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Accelerated Arteriosclerosis in Heart Transplant Recipients Is Associated with a T-lymphocyte–Mediated Endothelialitis

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Accelerated arteriosclerosis has emerged as a major life-threatening complication in long-term survivors of heart transplantation. It has been proposed that accelerated arteriosclerosis is an immune-mediated complication of rejection. We observed a striking endothelialitis in the coronary arteries of two explanted hearts obtained from patients with severe transplant-related accelerated arteriosclerosis. This finding prompted us to review the pathologic changes in the coronary arteries of 23 autopsied patients who had received heart transplants. The infiltrate in these vessels was characterized using immunohistochemical stains for lymphocytes (CD45), macrophages (MAC-387), T lymphocytes (CD45RO), B lymphocytes (L-26), and smooth muscle cells (actin). In addition, a full panel of monoclonal antibodies was used on the fresh-frozen tissue available from one of the two explanted hearts. Ten of the eleven recipients with accelerated arteriosclerosis had a moderate to marked lymphocytic endothelialitis compared to 3 of 14 without transplant-related arteriosclerosis ($P < 0.005$). Immunohistochemical staining of the paraffin-embedded material demonstrated that most of the lymphocytes in the subendothelial space of these vessels were T lymphocytes and that this infiltrate was associated with an accumulation of macrophages and a proliferation of smooth muscle cells in the intima. In the explanted heart from which fresh-frozen tissue was available for more detailed cell typing, the T cells marked predominantly as cytotoxic T lymphocytes (CD8+, CD2+). These results suggest that accelerated arteriosclerosis may be mediated, in part, by a cytotoxic T-lymphocyte–directed endothelialitis. (Am J Pathol 1990, 137: 871–882)

Long-term survival following heart transplantation has increased significantly in recent years.[1] With this improved survival, several new challenges have arisen, including the development of malignancies and graft arteriosclerosis.[1–3] Graft arteriosclerosis is a particularly disconcerting problem because it has persisted at a relatively high rate, despite the introduction of cyclosporine immunosuppression, and because patients with life-threatening accelerated arteriosclerosis often are asymptomatic.[4–8]

Graft arteriosclerosis can affect both children and adults and can occur as soon as 3 months after transplantation.[8,9] Angiographically accelerated arteriosclerosis is characterized by a diffuse concentric narrowing of the coronary arteries.[4–7,10] The lesions are present predominantly in the distal segments, and microscopically one usually finds a concentric fibrointimal thickening with a proliferation of smooth muscle cells and an accumulation of lipid-laden macrophages.[2,11–13]

Despite its striking appearance, the pathogenesis of accelerated arteriosclerosis has remained relatively obscure. A variety of mechanisms have been proposed to account for the rapid development of arteriosclerosis in transplanted organs. These include humoral immunity, T-lymphocyte–mediated cellular immunity, lymphostasis, and hyperlipidemia.[14–17]

We used immunohistochemical stains to characterize the cells in the arteriosclerotic lesions in transplanted hearts. Two surgically explanted hearts and 23 hearts obtained at autopsy were examined.

Patients and Methods

Between July 1983 and May 1990, 117 patients received a total of 119 heart transplants at The Johns Hopkins Hos-

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pital. Each heart transplant has been assigned a unique number (H-1 to H-119). One of the recipients, transplant H-21, was retransplanted 56 months after his first transplant because of severe arteriosclerosis in the transplanted heart. The results of immunohistochemical studies performed on fresh-frozen and paraffin-embedded material from that heart prompted us to review the autopsy material available to us from the 32 heart transplant recipients who have died. Autopsies were performed on 24 of these 32 patients and paraffin-embedded tissue, including sections of epicardial coronary arteries, was available from 23 of these autopsies. These 23 autopsy cases and 2 hearts (H-21 and H-36) explanted from patients with severe accelerated arteriosclerosis form the basis of this study.

All of the clinical and histologic material available was reviewed by two of us (RHH and GMH). Recipients were considered to have cytomegalovirus (CMV) infection if, following transplantation, they had a biopsy or culture positive for CMV. For purposes of this study, the number of rejection episodes were counted as the number of times the patient received supplemental immunosuppression for the finding of myocyte necrosis on endomyocardial biopsy. Accelerated arteriosclerosis was defined histologically as a diffuse luminal reduction by concentric fibrointimal thickening.[2,8,11,12] This process often, but not universally, involved both the large epicardial vessels and the smaller perforating arteries. Nontransplant-related atherosclerosis was defined as luminal reduction by focal asymmetrical plaques with a fibrous cap and a central zone of lipid-laden macrophages. This form of atherosclerosis tended to involve the more-proximal coronary arteries. The degree of transplant-related accelerated arteriosclerosis, nontransplant-related atherosclerosis, and the degree of lymphocytic endothelialitis present in each heart were graded by two of us (RHH and GMH) from 0 to 4+ (least to greatest). For purposes of data analysis, endothelialitis was considered to be significant only if it was given a grade of 2+ or more.

Ten control cases were chosen from hearts explanted from the 117 heart transplant recipients. Control hearts were selected to represent a variety of ischemic and nonischemic diseases.

Immunoperoxidase Techniques

Fresh-frozen Tissue

Fresh-frozen tissue was available in one case (H-21). Five-micron sections of frozen tissue from the coronary arteries were desiccated over silica overnight. The sections were then fixed in cold acetone and rinsed with phosphate-buffered saline containing 0.2% bovine serum albumin. Endogenous biotin receptors were blocked with phosphate-buffered saline containing 1% bovine serum albumin. The sections were then incubated overnight with antibodies to common leukocyte antigen (CD45, Hle, Becton-Dickinson, San Jose, CA), T lymphocytes (CD2, OKT-11, Ortho Diagnostics, Rariton, NJ), cytotoxic/suppressor T lymphocytes (CD8, OKT-8, Ortho Diagnostics), helper T lymphocytes (CD4, OKT-4, Ortho Diagnostics), monocytes (OKM-1, Ortho Diagnostics), class I antigens (HLA-ABC, Dako Co., Carpinteria, CA), class II antigen (HLA-DR, Becton-Dickinson), complement (C3, Behring Diagnostics, LaJolla, CA), immunoglobulin G (Tago Co., Burlingame, CA), and immunoglobulin M (Tago Co.). Sections then were washed and developed with the avidin-biotin complex (ABC) method with diaminobenzidine and copper sulfate. The slides were counterstained with the Giemsa stain.

Paraffin-embedded Tissue

Paraffin-embedded tissue was available on all 25 cases. Tissue from the coronary arteries was fixed in neutral buffered 4% formaldehyde solution and embedded in paraffin. Four-micron sections were deparaffinized with xylene and alcohol and washed briefly with TRIS-buffered saline. Endogenous peroxidase was inactivated with 3% hydrogen peroxide and TRIS-buffered saline, and endogenous biotin receptors were blocked with normal horse serum. The sections were incubated overnight with antibodies to common leukocyte antigen (CD45, Dako Co., Carpinteria, CA), Actin (Enzo Co., NY), B lymphocytes (L-26, Dako Co.), T lymphocytes (CD45RO, UCHL-1, Dako Co.), or macrophages (MAC-387, Dako Co.). They were washed and developed with the avidin-biotin complex immunoperoxidase procedure. Sections were colorized with 3-amino, 9-ethyl carbazole and counterstained with Mayer's Hematoxylin.

Case Reports

The following two cases illustrate both the typical clinical and pathologic findings in patients with accelerated arteriosclerosis and demonstrate the rapidity with which this process can occur.

H-21

This 21-year-old man underwent heart transplantation for viral myocarditis. The patient was maintained on cyclosporine (4.3 to 10.0 mg/kg/day Sandoz Inc., Basel, Switzerland) and methylprednisolone immunosuppression. During the year following his transplant, he experi-

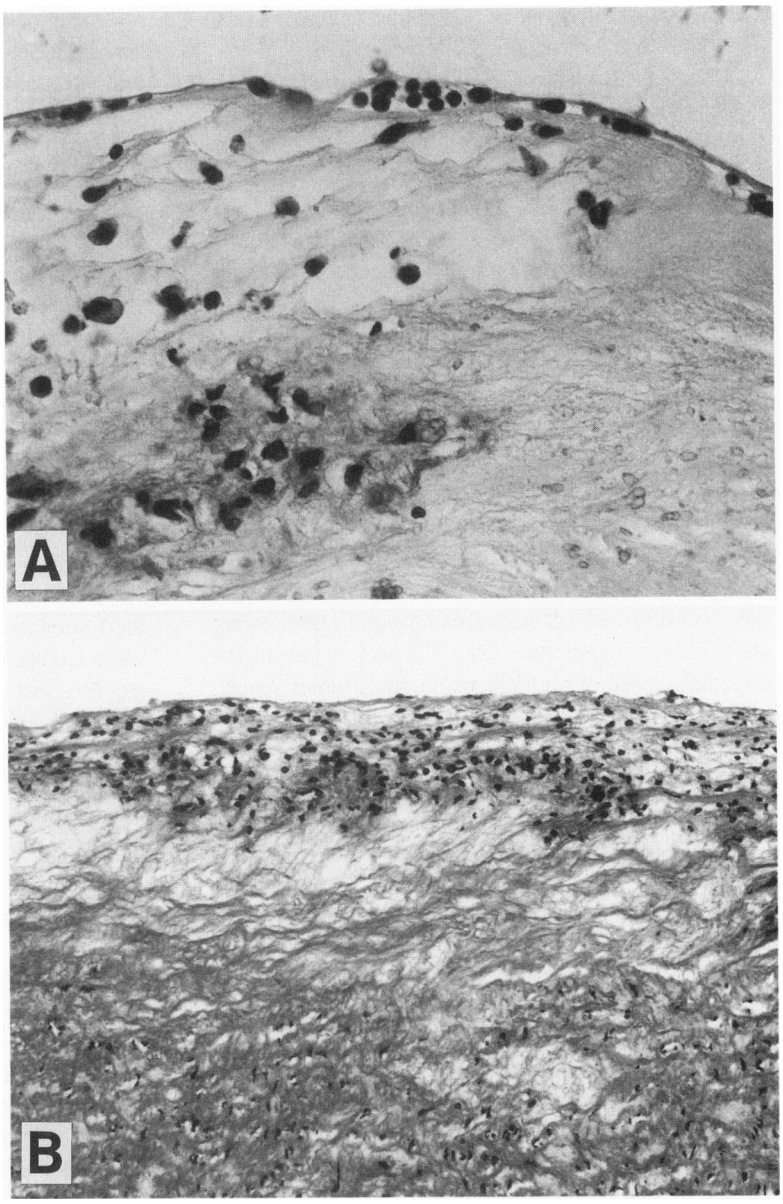


Figure 1. *H-21. A lymphocytic endothelialitis is present in the coronary arteries of this explanted heart with severe transplant-related accelerated arteriosclerosis* (A). *The endothelialitis also involved the donor segment* (B) *of the aorta. (*A, *400×;* B, *170×; both hematoxylin and eosin).*

enced five episodes of moderate acute heart rejection proved by biopsy. These episodes were treated with either methylprednisolone, equine ATGAM (Upjohn Co., Kalamazoo, MI), or prednisone. Cardiac angiography performed 1 and 2 years after his transplant was essentially unremarkable, while coronary angiography at 3 years revealed diffuse coronary artery disease of the type typically seen in transplant recipients.[4-6] Forty-three months after his transplant the patient developed severe congestive heart failure and an echocardiogram revealed anterior wall akinesis with an associated mural thrombus. Anticoagulation with warfarin was initiated, and a second heart transplant procedure was performed 56 months after the initial operation. The patient's postoperative course was complicated by a single episode of rejection for which he received methylprednisolone and by bradycardia that required the placement of a pacemaker. The patient remains in good condition 4 months later.

The explanted heart weighed 430 g and was remarkable for a large remote transmural myocardial infarct involving the intraventricular septum and anterior wall of the left ventricle. An adherent intraventricular thrombus was associated with the infarct. Examination of the coronary arteries revealed occlusion of both the right coronary artery and the left anterior descending coronary artery and stenosis (50%) of the left circumflex coronary artery. Microscopic examination of the coronary arteries revealed a diffuse concentric intimal thickening with many foamy macrophages and a prominent lymphocytic endothelialitis (Figure 1A). This endothelialitis was remarkable in that

it also involved the donor portions of the root of the aorta (Figure 1B) and the pulmonary artery.

Immunohistochemical stains were performed on both fresh-frozen and paraffin-embedded material from this case. These stains revealed a striking cytotoxic T-lymphocyte–mediated endothelialitis in the coronary arteries (Figure 2). This endothelialitis was accompanied by the accumulation of macrophages and the proliferation of smooth muscle cells in these vessels. The majority of the cells in the subendothelial space stained strongly for common leukocyte antigen (CD45), OKT-11 (CD2), and UCHL-1 (CD45RO), and OKT-8 (CD8, a marker of cytotoxic/suppressor cells), while moderate numbers of cells stained for MAC-387 and OKM-1 (macrophage/monocyte markers), and only some cells stained for OKT-4 (CD4, a marker of T-helper lymphocytes). L-26–positive cells (B lymphocytes) appeared to be confined to lymphoid aggregates in the epicardial connective tissues. A strong diffuse staining for immunoglobulin M (IgM) was noted in the intima and media of several of the blood vessels, while stains for immunoglobulin G and C3 were negative. The endothelial cells of both large and small blood vessels stained positively for class II (HLA-DR) and class I (HLA-ABC) antigens.

H-29

This 18-year-old man underwent heart transplantation for cardiomyopathy of unknown etiology. The patient was maintained on cyclosporine (6 to 10 mg/kg/day, Sandoz Inc.) and methylprednisolone immunosuppression. He experienced three episodes of rejection for which he received methylprednisolone, OKT-3, and rabbit anti-thymocyte serum. Cardiac catheterization performed 1 year after transplantation revealed normal coronary arteries. The patient remained stable and in good condition until 23 months after his transplant, when he collapsed without warning and died despite resuscitative efforts.

At autopsy, the heart weighed 430 g and was remarkable for marked stenosis (50% to 90%) of all of the epicardial coronary arteries. Histologic examination of those arteries showed a pronounced intimal thickening with many foamy macrophages and a lymphocytic endothelialitis (Figure 3). The myocardium was remarkable for a recent myocardial infarct in the posterior wall of the left ventricle. All suture lines were intact. Other autopsy findings of note included acute congestion and edema of the lungs and a small remote infarct in one of the kidneys.

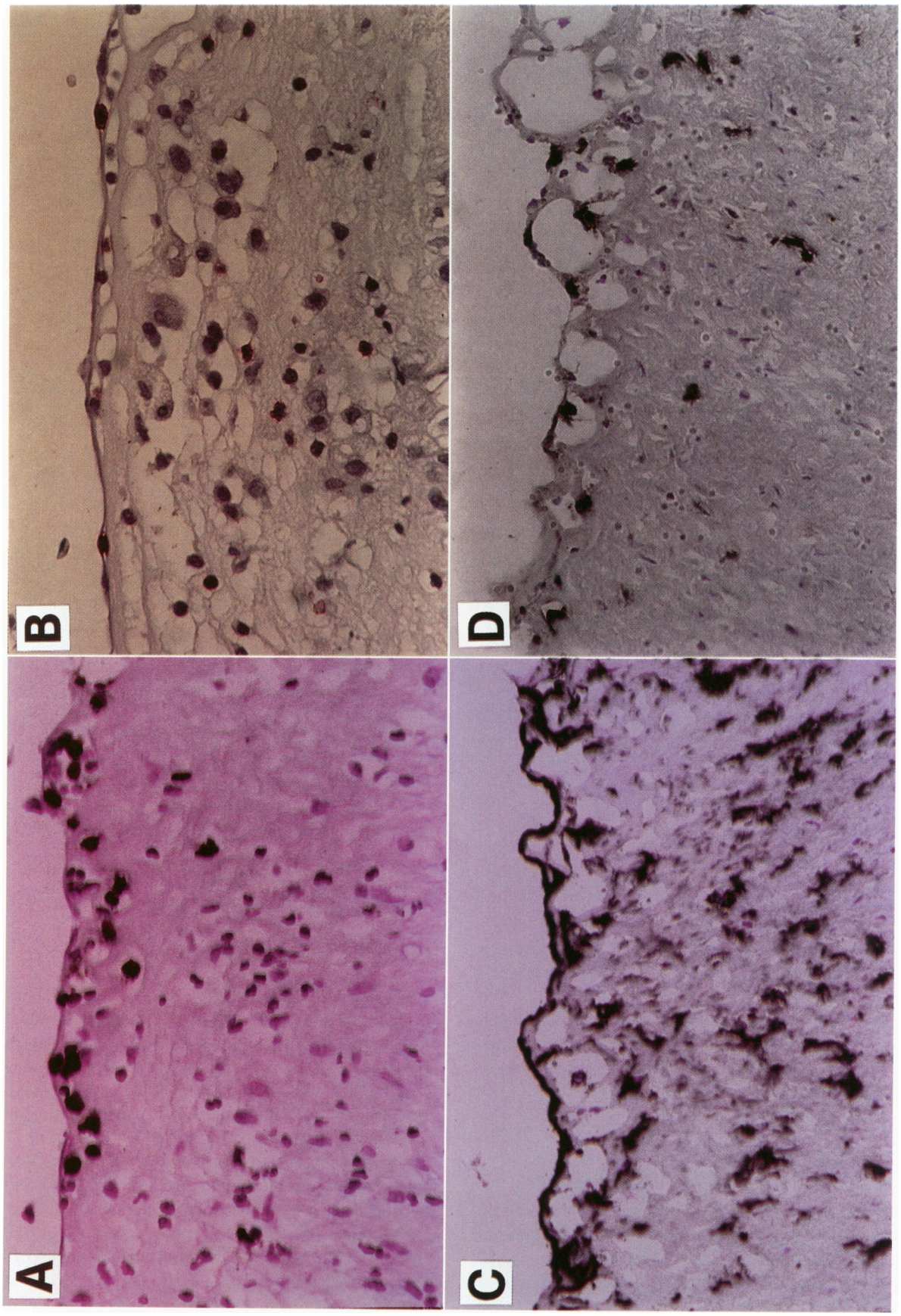
Immunohistochemical stains performed on paraffin-embedded tissue from this case demonstrated that most lymphocytes in the subendothelial space were T lymphocytes (L-26 negative, and CD45 and CD45RO positive). Many large foamy cells also were present in the subendothelial space and these stained positively for the macrophage marker (MAC-387). Staining for actin demonstrated a proliferation of smooth muscle cells in the intima, and a stain for LN-3 showed induced class II antigen expression on the endothelial cells. B lymphocytes (L-26 positive cells) appeared to be confined to epicardial lymphoid aggregates and were not associated with blood vessels.

Results

Table 1 summarizes the clinical findings. Eleven hearts were from patients with pathologic evidence of accelerated arteriosclerosis and 14 hearts were from patients without pathologic evidence of accelerated arteriosclerosis. The post-transplantation survival times for the 11 patients with accelerated arteriosclerosis ranged from 5.5 to 55 months (mean, 34 months). The mean donor age for these patients was 25 years (range, 15 to 36 years) and the mean recipient age was 39 years (range, 18 to 53 years). The mean ischemic time for these patients was 171 minutes (range, 75 to 250 minutes), and 7 of these 11 patients had culture- or biopsy-proved CMV infection at some time during their post-transplantation course. Two of the eleven patients had two class I major histocompatibility (MHC) antigen matches with their donor, four had one match, and five had no matches. Two of these eleven patients were HLA-DR matched with their donors. During the first post-transplantation year, this group of 11 patients experienced a mean of 3.3 rejection episodes per patient per year.

The post-transplantation survival times for the 14 patients without evidence of accelerated arteriosclerosis ranged from 0.1 to 17 months (mean, 6 months). The mean donor age for these patients was 29 years (range, 17 to 37 years) and the mean recipient age was 39 years (range, 13 to 62 years). The mean ischemic time for these 14 patients was 177 minutes (range, 87 to 259 minutes), and 7 of these patients had a CMV infection following their transplant operations. Eight of these fourteen patients (57%) had one class I MHC antigen match with their donor, and six had none. Five of the fourteen patients were HLA-DR matched with their donors. During the first post-

Figure 2. *H-21. Immunohistochemical staining demonstrated that the endothelialitis in the coronary arteries of this explanted heart with accelerated arteriosclerosis was composed of macrophages* (A) *and T lymphocytes* (B). *The endothelialitis was associated with induced class II major histocompatibility antigen expression on endothelial cells* (C), *and the T lymphocytes marked as cytotoxic T lymphocytes* (D). *(Immunohistochemical staining for* A: *MAC-387, 400×;* B: *CD45RO, 685×;* C: *HLA-DR, 575×;* D: *CD8, 575×).*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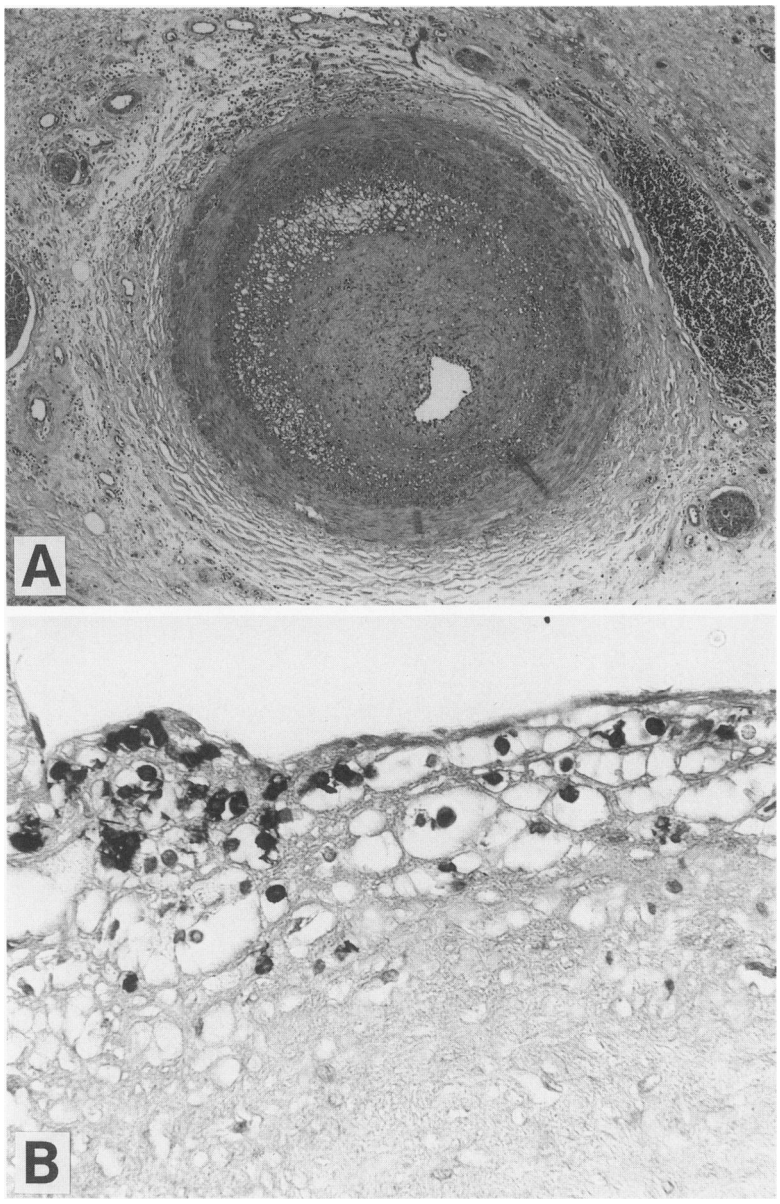

Figure 3. *H-29. The accelerated arteriosclerosis in this case was characterized by a concentric luminal narrowing* **(A)**. *The intimal thickening was associated with a T-lymphocyte endothelialitis* **(B)**. *(***A**: *60×, hematoxylin and eosin;* **B**: *400×, immunohistochemical staining for CD45RO).*

transplant year, this group of 14 patients experienced a mean of 3.3 rejection episodes per patient per year.

With the exception of graft survival ($P = 0.0002$, Student's *t*-test) there were no significant differences in the various clinical features examined between the two groups (with and without accelerated arteriosclerosis) of transplant recipients.

Control Patients

Primary diseases among the 10 controls included ischemic heart disease secondary to nontransplant-related atherosclerosis (six cases) and idiopathic cardiomyopathy with minimal coronary artery disease (four cases).

Lymphocytic Endothelialitis

Transplant-related accelerated arteriosclerosis was identified in 11 hearts. In 10 of these 11 cases, there was a marked lymphocytic endothelialitis. This lymphocytic endothelialitis was characterized by the presence of many lymphocytes and macrophages in the subendothelial space and by the vacuolization of this space, giving the appearance of a 'lifting' of the endothelial cells. In all 11 hearts with accelerated arteriosclerosis, the intima was thickened by a proliferation of spindle-shaped cells and by an increase in the amount of fibrous connective tissue.

A lymphocytic endothelialitis was identified in 3 (H-5, H-15, and H-105) of the 14 hearts without accelerated arteriosclerosis, a prevalence significantly less than that

Table 1. *Clinical Summary*

Heart transplant number	Recipient age (years)	Donor age (years)	Reason for transplant	Ischemic time (minutes)	Post-transplant CMV status	Graft survival (months)	Cause of death/reason for retransplantation
Recipients Without Accelerated Arteriosclerosis							
24	17	34	DCM	240	−	0.1	Pulmonary hemorrhage
15	24	32	DCM	87	−	1.0	ACR
66	45	21	DCM	165	−	1.1	ACR
41	13	33	DCM	159	−	1.1	Fungal sepsis
94	56	33	IHD	259	+	1.2	Bacterial sepsis
105	26	36	DCM	186	−	2.0	ACR
99	43	19	IHD	186	+	3.0	Lymphoproliferative disorder
91	62	37	DCM	190	+	4.0	Toxoplasmosis
5	49	19	DCM	195	+	4.2	ACR
102	54	27	IHD	178	−	8.0	Pancreatitis
84	43	29	DCM	181	+	9.5	CMV and PCP Pneumonia
1	28	27	DCM	109	−	11.9	Pneumonia and CVA
18	37	17	DCM	128	+	16.1	Sepsis, GVHD
8	47	35	IHD	209	+	17.1	Bacterial sepsis
Recipients with Accelerated Arteriosclerosis							
7	40	25	DCM	160	+	5.5	ACR, MI due to vasculitis
35	40	21	DCM	97	+	8.4	ACR
63	53	17	IHD	220	−	17.6	Bacterial mediastinitis
29	18	36	DCM	89	+	22.8	MI
33	42	23	IHD	228	−	23.3	Bacterial sepsis
57	40	21	DCM	150	−	42.5	ACR
23	50	30	DCM	250	+	44.5	Pneumonia
11	46	29	DCM	75	+	49.1	MI, embolic CVA
14	27	15	IHD	200	−	50.6	MI
36	48	33	DCM	197	+	54.6	Accelerated arteriosclerosis
21	21	21	DCM	210	+	55.5	MI

ACR, acute rejection; CMV, cytomegalovirus; CVA, Cerebral vascular accident; DCM, dilated cardiomyopathy; GVHD, graft-versus-host disease; IHD, ischemic heart disease; MI, myocardial infarction; PCP, pneumocystis carinii penumonia.

seen in the hearts with accelerated arteriosclerosis ($P < 0.005$, Fisher's exact test). The lymphocytic endothelialitis in these three hearts was associated with moderate acute rejection (many foci of myocyte necroses in the myocardium). In contrast to the lymphocytic endothelialitis seen in patients with accelerated arteriosclerosis, the lymphocytic endothelialitis in these three patients was not associated with a proliferation of spindle-shaped cells in the intima.

A lymphocytic endothelialitis was identified in one of the six control hearts with natural atherosclerosis and in none of the four control hearts without atherosclerosis.

Thrombosis of the Affected Vessel

In three cases (H-7, H-15, and H-36) the lymphocytic endothelialitis was associated with thrombosis of the affected vessels. Two of these patients had accelerated arteriosclerosis (H-7 and H-36) and the third did not. All three patients with vascular thromboses also had moderate acute rejection as evidenced by the presence of myocyte necrosis. Thromboses were not identified in the control hearts.

Medial Lymphocytic Infiltrate

A moderate to marked (2+ or greater) lymphocytic infiltrate was identified in the media of 5 (H-7, H-14, H-21, H-36, and H-63) of the 11 cases with accelerated arteriosclerosis and in 2 (H-5 and H-105) of the 14 patients without. The two patients without accelerated arteriosclerosis who had a medial lymphocytic infiltrate also had endothelialitis and myocyte necrosis. Lymphocytic infiltrates were not identified in the media of the vessels of the control hearts.

Presence of Moderate Acute Rejection (Myocyte Necrosis)

Eight patients (H-5, H-7, H-15, H-35, H-36, H-57, H-66, and H-105) had evidence of moderate acute rejection (myocyte necrosis) at autopsy. These patients survived from 1 to 54 months after transplantation (mean, 15 months). Four of these eight patients did not have accelerated arteriosclerosis. Six of these patients also had marked (grade 3+ or 4+) endothelialitis, one had moderate (grade 2+) endothelialitis, and one had mild (grade 1+) endothelialitis. Myocyte necroses were not identified in the 10 control hearts.

Table 2. *Pathologic Findings*

Heart transplant number	Graft survival (months)	Degree of natural atherosclerosis (0–4+)	Degree of accelerated arteriosclerosis (0–4+)	Degree of endothelialitis (0–4+)	Immunohistochemical stains*		
					UCHL-1	MAC-387	L-26
Patients without Accelerated Arteriosclerosis							
24	0.1	0	0	0	0	0	0
15	1.0	0	0	2†	1	1	0
66	1.1	0	0	1†	1	0	0
41	1.1	1	0	1	1	1	0
94	1.2	1	0	0	0	0	0
105	2.0	2	0	3†	3	3	0
99	3.0	1	0	0	0	0	0
91	4.0	2	0	1	1	1	0
5	4.2	0	0	3†	3	1	0
102	8.0	1	0	0	0	0	0
84	9.5	1	0	0	0	0	0
1	11.9	0	0	1	1	NI	0
18	16.1	1	0	0	0	0	0
8	17.1	0	0	0	0	0	0
Patients with Accelerated Arteriosclerosis							
7	5.5	0	1	3†	3	2	0
35	8.4	0	2	3†	4	NI	0
63	17.6	0	3	2	2	1	0
29	22.8	0	2	3	3	NI	0
33	23.3	0	2	3	4	2	0
57	42.5	0	2	3†	2	2	0
23	44.5	0	2	2	1	1	0
11	49.1	0	4	1	0	0	0
14	50.6	0	3	2	2	2	0
36	54.6	0	4	4†	4	3	1
21	55.5	0	4	4	4	4	0

* Relative number of positive staining cells (0 to 4+) in subendothelial space.
† Patient also had moderate acute rejection with myocyte necrosis.
NI, Immunoperoxidase staining not interpretable.

Immunohistochemical Staining

The results of the immunohistochemical staining performed on paraffin-embedded material from all 25 cases are summarized in Table 2. In the 13 cases with endothelialitis, the small cells in the subendothelial space stained positively for the lymphocyte and T-cell markers (CD45 and CD45RO), while the larger foamy cells stained with the macrophage marker (MAC-387). The spindle-shaped cells in the intima stained for actin, and many of the endothelial cells, particularly those in smaller blood vessels, stained positively for class II antigen (LN-3). B lymphocytes (L-26 positive cells) generally were not identified within blood vessels, but were found in lymphoid aggregates in the epicardial connective tissue. Most of the cells identified in the media of the blood vessels stained for common leukocyte antigen (CD45) and with the T-lymphocyte marker (CD45RO). The results of the immunohistochemical stains performed on the fresh-frozen material from H-21 are summarized in the description of that case above. Most of the subendothelial lymphocytes in this case marked as cytotoxic T lymphocytes (CD8+).

Immunohistochemical stains performed on the 10 control cases were remarkable only for a proliferation of smooth muscle cells (actin-positive cells) in the intima of the coronary arteries from the six cases with natural atherosclerosis and for the presence of T lymphocytes (CD45RO-positive cells) and macrophages (MAC-387 positive cells) in the intima of the one case with endothelialitis.

Discussion

Accelerated arteriosclerosis can occur as soon as 3 months after heart transplantation and it can affect recipients as young as 4 years of age.[8,9] Accelerated arteriosclerosis strikes approximately one third of all long-term survivors, it can be difficult to diagnose angiographically, and it can cause sudden unexpected death because cardiac allograft recipients do not experience typical angina pectoris due to graft denervation.[4] It has been hypothesized that accelerated arteriosclerosis is an immune-mediated complication of rejection.[14,17]

In this study we found a close relationship between accelerated arteriosclerosis and an endothelialitis. The inflammatory infiltrate in the endothelialitis was composed mostly of T lymphocytes and macrophages, and in the case in which fresh-frozen tissue was available for more detailed cell typing, most of the T lymphocytes marked as

cytotoxic/suppressor cells (CD8+). While these findings do not prove a causal relationship, they do provide evidence that an immune-mediated cytotoxic T-lymphocyte–directed endothelialitis plays a role in transplant-related accelerated arteriosclerosis.

It has long been hypothesized that endothelial cell injury is central to the development of accelerated arteriosclerosis in transplant recipients, and a growing body of evidence has accumulated showing that endothelial cells are injured in cardiac allograft recipients.[17,18] Endothelial denudation with superimposed thrombosis and associated endothelial mitoses, suggesting endothelial repair, have been described in heart allograft recipients.[19] These light microscopic observations have been confirmed by electron microscopic studies that have shown vesiculation and loss of endothelial cells in rabbit, canine, and human heart allografts.[13,20–22] Similarly immunohistochemical studies have demonstrated an induction of class II major histocompatibility antigens and the expression of 2G7 and 7A9, antigens associated with endothelial cell activation, in the endothelial cells of cardiac allograft recipients with rejection.[23–26] Injury to the endothelial cell may, in turn, promote the development of arteriosclerosis by a number of different mechanisms.

Endothelial cells normally provide a nonthrombogenic surface and permeability barrier to the blood stream and they produce a variety of growth factors and cytokines. An alteration in any of these functions could promote arteriosclerosis.

Injury to endothelial cells could promote arteriosclerosis through the adherence of platelets. In an experimental model of atherosclerosis, Stemerman et al[27] demonstrated that one of the first events following injury to endothelial cells is the binding and degranulation of platelets. Similarly electron microscopic observations in rabbit, rat, and dog models of cardiac allograft arteriosclerosis have demonstrated the adherence of platelets to injured endothelial cells.[13,20,22,28] These observations at the electron microscopic level parallel what is seen by light microscopy in human heart allograft recipients. Uys and Rose[2] identified vascular thrombosis in 10 of 14 cardiac allografts they examined, and Bieber et al[21] found it in five patients. Three of the patients we examined had significant coronary artery thrombosis. In addition to directly causing luminal reduction by thrombosis, platelet aggregation also may promote arteriosclerosis through the release of a variety of growth factors.[29] One of the growth factors released when platelets aggregate and degranulate is platelet-derived growth factor (PDGF). The release of PDGF can promote arteriosclerosis because PDGF acts as a mitogen for fibroblasts and smooth muscle cells.[30–33] Indeed Fellstrom et al[33] found increased staining for PDGF receptors in blood vessels of kidneys with transplant-related accelerated arteriosclerosis.

Endothelial cells also act as a permeability barrier to the blood stream, and the loss of this barrier and may promote arteriosclerosis.[22] Using a rabbit model of atherosclerosis, Hardin et al[34] demonstrated that lipids preferentially accumulate in areas of endothelial injury and that there is a synergy between immune-mediated endothelial injury and high serum-lipid levels in the development of atherosclerosis. This breakdown in the endothelial barrier to the accumulation of lipids may be particularly devastating in heart transplant recipients who are already prone to the development of hyperlipidemia.[16]

Our findings of a diffuse mural staining for IgM in a patient with accelerated arteriosclerosis also may reflect the breakdown of the endothelial cell barrier in these patients.[21,35] A diffuse staining of blood vessels for IgM not accompanied by the deposition of complement has been reported previously in heart transplant recipients and it is believed to represent nonspecific leakage of immunoglobulin.[35] Leakage, however, cannot account for the discrete deposition of immunoglobulin and complement described by Bieber et al[21] in 6 of the 12 transplanted hearts they examined. The identification of both specific and nonspecific patterns of immunoglobulin staining in the vessels of transplanted hearts suggests that humoral immunity may promote endothelial injury in some, but not all, transplanted hearts.

Injured endothelial cells may also directly produce and release growth factors and cytokines which promote the development of arteriosclerosis.[29] For example, human endothelial cells can express genes for interleukin-1 and PDGF.[17,31] Interleukin-1 can, in turn, promote the response of T lymphocytes to antigenic stimuli and, as was discussed earlier, PDGF can act as a powerful mitogen for fibroblasts and smooth muscle cells.[17,31,33] Injured endothelial cells also may nonspecifically release alloantigens that may promote vascular rejection. This hypothesis is supported by the finding of Cerilli et al[36] that renal allograft rejection is associated with the development of antivascular endothelial cell antibodies, and by a study of 118 human heart transplant recipients in which Reemtsma[37] demonstrated a correlation between the production of anti-HLA antibodies and the development of graft arteriosclerosis.

Although it has been suggested that humoral immunity, that is the development of cytotoxic antibodies, is the basis for the endothelial injury seen in heart transplant recipients, our findings of a T-lymphocyte–mediated endothelialitis associated with accelerated arteriosclerosis suggest that cellular immunity is also important in this process.[14] Our findings are not entirely surprising. Electron microscopic examination of the coronary arteries in human heart transplant recipients with accelerated arteriosclerosis has demonstrated an intimate association between lymphocytes in the subendothelial space and

injured endothelial cells.[3,13] Furthermore previous immunohistochemical studies have shown that most cells in the intima of these vessels are T lymphocytes, macrophages, and smooth muscle cells.[19,38,39]

Once present in the intima, T lymphocytes and macrophages, in addition to directly injuring the endothelium, may themselves promote arteriosclerosis.[29] Activated T lymphocytes can secrete a variety of bioactive substances, including tumor necrosis factor (TNF), which can promote smooth muscle cell growth.[17,29,40,41] Similarly macrophages can release PDGF, transforming growth factor beta, and tumor necrosis factor, factors that can act to promote arteriosclerosis.[29,31,42,43]

While an association does not prove a causal relationship, seven independent lines of evidence suggest that both the lymphocytic endothelialitis and the arteriosclerosis seen in cardiac allograft recipients are due to rejection: 1) lymphocytic endothelialitis and arteriosclerosis are not present in autografts or in hearts transplanted between genetically identical animals[20,28]; 2) coronary artery vasculitis and arteriosclerosis develop in hearts transplanted across histocompatibility barriers[28,44]; 3) lymphocytic endothelialitis and arteriosclerosis, as demonstrated by our case H-21, are usually limited to the graft; 4) graft arteriosclerosis can develop in the absence of cyclosporine therapy[28]; 5) coronary artery vasculitis and arteriosclerosis are enhanced by sensitization of the recipient to donor lymphocytes[44]; 6) the development of accelerated arteriosclerosis often correlates with the number of rejection episodes[6,45]; and 7) transplant related coronary artery disease can be reversed by increased immunosuppression.[46,47]

It is unlikely that the lymphocytic endothelialitis seen in cardiac allograft recipients is due to graft ischemia or to the transplant procedure itself. First we found no correlation between graft ischemia time and the development of lymphocytic endothelialitis. Second lymphocytic endothelialitis was not present in several of the patients who survived longer than 1 year (H-8, H-11, and H-18). Third, among the patients without accelerated arteriosclerosis, lymphocytic endothelialitis was present only in those who had acute rejection (H-5, H-15, and H-105). These findings suggest that lymphocytic endothelialitis is a result of graft rejection, and not a long-term manifestation of the transplant procedure itself. Animal studies also support this hypothesis because no pathologic changes are seen in the vessels of cardiac autografts.[20,28]

We believe that accelerated arteriosclerosis is mediated by a cytotoxic T lymphocyte directed endothelialitis and that this endothelialitis is a manifestation of rejection. Other factors, such as hyperlipidemia, cyclosporine therapy, cytomegalovirus infection, fluid hemodynamics, and lymphostasis, factors not rigorously addressed in this study, also may contribute to the development of accelerated arteriosclerosis.[15,16,48–53]

Finally, although natural atherosclerosis and accelerated transplant-related arteriosclerosis differ greatly in their speed of onset and angiographic appearances,[4–6] there are several parallels between these two processes. The intimal lesions in both diseases can contain smooth muscle cells, macrophages, and T lymphocytes,[19,38–40,54,55] both processes have been related to immune injury,[34,44] and both have been associated with cytomegalovirus infection.[51,53,56] It is interesting to speculate that low-grade endothelialitis plays a role in the development of natural atherosclerosis.[29]

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