1316

FK 506 Inhibits the Development of Transplant Arteriosclerosis

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TRANSPLANT arteriosclerosis is the most serious complication of successful cardiac transplantation.[1,2] Improvements in cardiac graft survival after transplantation have led to the recognition of a proliferative, occlusive arteriosclerosis in the coronary arteries of the graft. In rats, this arteriosclerosis is the result of chronic, immune-mediated rejection reaction.[3] Manipulation of the recipient immune responses results in alterations in the expression of the disease. Effective immunosuppression with cyclosporine (CyA), for example, can prevent the development of the lesions.[4] There have been recent reports, however, that FK 506 may be responsible for stimulating, rather than preventing, transplant arteriosclerosis in rats.[5] We have recently evaluated the influence of FK 506 treatment on the development of transplant arteriosclerosis in the LEW-to-F344 rat allograft model. These studies were designed to establish whether (1) FK 506 alone will induce arteriosclerosis in syngeneic grafts and (2) whether the treatment of cardiac allograft recipients with minimal levels of FK 506 will interfere with or prevent the development of transplant arteriosclerosis.

MATERIALS AND METHODS

Animals

Adult male LEW ($RT1^l$) and F344($RT1^{lvl}$) rats (Harlan Sprague Dawley, Indianapolis, Ind) weighing 220 to 240 g were used as donors and recipients for these experiments. The recipients of heterotopic cardiac grafts were assigned to four experimental groups: group 1 (N = 14), untreated controls (vehicle only); group 2 (N = 10), oral FK 506 (1.0 mg/kg/d); group 3 (N = 9), oral CyA (5 mg/kg/d); group 4 (N = 7), syngeneic (F344) cardiac grafts and oral FK 506 (1 mg/kg/d).

Cardiac Transplantation

The procedure of heterotopic cardiac transplantation used in this study is a modification of the technique originally described by Ono and Lindsey.[6] The graft was implanted in the abdominal cavity with both anastomoses done in a running end-to-side fashion with 10-0 Novafil on a TE-70 needle. Operative times ranged from 30 to 45 minutes with a success rate of approximately 90%. The grafts were evaluated for function by daily abdominal palpation daily and rejection confirmed by laparotomy. All grafts were removed for examination at day 90 posttransplantation and a detailed histopathologic examination of the lesions conducted.

Immunohistochemistry

Monoclonal antibodies (MAbs) specific for Pan T cells (OX19); T helper cells (W3/25); T cytotoxic/suppressor cells (OX8); macrophages (ED-1), and smooth muscle cells (ASM-1) were used to characterize the arteriosclerotic lesions. Immunoperoxidase studies were performed using a three-step indirect method.[7]

Histopathologic Evaluation

Histologic grades for recording severity of the lesions present in the donor myocardium consisted of scoring lesions as: 0, normal; 1, mild; 2, moderate; and 3, severe. Quantitation of the number of inflammatory cell subsets in the myocardium of the grafts was performed by light microscopic observation. Five randomly chosen fields were examined for each MAb and the mean cell count was expressed as the number of cells/high power field (HPF).

RESULTS

Treatment of allograft recipients with low levels of FK 506 or CyA was associated with improved graft survival and a marked reduction in the severity of the inflammatory infiltrate in the grafts. Allograft survival varied between the treatment groups with a high survival rate 90 days posttransplantation in group 2 (FK 506 treatment, 90%), group 3 (CyA treatment, 100%), and group 4 (syngeneic grafts, 100%). Of 14 animals included in the untreated controls (group 1), 8 grafts were rejected by the recipients at 50 to 70 days posttransplantation. Six (42.8%) grafts survived the full 90 days posttransplantation. The improved graft survival following immunosuppression with CyA and FK 506 was associated with a reduction in the degree of vascular intimal proliferation, perivascular infiltration, myocardial inflammation, and fibrosis (Table 1, Fig 1). There was no evidence of vascular intimal proliferation or other transplant-associated inflammatory lesions in the syngeneic F344 grafts treated with FK 506.

Immunohistochemical examination of the inflammatory cell subset distribution in the myocardium of the grafts demonstrated that both FK 506 and CyA decreased the number of infiltrating leukocytes, particularly macrophages and cytotoxic T cells, when compared with the control group.

DISCUSSION

Recent reports from both clinical and experimental studies have indicated that transplant arteriosclerosis is the result of a chronic, low-grade immune response of recipient to donor graft histocompatibility antigens.[7,8] The ability of immunosuppressive agents, such as CyA, to prevent the

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0041-1345/91/$3.00/+0

Table 1. Severity of the Pathologic Changes Observed in LEW-to-F344 Cardiac Allografts

Group (No.)	Histopathologic Lesions*			
	VIP	PVF	MI	MF
Group 1				
Control (6)	2.68 ± 0.18	1.92 ± 0.55	2.00 ± 0.54	2.17 ± 0.26
Group 2				
FK 506 (9)	1.64 ± 0.30†	1.06 ± 0.58	1.38 ± 0.47	1.30 ± 0.49
Group 3				
CyA (9)	1.74 ± 0.38†	1.25 ± 0.71	1.30 ± 0.41	1.35 ± 0.68
Group 4				
Syngeneic (7)	0.00	0.00	0.30 ± 0.20	0.00

Abbreviations: VIP, vascular intimal proliferation; PVF, perivascular fibrosis; MI, myocardial inflammation; MF, myocardial fibrosis.
*The values represent the mean score (0.0 to 3.0) for the lesions within each group ± SD.
†Indicates differences of $P < .05$ when compared with control values (Student's t test).

intimal proliferative lesions in experimental animals is consistent with therapeutic control of the chronic immune reaction. The same effect of CyA therapy has not been consistently observed in patients with successful heart grafts.[9] The severity of arteriosclerotic lesions frequently displays little correlation with the number of rejection episodes, the degree of HLA matching, or the use of immunosuppressive drugs. In rats, the most severe vascular lesions occur in strain combinations in which weak histocompatibility differences allow for prolonged survival of the graft with minimal evidence of active rejection. The apparent lack of correlation of immunosuppression with the development of arteriosclerosis in patients may represent weak histocompatibility differences that stimulate the proliferative lesions without precipitating clinically apparent episodes of acute rejection.

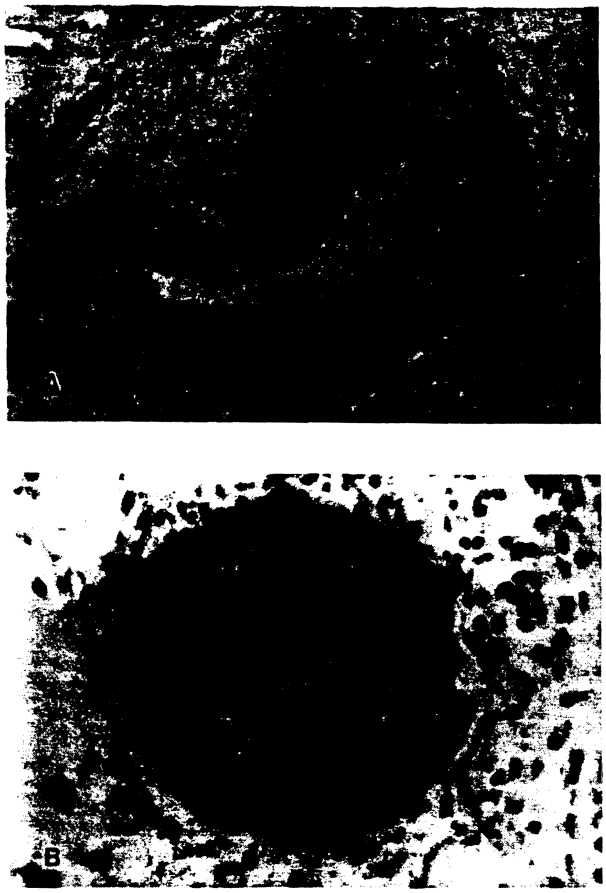

Fig 1. **(A)** Histopathologic changes present in allograft 90 days posttransplantation after treatment with FK 506 with mild vascular intimal proliferation and perivascular mononuclear cell infiltration (EVG stain; original magnification, ×400). **(B)** Untreated allografts exhibit severe intimal thickening in coronary arteries (immunoperoxidase stain for α actin; original magnification, ×400).

FK 506 is a new and potent immunosuppressive drug that is similar in its mode of action to CyA.[10,11] Recent reports, however, have provided evidence for the development of vasculitis in dogs[12] and transplant arteriosclerosis in rats.[5] The data presented here demonstrate that FK 506 inhibits the development of transplant arteriosclerosis in rats. These results are consistent with the immunosuppressive activity of the drug and the reduction in the severity of the pathologic lesions present in the grafts. They are also in general agreement with the expected results for a drug with immunosuppressive activity similar to that of CyA. The levels of CyA and FK 506 used in these studies were chosen to be minimally effective therapeutic doses to mimic the clinical management of the transplant patient. Both drugs significantly inhibited the development of the arteriosclerosis. Neither compound, however, completely prevented the development of the transplant lesions at the dose levels used. There was no evidence of the appearance of vascular lesions in syngeneic grafts of recipients treated with FK 506. These results demonstrate that FK 506 acts as an immunosuppressive agent to prevent transplant arteriosclerosis in experimental animals and does not independently stimulate the development of vascular lesions.

REFERENCES

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