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How Cyclosporine Modifies Histological and Molecular Events in the Vascular Wall during Chronic Rejection of Rat Cardiac Allografts

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Accelerated allograft arteriosclerosis (chronic rejection) has emerged as a major factor affecting long-term survival of human cardiac allografts. The underlying mechanism of this disorder remains unclear. The purpose of this study was to investigate the effect of cyclosporine on the development of cardiac allograft arteriosclerosis at the cellular and molecular level. Heterotopic rat cardiac allografts from DA donors to WF recipients, with a strong genetic disparity in major histocompatibility complex and non-major histocompatibility complex loci, were used. The allograft recipients received triple-drug immunosuppression consisting of methylprednisolone (0.5 mg/kg/day), azathioprine (2 mg/kg/day), and three different doses of cyclosporine A (CsA; 5, 10, and 20 mg/kg/day). The grafts were removed 3 months after transplantation and processed for histology and immunohistochemistry. Low dose CsA (5 mg/kg/day) was associated with a severe form of intimal cell accumulation and intimal thickening in epicardial arteries and in smaller intramyocardial arterioles with nearly occluded vessel lumens 3 months after transplantation. The intermediate dose CsA (10 mg/kg/day) significantly inhibited arterial intimal thickening but was not efficient in reducing intimal cell accumulation. Instead, high dose CsA (20 mg/kg/day) significantly inhibited all arteriosclerotic vascular wall changes in the allografts. Immunohistochemistry revealed that the occluded epicardial arteries of cardiac allografts with low dose CsA expressed VCAM-1 on the endothelium. Higher CsA doses significantly reduced the expression of endothelial VCAM-1. Neither ICAM-1 nor major histocompatibility complex class II were expressed. Perivascular arterial infiltrates consisting of T helper cells and monocytes/macrophages were a characteristic finding in the allograft with low dose CsA. In the allografts treated with higher doses of CsA, arterial perivascular infiltrates were seldom seen. Our results conclusively demonstrate that sufficient immunosuppression with CsA inhibits intimal thickening and intimal cell accumulation of long-surviving rat cardiac allografts in a dose-dependent fashion. Arteriosclerotic alterations associated with increased expression of arterial endothelial VCAM-1 were totally down-regulated by high doses of CsA. (Am J Pathol 1995, 146:972–980)

Accelerated allograft arteriosclerosis, chronic rejection, has emerged as a major factor affecting long-term survival of human cardiac allografts.[1–3] Microscopically, in autopsy material, the basic underlying lesion is diffuse, concentric intimal thickening[2] consisting mainly of proliferating smooth muscle cells and macrophages in the intimal space. Also, low level intramural and perivascular inflammation affects most of the intramyocardial arteries.[4]

Triple-drug immunosuppression consisting of cyclosporine A (CsA), azathioprine (AZA), and methylprednisolone (MP) is widely used in heart transplant patients. The frequency of cardiac allograft arteriosclerosis, however, is no different in CsA-treated recipients compared with the AZA-treated patients.[5] In fact, myocardial fibrosis and accelerated allograft arteriosclerosis are sometimes associated with the use of CsA.[6] It has been suggested that such changes

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may in part be due to the effects of CsA or its vehicle on plasma lipoproteins[7] or systemic blood pressure.[8] A recent prospective, randomized study of renal allograft recipients showed that triple-drug immunosuppression including CsA was better than any of the two-drug combinations (AZA plus MP, CsA plus MP, or CsA plus AZA) in preventing deterioration of renal graft function.[9] Another study applying post-transplant blood samples of kidney allograft recipients revealed that low CsA trough levels correlated with poor outcome of the transplants.[10]

In this communication we describe an inhibitory effect of CsA on the development of cardiac allograft arteriosclerosis, by applying heterotopic rat cardiac allografts under triple-drug immunosuppression with different doses of CsA. We also demonstrate a regulatory role of CsA on the expression of vascular cell adhesion molecule-1 (VCAM-1) suggesting that VCAM-1 may be linked with graft arteriosclerosis.

Materials and Methods

Experimental Design

Rat cardiac allografts from DA donors to WF recipients with a strong genetic disparity in major histocompatibility complex MHC and non-MHC loci, were used. The allograft recipients ($n = 6$) received triple-drug immunosuppression including AZA (2 mg/kg/day), MP (0.5 mg/kg/day), and CsA (5, 10, or 20 mg/kg/day) throughout the study. The grafts were removed 3 months after transplantation and processed for histology and immunohistochemistry. Our group has previously demonstrated that syngeneic DA-DA kidney grafts under CsA do not develop any vascular wall changes[11] and, thus, syngeneic controls were not performed.

Experimental Animals

Inbred DA (AG-B4, $RT1^a$) and WF (AG-B2, $RT1^u$) rat strains were used as donors and recipients, respectively. The animals were purchased from the Laboratory Animal Center, University of Helsinki. They were 2 to 3 months of age and weighed 200 to 300 g. The rats were fed with regular rat food (Altromin, standard diet, Chr. Petersen A/S, Ringsted, Denmark), and tap water *ad libitum*. All animals were maintained on a 12-hour light/dark cycle. The animals received humane care in compliance with the Principles of Laboratory Animal Care and the Guide for the Care and Use of Laboratory Animals prepared and formulated by the Institute of Laboratory Animal Resources and published by the National Institutes of Health (NIH Publication No. 86-23, revised 1985).

Immunosuppressive Regimens

The animals received triple-drug immunosuppression by mouth for the entire observation time. Perioperatively, the rats received also CsA (Sandimmun; Sandoz Pharma AG, Basel, Switzerland), 15 mg/kg subcutaneously in the neck as a single dose. For the injection, 50 mg/ml CsA infusion substance was dissolved in 200 mg/ml Intralipid (KabiVitrum, Stockholm, Sweden) to a final concentration of 3 mg/ml. Thereafter, CsA (Sandimmun mixture, 100 mg/ml; Sandoz) at a dose of either 5, 10, or 20 mg/kg/day was given by mouth with regular rat food. MP, 0.5 mg/kg/day (40 mg/ml Solu-Medrol; Upjohn, Puurs, Belgium), and AZA, 2 mg/kg/day (Imuran; Wellcome, London, UK), were administered in drinking water. Radioimmunoassay (Sandimmun-Kit, Sandoz) was applied to determine whole blood CsA levels. Blood was drawn from the tail tip of the rat once a week for the first month and then once every month for 3 months.

Heterotopic Cardiac Allografts

Intra-abdominal heterotopic cardiac allografts were transplanted by using a modified technique of Ono and Lindsey.[12] In short, the donor rats were anesthetized by ether. After perfusion of 200 IU of heparin in 1 ml of ice-cold phosphate-buffered saline into the inferior vena cava, it was ligated with 6–0 silk. The superior vena cavae and pulmonary veins were ligated *en bloc* with 6–0 silk, and the pulmonary artery and aorta were transected 2 to 3 mm above their origin in the heart. Recipient animals were anesthetized with chloral hydrate (240 mg/kg intraperitoneal) and were given 0.25 mg/kg buprenorphine subcutaneously (Temgesic; Reckitt & Colman, Hull, UK) for postoperative pain relief. A midline incision was made, the great abdominal vessels were dissected free from the surroundings, and the graft was implanted in the abdominal cavity. The aorta and pulmonary artery were anastomosed with abdominal aorta and inferior vena cava in a running end-to-side fashion with 9–0 nylon suture. Total ischemic time varied from 45 to 60 minutes, during which time the graft was kept in an ice bath of 4 C phosphate-buffered saline for 15 minutes. Hearts were cooled throughout the procedure with frequent changes of saline-cooled gauzes. The grafts were evaluated for function by abdominal palpation. At removal, the grafts were immediately washed with

phosphate-buffered saline and sectioned into four to five cross sections and processed for histology and immunohistochemistry.

Histology

At least two midsections of the allografts were fixed in 10% phosphate-buffered formalin for 24 hours, routinely processed, and embedded in paraffin. Four-micron-thick cross sections of cardiac allografts were stained with Mayer's hematoxylin and eosin, Masson's trichrome, Weigert van Gieson (for elastin), and for pyroninophilic cells with Unna Pappenheim. Rejections were diagnosed and graded according to the Working Formulation of the International Society for Heart and Lung Transplantation.[13] Slides were examined by light microscopy by two observers in blind review, and the score assigned was determined by consensus of the observers. The analysis was done semiquantitatively by scoring the histological parameters from 0 to 3 (0, normal; 1, mild; 2, moderate; and 3, severe changes).

Definition of Cardiac Allograft Vessels

Vessels with a well defined smooth muscle cell layer in the vascular wall were identified as arteries. Epicardial arteries had a large luminal diameter and thick vascular wall. Intramyocardial arterioles had a small luminal diameter and thin vascular wall. Capillaries and veins were excluded from the study, and only epicardial arteries and intramyocardial arterioles were evaluated for the histological parameters given below.

For intimal cell accumulation in the luminal part of the intima,[14] score 1 was given when intimal cells were closely packed in one layer and scores 2 and 3 were given when intimal cells were seen in two or three closely packed cell layers, respectively. The changes in intimal thickness were scored mild (score 1, <25% occlusion of the lumen) when the intima was readily discernible and moderate (score 2, 25 to 50% occlusion) to severe (score 3, >50% occlusion) when the lumen was encroached upon.

Immunohistochemistry

One of the midsections of the allografts was embedded in OCT (Tissue-Tek, Miles, Elkhart, IN), snap-frozen, and stored at −70 C. Frozen sections were air dried onto poly-D-lysine-coated slides, fixed in acetone for 20 minutes at −20 C, and stored at −20 C until used. Before immunostaining, the slides were refixed with chloroform and then air dried. Four-micron-thick cross sections were incubated with the following monoclonal antibodies by using a three-layer indirect immunoperoxidase technique: 1A29 (Seikagaku Co., Tokyo, Japan), a mouse immunoglobulin (Ig)G1 monoclonal antibody to rat intercellular adhesion molecule-1 (ICAM-1, CD54); 5F10 (a generous gift from Dr. Roy Lobb, Biogen, Cambridge, MA), a mouse IgG2a monoclonal antibody to rat VCAM-1; WT.1 (Seikagaku), a mouse IgG2a monoclonal antibody to rat leukocyte function-associated antigen-1 (LFA-1)α-chain (CD11a); HP2.1 (Immunotech SA, Marseilles, France), a mouse IgG1 monoclonal antibody to a human very late antigen (VLA-4, CD49d/29) that cross-reacts with a rat VLA-4[15]; W3/25 (Sera Lab, Sussex, UK), a mouse IgG1 monoclonal antibody to rat T helper cells (CD4 equivalent); OX8 (Sera Lab), a mouse IgG1 monoclonal antibody to rat T cells (nonhelper subset; Lyt2/Lyt3, CD8 equivalent); OX42 (Sera Lab), a mouse IgG2a monoclonal antibody to rat macrophages (160-, 103-, 96-kd polypeptide); OX 6 (Sera Lab), a mouse IgG1 monoclonal antibody to rat major histocompatibility complex (MHC) class II common determinant; interleukin 2 receptor (IL-2R; CD25), a mouse monoclonal antibody to rat IL-2R (a generous gift from Dr. J. Kupiec-Weglinski, Harvard Medical School, Boston, MA); and α-smooth muscle actin (Biomakor, Rehovat, Israel), a mouse monoclonal antibody to rat smooth muscle cells.

The primary antibodies were used at a dilution of 1:100 in Tris/1% bovine serum albumin. After a 30-minute incubation at room temperature, the sections were washed in Tris buffer and incubated for 30 minutes with a peroxidase-conjugated rabbit anti-mouse Ig (Dako Immunoglobulins A/S, Copenhagen, Denmark). After washing in Tris, the sections were incubated with goat anti-rabbit Ig (Caltag Laboratories, San Francisco, CA). The reaction was revealed by the chromogen 3-amino-9-ethylcarbazole containing hydrogen peroxidase. The specimens were counterstained with hematoxylin and coverslips were aqua-mounted (Aquamount; BDH Ltd., Poole, England).

Quantitation of Immunostaining

The scoring of expression of adhesion molecules (ICAM-1, VCAM-1, and MHC class II) was focused on endothelial cells of epicardial arteries and intramyocardial arterioles. Expression of ligands for adhesion molecules (LFA-1, Mac-1, and VLA-4) and that of inflammatory cell subsets (CD4, CD8, and monocytes/macrophages) and activation markers (MHC class II

and IL-2R) was assessed from inflammatory cells. The blinded analysis was done semiquantitatively by scoring the intensity of the staining from 0 to 3 as follows: 0, no visible staining; 1, few cells with faint staining; 2, moderate intensity with multifocal staining; and 3, intense diffuse staining of the cells analyzed.

Statistics

All data are expressed as mean ± SEM. The Kruskal-Wallis test (Z corrected for ties) was used to evaluate the significances. P values < 0.05 were regarded as statistically significant.

Results

Myocardial Rejection in Allografts with Different Doses of CsA under Triple-Drug Immunosuppression

In the group of recipients with 5 mg/kg CsA, three allografts were lost 25 ± 3 (mean ± SE) days after transplantation due to irreversible rejection. All of the surviving allografts showed grade 3 rejection (Table 1) with diffuse intramyocardial inflammatory cell infiltrates consisting of T helper cells (CD4), monocytes/macrophages (OX42), and to a lesser extent of T cytotoxic cells (CD8) (not shown). Many of the inflammatory cells also expressed MHC class II and IL-2R (not shown). In recipients with 10 mg/kg CsA, one grade 1A and one grade 1B rejection was seen (Table 1). In the group of recipients with 20 mg/kg CsA, the grafts were free from rejection 3 months after transplantation (Table 1).

Histological Obliterative Findings of Cardiac Allograft Arteries Treated with Different Doses of CsA under Triple-Drug Immunosuppression

In the group of recipients with 5 mg/kg CsA, the epicardial arteries and intramyocardial arterioles were nearly occluded by thickened intimas 3 months after transplantation (Figures 1 and 2). In recipients with 10 mg/kg CsA, arterial intimas were mildly occluded but significantly less than in the group with 5 mg/kg CsA ($P < 0.01$; Figures 1 and 2). In the group of recipients with 20 mg/kg CsA, arterial intimal thickening was almost nonexistent ($P < 0.01$) (Figures 1 and 2).

Arterial intimal cell accumulation in the luminal part of the intima was scored from mild to moderate in the group with 5 mg/kg CsA (Figure 1). In the group with 10 mg/kg CsA, even somewhat higher intimal cell accumulation scores were observed, but this was not statistically significant (Figure 1). However, in the group with 20 mg/kg CsA, significantly less intimal cell accumulation was scored in epicardial arteries ($P < 0.01$) and almost significantly less in the intramyocardial arterioles ($P < 0.06$; Figure 1).

A clear inverse correlation between mean blood CsA levels and mean intimal thickness, but not between intimal cell accumulation in epicardial arteries and intramyocardial arterioles, could be demonstrated, as shown in Figure 3.

Effect of CsA on the Perivascular Inflammatory Cell Response

Perivascular infiltrates of moderate intensity were recorded around epicardial arteries. They consisted of mainly T helper cells (CD4) and monocytes/macrophages (OX42) in the group with 5 mg/kg CsA (Table 2). Only few T cytotoxic cells (CD8) were seen. In the group with 10 to 20 mg/kg CsA, periarterial inflammation was seldom seen (Table 2). Thus, higher doses of CsA significantly reduced the number of periarterial CD4- and OX42-positive cells ($P < 0.05$; Table 2). Only in the group with 5 mg/kg CsA, few class II-positive and IL-2R-positive inflammatory cells were observed periarterially (Table 2). In the perivascular space of intramyocardial arterioles, the same distribution of inflammatory cells and activation markers was observed, but statistical difference between the groups was not reached (Table 2).

Table 1. *Myocardial Rejection in Allografts with Different Doses of CsA 3 Months after Transplantation under Triple-Drug Immunosuppression*

CsA (mg/kg)	Number of transplants	Irreversible rejection	Grade				
			0	1A	1B	3A	3B
5	6	3*	0	0	0	1	2
10	6	0	4	1	1	0	0
20	6	0	6	0	0	0	0

*The grafts were lost 25 ± 3 (mean ± SE) days after transplantation due to irreversible rejection and excluded from the study.

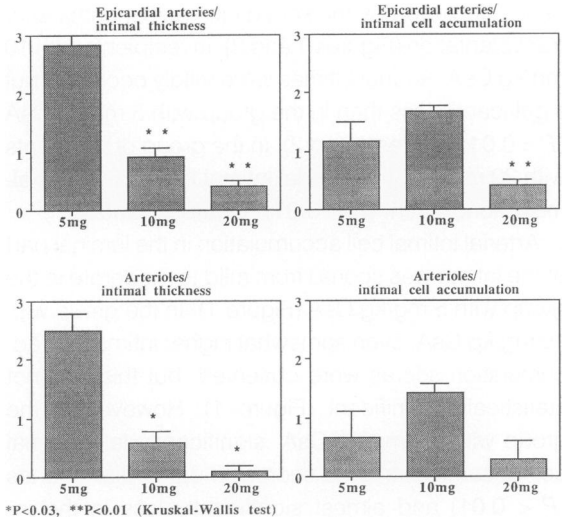

Figure 1. Left panels: *intimal thickness in epicardial arteries and in intramyocardial arterioles in cardiac allografts under triple-drug immunosuppression treated with three different doses of CsA.* Right panels: *intimal cell accumulation in epicardial arteries and in intramyocardial arterioles in the same constellation 3 months after transplantation. Semiquantitative scoring from 0 to 3.*

Effect of CsA on Adhesion Molecule and Counter-Ligand Expression

ICAM-1 was usually not expressed on arterial endothelium of allografts under triple-drug immunosuppression (Table 3). On the other hand, the counter-ligand LFA-1 was highly expressed in recipients with 5 mg/kg CsA compared with the recipients with 10 to 20 mg/kg CsA (Table 3). In other words, increasing doses of CsA significantly reduced the number of LFA-1-positive inflammatory cells around epicardial arteries ($P < 0.02$) and intramyocardial arterioles ($P < 0.05$).

A striking difference in VCAM-1 expression on the endothelia of epicardial arteries was seen between the groups with different CsA doses (Table 3). In the occluded epicardial arteries of the group with 5 mg/kg CsA, VCAM-1 was moderately to strongly expressed on the epicardial endothelium (Figure 4). In the groups with 10 to 20 mg/kg CsA, hardly any VCAM-1 expression was seen ($P < 0.03$; Table 3 and Figure 4). The occluded intimas of the group with 5 mg/kg CsA consisted of α-actin-positive smooth muscle cells (Figure 5). The counter-ligand VLA-4 expression to VCAM-1 was not detected in allografts (Table 3). In the recipients with 5 mg/kg CsA, the endothelia of epicardial arteries and intramyocardial arterioles expressed MHC class II with a mild intensity. No endothelial MHC class II expression was seen in the groups with higher doses of CsA (Table 3).

Discussion

In this communication we used a heterotopic rat cardiac transplant model (DA to WF) under triple-drug

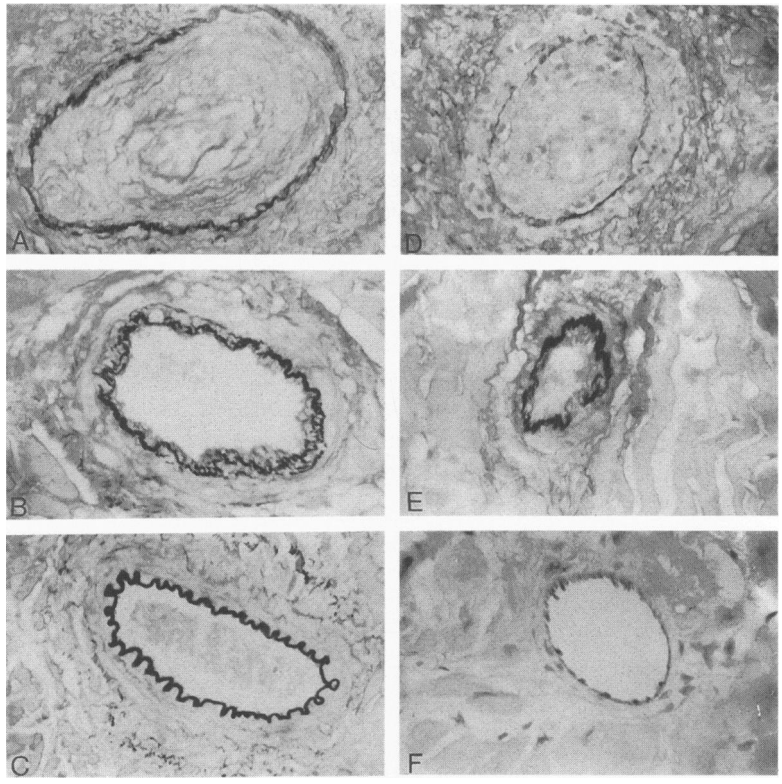

Figure 2. A *to* C: *Photomicrographs of epicardial arteries.* D *to* F: *Photomicrographs of intramyocardial arterioles.* A *and* D: *Severe intimal thickening (score 3) compromising the vascular lumen in cardiac allograft under 5 mg/kg CsA.* B *and* E: *Slight intimal thickening and intimal cell accumulation in cardiac allograft under 10 mg/kg CsA.* C *and* F: *Intact arteries of cardiac allograft under 20 mg/kg CsA 3 months after transplantation (Wiegert van Gieson; original magnification, ×40).*

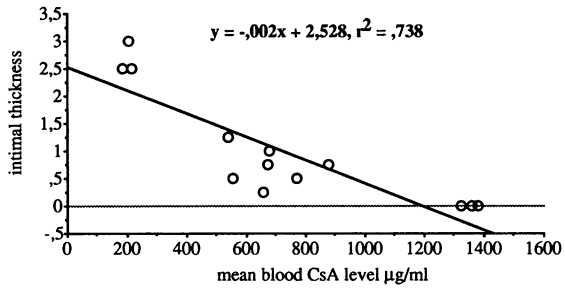

Figure 3. *The inverse correlation between mean CsA blood level and mean intimal thickness of epicardial arteries and intramyocardial arterioles. Semiquantitative scoring from 0 to 3.*

immunosuppression, similar to most regimens for humans, and investigated the association between CsA and transplant arteriosclerosis by using three different doses of CsA. Our data demonstrate that low level CsA (5 mg/kg) was associated with severe intimal cell accumulation and intimal thickening in epicardial arteries and intramyocardial arterioles. This process of allograft arteriosclerosis (chronic rejection) nearly occluded the lumens of the vessels 3 months after transplantation. The dose of 10 mg/kg CsA significantly inhibited intimal thickening in arteries but did not reduce intimal cell accumulation. The dose of 20 mg/kg CsA significantly inhibited vascular wall changes in arteries but less in arterioles.

Immunohistochemistry revealed that the endothelium of occluded epicardial arteries in cardiac allografts with low dose CsA strongly expressed VCAM-1. Higher doses of CsA significantly decreased the expression of endothelial VCAM-1. ICAM-1 was not expressed in arterial endothelium under any dose regimen. Endothelial MHC class II expression was seen only in allografts with low dose CsA. The activation, ie, expression of MHC class II and IL-2R on peripheral inflammatory cells, was effectively suppressed by CsA in all dose regimens. Perivascular arterial infiltrates consisting of T helper cells (CD4) and monocytes/macrophages (OX42) were a characteristic finding in allografts with low dose CsA. In allografts treated with higher doses of CsA, arterial perivascular infiltrates were sparce. Thus, high dose CsA was associated with significantly decreased endothelial expression of adhesion molecules, particularly VCAM-1, and with significantly reduced perivascular inflammation in arterial sites of the allografts. Recently, VCAM-1 has warranted particular interest in the context of atherosclerosis, because of its early and focal expression on endothelial cells where monocytes first accumulate at the sites of fatty streak formation in a rabbit model.[16,17] The induction of VCAM-1 during rejection has also been reported in heart allograft patients.[18,19]

Previous *in vitro* studies by Ross et al[20] have revealed that CsA causes a dose-dependent inhibition of endothelial cell growth in rabbit aorta. In addition, smooth muscle cells were particularly susceptible to the cytostatic effect of CsA *in vitro* and were also inhibited from proliferation in a dose-dependent fashion. However, the same authors failed to show similar effects *in vivo*.[20] On the contrary, Jonasson et al[21] demonstrated, in rats with balloon catheter-injured carotid arteries, the antiproliferative effect of CsA on smooth muscle cell replication *in vivo*. Thyberg and Hansson[22] suggested that CsA, at least in part, is directly inhibitory to smooth muscle cell proliferation *in vivo*, especially as IFN-γ, a product of activated T lymphocytes that are suppressed by CsA, is also antiproliferative to smooth muscle cells. Our results support the finding that CsA has an inhibitory effect on the development of arteriosclerotic vascular wall changes. In our rat cardiac allografts, CsA clearly inhibited *in vivo* intimal thickening in a dose-dependent fashion.

It should be stressed that our model deals with transplantation-associated arteriosclerosis, most evidently induced by the inflammatory response against the allograft. We hypothesized that CsA downregulates this inflammation and thereby inhibits allograft arteriosclerosis. Thus the mechanism may be different from the previous *in vitro* and *in vivo* studies

Table 2. *Effect of CsA on the Structure of Inflammation in the Perivasculature of Epicardial Arteries and Intramyocardial Arterioles*

Vessel	CsA (mg/kg)	Inflammatory cells				
		CD4	CD8	OX42	MHC class II	IL-2R
Epicardial arteries	5	1.7 ± 0.6*	0.5 ± 0.3	1.8 ± 0.2*	0.6 ± 0.3	0.6 ± 0.3
	10	0.5 ± 0.2	0 ± 0	0.5 ± 0.5	0 ± 0	0 ± 0
	20	0.1 ± 0.1	0 ± 0	0.3 ± 0.3	0 ± 0	0 ± 0
Intramyocardial arterioles	5	1.7 ± 0.3	0.5 ± 0.2	1.5 ± 0.5	0.6 ± 0.3	0.6 ± 0.3
	10	0.9 ± 0.2	0 ± 0	0.7 ± 0.2	0 ± 0	0 ± 0
	20	0.8 ± 0.1	0 ± 0	0.8 ± 0.3	0 ± 0	0 ± 0

*$P < 0.05$ (Kruskal-Wallis test).

Table 3. *Effect of CsA on Adhesion Molecule and Counter-Ligand Expression in Epicardial Arteries and Intramyocardial Arterioles*

		Endothelial cells			Inflammatory cells	
Vessel	CsA (mg/kg)	MHC class II	ICAM-1	VCAM-1	LFA-1	VLA-4
Epicardial arteries	5	0.8 ± 0.6	0 ± 0	1.8 ± 0.2*	1.3 ± 0.7†	0 ± 0
	10	0 ± 0	0.2 ± 0.2	0.3 ± 0.2	0.1 ± 0.1	0 ± 0
	20	0 ± 0	0 ± 0	0.2 ± 0.2	0.1 ± 0.1	0 ± 0
Intra myocardial arterioles	5	0.5 ± 0.5	0 ± 0	1 ± 0.3	1.3 ± 0.7†	0 ± 0
	10	0 ± 0	0.2 ± 0.2	0.7 ± 0.2	0.1 ± 0.1	0 ± 0
	20	0 ± 0	0 ± 0	0.3 ± 0.2	0.1 ± 0.1	0 ± 0

*$P < 0.03$, †$P < 0.02$ (Kruskal-Wallis test)

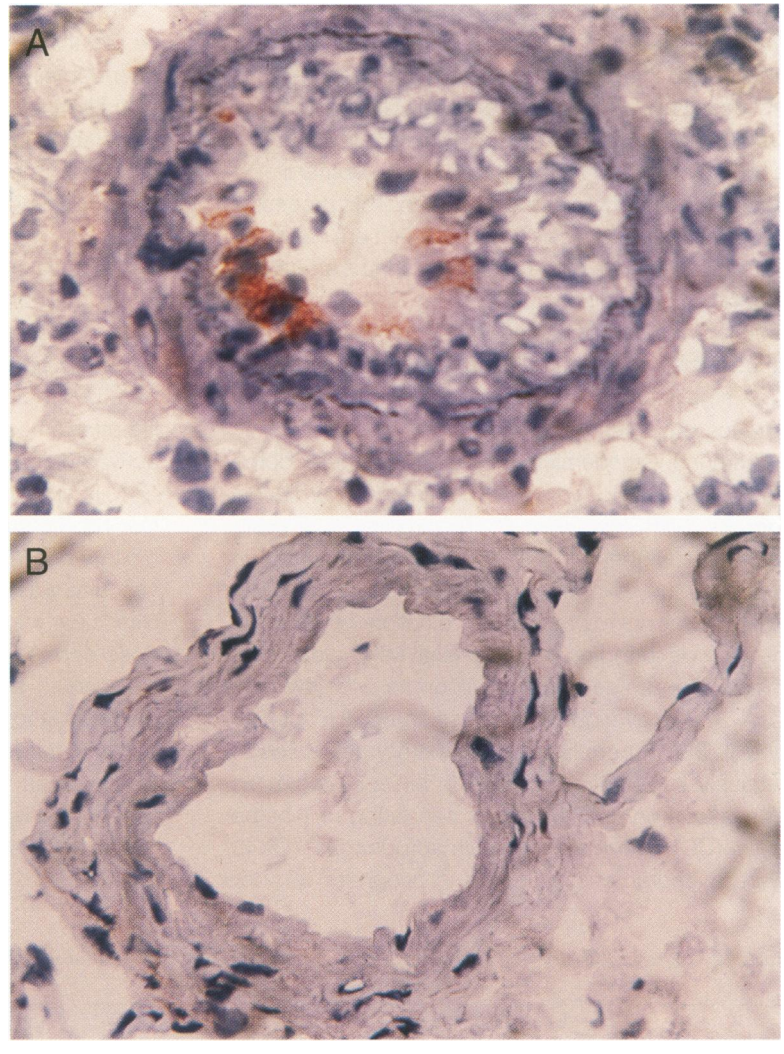

Figure 4. A: *Photomicrographs showing VCAM-1 expression 3 months after transplantation on endothelium of epicardial arteries in cardiac allograft under 5 mg/kg CsA. Note the increased intimal thickness compromising the vascular lumen.* **B:** *VCAM-1 expression in cardiac allograft under 20 mg/kg CsA. Note that endothelial cells stain negative for VCAM-1 and that no accelerated arteriosclerotic changes are seen (immunoperoxidase staining with monoclonal antibody to VCAM-1; original magnification, ×40).*

of Ross et al[20] and Jonasson et al,[21] who evaluated the effect of CsA in a restenosis model in which the atherosclerotic lesion is induced by mechanical injury. This difference in the underlying mechanisms may, at least in part, explain the different effects of CsA in these models.

Mennander et al,[23] using rat aortic allografts immunosuppressed with CsA alone, demonstrated that CsA was associated with an accelerated type of transplant arteriosclerosis particularly early after transplantation. It was not linked to a high level of periarterial inflammation but instead effectively sup-

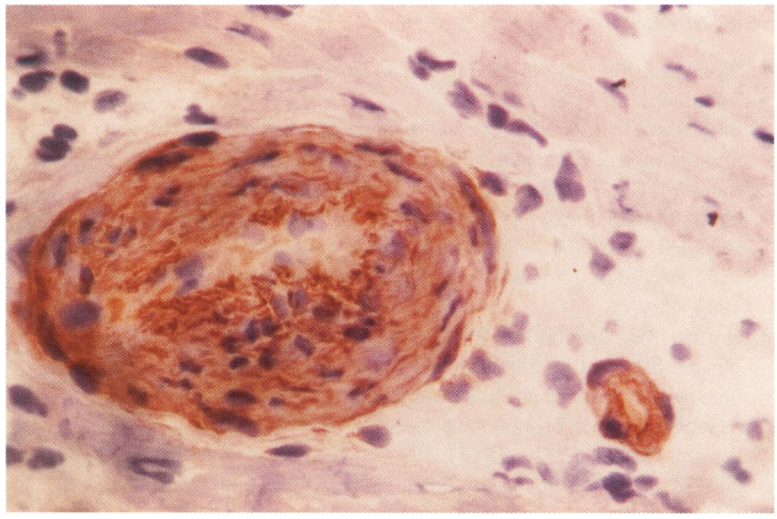

Figure 5. *Photomicrograph demonstrating α-actin-positive smooth muscle cells in the thickened intimas of occluded epicardial arteries of cardiac allografts under 5 mg/kg CsA (immunoperoxidase staining with monoclonal antibody to α-smooth muscle actin; original magnification, ×40).*

pressed the perivascular inflammatory response compared with AZA. However, a subendothelial inflammatory cell response (endothelialitis) and early influx of proliferating smooth muscle cells to the intimal space was seen when 5 mg/kg/day CsA was administered as monotheraphy. When triple-drug immunosuppression was used, both endothelialitis and accelerated arteriosclerosis were suppressed.[23] It was suggested that CsA would directly affect the vascular wall, either endothelium or smooth muscle cells or both. Steroids would oppose the proliferative effect of CsA. Our current findings demonstrate that CsA given with AZA and steroids does not accelerate allograft arteriosclerosis but rather inhibits it in a dose-dependent fashion, though in doses higher than in Mennander's study.[23]

Guttmann et al[24] reported that CsA at a dose of 15 mg/kg started at the day of transplantation completely prevented vascular lesions of rat cardiac allografts at 2 months after transplantation. When initiation of CsA was postponed to 40 days, when vascular changes were already present, there were not significant vascular lesions. The authors suggested that CsA interferes with the ongoing persisting immune response and allows host mechanisms to reverse vascular damage until irreversible occlusive disease has been established.

Herskowitz and his colleagues[25] reported in rat heart allografts infected with rat cytomegalovirus that short-term therapeutic doses of 10 mg/kg CsA, started after allograft arteriosclerosis was already established, significantly decreased the development of graft arteriosclerosis. The early features of graft vascular disease, leukocyte adhesion to the intimal endothelium and focal intimal hyperplasia, were essentially completely reversed by high dose, short-term CsA therapy.

In a clinical setting, Almond et al[26] investigated risk factors for biopsy-proven chronic rejection in kidney transplant patients. Their data revealed that a major risk factor for chronic rejection was a CsA dose below 5 mg/kg/day at 1 year postoperatively. Another study by Pfaff et al[27] showed that kidney transplant patients on triple therapy who had CsA levels below 400 ng/ml had a significantly increased incidence of rejection, compared with a CsA level over 400 ng/ml. In heart transplant patients, however, the frequency of cardiac allograft arteriosclerosis, chronic rejection, was no different in CsA-treated recipients compared with the AZA-treated patients when evaluating coronary angiography data.[5]

In conclusion, our results indicate that CsA decreases the development of heart allograft arteriosclerosis in a dose-dependent manner, as judged by diminished arterial intimal thickness and cell accumulation. The possible mechanism behind this inhibition is that CsA reduced the intensity and cellular components of the perivascular inflammatory response of the allograft arteries. Thus, less cytokines up-regulating adhesion molecules, ie, VCAM-1 and growth factors triggering smooth muscle cell migration and proliferation, were produced in these rat cardiac allografts.

Acknowledgments

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