NOTE *Pathology*

Acute Vascular and Interstitial Rejection Following Renal Allograft Transplantation in Dogs

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ABSTRACT. Renal allograft transplantation was performed in four beagles. Immunosuppressive treatment using cyclosporine, mizoribin and prednisolone was continued from Day 5 pre- until Day 20 post-transplantation. Between Days 28 and 32 post-transplantation, an abrupt elevation of the serum creatinine values followed by the development of uremia was seen in all recipients. Histopatholog y of the allografts examined between Days 28 and 37 revealed edema, necrosis, hemorrhage and severe diffuse interstitial cellular infiltration as well as tubulitis. Glomerular changes notably included swelling of the tufts due to hypercellularity, which was consistent with transplant glomerulitis. The intrarenal arteries exhibited fibrinoid necrosis of the walls and intimal or transmural cellular infiltration. These renal lesions were consistent with those of acute vascular and interstitial rejection in humans.

KEY WORDS: kidney, rejection, renal transplantation.

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Chronic renal failure (CRF) is one of the most important causes of mortality in dogs and cats. Renal transplantation has been successful in human patients with CRF. In veterinary medicine, trials of renal allograft transplantation have been performed in dogs [3–5] and cats [4, 6], and although the clinical application of renal allograft transplantation to affected animals is not common, it may be one of the choices for the treatment of end-stage CRF.

The critically important aims of post-operative care are to prevent rejection of the allograft by immunosuppressive therapies and to control infection. The early diagnosis of rejection based on clinical and/or histopathological findings is essential for postoperative management in dogs and cats as in humans. International standardization of the criteria for the histologic diagnosis of renal allograft rejection has been developed to guide therapy in transplant patients and to help establish an objective rejection end point in clinical trials in humans [11]. On the other hand, little is known about the histopathological changes in renal allograft rejection in dogs as well as in cats. Further information is necessary to develop the clinical application of renal transplantation for end-stage renal diseases in these animals.

In this study, we performed experiments to establish clinical procedures for the detection of early allograft rejection in dogs. In this study, all recipient dogs showed severe renal failure after discontinuation of the immunosuppressive therapy, suggesting acute rejection. The aim of this report is to describe the characteristic histopathological changes of acute renal allograft rejection in dogs. The detailed clinical data obtained in these experiments will be published elsewhere.

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Four pairs of clinically normal beagles (Table 1) were selected for renal transplantation on the basis of the direct cross-matched test [9]. The left kidneys of the donor dogs were resected and transplanted in the right iliac fossa of the recipient dogs (Nos. 1–4). End-to-side anastomosis of the renal and common iliac veins and end-to-end anastomosis of the iliac and renal arteries were carried out. A ureteroneocystostomy was performed after renal blood flow and urine production were confirmed in the allograft. Thereafter, the right and left kidneys of the recipients were resected for use in other experiments (data not shown).

Cyclosporine, mizoribin and prednisolone were used for immunosuppressive treatment. The doses and schedule of medicine administration followed procedures modified from those reported by Minami *et al.* [9] as shown in Fig. 1.

Serum creatinine (SCr) and blood urea nitrogen (BUN) were monitored as indicators of renal function throughout the experiment. The values of SCr (Fig. 2) and BUN(data not shown) were moderately elevated soon after transplantation, and then stabilized for about 3 weeks. One recipient dog developed a fever Day 20 post-transplantation. An abrupt increase in the SCr and BUN values of all dogs was seen between Days 28 and 32 post-transplantation. All recipient dogs showed signs of uremia immediately after the abrupt elevation of SCr and BUN. From Days 29 to 37 post-transplantation, the recipient dogs became moribund and were euthanized. The transplanted kidneys as well as the right kidney of each donor were examined histopathologically.

The renal allografts showed essentially the same histologic changes in all recipients. The interstitial lesions were characterized by widening due to edema, hemorrhage and severe diffuse cellular infiltration of lymphocytes, plasma cells, macrophages and neutrophils (Fig. 3).

Table. 1. Recipient and donor dogs for renal transplantation

	Recipients		Donors		Racipient/Donor
No.	BW(kg)	Sex	BW(kg)	Sex	B.W. ratio
1	11.0	♂	10.0	♀	1.1
2	10.0	♂	9.0	♀	1.1
3	10.0	♂	10.0	♀	1.0
4	10.0	♀	8.5	♀	1.2

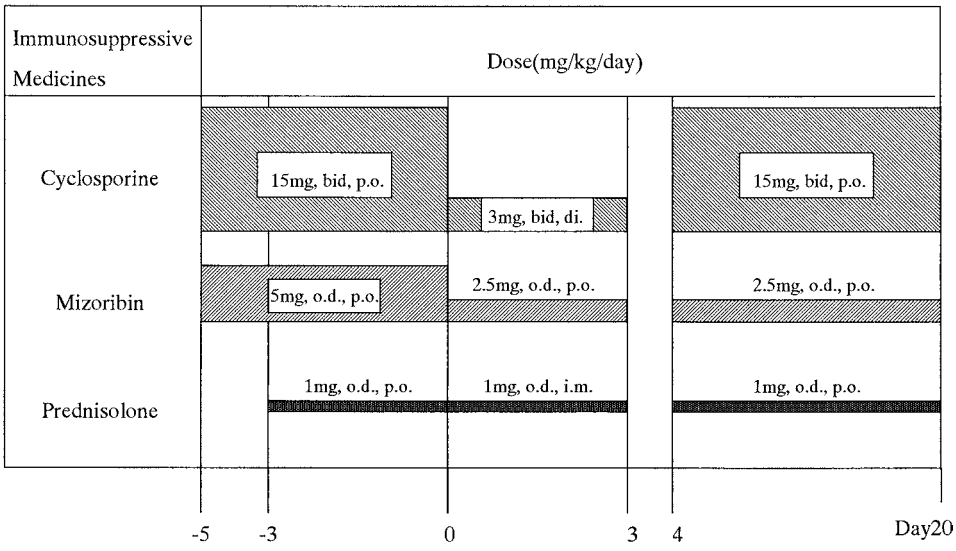

Fig. 1. Schedule of immunosuppressive treatment. 0: The day of transplantation.

The combination of these inflammatory cells varied among cases, however, lymphocytes and plasma cells predominated in all allografts. Necrosis in the cortical tissues varied among allografts. The proliferation of fibroblasts with a slight increase in collagen fibers was occasionally observed in the edematous interstitium. Tubulitis was the most characteristic change in the cortical tubules (Fig. 4). The glomeruli showed prominent swelling and hypercellularity due to the accumulation of mononuclear cells and a few neutrophils in the capillary lumens (Fig. 5). Intrarenal arteries showed fibrinoid necrosis of the walls and intimal or transmural cellular infiltration (Fig. 6). Fibrin thrombi were often formed in the lumens of the affected vessels. Focal infarction developed in association with these vascular lesions.

Immunohistochemical examinations were performed to assess the deposition of IgG and IgM in all allografts as well as the right kidneys of each donor, using paraffin sections with anti-dog IgG (γ chain) (Kirkegaard & Perry Lab. Inc., Gaithersburg, MD) and anti-dog IgM (μ chain) (Kirkegaard & Perry Lab.Inc., Gaithersburg, MD). Markers of T-lymphocytes infiltrating the interstitium were also examined in the allografts (Nos. 1–3) using frozen sections with anti-dog CD4 (clone YKIX 302.9: BIOSOURCE Int., Camarillo, CA) and CD8 (clone YCATE 55.9: BIOSOURCE Int., Camarillo, CA). Immunostaining was carried out using a Histofine kit (NICHIREI Corp., Tokyo, Japan) or a VECTASTAIN ABC kit (Vector Laboratories, Burlingame, CA). IgG and IgM deposition was seen on the surface of the endothelium of the glomerular capillaries and interstitial vessels. IgG or IgM positive lymphocytes were numerous in the interstitium. CD8+ T-lymphocytes were more numerous than CD4+ cells in the interstitium. None of the right-side kidneys of the donor dogs showed any significant lesions.

The diagnosis of human allograft rejection is based on the histological evaluation of renal biopsies, and the rejection is categorized into three types : hyperacute, acute and chronic [1]. Among these, acute rejection is morphologically classified mainly into two types: interstitial and vascular rejection, which often develops simultaneously in the renal allografts [1].

Crowell *et al.* [3] described renal allograft lesions in dogs, which were consistent with the lesions of acute rejection in humans. However, the glomeruli were essentially normal, and tubulitis development was not mentioned in their report. The diffuse interstitial cellular infiltration and arterial lesions characterized by intimal and transmural cellular infiltration seen in the allografts in this study might be highly indicative of acute rejection, and also suggest the

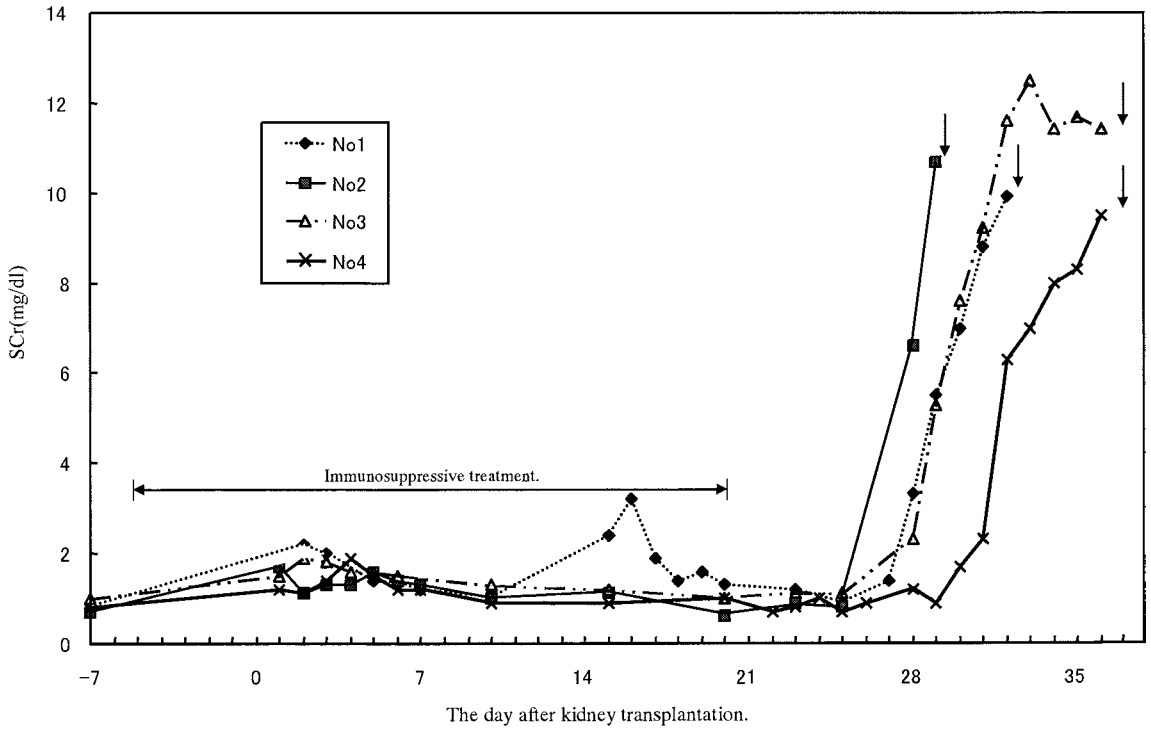

Fig. 2. Alterations of serum creatinine in renal transplantation. 0: The day of transplantation. ↓: The day of necropsy.

simultaneous vascular and interstitial rejection seen in human renal allografts [1]. Glomerular involvement, known as transplant glomerulitis, and tubulitis are also characteristic to and highly correlated with acute renal allograft rejection in humans [1, 2, 7, 10]. The glomerular changes characterized by hypercellularity were consistent with transplant glomerulitis in humans, and tubulitis was quite common in our cases. These glomerular and tubular lesions have not previously been reported in canine renal allografts.

Immunohistochemical examination revealed IgG and IgM deposition on the vascular endothelium in the renal interstitium and glomeruli in this study. However, the depositions of immunoglobulins and compliment components are reported to be parameters with minor significance in acute rejection [1]. We also showed the infiltration of CD4+ or CD8+ T-lymphocytes in the allografts, which are considered to be highly correlated with acute rejection [1]. In this study, CD8+ T-lymphocytes were more numerous than CD4+, however, the significance of the ratio of CD4+/CD8+ T-lymphocytes was controversial [1].

Among the immunosuppressive agents used in this study, cyclosporine has been shown to be nephrotoxic [8, 10], and it is sometimes difficult to differentiate between rejection and cyclosporine nephrotoxicity. We did not perform a control experiment for cyclosporine nephrotoxicity in this study, however, there were no changes strongly suggestive of cyclosporine nephrotoxicity, such as arteriolopathy in the renal allografts.

The clinical and the histopathological evaluations were not always in agreement with the diagnosis of acute rejection [1]. Acute rejection occurs in most allograft recipients in spite of continuous immunosuppressive treatment [3]. In our cases, the acute onset of azotemia and renal morphological changes were consistent with acute rejection. The abrupt increases in the values of SCr and BUN with the rapid development of uremia might have been due mostly to arterial changes resulting in parenchymal necrosis. Interstitial cellular infiltration might have developed during immunosuppressive therapy.

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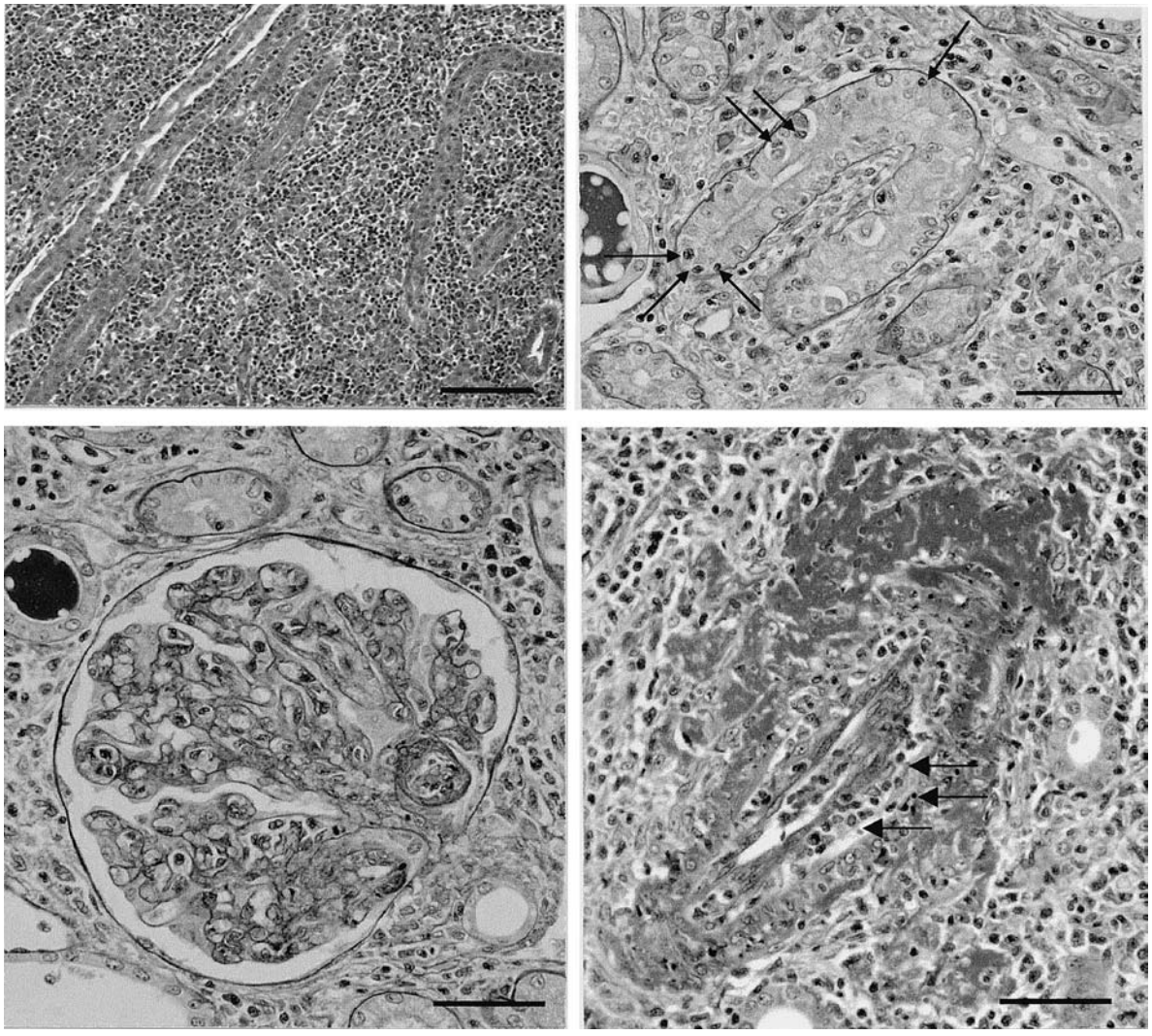

Fig. 3. Dog No. 4. Severe interstitial cellular infiltration of lymphocytes, plasma cells, macrophages and neutrophils. Hematoxylin-eosin stain. Bar =50 μm.
Fig. 4. Dog No. 3. Cortical tubulus infiltrated by mononuclear cells (arrows). Periodic acid-Schiff reaction. Bar =50 μm.
Fig. 5. Dog No. 3. The glomerulus showing swelling due to infiltration of mononuclear cells and a few neutrophiles in dilated glomerular capillary lumens. Periodic acid-Schiff reaction. Bar =50 μm.
Fig. 6. Dog. No. 3. Small artery in the cortex showing endothelial swelling, subendothelial infiltration with mononuclear cells (arrows) and fibrinoid necrosis of the walls. Hematoxylin-eosin stain. Bar =50 μm.

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