Atherosclerosis and autoimmunity: a growing relationship

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Abstract

Atherosclerosis is regarded as one of the leading causes of mortality and morbidity in the world. Nowadays, it seems that atherosclerosis cannot be defined merely through the Framingham traditional risk factors and that autoimmunity settings exert a remarkable role in its mechanobiology. Individuals with autoimmune disorders show enhanced occurrence of cardiovascular complications and subclinical atherosclerosis. The mechanisms underlying the atherosclerosis in disorders like rheumatoid arthritis, systemic lupus erythematosus, antiphospholipid syndrome, systemic sclerosis and Sjögren’s syndrome, seem to be the classical risk factors. However, chronic inflammatory processes and abnormal immune function may also be involved in atherosclerosis development. Autoantigens, autoantibodies, infectious agents and pro-inflammatory mediators exert a role in that process. Being armed with the mechanisms underlying autoimmunity in the etiopathogenesis of atherosclerosis in rheumatic autoimmune disorders and the shared etiologic pathway may result in substantial developing therapeutics for these patients.

Key words: atherosclerosis, autoimmune disease, immune system.

INTRODUCTION

Atherosclerosis is a multifactorial disorder that starts early in life and finally is clinically presented later in life. This complication is primarily regarded as an impairment in the immune system in association with the vascular system. Isolation of immune cells such as lymphocytes and macrophages from atherosclerotic lesions implies involvement of the immune system in the etiopathogenesis of atherosclerosis. 1,2 Inflammation may exacerbate atherosclerosis through various approaches after infectious diseases, autoimmunity and other proatherogenic modifications during the inflammatory settings.

Autoimmune rheumatic diseases (AIRDs) have been accompanied with increased risks of cardiovascular-associated mortality and morbidity, mainly after atherosclerosis events. This can be attributed to traditional risk factors of atherosclerosis and therapy with specific drugs like corticosteroids. Several AIRDs are clearly associated with higher cardiovascular disease (CVD) as well as progression of subclinical atherosclerosis, which may occur prior to the manifestation of clinical disease and, therefore, be a target of early diagnosis and preventive therapeutic strategies. 3,4

In this review article, we intend to go through the findings about atherosclerosis and CVD events occurring during AIRDs.
IMMUNOPATHOGENESIS OF Atherosclerosis

Immune system cells are present in atherosclerotic plaques, proposing that they play a role in the mechanobiology of atherosclerosis. Infiltration of immune cells to the plaque lesions as well as their activation can occur after several triggering factors like immune response to infectious microbes. These immune cells are believed to exacerbate atherosclerosis progression, since depletion of CD4+ and CD8+ T cells significantly declined the formation of fatty streaks in C57BL/6 mice. Moreover, breeding of mice with severe combined immunodeficiency (SCID) and apolipoprotein E (ApoE)-knockout mice resulted in a 73% decline in aortic fatty streaks in offspring in comparison to mice with normal healthy immune systems. In addition, transferring of CD4+ T cells to immunodeficient mice led to increased lesion areas. Thus, it is clear that atherosclerosis has an autoimmune setting, in which the immune cells within plaques release several cytokines, such as interleukins (IL), tumor necrosis factor (TNF)-α, and platelet-derived growth factor (PDGF) (Fig. 1).

During atherosclerosis development, a cellular immune response specifically against oxidized low-density lipoprotein (oxLDL), heat-shock proteins (HSPs) and 2-glycoprotein-I (2GPI) has been identified, implying the role of these molecules in atherosclerosis processes. Atherosclerotic lesions obtained from carotid endarterectomies possess 2GPI, which is highly expressed within the intimal-medial border of human atherosclerotic plaques and the subendothelial area. Upon engraftment of lymphocytes from 2GPI-immunized LDL-receptor-deficient mice into syngeneic mice, the host mice demonstrated aggravated fatty streaks in comparison to mice obtaining lymphocytes from healthy mice. T cells against 2GPI can worsen atherosclerosis, proposing 2GPI as a autoantigen during atherosclerosis development. It seems that several specific cell lines responding to specific antigens can modify atherosclerosis through exacerbation or amelioration of its procedure.

On the other hand, a number of autoantibodies has been associated with increased risk of atherosclerosis and its clinical picture. In an animal study, it was observed that administration of anti-cardiolipin antibodies to LDL-receptor-deficient mice led to elevation of anti-cardiolipin production and increased atherosclerosis lesions. Moreover, immunization of mice with 2GPI eventuated in intense cellular (CD4+ cells) responses against 2GPI, as well as increased levels of

Figure 1 The balance of pro- and anti-atherogenic cytokine networks in atherosclerosis. IFN, interferon; IL, interleukin; MCP, monocyte chemotactic protein; TGF, transforming growth factor; TNF, tumor necrosis factor.
anti-2GPI antibodies that were accompanied with aggravated atherosclerotic lesions.

Oxidized low-density lipoprotein is up-taken by macrophages, which then are modified to foam cells and develop atherosclerotic events. Anti-oxLDL antibodies are seen in healthy individuals as well as in AIRD patients with atherosclerosis. Among the evaluations of various lipoproteins, anti-oxLDL autoantibodies distinguished properly between patients with peripheral vascular disorder and a control group. Moreover, there was a positive trend for increased autoantibody levels in subjects with more intense atherosclerosis. In several AIRDs, including systemic vasculitides, systemic sclerosis (SSc), and systemic lupus erythematosus (SLE), the autoantibodies against oxLDL were higher in comparison to controls. Furthermore, a correlation was observed between the level of antibodies to oxLDL and total immunoglobulin levels in SLE patients, while there was no correlation between the total immunoglobulin levels and antibodies to other antigens. This issue demonstrates that there is an increased total immunoglobulin level in patients with SLE that is against to some specific antigens, such as oxLDL.

ATHEROSCLEROSIS IN AUTOIMMUNITY

Atherosclerosis and rheumatoid arthritis

Rheumatoid arthritis (RA) patients show declined life expectancy and mortality ratios range from 0.87 to 3.0 compared with the healthy population. Studies have reported that CVDs are responsible for main cause of mortality in various cohorts of RA populations. Moreover, evidence implies that mechanisms underlying increased CVD mortality in RA are not completely understood. Various types of cardiac complications have been reported in RA individuals. However, ischemic heart disease occurring after atherosclerosis appears to be the main reason of CVD-associated deaths in RA cases. Cigarette smoking has been known as a risk factor for RA onset and progression that increases the disease severity and rheumatoid factor levels in a dose-dependent manner. Nonetheless, several researches could not determine smoking as a predictor of CVD-associated deaths in inflammatory polyarthritis and seropositive RA. RA treatment approaches and lifestyle of these patients may result in hypertension, diabetes mellitus, physical inactivity and obesity; however, evidence does not support clearly the implication of such factors in the accelerated atherosclerosis development in RA cases. As a frequently used drug in RA treatment, methotrexate increases homocysteine levels in plasma, which has been recognized as a new and modifiable risk factor for CVD development in the general population. During therapy with methotrexate treatment, continuous folate supplementation reduced homocysteine levels and declined CVD-associated mortality in patients with RA. Findings about the role of dyslipidemia in RA patients are incongruous. However, more reliable data demonstrated that an increase in small LDL and decreased high-density lipoprotein (HDL) levels occurred after chronic inflammation development rather than being a primary metabolic modification in RA. Rheumatoid arthritis by itself confers a remarkable risk factor for development of early atherosclerosis as well as CVD. In this context, several researchers proposed that impaired immune system and chronic inflammation in RA play key roles in development of accelerated atherosclerosis. Similar to the RA joint, atherosclerotic lesions are manifested through overexpression of adhesion molecules as well as increased cells secreting proinflammatory cytokines that infiltrated to the plaques by responding to chemotactic mediators released by locally activated endothelia. It seems that collagen-degrading mediators are involved in destabilization of atherosclerotic plaques and cartilage and bone erosion of the joints in RA patients. As a result, it can be concluded that the chronic and systemic inflammation in RA may induce early events and hence, development of accelerated atherosclerosis. Redundant cardiovascular-related mortality occurs frequently in RA patients alongside with more widely diffuse and systemic complications, which may manifest through vasculitis and lung involvement.

Despite this observation of rheumatoid vasculitis having a role in developing atherosclerosis, there is evidence proposing that vessel dysfunction is an important event during early endothelial damage in RA cases. Endothelial dysfunction has been observed in some cohort studies of RA patients, unrelated to age of patients, disease duration, disease activity levels and seropositive status. Although various factors could modify the mechanobiology of the endothelium, accumulating lines of evidence provide the view that endothelial dysfunction in RA patients is substantially associated with inflammation. It was observed that abnormal function of endothelium in young RA patients with low disease activity was predicted via C-reactive protein (CRP) and LDL levels. Chronic abnormal function of endothelium causes susceptibility to vascular wall damage, which can be diagnosed through
carotid intima-media thicknesses (IMT) during the preclinical stage, prior to clear disease manifestation. A bulk of studies have reported enhanced carotid IMT in RA patients. However, this observation could not be justified by corticosteroid therapy but seems to be relevant to systemic inflammation manifestations and disease duration, assigning a role for RA as a risk factor for atherosclerosis development. As an immunological factor exerting a common pathogenic player in both RA and atherosclerosis, a specific category of CD4+ T cells (CD4+ CD28null T cells) has attracted highlighted concern. After stimulation via endothelial autoantigens present in the peripheral blood of RA patients with unstable angina pectoris, CD4+ CD28null T cells are expanded. In addition, these cells infiltrate into the atherosclerotic plaques and exhibit a proinflammatory phenotype with the potential of damaging tissue, which mediates vascular injury. It has been shown that CD4+ CD28null T cells exert a contributory role in early development of atherosclerosis in RA patients. RA patients with increased proliferation of CD4+ CD28null T cells show an enhanced level of abnormal endothelial function as well as increased carotid IMT in relation to RA patients lacking proliferation of these cells.

Atherosclerosis and SLE
Systemic lupus erythematosus is a chronic autoimmune disorder that affects several organs and exhibits a wide spectrum of clinical presentations. Young women are predominantly affected with SLE, which generally does not demonstrate atherosclerosis development. Nonetheless, CVDs have been reported to be an important cause of mortality and morbidity in patients with SLE. In SLE patients, coronary artery disease has been reported with a prevalence of 6–10% and the development risk 4–8 times higher in relation to the normal population. As well, 3–25% of deaths in SLE patients has been reported to have underlying acute myocardial infarction. In a bimodal study describing the death causes due to SLE, it was demonstrated that early causes were associated with SLE itself or infections, and late causes were mediated by CVD. Furthermore, substantial atherosclerotic complications were seen in over 50% of fatalities in postmortem studies but were unrelated to the cause of death. Atherosclerosis has frequently been reported in SLE patients in comparison to the general population, and it represents accelerated development in both SLE and diabetes mellitus patients.

Despite atherosclerosis development being frequently observed early in the course of SLE, later age occurrence appears to be the main atherosclerosis determinant in these patients. In addition, the average count of changeable traditional risk factors about atherosclerosis, such as arterial hypertension, hypercholesterolemia, diabetes mellitus, smoking and obesity, is higher in SLE patients in relation to the normal population. Clinical studies on atherosclerosis demonstrated that older age at disease diagnosis, hypertension and hypercholesterolemia were the traditional risk factors predicting clinical manifestations. Nonetheless, Framingham risk factors, including age, sex, LDL, HDL and blood pressure, cannot explain atherosclerosis, which has been attributed to complicated interactions between traditional risk factors and factors related to the disease per se or its therapies. Cumulative dosage and prolonged corticosteroid treatment as well as longer disease duration are nontraditional risk factors that probably are the major predictors of atherosclerosis in SLE patients. Some recently identified risk factors contributing to atherosclerosis development are immunological factors such as anti-cardiolipin, anti-oxLDL, anti-2GPI, anti-HSP antibodies, inflammatory markers such as fibrinogen, CRP, inteleukin (IL)-6, CD40/CD40L, adhesion molecules, coagulation factors, such as plasminogen activator inhibitor-1 (PAI-1) and homocysteine. Researchers trying to identify diagnostic approaches are able to disclose an increased prevalence of cardiovascular lesions, since they potentially identify subclinical atherosclerosis.

Impaired mechanobiology of the coronary circulation has been observed in 40% of patients with SLE that could be even higher. Calculifications of the coronary artery were identified in 31% of SLE patients and were significantly increased in SLE patients in comparison to control subjects. The most frequently used approach for the diagnosis of subclinical atherosclerosis is through carotid B-mode ultrasound, which detected carotid plaques in 17–65% of SLE patients. That notwithstanding, carotid ultrasound directly examines only the carotid artery; this method supplies a precise evaluation of subclinical atherosclerosis.

The examination of risk factors for clinical atherosclerosis seems to be difficult in SLE since the cardiovascular complications are less frequently detected due to low SLE prevalence. The investigation of subclinical atherosclerosis has the benefit of sullying an increased number of lesions, resulting in suitable measurement of risk factors. A study demonstrated that focal atherosclerotic plaques were as frequent as 40% out of 175 SLE women, as diagnosed by B-mode ultrasound. Moreover, the study detected a negative correlation between...
disease activity and plaques.\textsuperscript{37} By ultrasound approach for detecting the common carotid artery in 26 SLE patients with previous history of CVDs, SLE patients without previous history CVD, and 26 healthy control cases, plaques were identified in 65% of SLE cases with CVD, 38% of SLE subjects without CVD, and 11% of the healthy control cases.\textsuperscript{38} Among the items identified more frequently in SLE cases with CVD in comparison to SLE patients without CVD that were associated with CVD were lupus anticoagulant, increased steroid cumulative dosage, osteoporosis-increased triglyceride levels, 1-antitrypsin, oxLDL, anti-oxLDL, lipoprotein(a), homocysteine and low HDL levels.

In a case–control study applying carotid ultrasound, plaques were found in 37% and 15% of SLE patients and healthy control, respectively.\textsuperscript{39} It was demonstrated that two factors were associated with plaques using the multivariate analysis of risk factors, namely age and SLE diagnosis. On the other hand, factors associating with plaques in SLE patients were longer duration of disease, older age at diagnosis, higher damage-index score, anti-Sm antibody absence, and no use of hydroxychloroquine and cyclophosphamide. It was suggested that attenuated disease severity and therefore, low administration of immunosuppressive drugs increased the risk of plaque development. Nonetheless, the patients with less severe disease activity were older than patients with high disease intensity. Thus, the higher prevalence of plaques in this group could be related not to milder disease but rather to age itself, a factor not considered by the authors in the multiple regression analysis for disease-related factors. On the other side, in a study evaluating female SLE cases without CVD, plaques were identified in 32% of patients and determinants of plaques were higher systolic blood pressure, lower HDL levels, use of antidepressants and older age.\textsuperscript{41}

In research to prospectively evaluate the implications of the traditional and nontraditional factors related to subclinical atherosclerosis in SLE patients without CVD, the intima was found to be thickened in 28% of patients and plaques were identified in 16.6% of them. Moreover, age and cumulative prednisone intake were found to be associated with abnormalities in the carotid artery. It seems that an interaction between traditional risk factors, most importantly age, and nontraditional risk factors, particularly cumulative prednisone intake, could be associate with atherosclerosis in SLE patients.\textsuperscript{40} As a predictor of atherosclerosis, age of cases appeared to be of greater importance in SLE patients compared with the general population. This may stem from adverse effects in older patients due to disease severity and treatment.

Atherosclerosis and SSc

Systemic sclerosis mainly affects the microcirculation and also microvascular endothelial cells, which lead to blood vessel obstruction and tissue anoxia.\textsuperscript{42} Furthermore, SSc considerably accelerates the endurance of the vessel wall of the macrocirculation, raising the risk of vascular obstructive disorder.\textsuperscript{43} The relationship between atherosclerosis and SSc was first elucidated in some SSc patients who experienced lower limbs amputation due to peripheral macrovascular disorder.\textsuperscript{44} Four patients with SSc were described with extensive macrovascular involvement of the lower limbs. However, the ulnar artery biopsy demonstrated a distinct vessel narrowing without plaques in these patients.\textsuperscript{43} In limited cutaneous SSc (lSSc), macrovascular disorder was identified in 58% of patients (18 of 31 patients), and 16% (five patients) of patients underwent lower limbs amputation. Biopsies demonstrated severe intimal thickening, extensive proliferation with degeneration of the internal elastic lamina, and plasma cellular and transmural lymphocytic infiltrate.\textsuperscript{45} In 10 of 53 SSc patients, 6.5% had cerebrovascular disease, 15.2% coexistent ischemic heart disease and 21.7% intermittent claudication (21.7%).\textsuperscript{44} The study by Soriano et al. of the main arteries of the abdomen, limbs and neck, showed that initially the ulnar artery was obstructed, which was ascertained by angiography in 15 patients and subtraction angiography in nine of 26 SSc patients. Angiography detected lower-limb involvement and also a raised stiffness of the radial artery.\textsuperscript{46} In approximately 64% of SSc patients versus 35% of healthy subjects, the carotid artery was affected.\textsuperscript{45} In addition, the involvement of the carotid artery was identified in 53 SSc patients.\textsuperscript{47} The evaluation of IMT in the common carotid artery by ultrasound showed significant increase. The IMT was significantly correlated with the existence of the D allele of the angiotensin-converting-enzyme (ACE) gene, which facilitates atherosclerosis.\textsuperscript{45} The increased frequency of the D allele significantly correlates with the incidence of SSc.\textsuperscript{48} These studies propose that considerably higher IMT and deletion/deletion (DD) or insertion/deletion (ID) variants of ACE gene are associated and increase the risk of macrovascular involvement in SSc.

In diffuse SSc the involvement of vessel network broadens from the microcirculation to the macrocirculation. The diffuse involvement of the vascular tree could be related first with two factors: the tendency of
the single case to atherosclerosis and disease pathogenesis. These two key factors can be synergized, thus affecting the integrity of the vessel network. In genetically susceptible SSC cases, determined by increased levels of LDL in oxidation state, oxidative stress and ischemia, vessel wall inflammation and fibrosis are induced, which eventually leads to SSC-associated vascular endothelial damage and finally generates a noxious loop involving the macrocirculation and microcirculation. In this situation, pathogenetic elements contributing to endothelial destruction, including dysfunction of the coagulation and fibrinolytic system, anti-endothelial cell antibodies, soluble intercellular adhesion molecule-1, CRP, and a rise of serum levels of homocysteine, may significantly promote the risk of accelerated macrovascular disorder. From the therapeutic perspective, the vessel wall protecting agents, such as antioxidants and statins, might become a possible options for the management of macrovascular and microvascular involvement in SSC patients. Overall, the extent of increased risk of atherosclerosis in SSC is not yet fully clear. Further studies are needed to address this specific question.

Atherosclerosis and Sjögren’s syndrome

Sjögren’s syndrome (SS) is a heterogeneous systemic autoimmune disorder determined by chronic lymphocyte infiltration of exocrine gland tissues and autoantibody production. The common manifestation of SS is highlighted by systemic organ involvement including nervous system, gastric, musculoskeletal, renal and pulmonary diseases. Furthermore, sicca syndromes frequently have been reported is SS patients. However, cardiac muscle involvement is very rare among SS patients. In a recent study, evaluation of SS patients and those with SS secondary to SLE, none of them had cardiac involvement. Moreover, the association between atherosclerotic plaque expansion and the occurrence of secondary SS in RA and SLE patients did not reach a statistically significant threshold.

There are some case report studies that demonstrated the occurrence of cardiac stroke among young patients with primary SS. Nevertheless, those cardiac strokes were mostly associated with vasculitis and not attributed to accelerated atherosclerotic plaque development. It has been reported that patients with primary SS were identified as having lower circulating anti-lipoprotein lipase autoantibodies compared with SLE or RA patients and healthy individuals. These specific autoantibodies have been related to increased levels of triglycerides in serum and probably an elevated risk of atherosclerosis. Taken together, the number of studies evaluating the risk and incidence of atherosclerosis between primary SS patients is not adequate. Thus, we propose that further studies are warranted to assess atherosclerosis susceptibility among primary SS patients.

THERAPEUTIC APPROACHES

Therapeutic strategies (Fig. 2) for atherosclerosis and other CVDs should be managed according to the clinical manifestation. Many patients who are susceptible to thrombosis and vascular occlusion, such as antiphospholipid syndrome and SLE patients, are prescribed aspirin which can reduce the possibility of vascular thrombosis, beyond the pro-coagulant activity of autoantibodies and underlying mechanisms, the increased atherosclerosis development characterizing these diseases. Still, the key point primarily should be that the best therapeutic approach is prevention. In patients with autoimmune disorders the risk of atherosclerosis and other CVDs are increased not only because of the autoimmune state, but also due to disease consequences, such as nephritis, which lead to nephrotic syndrome and hypertension, as well as drugs such as steroids. In addition, other possible risk factors should be elucidated in autoimmunity. In patients with SLE, increased levels of very LDL cholesterol, triglycerides, and homocysteine and also decreased levels of apolipoprotein A-1 and HDL cholesterol have been described. Accordingly, atherosclerosis patients should take preventive therapy based on instructions for blood pressure control, regular exercise, and when the usage of folic acid and statins is necessary. Immunosuppression of atherosclerosis is an interesting option, although it is still in the infancy stage. Immunomodulation approaches have been developed based on several methods, including immunosuppression, use of intravenous immunoglobulins (IV Ig), induction of oral tolerance with autoantigens (for instance oxLDL), cytokine inhibitors, gene therapy and bone-marrow transplantation.

Vaccination against atherosclerosis?

The finding of atheroprotective immunity has shed a new light on the possibilities for immunoprevention or immunotherapy. The application of atherosclerosis-related antigen should specifically induce those effective T cell-dependent antibodies that operate to decrease atherogenesis. At least theoretically, atheroprotective immunity should be induced without activation or
interference of other elements of the immune system. The principle explanation behind preventive immunity in atherosclerosis is to promote a specific immune response that would decrease the progression of atherosclerosis. Immunization with modified epitopes of LDL led to decreases in atherosclerosis lesions. These data determine that the humoral immune response against oxLDL is protective. Furthermore, immunization with oxLDL also led to decreased neo-intimal formation following balloon damage. However, the findings of different studies about the importance of oxLDL antibodies in association or prediction of atherosclerosis are inconsistent and inconclusive. The increased levels of anti-oxLDL antibodies were predictive of mortality, myocardial infarction occurrence and carotid atherosclerosis progression. The autoantibodies directed against oxLDL are likely belong to two separate families of antibodies. On one side, ‘pathogenic’ antibodies increase atherosclerosis progression and significantly correlate with atherosclerotic plaque formation. On the other side, immunization with oxLDL could stimulate the production of autoantibodies directed against large particle antigens, which play atheroprotective rather than atherogenesis roles in atherosclerosis. These particular antibodies can operate as promoting the clearance of the antigen–autoantibody complex rather than increasing its deposition in the arterial intima. The development of monoclonal antibodies against specific epitopes of oxLDL have shown that these monoclonal antibodies could inhibit the uptake of oxLDL by macrophages. In addition, anti-oxLDL antibodies could inhibit the uptake of apoptotic cells by macrophages. In Watanabe hyperlipidemic rabbits, the study by Palinski et al. described that parenteral vaccination with oxLDL particles decrease atherosclerosis. Subsequently, in fat-fed NZ White rabbits, LDL receptor (Idlr)^−/− mice and ApoE^−/− mice similar results were reported. Thus, these results illustrated that atheroprotective immunity can be achieved by vaccination. It seems that this active immunization operates through humoral as well as cellular
immunity and protection is achieved by secretion of natural IgM antibodies against phosphocholine and as well as T cell-dependent IgG antibodies against oxLDL. In particular, active immunization with oligopeptides of the ApoB100 also modulates atherosclerotic plaque formation. This specific manipulation in the immune system generally leads to activation of antigen-specific T helper cells by presentation of antigen through the major histocompatibility complex class II pathway. Accordingly, the possible contribution of T helper cell responses in atheroprotection can be deduced. However, it should be elucidated whether T helper cell responses operate via induction of high-titer antibodies against oxLDL or by immunoregulatory loops, which are mediated through Treg cells. In any event, up to now immunization data are promising and further efforts should be made in development of effective vaccines against atherosclerosis.

**Immunosuppression**

When atherosclerosis is attributed to an immunological process, it is essential to demonstrate whether this active immunological process leads to or instead protects from atherosclerosis. Another scenario is also possible, as in terms of disease or health statuses, both activities of the immune response could be happening in atherosclerosis; in other words it is a double-edged sword. Limited data emphasize this scenario. The treatment of C57BL/6 mice with cyclosporin A (an immunosuppressive agent) led to accelerated atherosclerosis, which suggests a preventive function of T cell-mediated response at the fatty streak stage. Paradoxically, the depletion of CD4+ and CD8+ T cells decreased fatty streak formation in C57BL/6 mice, representing the aggravated role of T cells in fatty streak formation. The offspring from the cross of immunodeficient scid/scid mice with ApoE knockout mice had a 73% decrease in aortic fatty streak lesions formation compared to healthy control mice. The transfer of CD4+ T cells from immunocompetent to the immunodeficient mice, led to 164% increase in fatty streak lesions formation. This finding was associated with transferred T cells into the atherosclerosis lesions. Another study also pointed to the influence of T cells in atherosclerosis: the intraperitoneal transfer of lymphocytes from LDL receptor-deficient mice which were immunized with β2-glycoprotein-1 into syngeneic mice lead to the formation of larger fatty streak lesions in the recipients mice, compared with mice that were transferred with lymphocytes from healthy control mice. The depletion of T cells from whole lymphocyte populations failed to induce fatty streak lesions. Therefore, β2-glycoprotein-I reactive T cells play a critical role in atherosclerotic plaque formation and immunosuppression could be a more interesting option in the treatment of atherosclerosis. The infiltrated leukocytes in atherosclerotic plaques express CD40 and CD40 ligand molecules. In LDL receptor-deficient mice, the treatment with anti-CD40 ligand antibody significantly modulates atherosclerotic plaque formation. These findings propose that depletion of T cell populations and/or inhibition of T cell responses could help to reduce atherosclerosis. This situation also occurs in cytotoxic agent administration during bone marrow transplantation or cancer therapy. Nevertheless, the problem is considerably more complicated, because these specific antibodies not only predispose us to infections but also have noxious effects on the vascular wall components that could facilitate atherosclerosis progression. Thus, the real consequence of immunosuppression might be even progression of atherosclerosis instead of inhibition.

**Induction of tolerance**

Beyond the role of oxLDL particles in immunization, intervention in mice at earlier stages with this antigen might induce tolerance. In the study by Nicoletti et al. the intervention of newborn ApoE knockout mice with oxLDL and a high-cholesterol diet lead to decrease of the immune response against oxLDL and also atherosclerosis susceptibility in mice. The intervention with oxLDL lead to clonal deletion and T cell tolerance instead of T cell anergy.

**Intravenous immunoglobulin**

Intravenous immunoglobulins which is prepared from serum immunoglobulins of numerous donors is mainly composed of the IgG isotype. It has several indications, including autoimmune diseases, alloimmune thrombocytopenia, idiopathic thrombocytopenic purpura, Guillain–Barré syndrome and various immunodeficiency states. Because atherosclerosis is an active immunological process, IVIg containing anti-idiotypic antibodies and natural antibodies could modulate the atherosclerosis process. Hence, inoculations of 49-day-old ApoE knockout mice with IVIg for 5 days significantly decreased fatty streak lesion formation by 35% in about 60 days. Moreover, the inoculation of mice with IVIg, significantly decreased fibrofatty lesions induced by 4 months of regime treatment. Therefore, IVIg was reported be effective not only during plaque formation, but also during fatty streak lesion formation in Atherosclerosis and autoimmunity.
atherosclerosis. Finally, IVIg therapy induced the inactivation of lymph nodes and spleen T cells, and led to a significant decrease in anti-oxLDL IgM antibodies.

Gene therapy

Gene therapy is a potential new strategy to target several factors participating in progression and development of atherosclerosis. Several genes that participate in the progression of atherosclerosis have been recognized and have been evaluated as potential new options for therapy (Fig. 1). The importance of using gene therapy to modulate immune responses actually depend on specifically targeting the expression of adhesion molecules on the vascular wall and chemokines to affect the progression of atherosclerosis. In humans, there are few clinical trials of gene therapy for different cardiovascular disorders. Several vector systems for gene therapy in CVD have been developed (Table 1). Retroviral-mediated vector of the encoding LDL receptor gene to the liver of familial hypercholesterolemia patients was effectively delivered, although LDL levels remained raised. Another study about a phase 1 clinical trial of intramuscular delivery of VEGF (vascular endothelial growth factor) encoding vector in the severe peripheral vascular disease milieu has been conducted. The delivery of VEGF encoding vector was implemented in 10 organs in nine cases suffering from non-healing ischemic foot ulcers. In that study the elevated levels of circulating VEGF and subsequently, objective evidence of improved distal flow in limbs was described. In some of the patients where conventional therapy was not effective, the direct delivery of VEGF plasmid into the myocardium was conducted and significant improvement in angina and also reduced ischemia was reported in all patients. Furthermore, similar findings were obtained within adenoviral base vector for delivery of VEGF to ischemic myocardium.

Cytokine network manipulation

The cellular components of atherosclerotic lesions, like autoimmune diseases, secrete numerous inflammatory cytokines including TNF, PDGF, interferon (INF)-γ, IL1, IL-2, IL-6, IL-8 and IL-12. Accordingly, several studies have been designed to inhibit the detrimental effect of these cytokines in atherosclerosis and they provide an effective approach for treatment of atherosclerosis (Fig. 2). Fortunately, in some of them encouraging results have been reported. In a cohort study of 130 elderly man, there was a significant association between circulating TNF-α levels and atherosclerotic plaque formation. Vascular calcification is an ectopic phenomenon that usually happens in atherosclerosis. TNF-α within atherosclerotic plaque lesions also contributes to bone formation. It has been shown that treatment of bovine aortic smooth muscle cells (which are able to stimulate osteoblastic differentiation) with

Table 1 Vector systems for gene therapy of cardiovascular disease

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<td>Adenovirus</td>
<td>Transduce dividing and nondividing cells</td>
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<td>High-level expression</td>
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<td>Adeno-associated</td>
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<td>Lentivirus</td>
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<td>Retroviruses</td>
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<td>Liposomes</td>
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TNF-α led to morphological alterations into osteo-
blast-like cells. Moreover, they raised the expression of
matrix mineralization and alkaline phosphatase as
evaluated by incorporation of calcium into the matrix.
In particular, pretreatment of bovine aortic smooth
muscle cells with KT5720 (protein kinase A specific
inhibitor) reduced TNF-α-induced mineralization and
cell morphology. This finding recommends that
TNF-α inhibition might attenuate calcification of
atherosclerosis. Targeting of other cytokine families
also has a critical role in modulation of atheroscle-
sis. In a rat model of the carotid artery, decrease in
transforming growth factor (TGF)-β levels through
ribozyme oligonucleotide targeting of the TGF-β gene
led to a substantial suppression of neointimal forma-
tion after vascular damage. This suppression of
neointimal formation was associated with significant
decrease of collagen synthesis. Furthermore, ApoE
and INF-γ knockout mice (double knockout) had a
considerable reduction in the size of atherosclerotic
lesions, compared to ApoE wild type mice. A sub-
stantial reduction in lesion cellularity and lesion lipid
accumulation was described. However, collagen con-
tent of lesions was elevated. In mice with the INF-γ
knockout gene, a significant elevation in atheroprotect-
egg particles including phospholipid/ApoA-IV has
been shown. Thus, INF-γ promotes atherosclerosis
by systemic as well as local modification of plasma
lipoproteins and the arterial wall, respectively. By
applying the immunodeficient mouse model, it has
been shown that INF-γ can trigger atherosclerotic
modifications in the absence of competent immune
response. This process is conducted through vascular
smooth muscle cells to trigger growth-factor-stimu-
lated mitogenesis. IL-8, as an inflammatory and
chemotactic cytokine, stimulates the proliferation and
migration of smooth muscle cells as well as vascular
endothelial cells. It has been reported that
increased baseline IL-8 plasma levels are related with
an elevated susceptibility of long-term all-cause com-
plications in cases with acute coronary syndrome.
Moreover, it has been elucidated that in acute coro-
nary syndrome, IL-8 via its angiogenic properties, may
contribute to atherosclerotic plaque formation. The
IL-8 receptor, C-X-C chemokine receptor (CXCR2) is
highly expressed on macrophages within atherscle-
rotic plaque lesions. In mice with CXCR2 defi-
ciency, the progression of accelerated atherosclerosis is
significantly decreased; hence, it is possibly a pro-
atherogenic factor.

Statins as immunomodulators
Human leukocyte antigen (HLA)-II molecules have a
key role in T lymphocytes activation and immune
response hemostasis. Statins directly inhibit IFN-γ-
induced expression of HLA-II, and therefore act as a
direct inhibitor of HLA-II-mediated T cell activation.
This effect of statins is mediated by suppression of the
inducible promoter IV of the HLA-II transactivator
CIITA, which is found in different cells of the immune
system, such as monocyte and endothelial cells. The
results of this study provide a defined mechanism for
the use of statins in the treatment of atherosclerosis.

CONCLUSION
Accelerated and early atherosclerosis is a distinct fea-
ture of some inflammatory and autoimmune disorders
due to more specific autoimmune mechanisms and
persistent inflammation. SLE and RA carry an elevated
susceptibility to atherosclerosis. In addition, SLE and
RA are highlighted by dominance of typical risk fac-
tors for coronary artery disorders and an elevated risk
of these disorders as well as an increased incidence of
subclinical atherosclerosis. However, in SSc and SS
patients, while there is a high frequency of macrovas-
cular disorders, the extent of increased risk of
atherosclerosis in these conditions is not yet fully
determined. Moreover, still no data exist to validate
elevated atherosclerosis in SSc and SS. The under-
standing of atherosclerosis as an active immune-asso-
ciated and autoimmune process is of critical
significance, not only for perception of its etiology
and pathogenesis, but also to facilitate the develop-
ment of effective therapeutic and preventive agents.
Up to now, most research is primarily in animal
models to modulate the development of atherosclero-
sis through the immune response. Therefore, it is
rational that applying the same strategies (cytokine
network manipulation, induction of tolerance, IV Ig,
active immunization and immunosuppression) which
have been used in the treatment of autoimmune dis-
ases, maybe effective in modulation or prevention of
atherosclerosis. The human application of these strate-
gies and their substitutes in reduction of morbidity
and mortality, would be an immense achievement in
medicine.

DISCLOSURE OF CONFLICT OF INTEREST
None.
REFERENCES

Atherosclerosis and autoimmunity

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