Effect of Cyclosporine on Arterial Balloon Injury Lesions in Cholesterol-Clamped Rabbits

T Lymphocyte–Mediated Immune Responses Not Involved in Balloon Injury–Induced Neointimal Proliferation

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Abstract—Restenosis after balloon dilatation of stenosed coronary arteries is a major clinical problem. Because T lymphocytes occur in neointima and because cyclosporine inhibits T-lymphocyte proliferation, we tested the hypothesis that cyclosporine would attenuate neointimal proliferation after balloon dilatation injury. Rabbits with a balloon-injured aorta, randomized to cyclosporine in the human therapeutic range (n=13) or vehicle (n=14) were followed up for 5 weeks; as a control for the effect of cyclosporine, half the rabbits received in addition an aorta allograft. Rabbits were clamped at a human plasma cholesterol level of 5 to 7 mmol/L. Cyclosporine did not affect aorta cholesterol accumulation or neointimal proliferation in balloon-injured aortas; however, it attenuated both in transplanted aortas. Likewise, cyclosporine had no effect on endothelial cells at balloon-injured sites, but protected these cells in the transplanted aortas. Infiltration of smooth muscle cells, T lymphocytes, and macrophages were unaffected by cyclosporine in balloon-injured aortas; however, in transplanted aortas, cyclosporine reduced the relative number of T lymphocytes and macrophages but increased the relative number of smooth muscle cells. Finally, in balloon-injured aortas, cyclosporine did not affect expression of vascular adhesion molecule-1, intercellular adhesion molecule-1, or major histocompatibility complex II, but all these cellular activation markers were attenuated by cyclosporine in transplanted aortas. These results suggest that cyclosporine does not attenuate neointimal proliferation after balloon dilatation, and that T lymphocyte—mediated immune responses are not involved in neointimal proliferation after balloon dilatation.

Key Words: balloon injury ■ restenosis ■ cyclosporine ■ endothelial activation ■ chronic rejection

Balloon dilatation of stenosed arteries, ie, percutaneous transluminal coronary angioplasty is used more and more in the treatment of ischemic heart disease. Although implantation of stents at the site of percutaneous transluminal coronary angioplasty reduces the restenosis rate1 long-term complications from this treatment are yet unresolved and restenosis still occurs. It would be of great clinical importance if a harmless medical treatment given around the time of percutaneous transluminal coronary angioplasty could attenuate or prevent restenosis.

One candidate for such a beneficial effect is cyclosporine, an immunosuppressive agent that inhibits T-lymphocyte proliferation.2 In experimental studies, cyclosporine has been shown to inhibit early atherosclerosis in the cholesterol-fed rabbit3 as well as the development of transplant arteriosclerosis after immune injury.4–6 As the histopathological events occurring after a balloon dilatation injury have many common features with that seen after cholesterol feeding and immune injury,7–13 including the presence of T lymphocytes,10,11 it could be hypothesized that cyclosporine would also inhibit neointimal proliferation after a balloon dilatation injury. Previous studies of this hypothesis gave equivocal results14–16; however, these studies were all performed in animal models with plasma cholesterol at ≈0.5 to 1.5 mmol/L, levels below that seen in most humans, and in only 1 was a clinically relevant dose of cyclosporine used.15

We tested the hypothesis that a clinically relevant dose of cyclosporine would attenuate aortic neointimal proliferation after a balloon dilatation injury in rabbits with plasma cholesterol levels clamped at a human level of 5 to 7 mmol/L; as a positive control of the beneficial effect of cyclosporine on neointimal proliferation,4,5 half the rabbits also received an aorta allograft4 in addition to the balloon injury. To test this hypothesis, we measured aortic cholesterol accumulation, neointimal proliferation, integrity of the endothelial cell layer, and intimal infiltration with smooth muscle cells, T
lymphocytes, macrophages, and mast cells, as well as upregulation of the cellular activation markers vascular adhesion molecule-1 (VCAM-1), intercellular adhesion molecule-1 (ICAM-1), and major histocompatibility complex II (MHCII).

**Methods**

**Animals and Surgical Procedures**

Outbred male White rabbits of the Danish Country Strain from Statens Serum Institut, Copenhagen, Denmark, were used. Donors and recipients of aortic allografts belonged to different litters from this strain. All experimental procedures were performed in accordance with Danish regulations for experiments on animals. Animals were randomized to either (1) balloon injury of the descending thoracic aorta alone or (2) balloon injury combined with an aorta allograft of a thoracic aorta as a bypass to the abdominal aorta; the allograft was included as a positive control for the effect of cyclosporine to attenuating the neointimal proliferation in transplanted aortas. To exclude that the allograft was influencing events seen at the balloon-injured site, only half of the rabbits received an allograft. All rabbits were anesthetized with repeated small doses of an intravenous 30% pentobarbital solution. On average, a total dose of \( \approx 50 \text{ mg/kg of body weight} \) was given to each rabbit.

**Balloon Injury Alone**

After systemic heparinization (100 IU/kg), a 4-F embolectomy catheter (Baxter Health Care Corp) was introduced through the superficial femoral artery to the descending thoracic aorta. The placement was verified by x-ray. The balloon was inflated with 0.6 mL of saline (distention, 9.0 mm) and the catheter retracted 3 cm. Finally, the balloon was deflated and withdrawn.

**Balloon Injury Combined With an Aorta Allograft**

The abdomen of the recipient rabbit was opened through a midline incision and the 4-F embolectomy catheter was inserted into the aorta via an incision prepared for the proximal anastomosis between the donor thoracic aorta and the recipient abdominal aorta. Balloon injury was performed exactly as described above. The thoracic aorta of the donor rabbit was removed and transplanted as a bypass graft onto the abdominal aorta of the recipient. Finally, the abdominal aorta of the recipient rabbit was ligated between the 2 anastomoses, thereby directing the entire arterial blood flow through the graft.

**Cyclosporine Treatment**

Rabbits with a balloon injury and rabbits with balloon injury combined with an aorta allograft were randomly assigned to intramuscular injections of cyclosporine or vehicle. Cyclosporine (10 mg/kg) (Sandimmun, Sandoz), or an equivalent volume of the cyclosporine vehicle (cremophor/ethanol, 66:33%), was administered intramuscularly at the completion of the balloon injury/transplantation and subsequently once daily in individualized doses designed to give whole blood trough levels in the human therapeutic range of 0.08 to 0.33 \( \mu \text{mol/L} \); blood cyclosporine concentrations were determined once per week (Emit Cyclosporine Assay, Syva Company).

**Cholesterol Feeding**

After a recovery period of 2 weeks when the rabbits were fed ordinary chow, each rabbit was fed individually adjusted cholesterolenriched pellets (0 to 1 g of cholesterol per rabbit per day) to achieve a mean plasma cholesterol concentration in the average human range of 5 to 7 mmol/L; plasma cholesterol concentrations were determined twice before, and every second day during, the cholesterol feeding period. Lipoprotein cholesterol concentrations were measured with an enzymatic kit (CHOD-PAP, Boehringer Mannheim). HDL (HDL, \( d>1.063 \text{ g/mL} \)), LDL (1.063\( \text{g/mL} \) - 1.019 g/mL), and VLDL (\( d<1.019 \text{ g/mL} \)) were separated by using ultracentrifugation as described previously.19

**Neointimal Proliferation**

Five weeks after the surgical procedures, rabbits were killed with intravenous pentobarbital (50 to 100 mg/kg). To delineate the region of the balloon injury, rabbits were injected intravenously with 5 mL of Evans Blue, 5 minutes before they were killed; this permitted the native thoracic aorta to be divided into 2 segments, a blue-stained balloon-injured part and the remaining normal aorta. A catheter was introduced into the left ventricle of the heart, and the vascular system perfused with 500 mL of saline; blood and perfusate left through an incision in the inferior vena cava. After perfusion, the entire aorta was dissected free and a 3- to 5-mm-long specimen of unopened aorta was taken from the native aorta (central part of white area above the blue-stained area), the balloon-injured aorta (central part of blue stained area), and the transplanted aorta (central part); after fixation in formalin, these specimens were embedded in paraffin, and 2 serial sections were stained with elastic van Gieson and elastic hematoxylin and eosin, respectively. Another 3- to 5-mm-long specimen from native, balloon-injured, and transplanted aortas (next to the specimen already taken) was immediately frozen in isopentane cooled with dry ice, and kept at \(-80^{\circ}\)C until further processing. Of the remaining native, balloon-injured, and transplanted aortas, the luminal surface areas were outlined, and the tissues divided into intima inner media layers and outer media layers. Each of these parts were weighed and the tissues stored at \(-20^{\circ}\)C until further processing. Total, free, and esterified cholesterol content was determined as described.19

Histomorphometric studies were performed blinded in all animals by 1 of the investigators (H.Ø.A.); neointimal proliferation and medial area were quantified by point counting.20 In addition, cross sections were evaluated independently and blinded for qualitative morphological features by 1 of the authors (H.Ø.A.).

**Immunohistochemistry**

Immunohistochemical demonstration of macrophages, T lymphocytes, smooth muscle cells, and endothelial cells was performed by the avidin-biotin method on formalin-fixed, paraffin-embedded aortic tissue, using the following monoclonal antibodies: RAM11 (DAKO Corporation), which recognizes an uncharacterized cytoplasmic antigen expressed by rabbit alveolar macrophages,21 L11/135 (Serotec), which is a pan-T-lymphocyte marker that recognizes rabbit T lymphocytes in blood and tissues but does not cross-react with other leukocytes or other cell types, HB/F35 (DAKO A/S), which is a specific marker for smooth muscle cell–specific actin in rabbits,22 and CD31 (DAKO A/S), a monoclonal antibody that reacts with a 100-kDa glycoprotein in endothelial cells. Because this latter antibody can cross-react with other cell types, it was a requirement that the staining should be found luminally, before the presence of endothelial cells could be accepted. Mast cells were visualized by an enzymatic stain (LEDER, naphthol AS-D-chloroacetate).

In cryostat sections of aortas, the 2C4 antibody (Serotec), which binds to the rabbit homolog of the class II MHC antigen, was used as a marker of inflammation with activation of the immune system. Furthermore, VCAM-1 and ICAM-1 were recognized by using the monoclonal antibodies Rb1/9 (mouse IgG, hybridoma supernatant) and Rb2/3 (mouse IgG, hybridoma supernatant), respectively. Both antibodies were kindly provided by Myron I. Cybulsky, Brigham and Women’s Hospital, Harvard Medical School.

To quantify the immunohistochemical changes in the different groups of rabbits the following method, performed “blind” by 1 of the investigators (H.Ø.A.), was used for the detection of macrophages (RAM11), T lymphocytes (L11/135), smooth muscle cells (HB/F35), and mast cells (LEDER): In a representative field (magnification, \( \times 400 \)), antibody-positive cells were expressed as percentages of the total number of cells (counted as nuclei) in the intimal layer. The number of a given cell type in the field chosen for counting varied from 0 to 127 cells, whereas the highest total number of cells in 1 field was 336. The mean bias ± standard error values for the relative number of smooth muscle cells, T lymphocytes, and macrophages, based on counting in 2 different representative fields of the same 13 aortic allografts, were 2.2 ± 1.5%, 1.2 ± 0.8%, and 0.1 ± 0.2%, respectively. For class II MHC (2C4), VCAM-1 (Rb1/9), ICAM-1 (Rb2/3), and endothelial cells (CD31), the intensity of staining was graded...
numerically on a scale from 0 to 5 as follows: grade 0, no staining; grade 1, patchy and weak staining; grade 2, uniform and weak staining; grade 3, patchy and moderate staining; grade 4, uniform and less intense staining; and grade 5, uniform and intense staining. 23

Statistics
All results are given as mean ± SEM values. Wilcoxon’s test for paired samples was used to evaluate the change in body weight during the experiment. For comparison between 2 groups, the Mann–Whitney U test was used. For comparison between 3 or more groups, the Kruskal–Wallis analysis of variance 24 was used. In case of a significant analysis of variance, post hoc analysis was performed with the Mann–Whitney U test. For categorical data, Fisher’s exact test was used. No correction for multiple comparisons was performed. P < 0.05, on 2-sided tests was chosen as the level of significance.

Results
All rabbits thrived and the body weight stayed constant throughout the 5-week study period (data not shown). Plasma cholesterol concentration curves were similar in all 4 groups of rabbits throughout the study (Figure 1). Mean plasma cholesterol concentrations during the entire study period as well as during the cholesterol feeding period were similar in all 4 groups (data not shown). Lipoprotein cholesterol levels were similar in all 4 groups except LDL cholesterol between cyclosporine- and vehicle-treated rabbits from the groups of rabbits with the combined procedure (2.6 ± 0.2 versus 1.7 ± 0.2 mmol/L, P = 0.01). To achieve the desired plasma cholesterol level, cyclosporine-treated animals needed significantly less dietary cholesterol than did vehicle-treated animals (balloon injury, 0.10 ± 0.01 versus 0.04 ± 0.01 g · kg⁻¹ · d⁻¹, P = 0.001; balloon injury + transplantation, 0.08 ± 0.01 versus 0.04 ± 0.01 g · kg⁻¹ · d⁻¹, P = 0.001), which is in agreement with findings in earlier studies. 4,5

Doses of cyclosporine in rabbits with balloon injury alone and in rabbits with balloon injury combined with an aorta allograft were 6.9 ± 0.1 and 7.5 ± 0.4 g · kg⁻¹ · d⁻¹. Mean trough levels of cyclosporine were held within the human therapeutic level of 0.08 to 0.33 μmol/L in both cyclosporine-treated groups (0.17 ± 0.01 and 0.14 ± 0.01 μmol/L).

Aortic Cholesterol Accumulation
Cyclosporine had no effect on intimal cholesterol accumulation in native or balloon-injured aortas (Figure 1). In transplanted aortas, however, cyclosporine reduced intimal cholesterol accumulation (P = 0.005).

Neointimal Proliferation
Cyclosporine had no effect on neointimal proliferation in native or balloon-injured aortas (Figures 1 and 2). However, in transplanted aortas, cyclosporine inhibited neointimal proliferation (P = 0.04).

Endothelial Cells
In 25 of 27 native aortas, we found a thin luminal staining representing endothelial cells in the entire circumference.
There was no difference between vehicle- and cyclosporine-treated rabbits (Figure 3) (data not shown).

In balloon-injured aortas, luminal staining was only seen in areas without neointimal proliferation, reaching and often creeping only a little way up the slope of the neointimal proliferation humps (Figure 3). There was no difference between cyclosporine- and vehicle-treated groups in staining intensity (Figure 3) (data not shown).

In transplanted aortas, however, only 1 of 7 transplants from the vehicle-treated group had staining, whereas all 6 from the cyclosporine-treated group had endothelial staining (Figure 3) \((P<0.005, \text{Fisher’s exact test})\).

**Smooth Muscle Cells, T Lymphocytes, and Macrophages**

Neointimal proliferation in balloon-injured aortas from vehicle-treated rabbits mainly consisted of smooth muscle cells and only a low number of macrophages and T lymphocytes were demonstrated (Figures 3 and 4). There was no difference between cyclosporine- and vehicle-treated groups in the relative number of smooth muscle cells, T lymphocytes, or macrophages.

In transplanted aortas from vehicle-treated rabbits, macrophages, smooth muscle cells, and T lymphocytes were all abundant (Figures 3 and 4). The numbers of both macrophages and T lymphocytes were significantly reduced in aortic allografts by cyclosporine, whereas cyclosporine caused a relative, but not absolute, increase in the number of smooth muscle cells.

**Mast Cells**

Only 2 mast cells were seen in the adventitia of 1 of the balloon-injured aortas, but none in the intimas of native, balloon-injured, or transplanted aortas.

**VCAM-1, ICAM-1, and MHCII**

In the intima of native and balloon-injured aortas, staining intensity for VCAM-1, ICAM-1, and MHCII did not differ between vehicle- and cyclosporine-treated rabbits (Figures 5 and 6). In transplanted aortas, however, staining intensities for VCAM-1, ICAM-1, and MHCII were lower in cyclosporine- than in vehicle-treated rabbits.

**Discussion**

In the rabbit, neointimal proliferation in arteries occurs after intimal balloon injury,\(^{10,13,25,26}\) where the process is accelerated by increased plasma cholesterol levels,\(^{25,26}\) as well as in cholesterol-induced atherogenesis,\(^{7,9}\) and after transplantation of solid organs or arteries.\(^{4,6,12}\) T lymphocytes, macrophages, and smooth muscle cells, and upregulation of VCAM-1,
ICAM-1, and MHCII, are all components of these 3 types of neointimal proliferation.\textsuperscript{7–13} Cyclosporine has been shown to inhibit early atherosclerosis in the cholesterol-fed rabbit.\textsuperscript{3} Furthermore, cyclosporine has been shown to inhibit development of transplant arteriosclerosis\textsuperscript{4,5,18} and to reduce an otherwise increased occurrence of T lymphocytes, macrophages, and smooth muscle cells and upregulation of VCAM-1, ICAM-1, and MHCII in transplanted arteries.\textsuperscript{5,6,27} We therefore hypothesized that cyclosporine would also inhibit neointimal proliferation occurring after aortic balloon dilatation injury. However, in the present study, using a clinically relevant dose of cyclosporine, rabbits with plasma cholesterol clamped at a mean level of 5 to 7 mmol/L, and a 5-week experimental period, cyclosporine had no effect on any of these changes in balloon-injured aortas, but significantly inhibited all these changes in the transplanted aortas, the positive controls.

To ensure that rabbits with a combined balloon injury and aorta had fully recovered from the surgical intervention and regained a normal gastrointestinal function before the start of the individualized cholesterol feeding, cholesterol feeding was not initiated before 2 weeks after surgery. It could be suggested that many of the proliferation and migration processes leading to intimal hyperplasia after balloon injury may have already been completed at this time. However, the significant upregulation of cellular activation parameters such as VCAM-1, ICAM-1, and MHCII as well as proliferation of smooth muscle cells and macrophages were seen 4 to 5 weeks after balloon injury in the present study as well as in earlier studies.\textsuperscript{10,13} Furthermore, intimal proliferation has been reported to proceed from 4 weeks up to as much as 20 weeks after balloon injury.\textsuperscript{10,14,28–32} Finally, cholesterol accumulation in both deendothelialized and reendothelialized areas has been reported to be an ongoing process from day 2,\textsuperscript{33} through to day 30,\textsuperscript{34} and up to day 119 after balloon injury.\textsuperscript{35} We therefore found it justifiable to delay the initiation of the cholesterol feeding until 2 weeks after the balloon injury and transplantation.

Upregulation of VCAM-1, ICAM-1, and MHCII in balloon-injured\textsuperscript{10} and transplanted aortas\textsuperscript{5,12,36} have been described earlier. Cyclosporine had no significant effect on VCAM-1, ICAM-1, and MHCII in the balloon-injured aorta, but a significant inhibitory effect on the very same cellular activation markers in the transplanted aorta. The inhibitory effect of cyclosporine on these markers in allografted arteries has been observed by others,\textsuperscript{6,27,36,37} whereas the lack of effect of cyclosporine on upregulation of VCAM-1, ICAM-1, and MHCII in arteries after a balloon injury has not been observed previously. This difference suggests that different mechanisms are responsible for upregulation of these cellular activation markers in the 2 forms of neointimal proliferation. In transplanted arteries from rabbits receiving no immunosuppression, the main stimulus for the upregulation is supposed to come from a high number of activated T lymphocytes\textsuperscript{38} and macrophages\textsuperscript{38} excreting the cytokines that
induces upregulation. Among these cytokines are interferon-γ (IFN-γ) and tumor necrosis factor-α (TNF-α). IFN-γ increases upregulation of the adherence proteins VCAM-1,3,8–40 and ICAM-1,3,8,39 as well as upregulation of MHCII.38 Furthermore, IFN-γ stimulates the activation of macrophages38 and augments the effects of TNF-α.40 TNF-α itself induces upregulation of adherence molecules on endothelial cells.38–40 Cyclosporine inhibits the excretion of IFN-γ indirectly by an inhibition of T-lymphocyte proliferation and via a direct inhibitory effect on the production.41 Thus, a lowered presence of IFN-γ per se would reduce the upregulation of VCAM-1, ICAM-1, and MHCII in the transplanted artery. In the balloon-injured aorta, however, other mechanisms such as increased levels of plasma cholesterol,8,9 altered shear stress,42 or local paracrine induction by TNF-α from smooth muscle cells43 may be more important stimuli for upregulation of at least VCAM-1 and ICAM-1. This could explain why cyclosporine is without effect on the expression of VCAM-1 and ICAM-1 as well as on the neointimal proliferation after a balloon injury. Upregulation of MHCII is normally believed to be induced by stimulation with IFN-γ.38 Cholesterol feeding in rabbits, however, also induces upregulation of MHCII10;22; the exact mechanism for this is not known. That cyclosporine had no effect on upregulation of MHCII in balloon-injured aortas suggests that IFN-γ may not be the stimulus for upregulation after a balloon injury.

We have shown in previous studies that cyclosporine has a powerful inhibitory effect on the development of transplant arteriosclerosis in aorta allografts in rabbits with plasma cholesterol clamped at a human level of 5 to 7 mmol/L.4,5,17

The cells seen in connection with neointimal proliferation in transplanted aortas from rabbits were immunological competent cells, ie, T lymphocytes and macrophages, as well as smooth muscle cells.5,18 Transmission electron microscopic investigations of allografted aortas within 2 weeks after transplantation showed that although cyclosporine exerted a powerful inhibition of the occurrence of T lymphocytes and macrophages, there still was some smooth muscle cell proliferation in the allograft.18 In the present study, we found a relative, but not an absolute, increase of smooth muscle cells in aortic allografts from cyclosporine-treated rabbits. The response of the artery to balloon injury consists mainly of proliferation of smooth muscle cells and, to a lesser extent, of infiltration of T lymphocytes and macrophages.10,26 In vitro studies investigating whether cyclosporine may have an inhibitory effect on smooth muscle cell proliferation have given equivocal results; in 1 study cyclosporine exhibited a dose-dependent inhibitory effect on smooth muscle cell proliferation,15 in another study the effect was either stimulatory or inhibitory, dependent on the dose of cyclosporine,43 and in a third study cyclosporine was without any effect.16 Finally, in a fourth study, cyclosporine was reported to inhibit smooth muscle cell proliferation indirectly via endothelial cell–derived factors.44 The in vitro results from the 2 latter studies16,44 are compatible with our present and most,4,5,18 but not all,45 former in vivo findings supporting the notion that cyclosporine has none or only a minor inhibitory effect on smooth muscle cell proliferation in vivo.

Ferns et al.15 using rabbits with plasma cholesterol at ≈2 mmol/L and cyclosporine in a clinically relevant dose, likewise found no effect on carotid neointimal proliferation 2 weeks after balloon injury, which is in agreement with our results. In a similar manner, Gregory et al.14 using very low doses of cyclosporine intraperitoneally in rats for a period of 2 weeks, found that cyclosporine had no effect on balloon injury–induced neointimal proliferation in carotid arteries. Jonasson et al.16 using supr clerical doses for only 2 days followed by 12 days without any treatment in rats, however, found that cyclosporine inhibited neointimal proliferation in balloon-injured carotid arteries examined 2 weeks after injury. They found no direct effect of cyclosporine on smooth muscle cell proliferation in vitro and therefore suggested that the inhibitory effect of cyclosporine seen in vivo was mediated through an inhibition of T-lymphocyte proliferation.16 The present study, however, could not demonstrate any difference in the occurrence of T lymphocytes in balloon-injured aortas between cyclosporine- and vehicle-treated rabbits. In addition, the scarce presence of T lymphocytes in the neointima formed as a consequence of balloon injury would not suggest that these cells would have a major impact on this type of lesion. Results from studies using athymic nude rats46,47 or T lymphocyte–depleted rats46 have given equivocal results concerning the role of T lymphocytes in the pathogenic process of balloon injury–induced neointimal proliferation. Thus, present and previous results taken together do not support a significant influence of T lymphocytes on neointimal proliferation in the artery after balloon injury.

In balloon-injured rats, Hancock et al.48 reported that mononuclear phagocytes, but not T lymphocytes, had a major impact on the development of intimal proliferation. Mononuclear leukocytes also seem to be the principal cell type when neointimal proliferation is induced by a perivascular electrical injury. In this latter model, an inflammatory reaction with mononuclear leukocytes eventually leads to smooth muscle cell migration and proliferation.49,50

Although the lesions formed after balloon injury and transplantation have several common features, it is quite possible that the mechanism at work differs in the 2 situations. This is illustrated clearly in the present study by the inhibitory effect of cyclosporine on neointimal proliferation in the transplanted aorta, but not in the balloon-injured aorta. In the transplanted artery, alloimmunity involving T lymphocytes and their consequent recruitment of macrophages seem to be of major importance51; cyclosporine inhibits T lymphocytes. In the balloon-injured artery, smooth muscle cell proliferation10,26 in response to wound healing and possibly macrophages under conditions with hypercholesterolemia3,25,30,48 may be of more importance.

In native and balloon-injured aortas, cyclosporine had no effect on endothelial coverage. In the transplanted aorta, however, cyclosporine significantly inhibited the disappearance of the endothelial coverage, in accordance with previous results.18 The mechanism behind this effect is not completely clear. However, it is believed that cyclosporine inhibits T cell–mediated endothelial injury.18 In a recent study by Walter et al.52 cyclosporine reduced oxidized LDL-induced apoptosis of human endothelial cells. In the present study, cholesterol feeding may have led to endothelial cell exposure, to increased levels of oxidized LDL, and thereby to an increased endothelial cell apoptosis. In native and balloon-injured aortas, this kind of injury may not have reached a
significant or measurable extent, whereas this mechanism in concert with the immunological injury may have been substantial in transplanted aortas.

In conclusion, the present data suggest that (1) cyclosporine does not influence the response of arteries to balloon injury, whereas it reduces allograft arteriosclerosis; (2) the expression of VCAM-1, ICAM-1, and MHCI after arterial balloon injury is not influenced by cyclosporine; (3) T lymphocytes do not play a crucial role in the arterial response to balloon injury; and (4) this response is quite distinct from allograft arteriosclerosis. In the present study, balloon injury was performed in a healthy artery, which is not the case when balloon dilatation is performed on an atherosclerotic stenosis of a human artery. Therefore, it cannot be ruled out entirely that cyclosporine may influence human restenosis, which is a far more complex process than the response of a normal rabbit aorta to balloon injury.\(^3\)

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References


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