Interaction of the Allogeneic State and Hypercholesterolemia in Arterial Lesion Formation in Experimental Cardiac Allografts

Hiroyuki Tanaka, Galina K. Sukhova, Peter Libby

Abstract To learn more about the interaction of allogeneic transplantation and hypercholesterolemia in the formation of arterial lesions, we performed heterotopic cardiac transplantation in rabbits. We analyzed lesions in both the coronary arteries and the proximal ascending aorta 6 weeks after surgery in both transplanted and native hearts of normocholesteremic rabbits and those with diet-induced hypercholesterolemia (serum cholesterol, 1638±366 mg/dL, n=6, 6 weeks after transplantation). All animals received cyclosporin A (5 mg · kg⁻¹ · d⁻¹) for immunosuppression. The transplanted aortas of hypercholesterolemic animals had thicker intimal lesions than did the native aortas (intima/media ratio, 0.67±0.4 versus 0.08±0.1, P<.05) and contained more T cells (37.4±12.8 versus 5.7±6.2 per high-power field, P<.001). In normocholesterolemic animals (n=5) the coronary arteries had negligible lesions in the native heart and only slight and inconsistent intimal lesions in the transplanted heart. In the hypercholesterolemic animals, more coronary arteries had intimal lesions in the transplanted hearts than in the native hearts (74% versus 43%). Coronary artery lesions in the native hearts consisted mostly of foam cells, while those in transplanted hearts had more abundant smooth muscle cells as determined by α-actin staining. Intimal endothelial cells in transplanted aortas expressed increased levels of vascular cell adhesion molecule-1 and intracellular adhesion molecule-1 compared with the native vessels subjected to identical levels of cholesterol. Medial smooth muscle cells in transplanted aortas contained much higher levels of immunoreactive tumor necrosis factor-α than did medial cells of the native aorta in the same hypercholesterolemic animals. The intima of transplanted aortas contained prominent microvessels compared with the native aorta of the hypercholesterolemic rabbits. We conclude that even during treatment with doses of cyclosporine that control acute myocardial rejection, hypercholesterolemia and the allogeneic state act together to augment allograft atherosclerosis. T-cell accumulation, intimal neovascularization, local cytokine expression, and indices of cell activation in arteries.

Key Words • transplantation • cyclosporine • vascular cell adhesion molecule-1 • hypercholesterolemia • graft arteriosclerosis

Currently, the development of an accelerated form of arteriosclerosis presents the major obstacle to the long-term success of cardiac transplantation. The pathogenesis of this transplantation-associated arteriosclerosis remains uncertain but probably involves multiple factors. Immunological differences between the host and donor tissues may contribute to its pathogenesis. The formation of anti-endothelial cell antibodies illustrates a potential humoral mechanism predisposing to the formation of allograft arterial disease. We and others have presented evidence that the development of transplantation-associated arteriosclerosis involves a cellular immune reaction to alloantigens on graft arterial cells. We have hypothesized that activation of a cascade of cytokines during the allogeneic response to the foreign tissue contributes to the development of the fibroproliferative arterial disease.

Hypercholesterolemia can contribute to the development of native atherosclerosis. Transplant recipients with end-stage atherosclerotic heart disease often have preexisting dyslipidemia, whereas those with nonischemic cardiomyopathy most often have no premorbid lipoprotein abnormality. Regardless of the cause of the underlying heart disease, the immunosuppressive therapy instituted on transplantation, including administration of corticosteroids and cyclosporin A, may promote dyslipidemia, hence the importance of understanding the interaction of the allogeneic state and hypercholesterolemia in the context of the development of allograft arteriopathy.

Little is known about the cellular aspects of the effect of this interaction. Alonso et al in pioneering studies more than a decade ago convincingly demonstrated that hypercholesterolemia augments the coronary arterial response to transplantation. In contrast, a recent study of rats rendered hypercholesterolemic by a cholesterol- and cholic acid–supplemented diet showed no augmentation of arteriosclerosis in allografted aortas. Few have used contemporary tools or clinically current immunosuppressive agents such as cyclosporin A to investigate this issue. We therefore studied this process in rabbits receiving heterotopic cardiac allografts. This preparation permits study of the native, untransplanted vessels and the donor’s native vessels in the same animal, allowing internal control for the individual’s degree of hypercholesterolemia and the systemic re-
response to operative and pharmacological interventions or any episodes of infection or acute rejection. We chose rabbits because they readily develop hypercholesterolemia when placed on atherogenic diets. Using this preparation, we monitored lesion morphology, indices of activation of vascular cells, and the expression of certain cytokines to evaluate the interactions of the allogeneic state and hypercholesterolemia in the pathogenesis of allograft arterial disease.

Methods

Heterotopic Heart Transplantation

Under deep anesthesia with intravenous injection of sodium pentobarbital (15 mg/kg), the hearts of 1.5-kg Dutch Belted rabbits were exposed through a median sternotomy, cross-clamped with occlusion of the coronary arteries, and perfusion-fixed with a modified Carrel technique. A subcutaneous pocket was constructed to accommodate the heterotopically placed allograft, and the neck incision made to expose the neck vessels in the recipient animal was closed. The animals were treated with cyclosporin A, 5 mg/d, by subcutaneous injection. All experiments were performed in accordance with the “Guide for the Care and Use of Laboratory Animals” (NIH publication No. 80-23, revised in 1978).

Atherogenic Diets

The animals consumed a diet consisting of rabbit chow supplemented with 4.5% hydrogenated coconut oil and cholesterol 0.5%. Five animals consumed this diet for a total of 7 weeks (starting 1 week before transplantation and continued for 6 weeks after transplantation), a time known from other experiments to yield formation of arterial lesions. Another five animals underwent the same heterotopic transplantation but consumed a normal rabbit chow diet. We chose to study the coronary arteries because of their importance in allograft arterial disease in transplanted hearts. We studied the ascending aorta because this locale is a well-known site of early atherosclerotic involvement in cholesterol-fed rabbits.

Harvest and Preparation of Tissues

At 6 weeks after transplantation, animals were placed in a restraining cage and killed by infusion of pentobarbital (120 mg/kg IV) after systemic heparinization (2000 U IV). The transplanted hearts were then excised and flushed with lactated Ringer’s solution through the aorta to remove the blood in the coronary circulation. For immunohistochemical evaluation we prepared cross sections of the whole heart at the level of the papillary muscles and at the level of the ascending aorta because this locale is a well-known site of early atherosclerotic involvement in cholesterol-fed rabbits.

Immunohistochemical Analysis

The following monoclonal antibodies (mAbs) were used for immunohistochemical analysis: mAb Rb/19 (mouse IgG1, hybridoma supernatant) recognizes L11/135 is a mouse IgG1 antibody that detects a 120-kD glycoprotein determinant present on rabbit thymocytes and peripheral T lymphocytes; this hybridoma was obtained from the American Type Culture Collection and grown as mouse ascites.12 mAb HHF-35 (mouse IgG1, purchased from Enzo Diagnostic) recognizes muscle-specific actin.16 Endothelial cells were identified by expression of von Willebrand factor (vWF), a constitutive endothelial cell marker, using a polyclonal goat anti-human antibody (Atlantic Antibodies) that cross-reacts with rabbit (vWF). Mouse mAb anti-human tumor necrosis factor-α (TNF-α) (a mouse IgG1, UBI) and mouse mAb anti-human interleukin-1β (IL-1β) (mouse IgG1, Genzyme) detected rabbit IL-1β and TNF-α.

Immunohistochemical staining was performed as follows: Serial cryostat sections (6 μm) were cut, air-dried onto poly- t-lysin–coated slides, and fixed in acetone at −20°C for 5 minutes. Sections were preincubated in Dade Biochrome’s phosphate-buffered saline (PBS) containing 0.3% hydrogen peroxide to reduce endogenous peroxidase activity. The sections were then incubated with primary antibodies diluted in PBS with 10% horse serum at room temperature for 60 minutes. After washing, species-appropriate biotinylated secondary antibodies were applied, followed by avidin-biotin peroxidase complex (Vectastain ABC Kit, Vector Laboratories). Antibody binding was visualized with 3- amino-9-ethylcarbazole (AEC) (Sigma Chemical Co). Sections were counterstained with Gill’s hematoxylin. Omission of primary antibodies and staining with type- and class-matched irrelevant immunoglobulin served as a negative control for each antibody.

Analysis of Aortic Lesions of Cholesterol-Fed Rabbits

Morphometry of the specimens of both transplanted and native ascending aortas of cholesterol-fed rabbits (n=5) was performed to measure intimal thickening. The areas of the intima and media were measured by weighing paper tramings of photographic images of both areas. The degree of intimal thickening was expressed as the ratio of the intimal area to the medial area (intimal area/medial area). The mean±SEM was calculated for the transplanted and native aortas, and a paired Student’s t test was used for statistical analysis.

The number of T cells in the intimal lesion per high-power field (×400) was counted for at least five different fields selected at random in the intimal lesions of each specimen. The difference of these values of the transplanted or native aorta was statistically analyzed by the paired Student’s t test.

Analysis of Lesions of Coronary Arteries

The sections of transplanted and native hearts of cholesterol-fed (n=5) and normal (n=5) rabbits were examined to evaluate diseased coronary arteries. The whole-mounted cross sections permitted systematic analysis of all of the coronary artery profiles in the plane examined. Each section so examined generally contained five to eight en face profiles of epicardial coronary arteries and 10 to 20 profiles of intramural coronary arteries. Coronary arterial lesions were classified according to their location (epicardial or intramural). In epicardial coronary arteries we evaluated major large coronary arteries and their branches, and in intramural coronary arteries we evaluated all the coronary arteries except for small arteries (arterioles) with a single layer of medial smooth muscle cells. This analysis was done on sections stained for α-smooth muscle actin by mAb HHF-35. Each epicardial and intramural vessel observed was classified in a binary fashion as either normal or diseased according to the presence of any degree of intimal thickening. We did not attempt to quantify the extent of intimal thickening in these non–perfusion-fixed
frozen sections (optimized for cell and activation marker identification and not for morphometry) because coronary arteries were subject to compression artifacts during sectioning.

Statistics

χ² analysis (Statview) was initially used to evaluate the main effects of diet (control versus 0.5% cholesterol), cardiac tissue source (donor versus recipient), and vessel location (intramural versus epicardial) on the proportion of vessels exhibiting atheromatous lesions. The analysis of each main effect pooled the data across other treatment groups. Subsequent analysis compared individual treatment groups within diet, tissue, and vessel location subgroups.

Results

Incidence of Intimal Lesion Formation of Coronary Arteries

In normocholesterolemic animals the frequency of disease of epicardial coronary arteries did not differ significantly between the transplanted and native hearts, although the number of lesions was quite low, even in the grafted heart under these conditions (Table 1). Intramural coronary arteries of the transplanted hearts were more often diseased than were the epicardial large coronary arteries (36% versus 11%) and had more frequent intimal lesions than did intramural coronary arteries of native hearts (36% versus 3%, Table 1).

Consumption of the atherogenic diet for 6 weeks produced foam cell–rich intimal lesions in native hearts, and the frequency of these lesions in intramural coronary arteries exceeded that in epicardial arteries (56% versus 17%, Table 1). Hypercholesterolemia accentuated formation of intimal lesions in the transplanted hearts (in 66% of epicardial and 78% of intramural vessels, Table 1). These results indicate that even in the presence of cyclosporin A, the allogeneic state augments the effect of hypercholesterolemia on lesion development, particularly in intramural coronary arteries.

Intimal Changes in the Native and Transplanted Aorta in Normocholesterolemic Animals

The native aorta of normocholesterolemic animals retained a normal histological appearance and lacked evidence for expression of any of the activation antigens studied except for a low level of basal expression of ICAM-1 in endothelial cells (not shown). The aortic cuff of the transplanted heart in these normocholesterolemic animals contained occasional lymphoid aggregates (Fig 1). These small, raised intimal lesions contained T cells and retained an intact endothelial covering, as shown by staining for vWF in a characteristic punctate pattern (Fig 1, right). The endothelial cells overlying these intimal lymphoid aggregates strongly expressed VCAM-1 and exhibited increased expression of ICAM-1 (Fig 1, left). In addition to the endothelium, some of the medial smooth muscle cells appeared activated, as indicated by expression of VCAM-1 and ICAM-1 (Fig 1, left). None of the five rabbits in this group had significant fibromuscular intimal lesions of the aorta.

Alterations in Native and Transplanted Arteries of Hypercholesterolemic Animals

After 6 weeks on the atherogenic diet, the mean cholesterol level in these immunosuppressed animals was 1638±366 mg/dL. In contrast to the normocholes-
terolemic animals (cholesterol levels, 53±18 mg/dL), in the rabbits consuming the atherogenic diet the native coronary arteries and aorta developed some fatty streaks (Figs 2 through 4). The transplanted segments of aorta consistently had thicker intimas than did the native aorta of the same animal (Table 2, Fig 2).

Hypercholesterolemia itself can induce expression of VCAM-1 and ICAM-1 in intimal lesions of native aortas. However, the degree of immunoreactivity for these activation markers by cells within the intimal lesions in the transplanted vessels appeared consistently more intense than that in the native aorta of hypercholesterolemic animals (Fig 3) embedded in the same blocks and thus cut, fixed, and stained in an identical manner. T-lymphocyte accumulation in the transplanted aorta exceeded that seen in the native vessel (Fig 3, bottom, and Table 2). In hypercholesterolemic animals, affected portions of both transplanted and native coronary arteries, some endothelial cells, and neointimal smooth muscle cells expressed VCAM-1 and ICAM-1, but the expression of VCAM-1 and ICAM-1 was much more frequent and intense in transplanted coronary arteries than in native coronary arteries (data not shown).

Increased Intimal Neovascularization in Transplanted Arteries

One strikingly consistent but unexpected finding was an increase in intimal neovascularization in the transplanted aortas of the hypercholesterolemic group (Fig 5). The expanded intima in the donor aorta of hyper-
FIG 1. Color photomicrograph showing focal T-cell accumulations in the transplanted aortic segment of a normocholesterolemic rabbit. The ascending aortas of the transplanted hearts contained focal, raised lesions characterized by T-cell infiltration as shown here. Upper left, Low-power view (x 100) of such a lesion stained for T cells with monoclonal antibody L-11/135. Upper right, higher-power view (x400) of the same area stained with the same anti-T-cell antibody. The endothelium over this lesion remained intact, as demonstrated by von Willebrand factor (vWf) in a typical granular pattern (middle right, x400; lower right, X100). Within these raised, lymphocyte-rich lesions, endothelial cells and intimal cells as well as subjacent medial smooth muscle cells displayed increased levels of vascular cell adhesion molecule-1 (VCAM-1) (middle left) or intracellular adhesion molecule-1 (ICAM-1) (lower left).
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Increased Cytokine Expression in the Tunica Media of Transplanted Vessels

The luminal endothelial cells of normal rabbit arteries contain immunoreactive IL-1β (Fig 6 and data not shown). Interestingly, the endothelial cells lining the lumen of the transplanted aortas contained much lower levels of immunoreactive IL-1β than did the endothelial cells of the ascending aortas of the native hearts (Fig 6). Neointimal cells in both the donor and recipient aortas of cholesterol-fed rabbits contained substantial TNF-α, but the intensity of staining for TNF-α in transplanted aortas appeared greater than that in native aortas (Fig 6, third row and low-power insets). In normocholesterolemic animals, neither the intima nor media of the native aorta contained immunoreactive TNF-α, whereas the transplanted aorta contained foci of immunoreactive TNF-α in medial smooth muscle cells in four of five animals. Medial smooth muscle cells in the transplanted aortas of hypercholesterolemic rabbits contained much higher levels of immunoreactive TNF-α than did medial smooth muscle cells of the native aortas studied in parallel (Fig 6, bottom, and insets). In the tunica media of transplanted aortas of normocholesterolemic animals, smooth muscle cells expressed TNF-α only modestly and inconsistently (data not shown).

In transplanted coronary arteries of the hypercholesterolemic animals, neointimal and medial smooth muscle cells expressed TNF-α and TNF-α-positive medial smooth muscle cells localized just below the neointimal lesions in some cases (Fig 7), although the extent and intensity of positive staining for TNF-α varied from point to point in each coronary artery. In the native coronary arteries of the hypercholesterolemic rabbits, some neointimal and medial smooth muscle cells expressed TNF-α but much less frequently and intensely than did transplanted coronary arteries. In normocholesterolemic animals, the transplanted coronary arteries

Increased intimal lesion formation in the transplanted versus native aorta of hypercholesterolemic rabbits. Low-power (x20) cross sections at similar levels of the ascending aorta in representative animals studied at 6 weeks. These results were representative of five animals in each group (Table 2) (hematoxylin and eosin stain).

Fig 2, Cross sections showing increased intimal lesion formation in the transplanted versus native aorta of hypercholesterolemic rabbits. Low-power (x20) cross sections at similar levels of the ascending aorta in representative animals studied at 6 weeks. These results were representative of five animals in each group (Table 2) (hematoxylin and eosin stain).
FIG 4. Left, two lowest panels. Color photomicrographs showing coronary artery lesions in heterotopically transplanted and native hearts of hypercholesterolemic rabbits 6 weeks after transplantation stained for α-muscle actin with HHF-35. Note the greater concentration of smooth muscle cells in the intimal lesion of the transplanted coronary arteries (left) compared with the foam cell-rich lesions seen in the native coronary vessels from the recipient animal (right). These results are representative of those observed in five animals (Table 1).
with lesions showed some foci of increased TNF-α expression, but much less frequently than did transplanted coronary arteries of the hypercholesterolemic rabbits because of the lower incidence of lesion formation in this group.

Coronary Arterial Lesions in the Transplanted Heart Have a More Fibrous Nature Than in Native Vessels

After 6 weeks on the atherogenic diet, coronary arterial lesions not only were more frequent in the transplanted heart but also had a distinct character. The typical intramural coronary arterial lesion in the native hearts contained large foam cells (Fig 4, right). In contrast, the coronary arterial lesions in the transplanted heart contained abundant smooth muscle cells, as demonstrated by α-actin staining in addition to some foam cells (Fig 4, left). Intimal lesions of both transplanted and native coronary arteries had lipid deposition identified by oil red O staining (data not shown). In some cases, eccentric coronary arterial lesions with T-cell infiltration were noted in the transplanted heart. Interestingly, these intimal lesions often occurred in the portion of the vessel that subtended a perivascular lymphoid infiltrate (Fig 7). This pattern suggests an interaction between T cells and intimal lesions (Fig 7).

Discussion

Alonso and colleagues' highlighted the importance of hypercholesterolemia in the development of coronary arterial lesions in transplanted hearts some years ago. Despite the increasing clinical importance of allograft arteriopathy, the interactions between hypercholesterolemia and immune phenomena, often invoked as contributors to the pathogenesis of accelerated atherosclerosis in transplanted organs, remain poorly understood. Allografted arteries in hypercholesterolemic rabbits exhibit increased permeability to lipoproteins. Yet others find little atherogenic effect of a cholesterol- and cholic acid-supplemented diet in rat aortic allografts. This issue has considerable clinical relevance because of the high prevalence of dyslipidemia in transplant recipients, either endogenous or induced by immunosuppressive drugs.

Current immunosuppression regimens for transplant recipients almost always use cyclosporin A or other xenobiotics. Cyclosporin A may promote or limit intimal lesion formation in arteries, depending on the inciting stimulus and experimental conditions. Therefore, it is particularly interesting that we found that doses of this agent that control myocardial rejection did not prevent the formation of arterial lesions in the allografts. We could not study the effect of cyclosporine itself systematically, since in the absence of this agent the engrafted hearts cease to function and have histological grade IV rejection (International Society for Heart and Lung Transplant criteria) after about 1 week.

The heterotopic transplantation model we used has the advantage of exposing both the native and transplanted hearts to precisely the same duration and degree of dyslipidemia. The lack of appropriate strains of inbred rabbits renders isograft controls impossible, however. In this preparation, the transplanted heart continues to beat, although pressure development and flow are not the same as in the native heart. Even though the transplanted aorta should be subject to lower shear stress than the native aorta, the degree of intimal thickening clearly exceeded that in the native aorta. Likewise, the coronary arteries of the transplanted heart should have less compressive force during systole than did the native coronary arteries. Nonetheless, the transplanted coronary arteries were diseased more frequently and displayed more fibrous lesions.

The finding of increased neovascularization in the intima and media of transplanted arteries was unexpected. The signals for this new vessel formation are uncertain. Cytokines such as IL-1 or TNF can promote angiogenesis, perhaps by inducing local production of directly acting angiogenic substances. Levels of immunoreactive TNF-α appeared to be higher in the media of transplanted than native aortas. Therefore, increased expression of TNF-α might contribute to this neovascularization in the neointima of transplanted aortas. T cells, found in greater numbers in the intima of transplanted arteries, might also provide stimuli for neangiogenesis. Neovascularization appears to be a consistent feature of ordinary human atherosclerosis, but we are unaware of any studies that specifically address this issue in human transplant arteriosclerosis.

The increased TNF in medial smooth muscle cells in transplanted vessels might regulate replication of smooth muscle cells as well as angiogenesis. Activated smooth muscle cells can produce TNF. This cytokine colocalizes with smooth muscle cells in advanced human atheroma and in arteries of human renal allografts affected with chronic rejection. Thus, TNF is a possible autocrine stimulus to smooth muscle proliferation in transplanted coronary arteries. The decrease in levels of another cytokine, IL-1β, in the endothelium of transplanted vessels may seem paradoxical. However, the constitutively expressed immunoreactive IL-1β within normal endothelial cells probably represents an inactive precursor. The decrease in IL-1β in the endothelium of the transplanted vessel may actually reflect processing and release of this multipotent cytokine.

The levels of expression of the well-characterized leukocyte adhesion molecules VCAM-1 and ICAM-1 by both endothelial and smooth muscle cells appeared to be accentuated in the transplanted versus native arteries. Several groups have documented increased endothelial adhesion molecule expression in allografted organs. This finding further indicates enhanced activation of vascular cells in the allogeneic state. In addition, expression of these adhesion molecules may have functional roles in leukocyte recruitment. The greater accumulation of lymphocytes within the arteriosclerotic lesions of transplanted vessels might also re-

### Table 2. Morphometry of Intimal Lesions and T-Cell Infiltration in the Aorta of Cholesterol-Fed Rabbits

<table>
<thead>
<tr>
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<th>Intima/Media (Area)</th>
<th>Number of T Cells in High-Power Field (×400)</th>
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<tbody>
<tr>
<td>Donor aorta</td>
<td>0.67±0.4*</td>
<td>37.4±12.8t</td>
</tr>
<tr>
<td>Native aorta</td>
<td>0.8±0.1</td>
<td>5.7±6.2</td>
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*P<.05 vs native aorta.
†P<.001 vs native aorta.
Photomicrographs showing increased neovascularization of the expanded intima of the transplanted aorta in hypercholesterolemic rabbits. Representative frozen sections stained for von Willebrand factor demonstrate striking neovascularization of the thickened intima (I) and the adventitia (A) of the transplanted aorta. Left, Montage depicting at lower power (x 100) the full thickness of a transplanted aorta. The arrowheads point to the elastic laminae bounding the media (M). The microvessels are shown in cross section in the high-power view (x400), middle. Microvessels in the adventitia of the transplanted aorta are readily demonstrable in the high-power view (x400), lower middle. Right, Representative native aorta stained for von Willebrand factor at low power (x100), top, and at high power (x400), bottom.
FIG 6. Color photomicrographs showing expression of interleukin (IL)-1β and tumor necrosis factor (TNF)-α in donor and native aortas of cholesterol-fed rabbits. von Willebrand factor (vWF) staining demonstrates the integrity of the endothelial lining of the lumen of the aorta of donor, transplanted, or of native aorta. Immunoreactive IL-1β, readily demonstrable in the endothelium of the native aorta (right, second row), is much lower in the endothelial cells in the donor aorta (left, second row). Endothelial cells, smooth muscle cells, and possibly infiltrating lymphocytes all colocalize with TNF-α in the intimas of both donor and native aortas (third row). However, medial smooth muscle cells contain much higher levels of TNF-α in the transplanted aorta compared with the native aorta (bottom row and low-power insets, ×100); arrowheads point to the internal elastic lamina.
FIG 7. Color photomicrographs showing detail of an epicardial coronary artery in a heart transplanted into a hypercholesterolemic rabbit. This artery showed a perivascular lymphoid accumulation similar to that commonly seen in human transplantation arteriosclerosis (demonstrated by a T-cell stain in the upper left). The intimal thickening in this example was eccentric; smooth muscle cells and cells in the intimal lesion colocalize with immunostainable tumor necrosis factor (TNF) (bottom, ×100 on left and ×400 on right). This association between perivascular lymphoid aggregates (also frequently observed in human transplanted arteries) and regions of intimal thickening appeared quite consistent.
fect local T-cell multiplication, activation of lymphocytes to express the cognate ligand for the adhesion molecules, or the local production of lymphocyte-specific chemoattractants. Other adhesion molecules (e.g., ICAM-2 or -3 or others as yet uncharacterized) could also contribute to recruitment of lymphocytes. However, Sadahiro et al. have recently shown that treatment of rabbits with an antibody that neutralizes the cognate ligand for VCAM-1, VLA-4 (an αβ integrin expressed on the surface of mononuclear leukocytes), reduced the degree of early leukocyte accumulation on the intima of coronary arteries of transplanted hearts in a preparation very similar to that used here.

The colocalization of perivascular lymphoid aggregates and intimal lesions in the coronary arteries of transplanted hearts points to the importance of T-cell/vessel interactions in the pathogenesis of this experimental form of arteriosclerosis. We have previously suggested a role for ongoing T-cell/endothelial interactions in the pathogenesis of human transplant-associated arteriosclerosis. The experimental results presented here suggest that lymphokines derived from extravascular lymphoid nodules, commonly found in human transplantation arteriosclerosis, may also influence lesion formation.

The present observations in a well-defined animal model of cardiac transplantation indicate that hypercholesterolemia and the allogeneic state act together to enhance formation of intimal lesions and activation of medial smooth muscle cells in transplanted arteries, in agreement with the pioneering study of Alonso et al. before the cyclosporine era. Our finding of strikingly increased neovascularization of intimal lesions in transplanted arteries in hypercholesterolemic animals provides unanticipated evidence for the local presence of angiogenic stimuli under these conditions. The present observations highlight the utility of experimental models to help understand the pathogenesis of a challenging and important problem of accelerated arteriosclerosis after transplantation.

Acknowledgments

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