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RAT RENAL TRANSPLANTATION AND ITS IMMUNOLOGICAL STUDY

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ABSTRACT

By microsurgical technique, a syngeneic renal transplantation was performed on inbred M.P.* strain of the rat; and allotransplantation was made between M.P. and Sprague Dawley (S.D.) strains. Although all grafts showed severe rejection within 8 days in the group of allotransplantation, the grafted kidney survived up to 27 days in syngeneic transplantation. The kidney grafted together with the bladder which was anastomosed to the host bladder survived longer than that with only ureterostomy made in the groin of the host.

Immunological study failed to show any positive host immunological reaction when the kidney cell were injected or transplanted to the syngeneic animals. The cellular antibody, however, was demonstrated in the spleen cells of the host which had been immunized by the kidney cells of the allotype.

INTRODUCTION

In many laboratories renal transplantations have been tried on dogs and rabbits[1) 2) 3) 4) 5)]. These animals, however, are difficult in obtaining inbred strains. To investigate the immunological problem of graft versus host reaction, highly inbred strains of animals which are constant in genetic disparity are required.

In the present paper, a new technique of renal allotransplantation and immunological study are described by using inbred strains of a rat.

MATERIAL AND METHOD

Animals

Rats used throughout this experiment are male Sprague Dawley (S.D.) supplied from The central Laboratory of Experimental Animals, TOKYO JAPAN, and male M.P. strains of rats, which had originated in the Moriyama Psychiatry Hospital and having marker gene "ccaaBBDDhh".

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* See Material and Method.

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Renal Transplantation

Syngeneic renal transplantation was performed from M.P. to M.P. and allotransplantation from M.P. to S.D..

Rats weighing about 100 g were used as donors and rats weighing 300 g were used as host, so that the diameter of the vessels to be anastomosed could fit each other. The donor animal was anesthetized with pentothal sodium, given intraperitoneally in a dose of 35 mg per kg of body weight. Excepting the left renal artery and vein, all the branches of the aorta and vena cava were ligated and divided. Two polyethylene tubes were then inserted into the vessels at the origin of the iliac vessels; one with the outside diameter of the tube 0.5 mm was for the aorta and the other 1.0 mm for the vena cava. Through the arterial tubing the left kidney was perfused with 10 ml of heparinized physiological saline cooled to 2°C. Both tubes and vessels were divided craniad to the liver and caudad to the bifurcation of the iliac vessels.

The bladder was ligated and cut at its neck, and the kidney removed as shown in Fig. 1. The tubes protruding from the distal edge of the aorta and vena cava were inserted into the hosts' femoral artery and vein respectively which had been isolated and clamped by a bulldog clamp at the proximal por-

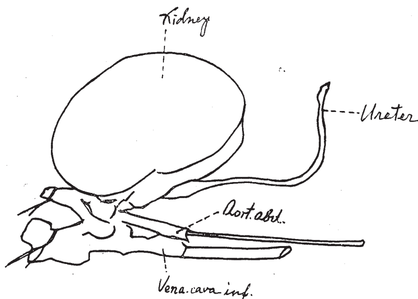
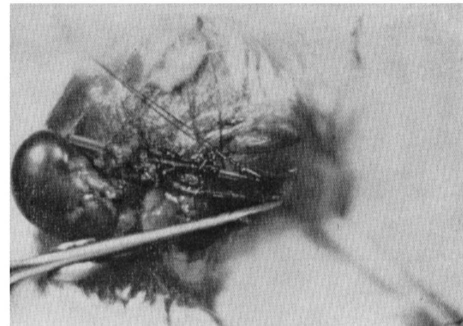


FIG. 1. Rat kidney after removal from the donor.
Two polyethylene tubes protruding from th distal edges.



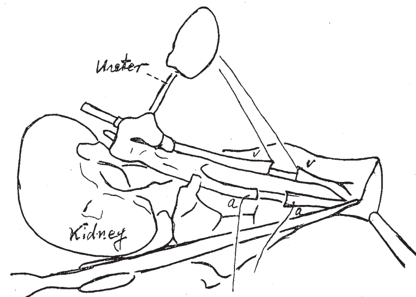


FIG. 2. The tubes were inserted into hosts' femoral artery and vein respectively.

tion, as shown in Fig. 2. Sustained by the tubes, an end to end anastomosis was made between the aorta and femoral artery and between the vena cava and femoral vein by continuous suture with 8-0 silk. After the anastomosed sites were ensured with paste "Alon alpha", the tubes were withdrawn through the open edge of the vessels and closed. When the bulldog clamp was removed from the vessels, the grafted kidney was flushed with blood and venous return was observed. Total ischemic time was mostly within 45 minutes. Then two experimental groups were subjected to urinary tract operation; in one the ureter was grafted to produce a fistula in the groin; in the other the bladder was anastomosed to that of the host, as shown in Fig. 3. In the latter group,

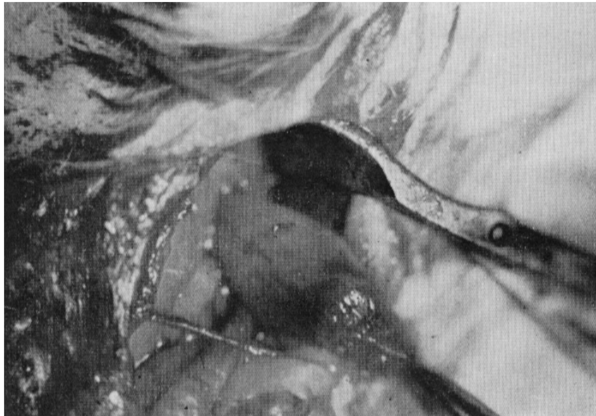


FIG. 3. Donor bladder is anastomosed to that of the host.

7 hosts were removed of their kidneys at the time of transplantation. None was nephrectomized in 12 trials of allotransplantations made between M.P. and S.D.. Every host rat was given water with saturated solution of tetracycline to prevent infection.

Immunological Studies

Immunization of Rat by Kidney cells

S.D. and M.P. rats received injection of kidney cells (2×10^6) of M.P. in an emulsion of Freund's complete adjuvant, both intraperitoneally and subcutaneously. These rats were bled on the 4th, 10th and 21st days after immunization; the sera and the spleen cells were harvested each time.

Method of Detecting the Cell Binding Antibody

Immediately after killing the immunized rats, the spleen was removed, cut and meshed through gauge to obtain a spleen cell suspension. After the spleen

cells were mixed and incubated at 37°C for 6 hours with the kidney cells of untreated M.P. as target cells, the cell mixtures were examined under a phase contrast microscope. The ratio of the spleen cells to the target cells ranged from 50:1 to 200:1.

Conjugation Method of Rat Serum with Fluorescein Isothiocyanate (FITC)

Rat γ-globulin fractions were dialysed against phosphate buffered solution after they were precipitated at 33% saturation of ammonium sulfate. The conjugation with fluorescein isothiocyanate[6)7)] on celite 10% manufactured by Nutritional Biochemical Corporation, Cleveland, U.S.A. was carried out by stirring at 20°C for 2 hours in 0.1 M of bicarbonate buffer (pH 9.5). The ratio of protein to FITC was 100 to 1. The supernatant fluid was applied directly to a sephadex G 25 column[8)9)] equilibrated with 0.02 M phosphate buffer at pH 6.2 to separate fluorescein antibody conjugates which appeared as a distinct band on the column from untreated isothiocyanate and its hydrolysis products.

Further fractionation of conjugates was made by elution with phosphate buffer containing 0.1 M NaCl on a column of DEAE-cellulose[10)11)12)] which had been activated by treatment with water, 1 N NaOH, water, 1 N HCl, and water in order.

Direct Immunofluoresceint Staining Method

Both stamped and cryotomed preparations of the kidney of all syngeneic and allogeneic transplantation were fixed by acetone for 10 minutes. After FITC labelled rat serum γ-globulin fraction was dropped on the preparations, the slides were incubated at 37°C for 30 minutes. They were then washed twice with phosphate buffer containing NaCl for 10 minutes, each time with gentle shaking. The stained preparations were examined under the Olympus Fluorescent Microscope. The grafted kidney sections were stained in the same way but FITC labelled rat γ-globulin was substituted by FITC labelled rabbit anti rat γ-globulin serum.

Rabbit anti Rat γ-Globulin Serum

The sera collected from 5 rats (Wister and Donryu) were precipitated at 33% saturation of ammonium sulfate; the globulin precipitate was dialysed against 1/15 M phosphate buffer solution. Gamma globulin fractions were obtained by passing through the sephadex G 25 column with elution of phosphate buffer solution. The injection of the globulin fractions was made on the rabbit foot pad once a week with Freund's complete adjuvant; 6 week after the first injection and 7 days after the last injection, the serum was tested by immunoelectrophoresis after absorption by rat liver powder. Three distinct precipitin lines of IgA, IgG and IgM appeared but no other lines were detected against whole rat serum.

RESULTS

Renal Transplantation

Except for immediate death due to hemorrhage and thrombosis of the renal artery, 8 syngeneic renal transplantations with ureterostomy in the groin of the host were performed. On the 4th postoperative day, the angiogram of a host showed patency of arterial supply of the graft as shown in Fig. 4. These grafts passed urine for about 7 days after transplantation as shown in Table 1.

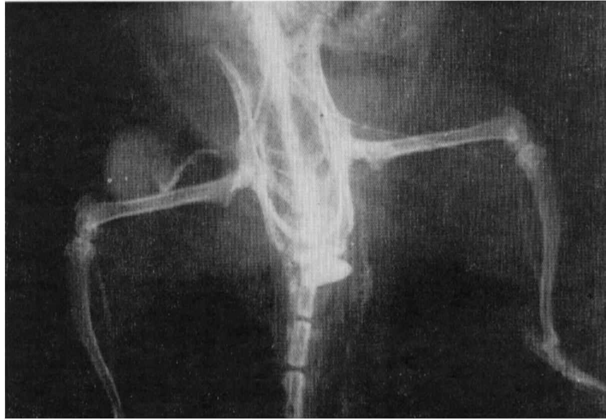


FIG. 4. 4th postoperative day: angiogram of host shows arterial supply to the graft.

TABLE 1. Kidney Transplantation with Ureterostomy

Donor	Host	Nephrectomy	Observed Urine Flow Post operative Days	No. Rat
M.P.	M.P.	(−)	7 8 9	6 1 1
M.P.	S.D.	(−)	2 3 4 8	2 5 4 1

The orifice of the ureter, however, was easily blocked by constriction of the skin, and hydronephrosis developed gradually due to obstruction. Reconstruction of the ureterostomy failed.

Twelve allotransplantation were made between M.P. and S.D.. Eleven grafted kidneys ceased to show flow of urine due to rejection occurring within a few days. Marked infiltration of lymphoid cells was noted in the grafted kidney sections, as shown in Fig. 5. Transplant reaction is apt to act in the small

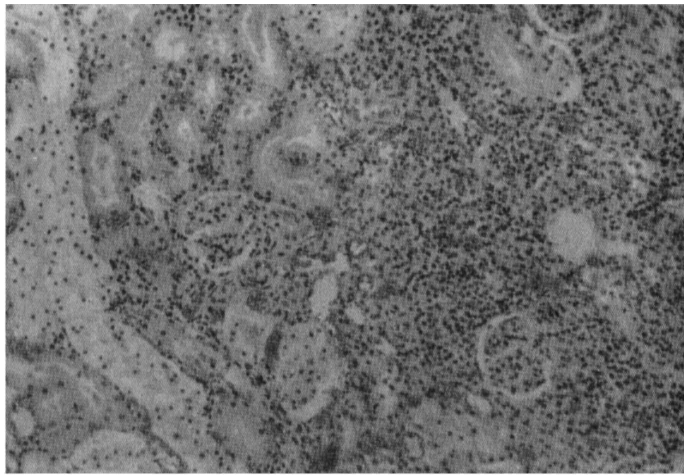


FIG. 5. 7th postoperative day: tissue section of allotransplant; marked lymphoid cell infiltration noted. (Hematoxylin and Eosin × 100)

vessels initially so that the grafts lack arterial supply in the early stage. The reason why the remaining one case was able to show of urine for 8 days is unknown, but the tissue section of this graft revealed severe rejection.

In case where both kidney and bladder were transplanted, 6 grafts showed hydronephrosis on the 7th postoperative day, as shown in Table 2. One, No.

TABLE 2. Both Kidney and Bladder Transplantation

Experiment	Donor	Host	Nephrectomy	Survival of Graft Postoperative Days
42	M.P.	M.P.	−	6
45	M.P.	M.P.	−	8
50	M.P.	M.P.	−	7
51	M.P.	M.P.	−	7
52	M.P.	M.P.	−	27
				Survival of Host Postoperative Days
56	M.P.	M.P.	+	4
57	M.P.	M.P.	+	7
60	M.P.	M.P.	+	4
61	M.P.	M.P.	+	10
62	M.P.	M.P.	+	4
67	M.P.	M.P.	+	11
68	M.P.	M.P.	+	10

52, survived for 27 days although the tissue section showed edema, tubular dilatation and granulation with rather minimum degeneration of the glomeruli. There was no transplant reaction observed in this tissue section as shown in

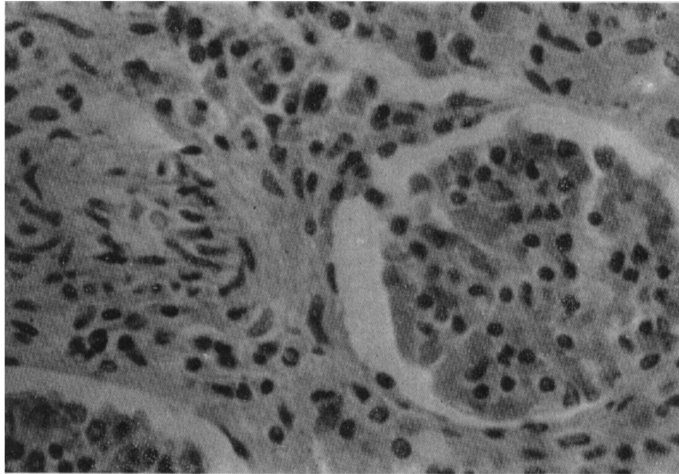


FIG. 6. 7th postoperative day: transplant reaction not marked: although thickened vessel walls noted; glomeruli still active. (Hematoxylin and Eosin × 400)

Fig. 6. When seven hosts were nephrectomized at the time of both kidney and bladder transplantation, all animals died within 11 days. The cause of death was presumably either adrenal insufficiency[13] or hydronephrosis due to hypofunction of the bladder, since the tissue section of these grafts showed only a slight degeneration of the kidney parenchyma and tubuli, as shown in Fig. 7. All control rats nephrectomized died within 48 hours.

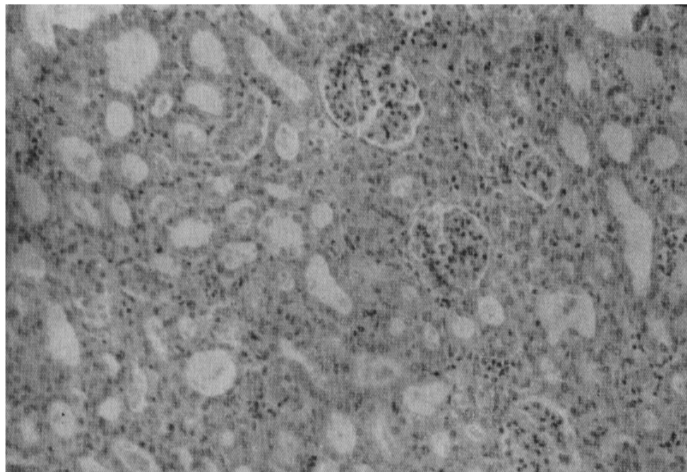


FIG. 7. Tissue section shows only slight degeneration of kidney parenchyma and tubuli. 10th postoperative day: syngeneic transplantation with nephrectomy of host. (Hematoxylin and Eosin × 100)

Immunological Studies

Both stamped preparations and cryotomed sections of the untreated kidney were stained by FITC labelled sera which had been harvested from either S.D. or M.P. rats on the 4th, 10th and 21st days after the immunization with M.P. kidney cells. All preparations of the grafted kidney failed to be stained with these sera, as shown in Table 3.

TABLE 3. Results of Immunological Study

Method	Donor	Recipient	Days after Immunization		
			4	10	21
Cell Adhesion	M.P. M.P.	S.D. M.P.	− −	+ −	± −
Immunofluorescent	M.P. M.P.	S.D. M.P.	− −	− −	− −

The grafted kidney sections were also stained by anti rat γ-globnlin serum in order to observe if the grafted kidney tissues adsorbed any host γ-globulin molecules. No grafted tissues were stained by anti rat γ-globulin serum, although infiltrated lymphoid cells or plasma cells which apparently produced γ-globulin, were stained with this serum.

The spleen cells of the S.D. rat which had been immunized with M.P. rat kidney cells 10 days previously, showed adherence, surrounding the target M.P. rat kidney cells as shown in Fig. 8. The spleen cells harvested on the 4th and 21st days after immunization, however, showed a slight degree of adherence to the target cells. The M.P. rat spleen cells immunized with the

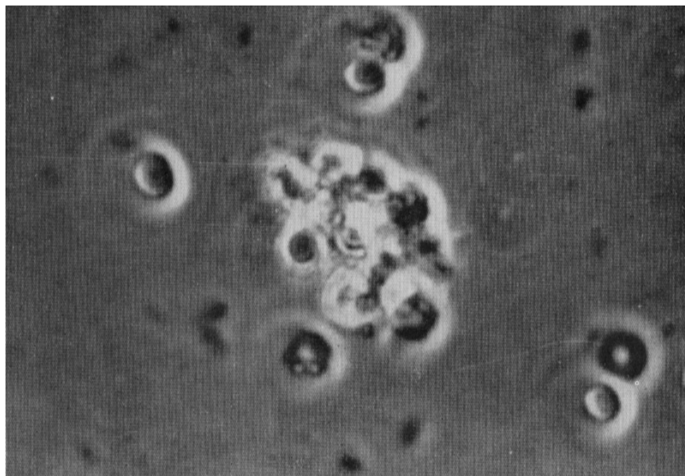


FIG. 8. Spleen cells adhere surrounding the target M.P. rat kidney cell. (×400)

syngeneic M.P. rat kidney cells failed to show any positive adherence test.

DISCUSSION

In the group where the ureter of the transplanted kidney was made into a fistula in the groin of the host, the urine flow continued for 7 days. The lumen of the ureter became gradually narrow and blocked by granulation. The graft developed hydronephrosis and finally pyelonephrosis. Gonzalez *et al.*[14] reported that ureterostomy of the grafted kidney at the neck of the host rat has to be reconstructed on the 7th to 10th postoperative days due to stenosis. At any rate ureterostomy was inevitably blocked and the graft ceased to function sooner or later.

Relatively long survival of graft could be expected in the group where both kidney and bladder were transplanted. In the study, the longest survival of the graft was the 27th postoperative day. The tissue section of this graft showed a comparatively low grade change in morphological feature. All hosts which had been nephrectomized at the time of renal transplantation died within 11 days. The cause of death was presumably either adrenal insufficiency or hypofunction of the grafted bladder due to denervation Sakai[15] described that denervation of the site of anastomosis of the bladder caused the renal graft to produce hyponephrosis in orthotopic rat renal transplantation.

The fact that untreated kidney tissues failed to be stained with rat serum immunized by syngeneic and allogeneic rat kidney cells may explain that the humoral factor does not act as a main role for the graft rejection in this experiment. By using anti rat γ-globulin to determine whether the grafted kidney tissues may adsorb the host globulin when the host reacted to the grafted kidney by means of the humoral factor, it was noted that the grafted kidney tissues were not stained by anti rat γ-globulin. Joseph *et al.*[16] described that fluorescent stains for IgG and IgM were consistently negative in all transplanted allogeneic and syngeneic kidneys and in host kidneys of all groups of rats.

Circulating antibody has been detected in human renal transplantation by Milgrom *et al.*[17] Horowitz *et al.*[18] showed fluoresceinated anti dog γ-globulin in the media of small vessels of the renal graft on the 4th postoperative day. However, no specific results was obtained to be involve in the immunological study of homologous transplantation.

Both humoral[19][20] and cellular[21][22] antibodies play an important role in the rejection mechanism, but the present studies indicate that in rat renal transplantation no localization of the humoral factor can be found. Cell-bound antibody production was observed as a cell adhesion phenomenon by the phase contrast microscopic examination in the group of allogeneic transplantation

although this phenomenon was not dominant. This accords with microscopic findings of allogeneic grafted kidney as shown in Fig. 5. The results here explain well that rat syngeneic kidney transplantation apparently diminishes the host immunological reaction.

If the technical problems of kidney transplantation can be removed, kidney grafts might be improved markedly. By various compositions of kidney allograft, considerable advantage may be expected also in the study of host immunological reactions in kidney transplantation.

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