348(9034):1079-82.
7. Ridker PM, Danielson E, Fonseca FA, Genest J, Gotto AM, Jr., Kastelein JJ, et al. Rosuvastatin to prevent vascular events in men and women with elevated C-reactive protein. *N Engl J Med*. 2008;359(21):2195-207.
8. Maxwell SR, Webb DJ. COX-2 selective inhibitors--important lessons learned. *Lancet*. 2005;365(9458):449-51.
9. McGettigan P, Henry D. Cardiovascular risk with non-steroidal anti-inflammatory drugs: systematic review of population-based controlled observational studies. *PLoS Med*. 2011;8(9):e1001098.
10. Nilsson J, Hansson GK. Autoimmunity in atherosclerosis: a protective response losing control? *J Intern Med*. 2008;263(5):464-78.
11. Combadiere C, Potteaux S, Rodero M, Simon T, Pezard A, Esposito B, et al. Combined inhibition of CCL2, CX3CR1, and CCR5 abrogates Ly6C(hi) and Ly6C(lo) monocytosis and almost abolishes atherosclerosis in hypercholesterolemic mice. *Circulation*. 2008;117(13):1649-57.
12. Saederup N, Chan L, Lira SA, Charo IF. Fractalkine deficiency markedly reduces macrophage accumulation and atherosclerotic lesion formation in CCR2^{-/-} mice: evidence for independent chemokine functions in atherogenesis. *Circulation*. 2008;117(13):1642-8.
13. Stoneman V, Braganza D, Figg N, Mercer J, Lang R, Goddard M, et al. Monocyte/macrophage suppression in CD11b diphtheria toxin receptor transgenic mice differentially affects atherogenesis and established plaques. *Circ Res*. 2007;100(6):884-93.
14. Jongstra-Bilen J, Haidari M, Zhu SN, Chen M, Guha D, Cybulsky MI. Low-grade chronic inflammation in regions of the normal mouse arterial intima predisposed to atherosclerosis. *J Exp Med*. 2006;203(9):2073-83.
15. Zhu SN, Chen M, Jongstra-Bilen J, Cybulsky MI. GM-CSF regulates intimal cell proliferation in nascent atherosclerotic lesions. *J Exp Med*. 2009;206(10):2141-9.
16. Drechsler M, Megens RT, van Zandvoort M, Weber C, Soehnlein O. Hyperlipidemia-triggered neutrophilia promotes early atherosclerosis. *Circulation*. 2010;122(18):1837-45.
17. Zerneck A, Bot I, Djalali-Talab Y, Shagdarsuren E, Bidzhekov K, Meiler S, et al. Protective role of CXC receptor 4/CXC ligand 12 unveils the importance of neutrophils in atherosclerosis. *Circ Res*. 2008;102(2):209-17.

18. Soehnlein O, Zernecke A, Eriksson EE, Rothfuchs AG, Pham CT, Herwald H, et al. Neutrophil secretion products pave the way for inflammatory monocytes. *Blood*. 2008;112(4):1461-71.
19. Lievens D, von Hundelshausen P. Platelets in atherosclerosis. *Thromb Haemost*. 2011;106(5):827-38.
20. Hsue PY, Deeks SG, Hunt PW. Immunologic basis of cardiovascular disease in HIV-infected adults. *J Infect Dis*. 2012;205 Suppl 3:S375-82.
21. Dansky HM, Charlton SA, Harper MM, Smith JD. T and B lymphocytes play a minor role in atherosclerotic plaque formation in the apolipoprotein E-deficient mouse. *Proc Natl Acad Sci U S A*. 1997;94(9):4642-6.
22. Daugherty A, Pure E, Delfel-Butteiger D, Chen S, Leferovich J, Roselaar SE, et al. The effects of total lymphocyte deficiency on the extent of atherosclerosis in apolipoprotein E^{-/-} mice. *J Clin Invest*. 1997;100(6):1575-80.
23. Zhou X, Nicoletti A, Elhage R, Hansson GK. Transfer of CD4(+) T cells aggravates atherosclerosis in immunodeficient apolipoprotein E knockout mice. *Circulation*. 2000;102(24):2919-22.
24. Binder CJ, Chang MK, Shaw PX, Miller YI, Hartvigsen K, Dewan A, et al. Innate and acquired immunity in atherogenesis. *Nat Med*. 2002;8(11):1218-26.
25. Buono C, Binder CJ, Stavrakis G, Witztum JL, Glimcher LH, Lichtman AH. T-bet deficiency reduces atherosclerosis and alters plaque antigen-specific immune responses. *Proc Natl Acad Sci U S A*. 2005;102(5):1596-601.
26. Lahoute C, Herbin O, Mallat Z, Tedgui A. Adaptive immunity in atherosclerosis: mechanisms and future therapeutic targets. *Nat Rev Cardiol*. 2011;8(6):348-58.
27. King VL, Szilvassy SJ, Daugherty A. Interleukin-4 deficiency decreases atherosclerotic lesion formation in a site-specific manner in female LDL receptor^{-/-} mice. *Arterioscler Thromb Vasc Biol*. 2002;22(3):456-61.
28. Mallat Z, Besnard S, Duriez M, Deleuze V, Emmanuel F, Bureau MF, et al. Protective role of interleukin-10 in atherosclerosis. *Circ Res*. 1999;85(8):e17-24.
29. Binder CJ, Hartvigsen K, Chang MK, Miller M, Broide D, Palinski W, et al. IL-5 links adaptive and natural immunity specific for epitopes of oxidized LDL and protects from atherosclerosis. *J Clin Invest*. 2004;114(3):427-37.
30. van Es T, van Puijvelde GH, Ramos OH, Segers FM, Joosten LA, van den Berg WB, et al. Attenuated atherosclerosis upon IL-17R signaling disruption in LDLr deficient mice. *Biochem Biophys Res Commun*. 2009;388(2):261-5.
31. Taleb S, Romain M, Ramkhalawon B, Uyttenhove C, Pasterkamp G, Herbin O, et al. Loss of SOCS3 expression in T cells reveals a regulatory role for interleukin-17 in atherosclerosis. *J Exp Med*. 2009;206(10):2067-77.
32. Erbel C, Chen L, Bea F, Wangler S, Celik S, Lasitschka F, et al. Inhibition of IL-17A attenuates atherosclerotic lesion development in apoE-deficient mice. *J Immunol*. 2009;183(12):8167-75.
33. Kolbus D, Ramos OH, Berg KE, Persson J, Wigren M, Bjorkbacka H, et al. CD8⁺ T cell activation predominate early immune responses to hypercholesterolemia in ApoE^(/) mice. *BMC Immunol*. 2010;11:58.
34. Olofsson PS, Soderstrom LA, Wagsater D, Sheikine Y, Ocaya P, Lang F, et al. CD137 is expressed in human atherosclerosis and promotes development of plaque inflammation in hypercholesterolemic mice. *Circulation*. 2008;117(10):1292-301.
35. Chyu KY, Zhao X, Dimayuga PC, Zhou J, Li X, Yano J, et al. CD8⁺ T cells mediate the athero-protective effect of immunization with an ApoB-100 peptide. *PLoS ONE*. 2012;7(2):e30780.
36. Kolbus D, Ljungcrantz I, Soderberg I, Alm R, Bjorkbacka H, Nilsson J, et al. TAP1-Deficiency Does Not Alter Atherosclerosis Development in ApoE^(-/-) Mice. *PLoS ONE*. 2012;7(3):e33932.

37. Ait-Oufella H, Salomon BL, Potteaux S, Robertson AK, Gourdy P, Zoll J, et al. Natural regulatory T cells control the development of atherosclerosis in mice. *Nat Med*. 2006;12(2):178-80.
38. Nakai Y, Iwabuchi K, Fujii S, Ishimori N, Dashtsoodol N, Watano K, et al. Natural killer T cells accelerate atherogenesis in mice. *Blood*. 2004;104(7):2051-9.
39. Tupin E, Nicoletti A, Elhage R, Rudling M, Ljunggren HG, Hansson GK, et al. CD1d-dependent Activation of NKT Cells Aggravates Atherosclerosis. *J Exp Med*. 2004;199(3):417-22.
40. Gotsman I, Grabie N, Gupta R, Dacosta R, MacConmara M, Lederer J, et al. Impaired regulatory T-cell response and enhanced atherosclerosis in the absence of inducible costimulatory molecule. *Circulation*. 2006;114(19):2047-55.
41. van Es T, van Puijvelde GH, Foks AC, Habets KL, Bot I, Gilboa E, et al. Vaccination against Foxp3(+) regulatory T cells aggravates atherosclerosis. *Atherosclerosis*. 2010;209(1):74-80.
42. Robertson AK, Rudling M, Zhou X, Gorelik L, Flavell RA, Hansson GK. Disruption of TGF-beta signaling in T cells accelerates atherosclerosis. *J Clin Invest*. 2003;112(9):1342-50.
43. Mallat Z, Gojova A, Brun V, Esposito B, Fournier N, Cottrez F, et al. Induction of a regulatory T cell type 1 response reduces the development of atherosclerosis in apolipoprotein E-knockout mice. *Circulation*. 2003;108(10):1232-7.
44. Steffens S, Burger F, Pelli G, Dean Y, Elson G, Kosco-Vilbois M, et al. Short-term treatment with anti-CD3 antibody reduces the development and progression of atherosclerosis in mice. *Circulation*. 2006;114(18):1977-84.
45. Klingenberg R, Ketelhuth DF, Strodtzoff D, Gregori S, Hansson GK. Subcutaneous immunization with heat shock protein-65 reduces atherosclerosis in Apoe(-)/(-) mice. *Immunobiology*. 2012;217(5):540-7.
46. van Puijvelde GH, Hauer AD, de Vos P, van den Heuvel R, van Herwijnen MJ, van der Zee R, et al. Induction of oral tolerance to oxidized low-density lipoprotein ameliorates atherosclerosis. *Circulation*. 2006;114(18):1968-76.
47. Klingenberg R, Lebens M, Hermansson A, Fredrikson GN, Strodtzoff D, Rudling M, et al. Intranasal immunization with an apolipoprotein B-100 fusion protein induces antigen-specific regulatory T cells and reduces atherosclerosis. *Arterioscler Thromb Vasc Biol*. 2010;30(5):946-52.
48. Stemme S, Faber B, Holm J, Wiklund O, Witztum JL, Hansson GK. T lymphocytes from human atherosclerotic plaques recognize oxidized low density lipoprotein. *Proc Natl Acad Sci U S A*. 1995;92(9):3893-7.
49. Hermansson A, Ketelhuth DF, Strodtzoff D, Wurm M, Hansson EM, Nicoletti A, et al. Inhibition of T cell response to native low-density lipoprotein reduces atherosclerosis. *J Exp Med*. 2010;207(5):1081-93.
50. Fredrikson GN, Soderberg I, Lindholm M, Dimayuga P, Chyu KY, Shah PK, et al. Inhibition of Atherosclerosis in ApoE-Null Mice by Immunization with ApoB-100 Peptide Sequences. *Arterioscler Thromb Vasc Biol*. 2003;23(5):879-84.
51. Maron R, Sukhova G, Faria AM, Hoffmann E, Mach F, Libby P, et al. Mucosal administration of heat shock protein-65 decreases atherosclerosis and inflammation in aortic arch of low-density lipoprotein receptor-deficient mice. *Circulation*. 2002;106(13):1708-15.
52. Wigren M, Kolbus D, Duner P, Ljungcrantz I, Soderberg I, Bjorkbacka H, et al. Evidence for a role of regulatory T cells in mediating the atheroprotective effect of apolipoprotein B peptide vaccine. *J Intern Med*. 2011;269(5):546-56.
53. Wigren M, Bengtsson D, Duner P, Olofsson K, Bjorkbacka H, Bengtsson E, et al. Atheroprotective effects of Alum are associated with capture of oxidized LDL antigens and activation of regulatory T cells. *Circ Res*. 2009;104(12):e62-70.

54. van Puijvelde GH, van Es T, van Wanrooij EJ, Habets KL, de Vos P, van der Zee R, et al. Induction of oral tolerance to HSP60 or an HSP60-peptide activates T cell regulation and reduces atherosclerosis. *Arterioscler Thromb Vasc Biol.* 2007;27(12):2677-83.
55. Herbin O, Ait-Oufella H, Yu W, Fredrikson GN, Aubier B, Perez N, et al. Regulatory T-cell response to apolipoprotein B100-derived peptides reduces the development and progression of atherosclerosis in mice. *Arterioscler Thromb Vasc Biol.* 2012;32(3):605-12.
56. Hermansson A, Johansson DK, Ketelhuth DF, Andersson J, Zhou X, Hansson GK. Immunotherapy with tolerogenic apolipoprotein B-100-loaded dendritic cells attenuates atherosclerosis in hypercholesterolemic mice. *Circulation.* 2011;123(10):1083-91.
57. Ketelhuth DF, Hansson GK. Cellular immunity, low-density lipoprotein and atherosclerosis: Break of tolerance in the artery wall. *Thromb Haemost.* 2011;106(5):779-86.
58. Mor A, Luboshits G, Planer D, Keren G, George J. Altered status of CD4(+)CD25(+) regulatory T cells in patients with acute coronary syndromes. *Eur Heart J.* 2006;27(21):2530-7.
59. Han SF, Liu P, Zhang W, Bu L, Shen M, Li H, et al. The opposite-direction modulation of CD4+CD25+ Tregs and T helper 1 cells in acute coronary syndromes. *Clin Immunol.* 2007;124(1):90-7.
60. Cheng X, Yu X, Ding YJ, Fu QQ, Xie JJ, Tang TT, et al. The Th17/Treg imbalance in patients with acute coronary syndrome. *Clin Immunol.* 2008;127(1):89-97.
61. Ammirati E, Cianflone D, Banfi M, Vecchio V, Palini A, De Metrio M, et al. Circulating CD4+CD25hiCD127lo regulatory T-Cell levels do not reflect the extent or severity of carotid and coronary atherosclerosis. *Arterioscler Thromb Vasc Biol.* 2010;30(9):1832-41.
62. Wigren M, Bjorkbacka H, Andersson L, Ljungcrantz I, Fredrikson GN, Persson M, et al. Low Levels of Circulating CD4+FoxP3+ T Cells Are Associated With an Increased Risk for Development of Myocardial Infarction But Not for Stroke. *Arterioscler Thromb Vasc Biol.* 2012.
63. Baecher-Allan C, Brown JA, Freeman GJ, Hafler DA. CD4+CD25high regulatory cells in human peripheral blood. *Journal of immunology.* 2001;167(3):1245-53.
64. Miyara M, Yoshioka Y, Kitoh A, Shima T, Wing K, Niwa A, et al. Functional delineation and differentiation dynamics of human CD4+ T cells expressing the FoxP3 transcription factor. *Immunity.* 2009;30(6):899-911.
65. Liu W, Putnam AL, Xu-Yu Z, Szot GL, Lee MR, Zhu S, et al. CD127 expression inversely correlates with FoxP3 and suppressive function of human CD4+ T reg cells. *The Journal of experimental medicine.* 2006;203(7):1701-11.
66. Zhou L, Chong MM, Littman DR. Plasticity of CD4+ T cell lineage differentiation. *Immunity.* 2009;30(5):646-55.
67. Caligiuri G, Nicoletti A, Poirier B, Hansson GK. Protective immunity against atherosclerosis carried by B cells of hypercholesterolemic mice. *J Clin Invest.* 2002;109(6):745-53.
68. Major AS, Fazio S, Linton MF. B-lymphocyte deficiency increases atherosclerosis in LDL receptor-null mice. *Arterioscler Thromb Vasc Biol.* 2002;22(11):1892-8.
69. Ait-Oufella H, Herbin O, Bouaziz JD, Binder CJ, Uyttenhove C, Laurans L, et al. B cell depletion reduces the development of atherosclerosis in mice. *J Exp Med.* 2010;207(8):1579-87.
70. Kyaw T, Tay C, Khan A, Dumouchel V, Cao A, To K, et al. Conventional B2 B cell depletion ameliorates whereas its adoptive transfer aggravates atherosclerosis. *J Immunol.* 2010;185(7):4410-9.
71. Kyaw T, Tay C, Krishnamurthi S, Kanellakis P, Agrotis A, Tipping P, et al. B1a B lymphocytes are atheroprotective by secreting natural IgM that increases IgM deposits and reduces necrotic cores in atherosclerotic lesions. *Circ Res.* 2011;109(8):830-40.

72. Kyaw T, Tay C, Hosseini H, Kanellakis P, Gadowski T, MacKay F, et al. Depletion of B2 but not B1a B cells in BAFF receptor-deficient ApoE mice attenuates atherosclerosis by potentially ameliorating arterial inflammation. *PLoS ONE*. 2012;7(1):e29371.
73. Sage AP, Tsiantoulas D, Baker L, Harrison J, Masters L, Murphy D, et al. BAFF Receptor Deficiency Reduces the Development of Atherosclerosis in Mice--Brief Report. *Arterioscler Thromb Vasc Biol*. 2012;32(7):1573-6.
74. Frostegard J. Low level natural antibodies against phosphorylcholine: a novel risk marker and potential mechanism in atherosclerosis and cardiovascular disease. *Clin Immunol*. 2010;134(1):47-54.
75. Caligiuri G, Khallou-Laschet J, Vandaele M, Gaston AT, Delignat S, Mandet C, et al. Phosphorylcholine-targeting immunization reduces atherosclerosis. *J Am Coll Cardiol*. 2007;50(6):540-6.
76. Nilsson J, Kovanen PT. Will autoantibodies help to determine severity and progression of atherosclerosis? *Curr Opin Lipidol*. 2004;15(5):499-503.
77. Fredrikson GN, Schioppa A, Berglund G, Alm R, Shah PK, Nilsson J. Autoantibody against the amino acid sequence 661-680 in apo B-100 is associated with decreased carotid stenosis and cardiovascular events. *Atherosclerosis*. 2007;194(2):e188-92.
78. Sjogren P, Fredrikson GN, Samnegard A, Ericsson CG, Ohrvik J, Fisher RM, et al. High plasma concentrations of autoantibodies against native peptide 210 of apoB-100 are related to less coronary atherosclerosis and lower risk of myocardial infarction. *Eur Heart J*. 2008;29(18):2218-26.
79. Schioppa A, Bengtsson J, Soderberg I, Janciauskiene S, Lindgren S, Ares MP, et al. Recombinant human antibodies against aldehyde-modified apolipoprotein B-100 peptide sequences inhibit atherosclerosis. *Circulation*. 2004;110(14):2047-52.
80. Schioppa A, Frendeus B, Jansson B, Soderberg I, Ljungcrantz I, Araya Z, et al. Recombinant antibodies to an oxidized low-density lipoprotein epitope induce rapid regression of atherosclerosis in apobec-1(-/-)/low-density lipoprotein receptor(-/-) mice. *J Am Coll Cardiol*. 2007;50(24):2313-8.
81. Geissmann F, Jung S, Littman DR. Blood monocytes consist of two principal subsets with distinct migratory properties. *Immunity*. 2003;19(1):71-82.
82. Swirski FK, Libby P, Aikawa E, Alcaide P, Luscinskas FW, Weissleder R, et al. Ly-6Chi monocytes dominate hypercholesterolemia-associated monocytosis and give rise to macrophages in atheromata. *J Clin Invest*. 2007;117(1):195-205.
83. Tacke F, Alvarez D, Kaplan TJ, Jakubzick C, Spanbroek R, Llodra J, et al. Monocyte subsets differentially employ CCR2, CCR5, and CX3CR1 to accumulate within atherosclerotic plaques. *J Clin Invest*. 2007;117(1):185-94.
84. Tsou CL, Peters W, Si Y, Slaymaker S, Aslanian AM, Weisberg SP, et al. Critical roles for CCR2 and MCP-3 in monocyte mobilization from bone marrow and recruitment to inflammatory sites. *J Clin Invest*. 2007;117(4):902-9.
85. Landsman L, Bar-On L, Zerneck A, Kim KW, Krauthgamer R, Shagdarsuren E, et al. CX3CR1 is required for monocyte homeostasis and atherogenesis by promoting cell survival. *Blood*. 2009;113(4):963-72.
86. Robbins CS, Chudnovskiy A, Rauch PJ, Figueiredo JL, Iwamoto Y, Gorbatov R, et al. Extramedullary hematopoiesis generates Ly-6C(high) monocytes that infiltrate atherosclerotic lesions. *Circulation*. 2012;125(2):364-74.
87. Dutta P, Courties G, Wei Y, Leuschner F, Gorbatov R, Robbins CS, et al. Myocardial infarction accelerates atherosclerosis. *Nature*. 2012; 487 (7407):325-9.

88. Ziegler-Heitbrock L, Ancuta P, Crowe S, Dalod M, Grau V, Hart DN, et al. Nomenclature of monocytes and dendritic cells in blood. *Blood*. 2010;116(16):e74-80.
89. Berg KE, Ljungcrantz I, Andersson L, Bryngelsson C, Hedblad B, Fredrikson GN, et al. Elevated CD14⁺⁺CD16⁻ Monocytes Predict Cardiovascular Events. *Circ Cardiovasc Genet*. 2012;5(1):122-31.
90. McDermott DH, Fong AM, Yang Q, Sechler JM, Cupples LA, Merrell MN, et al. Chemokine receptor mutant CX3CR1-M280 has impaired adhesive function and correlates with protection from cardiovascular disease in humans. *J Clin Invest*. 2003;111(8):1241-50.
91. Apostolakis S, Baritaki S, Kochiadakis GE, Igoumenidis NE, Panutsopoulos D, Spandidos DA. Effects of polymorphisms in chemokine ligands and receptors on susceptibility to coronary artery disease. *Thromb Res*. 2007;119(1):63-71.
92. Norata GD, Garlaschelli K, Ongari M, Raselli S, Grigore L, Catapano AL. Effects of fractalkine receptor variants on common carotid artery intima-media thickness. *Stroke*. 2006;37(6):1558-61.
93. Afzal AR, Kiechl S, Daryani YP, Weerasinghe A, Zhang Y, Reindl M, et al. Common CCR5-del32 frameshift mutation associated with serum levels of inflammatory markers and cardiovascular disease risk in the Bruneck population. *Stroke*. 2008;39(7):1972-8.
94. Koenen RR, Weber C. Chemokines: established and novel targets in atherosclerosis. *EMBO Mol Med*. 2011; 3 (12):713-25..
95. Weber C. Obstacles and options in the quest for drug candidates against vascular disease. *Thromb Haemost*. 2010;104(1):1-3.
96. Libby P, Ridker PM, Hansson GK, Leducq Transatlantic Network on A. Inflammation in atherosclerosis: from pathophysiology to practice. *J Am Coll Cardiol*. 2009;54(23):2129-38.
97. Ridker P, Rifai N, Pfeffer M, F S, Braunwald E. Long term effects of pravastatin on plasma concentration of c-reactive protein. The Cholesterol and Recurrent Events (CARE) investigators. *Circulation*. 1999;100:230-5.
98. Crisby M, Nordin-Fredriksson G, Shah PK, Yano J, Zhu J, Nilsson J. Pravastatin treatment increases collagen content and decreases lipid content, inflammation, metalloproteinases, and cell death in human carotid plaques: implications for plaque stabilization. *Circulation*. 2001;103(7):926-33.
99. Ridker PM. Testing the inflammatory hypothesis of atherothrombosis: scientific rationale for the cardiovascular inflammation reduction trial (CIRT). *J Thromb Haemost*. 2009;7 Suppl 1:332-9.
100. Ridker PM, Thuren T, Zalewski A, Libby P. Interleukin-1beta inhibition and the prevention of recurrent cardiovascular events: rationale and design of the Canakinumab Anti-inflammatory Thrombosis Outcomes Study (CANTOS). *Am Heart J*. 2011;162(4):597-605.
101. Schmitz G, Ruebsaamen K. Metabolism and atherogenic disease association of lysophosphatidylcholine. *Atherosclerosis*. 2010;208(1):10-8.
102. Goncalves I, Edsfeldt A, Ko NY, Grufman H, Berg K, Bjorkbacka H, et al. Evidence Supporting a Key Role of Lp-PLA2-Generated Lysophosphatidylcholine in Human Atherosclerotic Plaque Inflammation. *Arterioscler Thromb Vasc Biol*. 2012; 32 (6): 1505-12.
103. Corson MA, Jones PH, Davidson MH. Review of the evidence for the clinical utility of lipoprotein-associated phospholipase A2 as a cardiovascular risk marker. *Am J Cardiol*. 2008;101(12A):41F-50F.
104. Weber C, Noels H. Atherosclerosis: current pathogenesis and therapeutic options. *Nat Med*. 2011;17(11):1410-22.

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

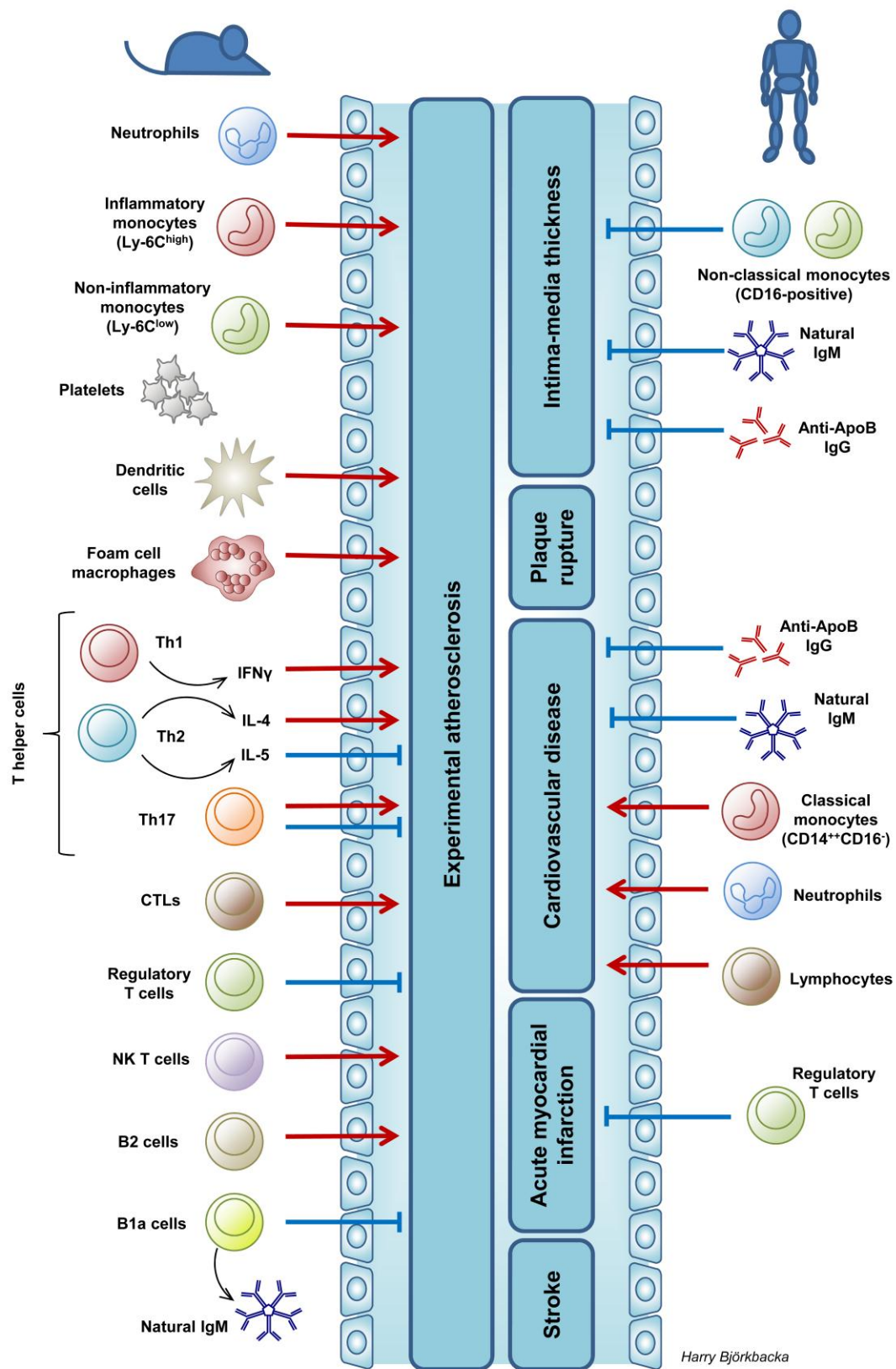


Figure 2

