Adaptive Response of T and B Cells in Atherosclerosis

Daniel F.J. Ketelhuth, Göran K. Hansson

Abstract: Atherosclerosis is a chronic inflammatory disease that is initiated by the retention and accumulation of cholesterol-containing lipoproteins, particularly low-density lipoprotein, in the artery wall. In the arterial intima, lipoprotein components that are generated through oxidative, lipolytic, and proteolytic activities lead to the formation of several danger-associated molecular patterns, which can activate innate immune cells as well as vascular cells. Moreover, self- and non-self-antigens, such as apolipoprotein B-100 and heat shock proteins, can contribute to vascular inflammation by triggering the response of T and B cells locally. This process can influence the initiation, progression, and stability of plaques. Substantial clinical and experimental data support that the modulation of adaptive immune system may be used for treating and preventing atherosclerosis. This may lead to the development of more selective and less harmful interventions, while keeping host defense mechanisms against infections and tumors intact. Approaches such as vaccination might become a realistic option for cardiovascular disease, especially if they can elicit regulatory T and B cells and the secretion of atheroprotective antibodies. Nevertheless, difficulties in translating certain experimental data into new clinical therapies remain a challenge. In this review, we discuss important studies on the function of T- and B-cell immunity in atherosclerosis and their manipulation to develop novel therapeutic strategies against cardiovascular disease. (Circ Res. 2016;118:668-678. DOI: 10.1161/CIRCRESAHA.115.306427.)

Key Words: atherosclerosis ■ B cells ■ immunomodulation ■ lipoproteins ■ T cells ■ therapy
Atherosclerosis is a chronic inflammatory disease that is initiated by the retention and accumulation of apolipoprotein B-containing lipoproteins, particularly low-density lipoprotein (LDL), in the intimal layer of the artery. In this process, extracellular matrix proteins in the innermost layer of the vessel, especially proteoglycans, are believed to play a significant role. LDL can bind to proteoglycans, for example, through ionic interactions between basic amino acids and negatively charged sulfate groups on proteoglycans, which keep LDL trapped in the artery.

In the early stages of atherosclerosis, the retention of LDL in the arterial intima triggers an inflammatory response in the vessel wall. It has been hypothesized that modifications to the trapped LDL, mediated by oxidative, lipolytic and proteolytic enzymes, and reactive oxygen species lead to the formation of several danger-associated molecular patterns (DAMPs), which can activate vascular and immune cells. In this context, other antigens in the plaque have been also suggested to trigger inflammation, including β-2 glycoprotein I, and heat shock proteins, both autologous and chlamydial heat shock protein 60 and mycobacterial heat shock protein 65.

Atherosclerotic plaques are preceded by the accumulation of lipid-laden cells that form fatty streaks in the intima. Although most cells in fatty streaks are macrophage-derived foam cells, T cells are also present in early disease. T cells and macrophages are found in all stages of disease, whereas B cells are only occasionally found in plaques. However, larger number of B cells may be found in the adventitial layer of the arterial wall, where tertiary lymphoid organs can be formed. T and B cells have been proposed to be key modulators of atherogenesis and plaque stability.

Stable plaques normally bear a fibrous cap, primarily composed of smooth muscle cells and collagen, formed as a response of the vessel wall to stabilize the lesion and prevent blood from making contact with prothrombotic molecules of the atherosclerotic plaque’s core. Hence, plaque growth and fibrous cap formation are associated with remodeling of the vessel wall, a complex process in which matrix metalloproteinases, cytokines, and lipid mediators are released by endothelial cells, smooth muscle cells, and leukocytes.

Elicited innate and adaptive immune responses may influence endothelial cell erosion, plaque stability, and thrombosis (Figure). T and B cells have been studied extensively, particularly in this century, due to advances in immune and molecular tools and genetic manipulation in animal models of atherosclerosis, which have allowed important disease mechanisms to be identified. In this review, we discuss key experimental studies that have increased our understanding of T and B cell immunity in atherosclerosis and the modulation of their responses in developing new potential treatment strategies against cardiovascular disease (CVD).

### Basic Concepts of T and B Cells in Immunity

T and B cells are the main constituents of the adaptive immune system. Unlike innate immunity, its reactions are typically highly specific and long-lasting, mainly through the generation of memory. Importantly, adaptive immunity has a key function in distinguishing foreign from self. In conjunction with innate responses, adaptive immune mechanisms are critical for the elimination of pathogens, to block toxic molecules and maintain homeostasis. If this system fails, it can react destructively against self-molecules, causing autoimmune disease.

Adaptive immunity is characterized by its exquisite specificity that permits recognition of millions of different molecular structures. Two structurally and functionally related surface receptors mediate specific recognition, the B-cell receptor (BCR) and the T-cell receptor (TCR). BCR and TCR, both members of the immunoglobulin superfamily of surface receptors, are generated by germ-line rearrangement during the differentiation of the B and T cells. T cells can be categorized according to their expression pattern of surface, intracellular proteins and function, which includes the release of cytokines and the capacity to help other cells, such as B cells and macrophages. T cells characteristically express an alpha/beta (αβ) or gamma/delta (γδ) TCR, express one of the mutually exclusive coreceptors CD4 or CD8, and CD3. In mammals, CD3 associates with TCR to form a protein complex that enables intracellular signal transduction on T cells upon the recognition of linear peptides, termed epitopes, bound to self major histocompatibility complex molecules (MHC or human leukocyte antigen [HLA] in humans) of an antigen presenting cell. CD4+ and CD8+ T cells recognize an epitope in association with MHC class II or I molecules, respectively. During their interactions with epitope-MHC and costimulatory molecules on antigen presenting cells, T cells become activated and undergo a broad range of responses, including proliferation, secretion of cytokines and other soluble mediators, and the expression of surface molecules.

Other T cells, named natural killer T (NKT) cells, can recognize complex lipid antigens, such as glycolipids that are often presented in the context of CD1, and initiate similar responses as those of classical MHC-restricted T cells. A large proportion of NKT cells express a conserved TCR,
generated from the invariant rearrangement of variable and joining regions of TCRα, Vα14-Jα18 TCR in mice, or its homolog Vα24-JαQ TCR in humans. These cells are named invariant NKT (iNKT) cells.

The main role of B cells in immunity is to mount antibody responses and act as antigen presenting cells for T cells. Recent development of B cell biology has demonstrated that these cells may also modulate immunity through the release of cytokines. Similar to their secreted form, antibodies are also expressed on the surface of B cells, in which case they are called BCRs. Like T cells, B cells also go through several developmental stages—they mature, differentiate, and give rise to various cell subsets on antigen recognition. Different, B-cell subtypes can be defined by their expression patterns of surface and intracellular proteins, as well as their distinct BCRs, and antibody and cytokine secretion profiles. Nearly all cells of the B cell lineage express CD19. Notably, certain B cells, such as marginal zone B cells can function as innate-like cells that can become activated on ligation of Toll-like receptor instead of BCR.

**T and B Cells in the Vessel Wall and Atherosclerotic Plaques**

T and B cells are present in atherosclerotic plaques in mice and humans. T cells constitute ≈10% of all cells in human plaques. Seventy percent of T cells in the plaque are described to be CD4+; the remaining largely CD8+. Most of CD4+ T cells in the vessel wall are T helper 1 CD4+ (Th1) cells, a major source of proatherogenic cytokines such as interferon gamma (IFN-γ) and tumor necrosis factor (TNF). Other T-cell subtypes have been reported in plaques as well, including Th2, regulatory T cells (Tregs), Th17 cells, and NKT cells.5

At late stages of disease, artery tertiary lymphoid organs (ATLOs) can be formed in the adventitial layer of the vessel wall. In mice, these lymphoid-like structures have been shown to harbor large numbers of T cells, ie, naïve, effector/memory, cytototoxic T cells, Tregs, and B cells at various stages of differentiation, indicating that the activation of T and B cells in the vessel wall may occur within ATLOs, in addition to plaques, which could be associated to disease in some cases.6,15,16 Still debated, the presence of ATLOs have been recently suggested to confer protection against advanced atherosclerosis in an age- and site-specific manner. Whether these structures should be considered a passive bystander of tissue inflammation or active players in disease requires further analysis.

**Global Deficiency of T and B Cells and Plaque Development**

The role of T and B cells in atherosclerosis has been examined experimentally using hypercholesterolemic mice that lack important elements of adaptive immunity, ie, recombination-activating genes (RAGs). RAG1 and RAG2 encode key enzymes for the rearrangement of the genes encoding the TCR and BCR that are essential for the generation and maturation of T and B cells. In the Apoe and Ldlr knockout strains (Ldlr<sup>−/−</sup> and Apo<sup>e<sup>−/−</sup></sup> respectively), global deficiency of T and B cells, for example, by being combined with Rag<sup>1<sup>−/−</sup></sup> or
Role of Different T-Cell Subtypes in Atherosclerosis

Previous studies showed that CD4+ T cell deficiency in Apoe−/− leads to atheroprotection, whereas the adoptive transfer of purified CD4+ T cells from old Apoe−/− mice or CD4+ T cells that are reactive to modified LDL into immunodeficient mice (Apoe−/−, nude/scid) accelerates disease. The latter of these findings were mirrored by increased peripheral blood levels of IFN-γ.21

The role of CD8+ T cells seems not as clear, both because these cells are less frequent in murine lesions and the fact that CD4 deficiency leads to compensatory changes in the CD8+ T cell population.24 Increased atherosclerosis and more CD8+ T cells have been found in plaques of mice fed high-fat diet,25 or injected with anti-CD137, a CD8+ T cell stimulating antibody.26 Surprisingly, CD8 deficiency had no effect in plaque size in Apoe−/− mice.24 Yet, the adoptive transfer of CD8+ T cells from mice immunized with the P210 peptide of ApoB100 was atheroprotective, suggesting that certain CD8+ T cells may even have a completely different function in atherosclerosis.27

The function of CD4+ T-cell subtypes, Th1 and Th2, in atherosclerosis continue to be studied since the initial reports were published over a decade ago. Depletion of the Th1 lineage-specific transcription factor T-bet, as well as interference with IFN-γ signaling, and absence of the Th1-promoting cytokines interleukin (IL)-12 and IL-18 have been shown to reduce the burden of atherosclerosis in hypercholesterolemic mice.28-35 Consistent with these findings, ablation of TGF-β signaling in CD4+ T cells of ApoE−/− mice, which aggravates Th1 responses, exacerbates the disease.36,37 Thus, there is a general consensus about CD4+ Th1 cells being proatherogenic.

Similar to Th1 cells, an unusual subset of T cells that lack CD28 and secrete high levels of IFN-γ and TNF were suggested to modulate atherosclerosis in humans.37-39 Unfortunately, CD4+CD28null T cells are not present in mice, and their contribution to disease cannot, therefore, be assessed by depletion or genetic experiments.

On the contrary to the Th1 cells, Th2 cells were initially considered to be antiatherogenic although this hypothesis remains debated. Targeted deletion of two Th2 cytokines, IL-5 and IL-13, accelerates disease progression, which suggests that Th2 cells are protective.40,41 Additionally, the induction of Th2 responses against ApoB100 through vaccination has been suggested to be the main mechanism mitigating atherosclerosis.42 However, hypercholesterolemic mice with a concomitant deficiency in IL-4, the signature Th2 cytokine, developed less severe disease,42,43 implying a proatherogenic function of these cells. The conflicting findings regarding the role of Th2 cells in atherogenesis could be attributed to various factors, including the capability of Th2 cytokines to induce immunoglobulin class switching, eg, IL-4 induction of IgE production. Notably, Fc epsilon receptor I (IgE receptor) deficiency reduces atherosclerosis in ApoE−/− mice.44 Th2 cytokines are known modulators of eosinophil and mast cell responses, both cells that could as well influence disease.45,46

Th17 cells have been implicated in autoimmune diseases, such as multiple sclerosis, and have been recently raising attention in atherosclerosis. Deficiency or depletion of IL-17, the signature Th17 cytokine, or its receptor was reported to be atheroprotective.47-51 Paradoxically, other work has suggested that IL-17 slows the development of atherosclerosis.52,53 IL-17A was found to be profibrotic, increasing collagen production and fibrous cap formation in Ldlr−/− mice. Although their role in plaque growth may remain unclear, data suggest that Th17 cells could be atheroprotective by promoting plaque stability.54

The role of NKT cells in atherosclerosis has been examined by different approaches experimentally, ie, CD1d-deficient hypercholesterolemic mice, administration of the prototypical CD1 ligand α-galactosylceramide (α-GalCer), and by adoptive transfer of iNKT cell-enriched splenocytes into immunodeficient Rag2−/−/− mice.55-59 In general, these studies indicate that NKT cells are proatherogenic. Notably, some studies indicated that NKT-dependent atherogenic mechanisms are pronounced only in the early stages of disease.57,59

An atheroprotective role to NKT has also been proposed. Injection of α-GalCer into Ldlr−/− mice fed western diet, and receiving collar placement around the carotid arteries, had antiatherogenic effects.60 The same study reported significantly more IL-10–producing T cells in the spleen and mediastinal lymph nodes of α-GalCer–injected mice, which mechanistically could be linked to anti-inflammatory mechanisms and the phenotype observed. Little is known about the natural ligands of NKT cells, and whether they could determine pro- and anti-inflammatory subtypes of NKTs, warranting further investigation on them.

Foxp3+ natural and inducible Tregs, and type 1 regulatory T cells (Tr1) exert immunoregulatory and suppressive activity through various mechanisms, the most common of which are the production of anti-inflammatory cytokines, such as TGF-β and IL-10, and contact-dependent cell-mediated inhibition (reviewed by Jäger et al).61 Although cellular immunity in atherosclerosis has been suggested to be generally deleterious, all Treg subtypes have been implicated in atheroprotection. Indeed, the balance between T-cell subsets might be crucial in the development of this disease. Depletion of CD25+CD4+ Tregs significantly exacerbates atherosclerosis.62 In line with these data, TGF-β blockade or loss of TGF-β signaling through disruption of TGF-β receptor II signaling in T cells, or IL-10 deficiency has been shown to accelerate plaque formation.63,64 Otherwise, the induction of Tr1 cells significantly reduced atherosclerotic lesions.65
In order to directly assess the function of Tregs in atherosclerosis, Klingenberg et al. used the depletion of regulatory T cell (DEREG) mouse model to study their role. The DEREG mouse expresses a fusion protein of the human diphtheria toxin receptor under control of the Foxp3 promoter and permits conditional depletion of Tregs in hyperlipidemic mice. Supporting previous findings, depletion of Foxp3 Tregs induced a robust increase in disease. Surprisingly, plasma cholesterol increased substantially because of impaired clearance of very-low-density lipoprotein on the depletion of Tregs. This study indicates that in addition to its importance in immunoregulation, Tregs are key players in the cross talk between immunity and lipoprotein metabolism, both of which can influence atherosclerosis and CVD.

A less common Treg, the CD8+ regulatory T cell, was recently described in the context of atherosclerosis. It has been proposed that these cells can regulate follicular T helper cell–germinal center B cell interactions and germinal center deleterious reactions, which result in atheroprotection. These data propose a unique mechanism by which Tregs could be atheroprotective.

The activity of indoleamine 2,3-dioxygenase (IDO) and the production of tryptophan metabolites from the kynurenine pathway have been proposed as important checkpoints influencing T-cell responses, particularly Tregs, inducing their stabilization and enhancing their regulatory phenotype. In experimental atherosclerosis, exogenous treatment with the tryptophan metabolite 3-hydroxyanthranilic acid substantially decreased plaque size, whereas pharmacological inhibition of the production of tryptophan metabolites from the kynurenine pathway has been shown to worsen atherosclerosis. Another study suggested that B cell–derived IL-10 is not essential to regulate atherosclerosis development. Indeed, studies on various mouse models have suggested that regulatory B cells can induce protection against autoimmune diseases, warranting additional studies to understand their role in atherogenesis and CVD.

Although most of the literature has indicated that B cells are protective in atherosclerosis, some studies have recently disputed this hypothesis. For instance, depletion of B2 cells using anti-CD20 and the lack of B cell-activating factor receptor (BAFFR), which is critical for maintaining mature B2 B cells, protect hypercholesterolemic mice against atherosclerosis. Of note, B2-cell depletion by anti-CD20 additionally impairs the antibody response to oxidized LDL (oxLDL) and T-cell activation, whereas B1a and plasma cells are not depleted completely by the treatment. Consistent with the proposition that some B-cell subtypes are deleterious in atherosclerosis, the overreactivation of T follicular helper–germinal center B cell axis has been suggested to be proatherogenic. Although several questions remain about the role of B cell responses in atherogenesis, data suggest that B2 cells are proatherogenic while B1a cells atheroprotective.

Both pro- and anti-atherosclerotic mechanisms of B cells could be driven by antibodies against oxidation-specific epitopes of LDL that are found in the circulation and in plaques of animals and humans. Nevertheless, active and passive immunization against LDL and other atherosclerosis-related antigens have shown atheroprotective effects in animals.

Role of B Cells in Atherosclerosis

B cells have been evaluated in various experimental disease models. Although a general pathological function has been attributed to B cells in numerous autoimmune diseases, particularly based on their ability to secrete autoantibodies, their role in atherosclerosis remains controversial.

It has been shown that the elimination of a large part of the B-cell population by splenectomy increases plaque formation, whereas adoptive transfer of B cells protected mice from developing disease. In line with these data, reconstitution of irradiated Ldlr−/− with B cell–deficient bone marrow has been shown to worsen atherosclerosis. Another study suggested that the lack of B1a cells and the consequent lowering of IgM was responsible for the deleterious effects of B-cell deficiency. The latter study proposed that natural IgM secreted by B1a cells can protect the vessel wall by inhibiting necrotic core formation. Similarly, the IgM response to oxidation-specific epitopes from B1b cells has been shown to protect against atherosclerosis. Recent data suggest that during the atherosclerotic process, atheroprotective immunity is elicited by oxidation-specific epitopes that are generated in the spleen during hypercholesterolemia. Such responses can include germinal center formation, increased number of antibody-secreting cells, and the induction of antiphosphocholine antibodies, all of which could attenuate hypercholesterolemia and reduce atherosclerosis.

The newly described regulatory B-cell subtype, which can secrete IL-10 and TGF-β and influence Treg development, was recently examined in the context of atherosclerosis. Strom et al. showed that adoptive transfer of lymph node-derived regulatory B cell enriched populations into Apoe−/− mice decreased inflammation and atherosclerosis, effects that were dependent on IL-10. Contrasting these data, another study suggested that B cell–derived IL-10 is not essential to regulate atherosclerosis development. Indeed, studies on various mouse models have suggested that regulatory B cells can induce protection against autoimmune diseases, warranting additional studies to understand their role in atherogenesis and CVD.

Immunomodulation of T- and B-Cell Responses in Experimental Atherosclerosis

Several immunization and tolerization protocols against antigens that are involved in atherosclerosis protect against disease in hypercholesterolemic animals. In addition to inducing B-cell responses, several of these protocols have been shown to also deviate deleterious effector T cell responses toward beneficial Treg activity. Therefore, immunotherapies that induce Tregs are under investigation by several groups worldwide.

In experimental atherosclerosis, the induction and expansion of Tregs by anti-CD3, CD31 receptor globulin treatment, administration of an active form of vitamin D3 (calcitriol), or II-2 and anti-IL-2 complexes have shown atheroprotective effects. Subcutaneous and mucosal administration of ApoB100 peptides; injection of ApoB100–loaded IL-10–induced
Immunomodulation of T- and B-Cell Responses in Human Disease

Despite the improvement in the efficacy of therapeutic agents against CVD, such as statins, two thirds of CVD cases cannot be prevented, and at least 10% of healthy individuals develop CVD in the absence of classical risk factors. None of the currently available clinical therapies directly target the immune reactions involved in atherosclerosis, although statins may also exert certain immunomodulatory effects.

Immunosuppressants and anti-inflammatory drugs, such as corticosteroids, cyclosporine, sirolimus, tacrolimus, azathioprine, cyclophosphamide, and methotrexate, remain valuable options for treating and preventing undesired response of T and B cells in the context of autoimmune diseases and transplant rejection. Modulation of T- and B-cell responses is an attractive target considering their specificity and long-lasting characteristics. Although certain immunosuppressants are used to prevent and treat atherosclerotic CVs, such as by local delivery using drug-eluting stents, their systemic use may result in undesirable side effects, including thrombosis, dyslipidemia, hypertension, and diabetes mellitus.

Methotrexate, which is a safe first-line treatment for some autoimmune conditions, decreases the risk of CVD in rheumatoid arthritis and psoriatic patients and is currently being examined with regard to its activity against CVD. Interestingly, methotrexate showed high activity against atherosclerosis in animal models suggesting that it may induce clinical benefits in the general human population. Similarly, TNF blockade, of which is also a standard therapy for autoimmune diseases, has been linked to decreased CVD risk in rheumatoid arthritis.

T cells, particularly the naïve, depend highly on costimulation to be activated. Thus, targeting surface costimulatory molecules has been considered as a method of controlling T-cell–mediated responses, which has been taken to humans. CTLA4-Ig is being examined as a first-line biological for the treatment of rheumatoid arthritis, where it has been shown to reduce disease activity and improve plasma lipid profiles in patients. Whether CTLA4-Ig can influence effector T cell responses in the vessel wall is unknown. Although it may be early to make conclusions about the advantages of biologics—drugs that are not chemically synthesized, eg, anticytokines, and fusion proteins—in preventing and treating CVD, the fact that some of these compounds may improve classical risk factors for CVD may facilitate that they can be used against atherosclerotic diseases.

With regard to anti-CD20–based depletion of B cells and its potential against CVD, there is a discussion over whether statins may reduce its efficacy. Hence, undesired drug–drug interactions, particularly those involving current first-line drugs for CVD, might limit their use.

There is compelling experimental data that support the use of anti-oxLDL to reduce vascular inflammation and to decrease and stabilize atherosclerotic plaques. The Goal of Oxidized LDL and Activated Macrophage Inhibition by Exposure to a Recombinant Antibody trial (GLACIER—NCT01258907) was a randomized, double-blind study that for the first time evaluated an anti-oxLDL–targeted monoclonal antibody in...
humans. Although it was well tolerated, the treatment did not significantly reduced 18F-fluorodeoxyglucose (FDG) uptake in the arterial wall, which was used as a biomarker for plaque inflammation, versus placebo. Moreover, no changes in plasma lipids or high-sensitivity C-reactive protein levels were observed. Although these results were disappointing, there may be several reasons for the failure of the GLACIER trial to meet its end point, including the selected study population, which had stable CVD on background statins, and the use of FDG-PET to assess plaque inflammation, as this method has several limitations and is not a standardized method between medical centers. New and better studies will be needed to properly access the potential of anti-oxLDL therapies for CVD in humans.

Conclusions

There is compelling evidence that T- and B-cell immune responses are involved in all phases of atherosclerosis, from the initiation to its end point complications. Despite their limitations, animal models have been instrumental in determining the molecular mechanisms that link these cells to CVD. However, translating the results into novel and more effective therapies remains challenging. More investments from the pharmaceutical industry, rather than academic support alone, should be made in the early stages of research and drug development to further develop this field. The success of certain immunomodulatory drugs in decreasing CVD risk in autoimmune disease patients indicate they could be used in a relatively large segment of the population. Yet, it remains unclear which patients could benefit most from these therapies, considering the chronic aspect of atherosclerosis, and the risk that long-term use of some drugs could result in immunosuppression-related complications. Vaccination is an attractive option because it specifically targets disease-associated antigens, while leaving the general host defense unhampered. It is encouraging that vaccine studies are being pursued with the aim of reaching the clinical trial phase in the near future.

Sources of Funding

The authors’ research is supported by grants from the Swedish Research Council-Medicine (grants 521-2009-4203—project grant and 349-2007-8703—CERIC Linnaeus Program [Center of Excellence for Research on Inflammation and Cardiovascular Disease]), the Swedish Heart-Lung Foundation, the Karolinska Institute Cardiovascular Program Career Development Grant, the Novo Nordisk Foundation Grant NNFI15CC0018346, Stockholm County Council, and European Union projects (Molstroke, AtheroRemo, VIA).

Disclosures

The authors have filed patents in the area of research.

References


Adaptive Response of T and B Cells in Atherosclerosis
Daniel F.J. Ketelhuth and Göran K. Hansson

Circ Res. 2016;118:668-678
doi: 10.1161/CIRCRESAHA.115.306427
Circulation Research is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
Copyright © 2016 American Heart Association, Inc. All rights reserved.
Print ISSN: 0009-7330. Online ISSN: 1524-4571

The online version of this article, along with updated information and services, is located on the World Wide Web at:
http://circres.ahajournals.org/content/118/4/668

Permissions: Requests for permissions to reproduce figures, tables, or portions of articles originally published in Circulation Research can be obtained via RightsLink, a service of the Copyright Clearance Center, not the Editorial Office. Once the online version of the published article for which permission is being requested is located, click Request Permissions in the middle column of the Web page under Services. Further information about this process is available in the Permissions and Rights Question and Answer document.

Reprints: Information about reprints can be found online at:
http://www.lww.com/reprints

Subscriptions: Information about subscribing to Circulation Research is online at:
http://circres.ahajournals.org//subscriptions/