Tohoku J. Exp. Med., 1998, **186**, 1-11

T Cell Hypofunctions and Glomerular Sclerotic and Angiogenic Changes Found Both in Rats Received Unilateral Nephrectomy plus Transplantation of Syngeneic Mesenteric Lymph Nodes and in Rats Received Unilateral Nephrectomy plus Splenectomy

TADAKO NAKATSUJI

Department of Transfusion, Hamamatsu University School of Medicine, Hamamatsu 431-3192

NAKATSUJI, T. *T Cell Hypofunctions and Glomerular Sclerotic and Angiogenic Changes Found Both in Rats Received Unilateral Nephrectomy plus Transplantation of Syngeneic Mesenteric Lymph Nodes and in Rats Received Unilateral Nephrectomy plus Splenectomy.* Tohoku J. Exp. Med., 1998, **186** (1), 1-11 —— Five male and 7 female Lewis rats unilaterally nephrectomized at the ages of 44-46 days were transplanted with the mesenteric lymph nodes (MLN) obtained from syngeneic rats 4 months after the nephrectomy. In addition, 6 males received unilateral nephrectomy plus splenectomy at the age of 38 days. All of these rats were observed for 8.5 months after the nephrectomy. Flow cytometry analyses indicated that 17-32% of MLN lymphocytes and 4-20% of peripheral blood (PB) lymphocytes lost CD2 antigen in the unilaterally nephrectomized rats. Approximately 20% of PB and MLN lymphocytes had lost CD2 antigen in the males with combined splenectomy and nephrectomy, one of which died of severe infection. The survival rates of the MLN graft after 4.5 months were 100% in the 5 nephrectomized males and 14% in the 7 nephrectomized females. The CD2 antigen on immunologically activated T cells was up-regulated. Twenty-eight% lymphocytes of the survived MLN graft and 51% PB T cells of the 7 females with MLN graft rejection showed the CD2 up-regulation. It was shown immunohistochemically that not only CD2-positive (+) cells but also CD4+ cells were increased in the cortex of the survived MLN graft. The hepatocyte growth factor receptor gene, c-met gene, was shown to be present in lymphocytes. The c-met was considered to cooperate with the CD2. Interstitial mononuclear cell infiltrations with glomerulosclerosis were found in the remaining kidneys of the males with unilateral nephrectomy plus MLN grafts. Glomerular angiogenesis and accompaning endothelial cell apoptosis were demonstrated without mesangial cell proliferation in the males with unilateral nephrectomy plus splenectomy. —— unilateral nephrectomy; CD2; c-met gene; splenectomy

Received June 18, 1998; revision accepted for publication September 12, 1998.

Address for reprints: Tadako Nakatsuji, M.D., Department of Transfusion, Hamamatsu University School of Medicine, Handa-cho 3600, Hamamatsu 431-3192, Japan.

e-mail: nh80415@hama-med.ac.jp

CD2 is a cell surface adhesion molecule on T cells and natural killer (NK) cells. CD2 has a long proline-rich cytoplasmic tail of the type bound to the Src family of tyrosine kinases, $p56^{lck}$ and $p59^{fyn}$ (Bell et al. 1996). Down-regulation of CD2 on T cells results in both diminished cell proliferation and disturbed interleukin (IL)-2 production (Fortner et al. 1998). Anti-CD2 monoclonal antibody (MAb) administration in vivo was reported to prolong graft survival and attenuate experimental autoimmunity (Schad et al. 1996; Hoffmann et al. 1997). It was shown in vitro that anti-CD2 MAb enhanced the production of transforming growth factor (TGF)-β, in which experiments NK cells served as the source of the TGF-β (Gray et al. 1998). Increased DNA synthesis of the renal tubular epithelium induces expression of mRNAs for collagens I, III and IV, and TGF-βI (Nath et al. 1998). The type II, but not type I, TGF-β receptor is reported to play a role in the formation of the renal vascular smooth muscle compartment (VSMC) (Liu and Ballermann 1998). On the other hand, hepatocyte growth factor (HGF) and HGF receptor (c-met) have been shown to be expressed on many target organs, including liver and kidney. The c-met oncogene encodes a transmembrane tyrosine kinase of the HGF receptor. The existence of a local HGF system (HGF and c-met) was reported in endothelial cells and VSMCs (Nakamura et al. 1995).

In this study, the regulation of CD2 on lymphocytes and the histopathological renal changes based on activation of cytokines were examined in the Lewis rat models of unilateral nephrectomy. Down-regulation of CD2 on lymphocytes was shown in the models. T cell hypofunctions were clear in the rats with combined splenectomy. Besides low numbers of CD2-positive (+) cells, splenectomized rats were infected more easily. The c-met functions on lymphocytes must be suppressed in the unilaterally nephrectomized rats. In rats with unilateral nephrectomy plus transplantation of mesenteric lymph nodes (MLN), interstitial mononuclear cell infiltration and glomerulosclerosis were detected in the remaining kidney. Activated TGF-βI production was shown indirectly in the rats with unilateral nephrectomy plus MLN transplantation. In rats with unilateral nephrectomy plus splenectomy, glomerular angiogenesis and apoptosis of endothelial cells were shown in the remaining kidney. Activated expression of TGF-βII receptor was suggested in the rats with combined splenectomy.

Materials and Methods

Animals

Lewis (LEW/Sea) rats maintained in the animal colony at the Hamamatsu University School of Medicine were used in this study.

Unilateral nephrectomy, transplantation of syngeneic MLN and splenectomy

In experimental (Exp.) A to E groups, the left kidney was removed from all

rats at 38–46 days of age. A dorsoventral incision was made on the left side. Renal vessels and a ureter were ligated and transected next to the left kidney (Waynforth and Flecknell 1992). Five males of Exp. A group and 7 females of Exp. B group were followed without any treatment for 8.2–8.3 months after the unilateral nephrectomy. MLN obtained from normal Lewis males and females was transplanted into 5 males of Exp. C group and 7 females of Exp. D group 4 months after the unilateral nephrectomy. The rats were followed for a further 4.5 months. For MLN transplantation, 1-cm long piece of syngeneic MLN was enclosed in the great omentum of the unilaterally nephrectomized rats. For each Exp. C rat, 0.33 mg of fibronectin fragment, H-Gly-Arg-Gly-Asp-Ser-OH, (BACHEM Feinchemikalien AG, Bubendorf, Switzerland) and 1.17 mg of hepatocyte growth factor (HGF), H-Gly-His-Ly-OH-AcOH-1.5H_2O, (BACHEM Feinchemikalien AG) were injected subcutaneously 4 days before the transplantation of syngeneic MLN. One mg of the fibronectin fragment H-Gly-Arg-Gly-Asp-Ser-OH (BACHEM Feinchemikalien AG) and 4 mg of HGF were injected subcutaneously on the day of transplantation into each Exp. C rat. Five mg of HGF was injected into each Exp. C rat on the first and second days posttransplantation. The fibronectin fragment H-Arg-Gly-Asp-Ser-OH (0.41 mg) and HGF (1.67 mg) were injected subcutaneously into each Exp. D rat on the day of transplantation. The HGF (2.62 mg to 2.86 mg per rat) was injected subcutaneously into Exp. D rats on the first and second days posttransplantation. Six male Exp. E rats were unilaterally nephrectomized with splenectomy at 38 days of age and followed for 8.5 months. Five non-nephrectomized Exp. F group females were transplanted with MLN obtained from normal Lewis rat females at 4.5 months of age. Exp. F rats were injected subcutaneously with 2 mg, 4 mg and 4 mg of HGF on the day of transplantation and on the third and fourth days posttransplantation, respectively. Exp. F females were followed for 4.5 months after the MLN transplantation.

Histopathological analyses

At the time of sacrifice, the body weights (BW) and right renal weights (r-RW) were measured in all the rats. The kidneys and host MLN of Exp. C and E rats and the surviving MLN grafts were fixed in 20% formalin to make tissue sections. The tissue sections were stained with hematoxylin-eosin (H-E). In Exp. C and E groups, PB was smeared and stained with May-Gruenwald-Giemsa. The right kidney of the Exp. E group was fixed with 2% glutaraldehyde for 2 hours and post-fixed with 1% osmium tetroxide. The pieces were embedded with epoxy resin and observed by a transmission electron microscope (JEM1220, JEOL, Tokyo).

Immunofluorescent tissue stains and flow cytometry (FCM) analyses

The sections of the MLN graft and the host MLN obtained from Exp. C were

stained with mouse anti-rat CD4 IgG1 conjugated with fluorescein isothiocyanate (FITC) and with mouse anti-rat CD2 IgG2a conjugated with FITC (Cedarlane Laboratories Ltd., Hornby, Ontario, Canada). The sections were incubated with anti-CD4 MAb or anti-CD2 MAb at room temperature for 20 minutes and at 37°C for 10 minutes. After washing with phosphate buffered saline (PBS), the sections were counter-stained with hematoxylin. The FITC-labelled sections were observed with a laser scanning confocal microscope (Bio-Rad Laboratories, Hercules, CL, USA).

The lymphocytes separated from PB, host MLN and MLN grafts were analyzed by direct immunofluorescent staining using an EPICS (R) Profile II Analyzer (Coulter, Miami, FL, USA). Red cells in the separated lymphocytes were removed using 1.5× lysing solution of Ylem code LYS100 (YLEM S.R.L., Roma, Italy). One million cells were incubated with 0.1–0.2 μg of mouse anti-rat CD2 IgG2a conjugated with FITC (Cedarlane Laboratories Ltd.) for 30 minutes at 4°C, and then fixed in 0.5% paraformaldehyde at 4°C over night. After washing with PBS, the FITC- labelled cells were analyzed by FCM. The cell mass of only lymphocytes was selected to calculate the percent of cells positive for CD2.

DNA extraction and polymerase chain reaction (PCR)

DNA was extracted from PB, bone marrow (BM), MLN and kidney. Template DNA preparation kits (Fermentas Ltd., Vilnius, Lithuania) were used for the DNA extraction. The primers specific for the c-met gene (5′-primer, 5′-TGATGAATGCCCCAGCGGTA-3′; 3′-primer, 5′-TTCATTGCCCATTGAGAT-3′) were synthesized by Katayama Chemical Co., Nagoya (Fukamachi et al. 1994). The cDNA was amplified in the reaction mixtures using Ampli Taq DNA polymerase (Perkin Elmer, Branchburg, NJ, USA). One, 10 and 30 cycles of the 3-step PCR were selected. The 3-step PCR consisted of thirty cycles of denaturing at 94°C for 1 minutes, annealing at 61°C for 1 minutes and extension at 72°C for 2 minutes. The PCR products were electrophoresed through a 2% agarose gel containing 0.5 mg/ml ethidium bromide. As markers, a 1-kbase DNA Ladder (Promega, Madison, WI, USA) and GeneRuler™ 100-bp DNA Ladder (Fermentas Ltd.) were subjected to electrophoresis on the agarose gels with Tris-Boric acid-EDTA (TBE) running solution.

Results

Table 1 shows the results of r-RW, r-RW/BW and 4.5 month survival ratios of the MLN grafts in the Exp. A to E rats that were followed for 8.2–8.5 months after the unilateral nephrectomy. Exp. F and control rats without nephrectomy were 9 and 8.5 months old. The r-RW ranged from 1.7 ± 0.3 g (Mean ± S.D.) to 1.9 ± 0.2 g in the males with unilateral nephrectomy. The r-RW was 1.2 ± 0.1 g in the females with unilateral nephrectomy. In the rats with unilateral nephrectomy, the remaining kidneys gained 0.5 g to 0.7 g in weight during the 8–9

TABLE 1. *Right(r)-RW, r-RW/BW and graft survival ratios in the Lewis rats with unilateral nephrectomy*

Exp. group-Sex (n)	Nephrectomy	r-RW M±S.D.(g)	r-RW/BW M±S.D.(%)	Graft survival ratio (%)
A-M (5)	(+)	1.8±0.1	0.52±0.02	Not done
B-F (7)	(+)	1.2±0.1	0.58±0.06	Not done
C-M (5)	(+)	1.9±0.2	0.52±0.03	100 (5/5)[a]
D-F (7)	(+)	1.2±0.1	0.54±0.03	14 (1/7)
E-M (5)	(+)	1.7±0.3	0.56±0.02	Not done[a,b]
F-F (5)	(−)	0.7±0.1	0.35±0.03	40 (2/5)
Cont-M (6)	(−)	1.2±0.1	0.34±0.01	Not done
Cont-F (4)	(−)	0.7±0.0	0.36±0.02	Not done

[a]Metarubricytes were recognized on the PB smears. [b]Splenectomy.

months. Among the males with unilateral nephrectomy, the males with splenectomy had the highest % of r-RW/BW, 0.56±0.02, which was 0.22% higher than the control. As one of the Exp. E males died of severe infection 4 months after the nephrectomy plus splenectomy, the weights could be measured in 5 rats. In the Exp. C group, 100% (5/5) of the MLN grafts survived for 4.5 months, although necrosis and fibrosis were found in the survived MLN grafts. In Exp. D and F females, 14% (1/7) and 40% (2/5) of the MLN grafts survived for 4.5 months, respectively. Metarubricytes (normoblasts) appeared often on the PB smears of Exp. C and E males. All the 5 kidneys of Exp. C group had focal findings of interstitial mononuclear cell infiltrations with glomerulosclerosis.

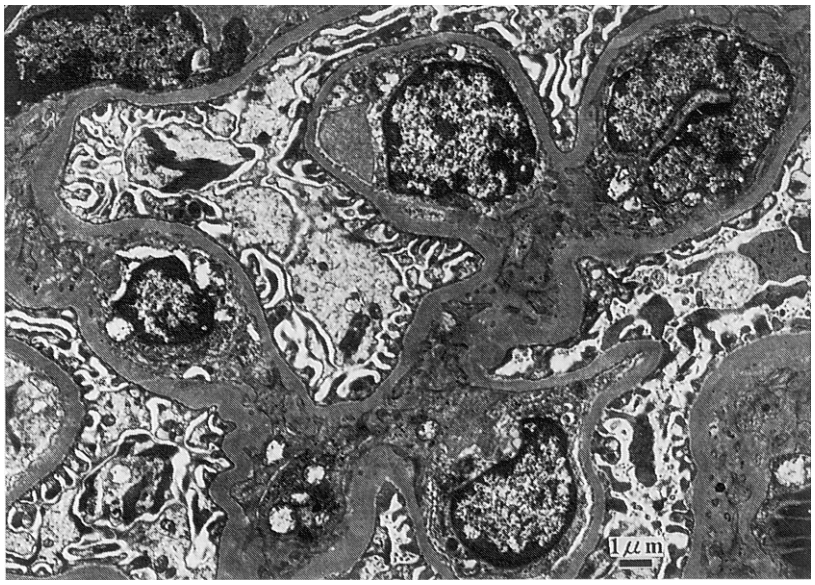

Fig. 1. Electron micrograph of the glomerulus in an Exp. E rat, which received unilateral nephrectomy plus splenectomy. Angiogenesis is seen. The capillary tubules are very narrow. At the corner on the lower right side, a thick subendothelial deposit and part of an apoptotic endothelial cell are seen. (Uranyl acetate-lead citrate double staining, ×6500).

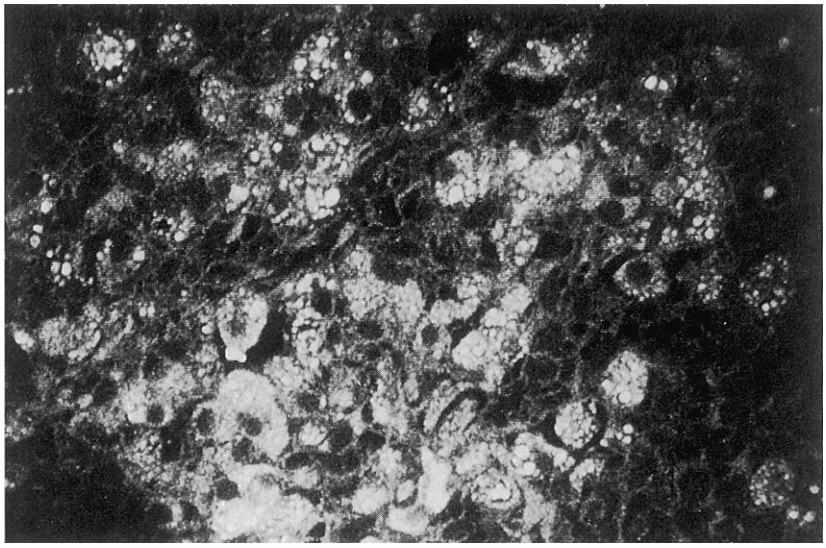

Fig. 2. The surviving MLN graft of an Exp. C rat. Many cortex lymphocytes are stained positively with anti-rat CD4 MAb conjugated with FITC. Compared with the host MLN, CD4+ T cells, which were stained positively by immunohistochemical method, were increased remarkably. (Immunofluorescent antibody staining).

But, in Exp. E group, only two kidneys had focal findings of interstitial mononuclear cell infiltration without glomerulosclelosis. Angiogenesis and endothelial cell apoptosis were demonstrated in the electron micrographs of Exp. E rat glomeruli (Fig. 1). Proximal convoluted tubules and some epithelial cells had many vacuoles in the cytoplasm. Brush borders of the proximal convoluted tubules were 3 μm in the length. The proliferation of mesangial cells was not found. Immunofluorescent staining with anti-CD4 MAb is shown in Fig. 2. In the MLN graft of Exp. C rats, many lymphocytes were stained positively with anti-CD4 MAb. Immunohistochemically, the numbers of CD4-positive (+) cells were high in the graft, but not in the host MLN. The anti-CD4 MAb bound more strongly to the lymphocytes of the graft than did anti-CD2 MAb.

The results of FCM are summarized in Table 2. The lymphocytes of the rats with unilateral nephrectomy had a reduced proportion of CD2-positive (+) cells. Exp. A and B rat lymphocytes had 9-10% few CD2+ cells than control rat lymphocytes in the MLN. Exp. C rats, which had graft survival of 100%, had a 13% reduction of CD2+ PB cells and a 17% reduction of CD2+ MLN cells. In the Exp. D females which had a 14% survival rate of the MLN graft, 32% of MLN lymphocytes lost the CD2 antigen. However, in the Exp. D females with 85 ± 7% of PB CD2+ cells, 51 ± 13% of the PB lymphocytes had up-regulation of CD2, which was indicated as a single peak shifted to the right in FCM (data not shown). The MLN grafts of the Exp. C, D and F groups had 76 ± 6% to 82% CD2+ cells, in which 28 ± 13% CD2+ cells were also shown as a single peak shifted to the right in FCM (data not shown). Unilaterally nephrectomized Exp. E rats with splenectomy had depressed CD2 expression, and 20% of the PB lymphocytes lost

TABLE 2. *Percent of CD2-positive (+) lymphocytes in PB and MLN in the Lewis rats with unilateral nephrectomy*

Exp. group-Sex (n)	CD2-positive PB cell M±S.D.(%)	CD2-positive MLN cell M±S.D.(%)
A[a]-M (5)	Not tested	70±14
B[a]-F (7)	84± 5	75± 6
C[b]-M (5)	74± 7	63± 4
MLN grafts (5)		76± 6[e]
D[b]-F (7)	85± 7[e]	52±11
MLN graft (1)		81[e]
E[c]-M (5)	67±11	59± 2 (n=3)
		88± 5 (n=2)
F[d]-F (5)	85± 1[e]	76± 8
MLN grafts (2)		82[e]
Cont-M (6)	87± 4	80± 6
Cont-F (4)	89± 1	84± 2

[a]Nephrectomy. [b]Nephrectomy and MLN transplantation. [c]Nephrectomy and splenectomy. [d]MLN transplantation. [e]Two peaks of CD2+ cells were seen.

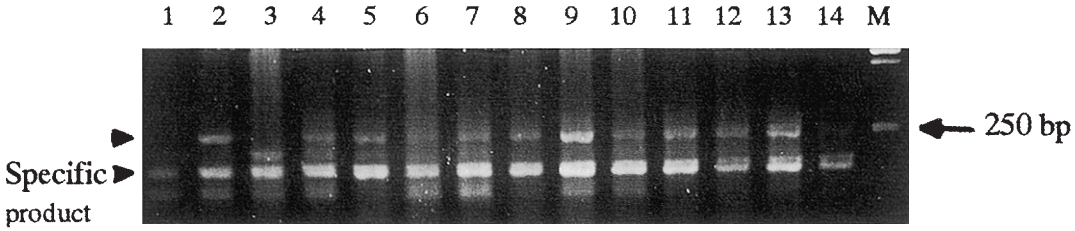

Fig. 3. PCR analysis of the c-met gene using 5′-primer. Specific products of 240 bp and 160 bp appear here. The DNA of lanes 1 to 10 was extracted from the kindeys of an Exp. A rat (lane 1), Exp. C rats (lanes 2–4), Exp. E rats (lanes 5–6), controls (lanes 7–9) and an Exp. F rat (lane 10). The DNA of lanes 11 to 14 was extracted from the BM cells of Exp. A rats (lanes 11–12) and controls (lanes 13–14). M is the marker with a 250 bp fragment.

CD2 expression. Three rats of the Exp. E group lost CD2 in 21% of MLN lymphocytes. Non-nephrectomized Exp. F females, with 40% of MLN graft survival, had 48±28% of up-regulated CD2+ cells in the PB (data not shown). The % of CD2+ MLN cells was near the normal level in the Exp. F rats.

Fig. 3 shows the PCR products of the c-met gene. The DNA used for the PCR was extracted from the kidney and BM of Exp. A, C, E and F group rats and control rats. The c-met gene products of 240 bp and 160 bp were amplified in the PCR using only 5′-primer. Fig. 4 shows the PCR products of the c-met gene using both 5′-primer and 3′-primer. The DNA analyzed by PCR was extracted from the MLN, PB and kidney of Exp. B, C and E group rats and control rats. The same c-met gene products of 240 bp and 160 bp were more specifically amplified. The sizes of the products were determined by comparison with sizes of

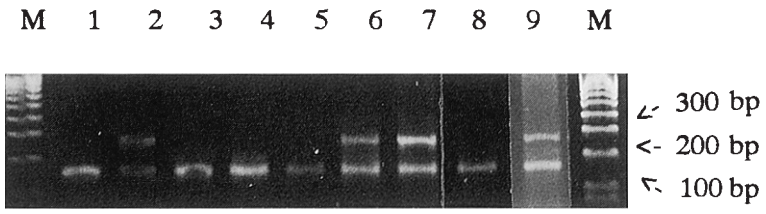

Fig. 4. PCR analysis of the c-met gene using both 5′-primer and 3′-primer. Specific products of 240 bp and 160 bp are amplified. The DNA of lanes 1 to 3 was extracted from the MLN cells of an Exp. C rat (lane 1), a control (lane 2) and an Exp. E rat (lane 3). The DNA of lanes 5 to 8 was extracted from the PB cells of controls (lanes 4–5 and 7) and Exp. B rats (lanes 6 and 8). The DNA of lane 9 was extracted from the kideny of an Exp. C rat.

the markers (M) used. The two specific products appeared in varying amounts, depending partially on minor differences of PCR conditions. Thus, the c-met gene was confirmed to be present in kidney, BM, MLN, and PB cells.

Discussion

The prolongation of graft survival and the amelioration of experimental autoimmunity have been reported to result from the in vivo administration of anti-CD2 MAb (Schad et al. 1996; Hoffmann et al. 1997). In the unilaterally nephrectomized males, who had a 100% survival rate of the MLN graft, the prolongation of the MLN graft survival was explained well by the down-regulation of CD2 antigen on lymphocytes. On the contrary, immunologically activated T cells showed up-regulation of CD2. In the unilaterally nephrectomized females, who had graft rejection of the transplanted MLN, PB lymphocytes had up-regulation of CD2 and host MLN lymphocytes lost CD2 antigen. The males received unilateral nephrectomy plus splenectomy had repeated pulmonary infections and in the rats, the down-regulation of CD2 was found in 20–21% of the PB and MLN lymphocytes. One of the splenectomized males died of severe infection. The body weight gains of all the splenectomized males were smaller than those of the other males, as indicated by the highest % of r-RW/BW. The c-met gene is expressed on lymphocytes, as shown in this study. The down-regulation of CD2 on T cells was considered to be related to the down-regulation of c-met on T cells. The cytoplasmic domains of CD2 and c-met bind to trans-membrane tyrosine kinases. Disturbed tyrosine phosphorylation of lymphocytes might have occurred in the rats with unilateral nephrectomy. The down-modulations of CD2 and c-met on lymphocytes reflected the hypofunctioning T cells. In the splenectomized rats, the tyrosine phosphorylation of the PB lymphocytes seemed to be disturbed more strongly than in the other rats. Increased infection due to T cell dysfunction occurred in the rats with splenectomy. Metarubricytes in the PB of the unilaterally nephrectomized males with MLN grafts were considered to depend on hypo-splenic states. In their passage through the interendothelial slits of the splenic sinus, the nephrectomized-

transplanted male spleen might have had the reduced phagocytic function of endothelial reticular cells.

The MLN grafts which survived for 4.5 months showed the necrosis, surrounded by fibrosis. The remaining cortex lymphocytes were stained strongly with anti-CD4 MAb. T cells with CD4 antigen were increased in the MLN graft. T cells with CD4 are important in inflammatory rejective reactions toward grafts. Cellular interactions of CD4+ T cells with macrophages and B cells must have led to the production of cytokines and immunoglobulins. In developing fetal MLN, there are CD4+CD3− and lymphotoxin (LT) β+ cells that can differentiate to dendritic antigen-presenting cells (APC), NK cells and follicular cells (Mebius et al. 1997). CD4+CD3−LTβ+ cells, which express LTβ and the chemokine receptor BLR1, are necessary to develop lymphoid organs. As CD4 antigen is expressed on many cortex cells, the CD4+CD3−LTβ+ cells, which are the precursors of NK cells, seemed to proliferate in the MLN graft. As CD2 expression on the lymphocytes of the grafts was also strong, as determined by FCM and immunohistochemically, NK cells, with expression of CD2, must proliferate in the MLN grafts.

The unilaterally nephrectomized males, with the 100% survival of the MLN graft received injections of both HGF and fibronectin fragments. The treatment of VSMCs with fibronectin activates the protein kinase C (PKC) pathway and induces cellular spreading via integrin receptors within 30 minutes (Haller et al. 1998). VSMC binding to fibronectin fragments probably helped the VSMC spreading and migration in the omentum, where the syngeneic MLN graft was transplanted. The expressions of HGF and HGF receptor (c-met) mRNA in controlling endothelial cells and VSMCs were also reported to have a positive role in vascular functions (Nakamura et al. 1995). Both fibronectin fragments and HGF might have helped to reconstruct the blood circulation in the grafts. Generally, not only high HGF synthesis, but also high production of fibronectin, are said to occur at the early stage of postnephrectomy, which is followed by the proliferative responsiveness of mesangial cells (Weissgarten et al. 1998; Tada and Isogai 1998). Thus, nephrectomy itself was concluded to exert a positive effect on the graft survival.

The kidneys of the unilaterally nephrectomized males with the MLN graft showed the focal findings of interstitial mononuclear cell infiltration with glomerulosclerosis. As it has been reported that the expression of the mRNAs of TGF-βI and collagens I, III and IV were promoted by increased DNA synthesis of renal tubular epithelium (Nath et al. 1998). Renal fibrosis has been caused by the high production of TGF-βI and collagens. In the rats with combined MLN transplantation, proliferated NK cells and CD4+ cells in the MLN grafts also served as an another source of the TGF-β production (Gray et al. 1998). From both the findings of remaining r-kidneys and surviving MLN grafts, high production of TGF-βI was concluded in the rats received nephrectomy plus MLN

transplantation. The remaining kidneys of the males with splenectomy showed glomerular angiogenesis and related endothelial cell apoptosis. However, glomerulosclerosis was not found in any of the male r-kindeys with splenectomy. TGF-β type I receptor was not detected in vascular elements, but TGF-β type II receptor was expressed in renal stromal VSMC precursors and developing blood vessels (Liu and Ballermann 1998). Increased expression of TGF-β type II receptor in renal VSMC precursors seemed to occur in the remaining kidney of the splenectomized rats with glomerular angiogenesis.

Acknowledgments

The author thanks all the members of the Experimental and Practical Equipment Center, Hamamatsu University School of Medicine, for excellent technical assistance. This work was supported partially by the Kidney Bank of Shizuoka Prefecture, Japan.

References

1) Bell, G.M., Fargnoli, J., Bolen, J.B., Kish, L. & Imboden, J.B. (1996) The SH3 domain of $p56^{lck}$ binds to proline-rich sequences in the cytoplasmic domain of CD2. *J. Exp. Med.*, **183**, 169–178.
2) Fortner, K.A., Russell, J.Q. & Budd, R.C. (1998) Down-modulation of CD2 delays deletion of superantigen-responsive T cells. *Eur. J. Immunol.*, **28**, 70–79.
3) Fukamachi, H., Ichinose M., Tsukada, S., Kakei, N., Suzuki, T., Miki, K., Kurokawa, K. & Masui, T. (1994) Hepatocyte growth factor region specifically stimulates gastro-intestinal epithelial growth in primary culture. *Biochem. Biophys. Res. Commun.*, **205**, 1445–1451.
4) Gray, J.D., Hirokawa, M., Ohtsuka, K. & Horwitz, D.A. (1998) Generation of an inhibitory circuit involving CD8+T cells, IL-2, and NK cell-derived TGF-β: Contrasting effects of anti-CD2 and anti-CD3. *J. Immunol.*, **160**, 2248–2254.
5) Haller, H., Lindschau, C., Maasch, C., Olthoff, H., Kurscheid, D. & Luft, F.C. (1998) Integrin-induced protein kinase Cα and Cε translocation to focal adhesions mediates vascular smooth muscle cell spreading. *Circ. Res.*, **82**, 157–165.
6) Hoffmann, J.C., Herklotz, C., Zeidler, H., Bayer, B. & Westermann, J. (1997) Anti-CD2 (OX34) MoAb treatment of adjuvant arthritic rats: Attenuation of established arthritis, selective depletion of CD4+T cells, and CD2 down-modulation. *Clin. Exp. Immunol.*, **110**, 63–71.
7) Liu, A. & Ballermann B.J. (1998) TGF-β type II receptor in rat vascular development: Localization to juxtaglomerular cells. *Kidney Int.*, **53**, 716–725.
8) Mebius, R.E., Rennert, P. & Weissman, I.L. (1997) Developing lymph nodes collect CD4+CD3−LTβ+ cells that can differentiate to APC, NK cells, and follicular cells but not T or B cells. *Immunity*, **7**, 493–504.
9) Nakamura, Y., Morishita, R., Higaki, J., Kida, I., Aoki, M., Moriguchi, A., Yamada, K., Hayashi, S., Yo, Y., Matsumoto, K., Nakamura, T. & Ogihara, T. (1995) Expression of local hepatocyte growth factor system in vascular tissues. *Biochem. Biophs. Res. Commun.*, **215**, 483–488.
10) Nath, K.A., Grande, J., Croatt, A., Haugen, J., Kim, Y. & Rosenberg, M.E. (1998) Redox regulation of renal DNA synthesis, transforming growth factor-β1 and collagen gene expression. *Kidney Int.*, **53**, 367–381.
11) Schad, V., Greenstein, J.L., Giovino-Barry, V., LeGuern, A., Matejic, T., Glaser, R., Dickerson, M., Xu, Y., Bazin, H., Latinne, D., Monroy, R. & White-Scharf, M.E. (1996) An anti-CD2 monoclonal antibody that elicits alloantigen-specific hyporesponsiveness.

Transplant. Proc., **28**, 2051–2053.

12) Tada, H. & Isogai, S. (1998) The fibronectin production is incresed by thrombospondin via activation of TGF-β in cultured human mesangial cells. *Nephron*, **79**, 38–43.

13) Waynforth, H.B. & Flecknell, P.A. (1992) *Experimental and Surgical Technique in the Rat*, Academic Press Limited, London.

14) Weissgarten, J., Modai, D., Cohn, M., Berman, S. & Averbukh, Z. (1998) The proliferative responsiveness of mesangial cells to postnephrectomy serum declines progressively with time elapsing after contralateral nephrectomy. *Nephron*, **78**, 212–214.