Circulating Autoantibodies to Oxidized LDL Correlate With Impaired Coronary Endothelial Function After Cardiac Transplantation

James C. Fang, Scott Kinlay, Dominik Behrendt, Hiroyuki Hikita, Joseph L. Witztum, Andrew P. Selwyn, Peter Ganz

Objective—The oxidative modification of low density lipoprotein (LDL) may play a role in the pathogenesis of transplant-associated arteriosclerosis. Oxidized LDL (OxLDL) is immunogenic as well as atherogenic, and the level of autoantibodies to OxLDL has been taken as an index of the oxidant state of LDL. Because endothelial dysfunction is key in the initiation of transplant-associated arteriosclerosis, we postulated that the level of OxLDL autoantibody is associated with the degree of impairment of coronary endothelial function.

Methods and Results—Coronary endothelium-dependent dilation was assessed by using intracoronary acetylcholine and endothelium-independent dilation by nitroglycerin in 36 cardiac transplant recipients within 1 year of transplantation. The coronary responses to acetylcholine ranged from −37% (vasoconstriction) to 31% (vasodilation), and the responses to nitroglycerin ranged from 0% to 42% (vasodilation). The coronary vasomotor response to acetylcholine was significantly and inversely related to OxLDL autoantibody levels ($r = 0.43, P < 0.01$) but not LDL levels ($r = 0.04, P = 0.83$) or circulating OxLDL levels detected by monoclonal antibody EO6 ($r = 0.27, P = 0.11$). The coronary artery response to nitroglycerin was not related to levels of OxLDL autoantibodies, LDL, or EO6 (all $P$ NS).

Conclusions—Autoantibodies to OxLDL are increased in patients with coronary endothelial dysfunction in the first year after cardiac transplantation. The oxidative modification of LDL by inducing endothelial dysfunction in cardiac transplant recipients may be a critical step in the atherogenic effects of LDL and may provide a potential target for therapy. (Arterioscler Thromb Vasc Biol. 2002;22:2044-2048.)

Key Words: arteriosclerosis transplantation endothelium oxidized LDL

Transplant-associated arteriosclerosis (TxA) remains the primary obstacle to long-term survival after heart transplantation. The pathogenesis of TxA involves immunologic and nonimmunologic mechanisms that create repetitive vascular injury and trigger diffuse intimal proliferation. The evidence for a pathological role of LDL cholesterol in the development of TxA is conflicting. In addition, although therapy with statins improved survival and slowed the progression of TxA in 2 recent trials, LDL did not fall appreciably, nor was it correlated with improved outcome. OxLDL rapidly induces endothelial dysfunction in vitro, and the enhanced susceptibility of LDL to oxidation is associated with coronary endothelial dysfunction in humans in vivo. Indeed, the plasma level of OxLDL is related to the angiographic severity of TxA. It also predicts the development of TxA, suggesting that OxLDL may play a role early in the disease process.

Endothelial dysfunction is also common early after cardiac transplantation and heralds the rapid development of TxA. OxLDL is one potential source of endothelial injury. The relationship between OxLDL and coronary endothelial function early after cardiac transplantation is unknown. It is particularly important to understand this relationship, as much as coronary endothelial dysfunction, an early sign of TxA, may be reversible if OxLDL is treated. Accordingly, we hypothesized that OxLDL is associated with the impairment of endothelial function after cardiac transplantation and depressed, and oxidant stress is increased by conditions such as hypertension, diabetes, and the allogeneic activation of lymphocytes and macrophages. The posttransplant setting is especially conducive to the oxidative modification of LDL, because the antioxidant capacity is depressed, and oxidant stress is increased by conditions such as hypertension, diabetes, and the allogeneic activation of lymphocytes and macrophages. Indeed, the plasma level of OxLDL is related to the angiographic severity of TxA. It also predicts the development of TxA, suggesting that OxLDL may play a role early in the disease process.

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The oxidative modification of LDL is important in the atherogenicity of this particle. The oxidation of LDL is required for its uptake by macrophages. Furthermore, oxidized LDL (OxLDL) decreases NO bioavailability, is directly cytotoxic, and induces proinflammatory cytokines and adhesion molecules. The posttransplant setting is especially conducive to the oxidative modification of LDL, because the antioxidant capacity is depressed, and oxidant stress is increased by conditions such as hypertension, diabetes, and the allogeneic activation of lymphocytes and macrophages. Indeed, the plasma level of OxLDL is related to the angiographic severity of TxA. It also predicts the development of TxA, suggesting that OxLDL may play a role early in the disease process.

The relationship between OxLDL and coronary endothelial function early after cardiac transplantation is unknown. It is particularly important to understand this relationship, as much as coronary endothelial dysfunction, an early sign of TxA, may be reversible if OxLDL is treated. Accordingly, we hypothesized that OxLDL is associated with the impairment of endothelial function after cardiac transplantation and...
might be a better predictor of endothelial dysfunction than native LDL cholesterol.

Methods

Patient Population

Thirty-six cardiac transplant recipients were studied at the time of surveillance coronary angiography within the first year after cardiac transplantation at Brigham and Women’s Hospital. The study protocol was approved by the Human Research Committee of the hospital. Their characteristics are described in Table 1. Year 0 patients were studied a median of 52 weeks after transplantation. Year 1 patients were studied a median of 52 weeks after transplantation. All patients were immunosuppressed with cyclosporine, azathioprine, and prednisone. Hypertension was managed with α-blockers (26 patients), hydralazine (5 patients), diltiazem (16 patients), and ACE inhibitors (2 patients).

Coronary Endothelial Function Studies

Vasoactive medications were held at least 24 hours before cardiac catheterization, and all subjects were pretreated with aspirin the night before the study. Endothelium-dependent vasomotion was assessed by intracoronary infusions of acetylcholine (Miochol-E, OMJ Pharmaceuticals), according to a previously established protocol, into the left anterior descending or circumflex artery for 2 minutes, with final estimated intracoronary concentrations of 10⁻³, 10⁻², and 10⁻¹ mol/L. Endothelium-independent vasomotion was assessed by intracoronary infusion of nitroglycerin at 25 μg/min for 2 minutes. The maximal responses of 2 coronary arterial segments were measured by using quantitative angiography and were averaged for each patient.

Measurement of OxLDL Autoantibodies and Epitopes

Blood was obtained from the inferior vena cava at the time of catheterization, and plasma was frozen at −70°C. Minimal ex vivo lipid peroxidation was expected because whole blood is a potent antioxidant. The oxidant state of LDL was assessed by the level of circulating autoantibodies to OxLDL and the level of circulating epitopes of OxLDL, with all measurements performed at the University of California at San Diego under the supervision of one of the authors (J.L.W).²²,²³

Measurement of IgG Autoantibodies

The level of IgG autoantibodies binding to malondialdehyde (MDA)-LDL (a model of OxLDL) was determined as previously described by use of a chemiluminescence ELISA.²²,²³ MDA is a highly reactive breakdown product of polyunsaturated fatty acid oxidation that forms adducts with lysine residues of apoB.²⁴ MDA-LDL antigen for the ELISA was generated as previously described.²² Antibody binding was determined by use of alkaline phosphatase–labeled goat anti-human IgG and a chemiluminescence technique using LuminPhos (Lumigen). Antibody binding was expressed as relative light units (RLU) in 100 ms.²²,²³

Measurement of OxLDL

The circulating level of oxidized phospholipids associated with apoB-100–containing particles was detected by the monoclonal autoantibody EO6, a measure of minimally modified LDL. Murine autoantibody EO6 recognizes an oxidized phospholipid epitope generated on LDL when it undergoes oxidation.²⁵,²⁶ In brief, monoclonal MB47, a monoclonal antibody to human apoB-100, is plated on microtiter wells as a capture antibody; EDTA plasma is added at a saturating concentration of apoB-100–containing particles; and then the degree of EO6 binding to the bound apoB-100–containing particles is determined by the addition of a biotinylated EO6 and by detection using chemiluminescence techniques. To validate that equal amounts of apoB-100–containing particles were captured, in parallel wells the number of apoB-100–containing particles captured was detected by use of MB24, another apoB-100–specific monoclonal antibody. The data were then expressed in arbitrary units as a ratio of the binding of EO6 (in RLU per millisecond) to the binding of MB24 (in RLU per millisecond). Because there is only 1 MB24 binding site per apoB-100, the data can be expressed as EO6/apoB-100, with the realization that the vast majority of apoB-100 particles are LDL.

Data Analysis

The baseline characteristics are presented as mean±SD or proportions, as appropriate. The distributions of levels of IgG to OxLDL and EO6 were highly skewed; therefore, nonparametric methods were used to test univariate relationships. The associations between IgG autoantibody to OxLDL, EO6, and LDL versus coronary vasomotor function were assessed by Spearman correlation coefficients, with statistical significance at the 5% level. Linear regression was used to determine the univariate relationships of other important variables to endothelial dysfunction. Multivariate linear regression was performed to assess whether plasma LDL affected the relationship between the OxLDL measurements and endothelial function.

Results

Patient characteristics are described in Tables 1 and 2. No patient had angiographic evidence of coronary arteriosclerosis. The coronary response to acetylcholine ranged from −37% (negative numbers indicate vasoconstriction) to 31%. The endothelium-dependent (ie, acetylcholine) vasomotor re-
TABLE 3. Relationship Between Ab (OxLDL), LDL, and EO6 vs Vasomotor Responses

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<th>Acetylcholine Response</th>
<th>Nitroglycerin Response</th>
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<tr>
<td>IgG autoantibody (OxLDL)</td>
<td>-0.43</td>
<td>0.01</td>
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<tr>
<td>LDL</td>
<td>-0.04</td>
<td>0.83</td>
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<td>EO6</td>
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Response was inversely correlated with the IgG autoantibody levels to OxLDL ($r = -0.43$, $P < 0.01$; Table 3). Patients with higher IgG autoantibodies tended to vasoconstrict, and those with lower values tended to vasodilate to acetylcholine (Figure). There was no relationship between the IgG autoantibodies and the endothelium-independent (ie, nitroglycerin) vasomotor response ($r = -0.17$, $P = 0.32$; Table 3), suggesting that IgG autoantibodies were related selectively to endothelial dysfunction. There was a trend for an inverse relationship between EO6 levels and endothelium-dependent responses, but it did not reach statistical significance ($r = -0.27$, $P = 0.11$; Table 3). In contrast, there was no relationship between LDL cholesterol and either endothelium-dependent or -independent coronary vasomotor response (Table 3).

Because it was possible that the relationship between the IgG autoantibody to OxLDL and endothelial function might take time to develop, an analysis of year 0 and year 1 patients was performed for each group. In the year 0 group of 26 patients, the correlation was $r = -0.41$ ($P = 0.038$), and for the 10 patients in year 1, the correlation was $r = -0.58$ ($P = 0.082$). These separate analyses suggested that the inverse correlation between IgG autoantibody to OxLDL and endothelial function was true of the entire cohort independent of time since transplantation.

In univariate analyses of the factors that determined endothelial function, only the IgG autoantibody to OxLDL was significant. Furthermore, IgG autoantibody to OxLDL remained significant in a multivariate regression model that included plasma LDL cholesterol ($P < 0.05$). Finally, transplant indication (ischemic versus nonischemic cardiomyopathy), donor age, or use of specific cardiovascular medications (ACE inhibitors, diltiazem, and α-blockers) had no impact on these analyses.

Discussion

TXAA is thought to result from a “response to injury” of the endothelium. Patients with evidence of coronary endothelial dysfunction, detected by vasoconstriction to acetylcholine, exhibit rapid progression of TXAA. However, the causes of endothelial dysfunction in the posttransplant setting are not well understood. Endothelial injury may be induced by a cellular delayed-type hypersensitivity response to major histocompatibility complex class II molecules expressed on endothelial cells. In addition, cytomegalovirus infection may contribute to endothelial injury. The role of dyslipidemia remains controversial.

In the present study, we found that an increased circulating level of autoantibodies to OxLDL was a stronger determinant of endothelial dysfunction after cardiac transplantation than was native LDL. This observation supports the notion that oxidative modification of LDL contributes to the genesis of endothelial dysfunction and, hence, TXAA.

OxLDL may be of interest as a potential source of endothelial injury in TXAA. Holvoet et al have shown that circulating OxLDL levels are independently correlated with the severity of TXAA, whereas such a correlation is absent for native LDL. Circulating OxLDL can predict the development of TXAA, suggesting that OxLDL is involved early in the disease process. OxLDL is capable of mediating dysfunction of endothelial cells. Experimental studies have established that endothelial cells exposed to OxLDL produce less NO and more proinflammatory chemoattractant proteins and leukocyte adhesion molecules. In patients with coronary atherosclerosis, coronary endothelium-dependent responses to acetylcholine are correlated with the susceptibility of LDL to oxidation. The findings of the present study indicate that OxLDL not only is predictive of the development of TXAA within several years of transplantation but also is linked to endothelial dysfunction in the first posttransplant year, consistent with its role as an initiating event in TXAA.

Current approaches, used in the present study, for investigating the pathobiological role of OxLDL in vivo in humans rely on measuring the levels of specific oxidation epitopes of LDL in circulating blood as well as assaying the level of OxLDL autoantibodies. OxLDL particles can be found in tissues with a broad spectrum of oxidative modification. Minimally modified LDL is characterized by oxidation involving principally its lipid components and is still recognized by LDL receptors. Fully oxidized LDL is recognized by scavenger receptors via the oxidative modification of apoB. Fully oxidized LDL is typically not found in circulating blood because it is rapidly removed by the reticuloendothelial system, a process accelerated by the binding of autoantibodies. However, several laboratories have reported the presence of oxidation-specific epitopes on LDL in blood, including those detectable by antibody EO6, thought to represent minimally modified LDL, and the presence of such epitopes is correlated with clinical syndromes. Thus, whether
oxidatively modified LDL is found in the blood and reflects tissue levels in a useful manner probably depends on its overall oxidation state. In the present study, we found a trend but not a statistically significant relationship between the increased level of the oxidation epitope measured by EO6 and coronary endothelial dysfunction (P=0.11).

In contrast, reflecting the fact that OxLDL is strongly immunogenic, autoantibodies to various epitopes of OxLDL are readily found in atherosclerotic lesions and circulating blood of animals and humans. The generation of circulating autoantibodies is likely induced in response to OxLDL within atherosclerotic tissue; indeed, in atherosclerotic mice, levels of OxLDL autoantibodies in the blood correlate well with the content of OxLDL in arterial tissue. Therefore, autoantibodies to OxLDL may better represent the oxidative burden seen by the vascular tissues than any single measure of a circulating OxLDL epitope. Consistently, elevated titers of OxLDL autoantibodies have been documented in patients with various forms of atherosclerosis, including those with myocardial infarction, carotid atherosclerosis, and peripheral vascular disease, and in hypercholesterolemic smokers with endothelial dysfunction. In the present study, we found that patients with higher IgG level against OxLDL (ie, MDA- LDL) had impaired coronary endothelial function. This relationship was independent of other known factors that can modify endothelial function. However, we cannot exclude the possibility that the level of OxLDL autoantibodies is also influenced by the intensity of immune and inflammatory responses to OxLDL. Atherosclerotic lesions are known to be rich in T cells. Furthermore, CD4+ cells isolated from human carotid plaques can respond to OxLDL in an HLA-DR—dependent manner. These observations may suggest that inflammatory responses within the artery wall, mediated by OxLDL-specific T cells, could also be involved in mediating the endothelial dysfunction.

We were limited in the size of the present study by the inherent nature of invasive physiological protocols in human cardiac transplant recipients. It is possible that a larger study would confirm or eliminate a significant relationship between endothelial function and EO6 (OxLDL). However, we suspect from our results that the relationship between endothelial function and EO6 would not be as strong as the relationship between endothelial function and autoantibodies to OxLDL. Furthermore, the relationship between EO6 and endothelial function may not be as critical, inasmuch as autoantibodies to OxLDL may better represent the content of OxLDL within vascular tissue than any single measure of a circulating OxLDL epitope. Although the correlation between EO6 and endothelial dysfunction did not reach statistical significance, the relationship did support the observation that autoantibodies to OxLDL are increased in cardiac transplant recipients with endothelial dysfunction. Finally, we recognize that autoantibodies to OxLDL do not account for the entire variability in endothelial function; other factors associated with endothelial dysfunction remain to be identified.

The new findings in the present study suggest that oxidative modification of LDL may contribute to coronary endothelial dysfunction in cardiac transplant recipients. This relationship is important because endothelial dysfunction is an early pathogenetic step in TXA2 that is potentially reversible if offending stimuli such as OxLDL can be removed before severe luminal encroachment develops. Further studies directed at limiting the oxidation of LDL to prevent endothelial dysfunction and subsequent TXA2 are warranted.

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