

## PAPER

## Lupus nephritis reoccurs following transplantation in the lupus prone mouse

P Hamar<sup>1,\*</sup>, M Wang<sup>1,2,3,\*</sup>, M Godó<sup>1</sup>, G Kökény<sup>1</sup>, L Rosivall<sup>1</sup>, N Ouyang<sup>4</sup> and U Heemann<sup>3</sup>  
<sup>1</sup>Institute of Pathophysiology, Department of Medicine, Semmelweis University, Budapest, Hungary; <sup>2</sup>Department of Surgery, Sun-Yat-Sen Memorial Hospital, Guangzhou, People's Republic of China; <sup>3</sup>Department of Nephrology/ Klinikum rechts der Isar, Munich Germany; and <sup>4</sup>Texas A&M University, Pathology, College Station, TX 77843, USA

The incidence and pathomechanism of recurrent lupus nephritis (RLN) after transplantation is not clearly understood. Burning out of the autoimmune process or local immunoregulatory mechanisms in the kidney may be responsible for the low incidence of recurrence. These mechanisms cannot be investigated in human subjects, due to post-transplant immunosuppression. To investigate the pathomechanisms of RLN, male and female kidneys were transplanted from FAS deficient lupus prone (LPR) or control (FAS intact) MRL mice into either LPR or MRL recipients. Urinary protein and blood urea were assessed. Double negative (DN) lymphocyte proliferation was determined by flow cytometry. Two months after transplantation inflammatory infiltration of the glomerular, vascular and interstitial compartments were determined. Renal function as demonstrated by blood urea levels was normal in MRL recipients, but elevated in LPR recipients, independent of the donor strain. Paralleling functional results, inflammatory infiltration was mild or absent in MRL recipients of MRL grafts, and mild to moderate in MRL recipients of LPR grafts, suggesting that kidney removal from the autoimmune (LPR) environment significantly reduced inflammation. Graft infiltration was most severe in LPR recipients: grafts were similarly inflamed independent of the donor. All LPR recipients had significantly less CD4<sup>+</sup> Th cells versus MRL mice. Transplantation of LPR grafts into MRL recipients reduced CD4<sup>+</sup> Th cell percentage, accompanied by a slight induction of lupus autoantibody production. Our results demonstrate that lupus nephritis is not kidney specific in the LPR model with recurrence after transplantation in the absence of immunosuppression. *Lupus* (2010) 19, 175–181.

**Key words:** LPR mice; lupus nephritis; transplantation

### Introduction

Recurrent lupus nephritis (RLN) following transplantation has been the subject of debate recently. Previously it has been held that systemic lupus erythematosus (SLE) is a disease induced by circulating antibodies, immune complexes and subsequent complement deposition. However, lupus nephritis (LN) could be a kidney-based disease, as recurrence of the systemic disease has been reported to rarely follow nephrectomy or dialysis.<sup>1,2</sup> Recurrence was previously reported to be 1–4%.

A 10-year analysis demonstrated that patient and graft survival was similar in patients transplanted due to LN or other causes of renal failure. It was not only LN, but also the systemic signs of SLE that diminished after transplantation,<sup>3</sup> suggesting that replacing the kidney may heal the disease or lupus may 'burn out' following a kidney transplant. Cytokines over-expressed by renal parenchymal or infiltrating cells may determine the course of human and experimental LN.<sup>4</sup> Furthermore, some data suggest that local immunoregulatory mechanisms in the kidney are involved in LN such as *in situ* formation of immune deposits. As these deposits reflect intrinsic glomerular antigens the localization may differ between individuals. This theory may also explain the large variety in clinical manifestations of LN.<sup>5</sup>

On the other hand recent data question the rare recurrence of LN after transplantation.<sup>6</sup>

Correspondence to: Péter Hamar, MD, PhD, Institute of Pathophysiology, Department of Medicine, Semmelweis University, Budapest, 1089 Nagyvarad ter 4, Hungary.

E-mail: [hampet@net.sote.hu](mailto:hampet@net.sote.hu)

\*These authors contributed equally to this paper.

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With more detailed immunohistologic and electromicroscopic investigations, Goral and colleagues demonstrated that RLN in the graft occurred in 52% of patients who underwent graft biopsy and systemic activity may happen even during dialysis.<sup>7</sup>

Furthermore, the basic mechanisms of SLE are also under reevaluation. The uniform theory of circulating antibody deposition in innocent bystander organs does not explain the wide variety of disease manifestations ranging from mild antiphospholipid syndrome in some patients, to severe LN or neuro-lupus in others. The central pathogenic role of autoantibodies in lupus pathogenesis also has been questioned recently, as more and more data accumulates in favor of a cellular immune response.<sup>8</sup> Thus, pathologic activation of helper Th cells by cytokines (such as interferons) may be central in SLE pathogenesis.<sup>9</sup> Local, organ-specific mechanisms and interactions between target tissue and immune effector pathways with distinct auto-antibody patterns may be important driving forces of the autoimmune process.<sup>10</sup>

The pathomechanism of SLE and RLN is obscured by post-transplant immunosuppression in human subjects.

In the present study we investigated whether LN is a kidney-specific or rather a systemic disease, and if RLN appears after removal of the own kidney in the lupus prone (LPR) mouse model of LN. If SLE is a kidney-specific disease, lupus could be induced in MRL mice by implantation of an LPR kidney graft. This question cannot be investigated in a human system, as kidneys from lupus patients are never used as grafts.

LPR mice, homozygous for the Fas<sup>lpr</sup> autosomal recessive mutation develop a systemic autoimmune disease, similar to human SLE,<sup>11</sup> leading to uremic death at age 3–6 months, whereas control (FAS intact) MRL mice develop signs of systemic autoimmunity only after 12 months of age.<sup>12</sup> Systemic lupus in the LPR model is held to be a consequence of autoreactive T-cell proliferation inducing auto-antibody formation by B-cell clonal expansion,<sup>13</sup> which can be healed by stem cell replacement.<sup>14</sup>

On the other hand, some studies suggest a role for *local immune responses* (mediated by IFN- $\gamma$  and MHC-II expression) in nephritis development in LPR mice.<sup>15,16</sup> Furthermore, it has been concluded, that renal antigens<sup>17</sup> or renal antigen-specific autoreactive T-cells contribute to nephritis in LPR mice.<sup>18</sup> Factors responsible for the development, trafficking, and effector functions of kidney-infiltrating T-cells are poorly defined in the LPR mouse.<sup>19</sup>

Thus, we aimed to examine whether the LPR mouse model is suitable to investigate local, renal factors of nephritis or is the disease mainly a systemic process driven by lymphoproliferation. We hypothesized that removing the native kidney of LPR mice and replacing it with a kidney donated from a syngenic non-autoimmune control MRL strain may suppress the nephritis.

Furthermore, our study allows deeper insight into the pathomechanism of nephritis in LPR mice. Thus, we aimed to test whether normal (young MRL) mice develop the autoimmune disease if a nephritic (LPR) kidney is transplanted, and whether a normal (MRL) kidney develops nephritis in an LPR mouse. As estrogen is a precipitating factor in LN in human subjects, as well as in the LPR mouse model, we tested our hypothesis on both male and female animals.

## Methods

### *Experimental animals*

Four-month-old inbred, male and female MRL/MpJ-*Tnfrsf6*<sup>lpr</sup> (previously MRL/MpJ-*Fas*<sup>lpr</sup>); LPR mice (weighing  $35 \pm 9$  g, blood urea  $8.0 \pm 5.2$  mmol/l, urinary protein  $1.0 \pm 0.2$  mg/day) and wild-type MRL/MpJ; MRL (weighing  $41 \pm 4$  g, blood urea  $3.0 \pm 0.4$  mmol/l, urinary protein  $0.4 \pm 0.1$  mg/day) mice were ordered from Jackson Laboratory (/JAX/ Bar Harbor, ME, USA) ( $n = 6$ /group) and housed under specified pathogen free (spf) conditions in an individually ventilated cage system (IVIG) (Charles River, Budapest, Hungary), under standard conditions (light on 08:00–20:00; 40–70% relative humidity,  $22 \pm 1^\circ\text{C}$ ). The mice had free access to chow (Charles River, Budapest, Hungary) and tap water. All experimental procedures were in accordance with guidelines set by the Institutional Animal Care and Use Committee of Semmelweis University, and the Hungarian law on animal care and protection (1998/XVIII, 243/1998(XII.31)), and this study complied with the NIH guidelines.<sup>20</sup>

### *Kidney transplantation*

Orthotopic kidney transplantation was performed as described previously.<sup>21</sup> Briefly, mice were anesthetized with ketamine (Ketanest 100 mg/kg; Essex Pharma GmbH, Munich, Germany) and xylazine (Rompun 2 mg/kg; Bayer GmbH, Leverkusen, Germany). From a median laparotomy, both donor and recipient left kidneys were exposed, and the



renal vessels and the inferior vena cava and aorta were isolated. The donor kidney was perfused with ice-cold saline, and the renal vessels were excised together with an aortic or vena cava patch. The donor ureter was excised in full length. Following removal of the recipient kidney, the graft was orthotopically placed, and the aorta and vena cava patches were sutured with 10.0 prolene (Ethicon/Johnson and Johnson, Brussels, Belgium). The end of the ureter was pulled into the urinary bladder through a small incision. The contralateral right native kidney was removed in a second operation 7 days after transplantation. To prevent infectious complications during the perioperative phase, mice received 20 mg/kg Cephtriaxone i.m. (Rocephine®) (Roche Hungary Ltd, Budaörs, Hungary) after both operations.

#### *Experimental design*

Male LPR donor kidneys were transplanted into either male MRL (mLPR → mMRL) or male LPR recipients (mLPR → mLPR), and male MRL donor kidneys into male LPR (mMRL → mLPR) or male MRL recipients (mMRL → mMRL). In addition, female MRL kidneys were transplanted either into female LPR (fMRL → fLPR) or female MRL recipients (fMRL → fMRL). Female LPR kidneys were not used for grafting due to technical difficulties (large lymph nodes in the renal hilum). In each group, four mice were harvested 8 weeks after transplantation for histological analysis.

#### *Functional measurements, Routine chemistry*

For protein analysis, mice were placed in metabolic cages (Techniplast, Buguggiate, Italy) for 24-hour urine collection, at the end of the study, before harvesting the kidneys. To prevent bacterial growth, gentamicin was added to collection tubes. Urinary protein excretion was determined with the Biureth method using Bio-Rad protein assay kit according to the manufacturer's instructions (BioRad Laboratories, Munich, Germany) and reported as milligrams of protein per mouse per day. Absorbance was determined at a Philips PU8700 spectrophotometer at a wavelength of 595 nm.

Blood urea concentrations were determined using a Reflotron IV automat (Boehringer Mannheim, Germany) with a fast-test-stripe, from 32 µl whole blood.

#### *Histological analysis*

Removed kidney grafts were immersion-fixed in buffered formalin, embedded in paraffin, and 4 µm

sections were stained with hematoxylin/eosin (HE) and periodic acid Schiff (PAS).

#### *Quantification of graft inflammation*

Graft inflammation was quantified according to Berden *et al.*<sup>22</sup> and as described previously.<sup>23</sup> Briefly the quantification was as follows. Glomerulonephritis: grade 0: no inflammation; 1: mesangial thickening and/or mesangial and glomerular hypercellularity; 2: all previous plus inflammatory exudate/hyaline and/or adhesion to Bowman's capsule. 3: glomerular obliteration >70% of glomeruli. Interstitial infiltration: 0: none; 1: mild; 2: moderate; 3: severe interstitial infiltration. Vascular changes: 0 normal endothelial monolayer, no deposition of PAS positive material or inflammatory cells; 1: minimal PAS positive deposition within the vessel wall; 2: PAS positive deposition with lumen narrowing in vessel wall; 3: myointimal thickening and vessel wall destruction. Perivascular infiltration: Number of inflammatory cell layers was scored semiquantitatively: 0: no infiltration; 1: mild (<5 cell layers surrounding the vessel); 2: moderate (5–10 inflammatory cell layers); 3: severe infiltration (>10 layers).

Total grade: 0 = 0; I = 1–4; II = 5–8; III = 9–12.

#### *Flow cytometry*

##### *Monoclonal antibodies (mAbs)*

The following anti-mouse mAbs were purchased from PharMingen (Kasztel Med, Budapest, Hungary): anti-CD3-complex (PE-conjugated / 17A2/); anti-CD4 (FITC-conjugated / GK 1.5/); anti-CD8 (APC-conjugated / 53-6.72/); anti-CD45/B220 isoform (PerCP-conjugated / RA3-6B2/).

##### *FACS analysis*

To determine the immune state of the animals, the percentage of CD4-, CD8- (DN) TCR+, B220+ (DN B220+) cells was analyzed in peripheral blood samples. Blood was collected from the aorta before harvesting. Surface antigen expression was analyzed using a Beckman FACScan®/FACS Calibur flow cytometer (Becton Dickinson Immunocytometry, San Jose, CA, USA) as described previously.<sup>24,25</sup> Briefly, 32 µl peripheral blood sample was incubated in 12 75-mm Falcon tubes (Becton Dickinson Labware Europe, Meylan Cedex, France) with saturating concentrations (1 µl) of mAbs in the dark at room temperature for 20 min, and then with 1 ml erythrocyte lysing solution (Becton Dickinson Labware Europe, Meylan Cedex, France) in the dark at room temperature for exactly 10 minutes. To remove unbound



antibodies, samples were washed in 2 ml phosphate buffered saline (PBS), then fixed for 1 h in 350  $\mu$ l cold 2% paraformaldehyde and analyzed. Fluorescence was measured on duplicate samples. List mode data were analyzed using a Cell Quest 1.1.1 software (Becton Dickinson, San Jose, CA, USA). Fluorescence signals were recorded producing histograms of the gated cells and the data were expressed as the mean of relative fluorescence.

#### Measurement of lupus autoantibody panel

Anti-smiths (Sm), anti-double stranded DNA (dsDNA) and anti-histone (H) antibodies were qualitatively measured by commercial enzyme-linked immunosorbent assay (ELISA; Orgentec, Mainz, Germany) according to the manufacturer's instructions. As detection antibody, horseradish peroxidase (HRP) conjugated anti-mouse IgG (Sigma-Aldrich, St Louis, MO, USA) was used (1:1000 dilution in PBS, 1% Tween, 1% BSA). The cut-off value for each ELISA was determined by testing sera of 12 healthy mice of the outbred NMRI strain. Data are given as average OD  $\pm$  SD.

#### Data analysis

Data are expressed as means  $\pm$  SD. Statistical analyses were performed by two-way analysis of variance (ANOVA) for repeated measures followed by the Tukey *post hoc* test or Student's *t*-test, as appropriate. Statistical significance was considered at  $p < 0.05$ .

## Results

#### Renal function

In 4-month-old male LPR mice the average blood urea was significantly higher as compared with MRL ( $8.0 \pm 5.2$  versus  $3.0 \pm 0.4$  mmol/l,  $p < 0.05$ ) indicating that in LPR mice the autoimmune disease had already affected the kidneys. Blood urea was even higher in female LPR mice, as estrogen is known to accelerate disease progression in the LPR model. Blood urea and proteinuria remained within the normal range in both female and male MRL recipients. LPR recipients of either sex had pathologic proteinuria. The highest blood urea and urinary protein excretion values were measured in female LPR recipients (Table 1).

#### Histology

Overall grading of the grafts demonstrated that inflammatory infiltration was practically absent in

both male and female MRL isografts, suggesting that transplantation per se did not induce significant inflammation, 2 months after transplantation. Grade III inflammation was only observed in LPR recipients (more severe in females). Graft infiltration was independent of the donor strain; kidneys from MRL donors also revealed signs of inflammation: grade III in females, and grade II–III in male LPR recipients. However, removing the kidney from the autoimmune (LPR) background significantly reduced inflammation in male mice (mLPR to mMRL).

As expected in a type III autoimmune process of immunocomplex deposition, inflammatory infiltration was localized to vascular structures. Specifically, inflammatory infiltration was severe (grade 3) in the glomerular and vascular compartments, with only mild (grade 1) interstitial inflammation in age matched non-transplanted LPR animals. On the other hand, only mild glomerular inflammatory infiltration was present in just one male and one female MRL recipients of MRL kidneys (Table 2), whereas LPR recipients of MRL kidneys had grade 3 glomerular inflammation in female and grade 2–3 glomerular inflammation in male mice. Surprisingly, whereas male LPR recipients of LPR kidneys had severe glomerular inflammation, this was practically absent in male MRL recipients of LPR kidneys, suggesting that despite

**Table 1** Renal function. Initial values at the time of transplantation of LPR mice were blood urea  $8.0 \pm 5.2$  mmol/l and urinary protein  $1.0 \pm 0.2$  mg/day and for MRL blood urea  $3.0 \pm 0.4$  mmol/l and urinary protein  $0.3 \pm 0.1$  mg/day

Group	Blood urea (harvest) mmol/l	Urinary protein (harvest) mg/mouse/day
fMRL $\rightarrow$ fMRL	$2.0 \pm 0.9$	$0.5 \pm 0.1$
fMRL $\rightarrow$ fLPR	$20.3 \pm 3.9$	$6.1 \pm 1.5$
mMRL $\rightarrow$ mMRL	$4.0 \pm 1.1$	$0.3 \pm 0.2$
mMRL $\rightarrow$ mLPR	$13.5 \pm 1.4$	$4.2 \pm 1.8$
mLPR $\rightarrow$ mMRL	$4.6 \pm 1.85$	$0.6 \pm 0.1$
mLPR $\rightarrow$ mLPR	$16.4 \pm 3.4$	$3.8 \pm 0.9$

**Table 2** Detailed histologic data. Data are mean  $\pm$  SEM

Group	Glomerular inflammation Grade 0–3	Vascular and perivascular inflammation Grade 0–3	Interstitial inflammation Grade 0–3
fMRL $\rightarrow$ fMRL	$0.75 \pm 0.5$	$0.0 \pm 0.0$	$0.0 \pm 0.0$
fMRL $\rightarrow$ fLPR	$2.75 \pm 0.2$	$2.75 \pm 0.1$	$2.75 \pm 0.2$
mMRL $\rightarrow$ mMRL	$0.5 \pm 0.5$	$0.1 \pm 0.1$	$0.0 \pm 0.0$
mMRL $\rightarrow$ mLPR	$1.75 \pm 0.4$	$2.1 \pm 0.3$	$2.0 \pm 0.3$
mLPR $\rightarrow$ mMRL	$0.75 \pm 0.5$	$0.75 \pm 0.4$	$0.5 \pm 0.5$
mLPR $\rightarrow$ mLPR	$1.4 \pm 0.5$	$1.6 \pm 1.2$	$1.2 \pm 0.5$



the surgical process of transplantation, inflammation disappeared after removal from the autoimmune environment.

Similarly, vascular and perivascular inflammation was absent in both male and female MRL recipients of MRL grafts, whereas maximal (grade 3) vascular inflammation was observed in all female and most male LPR recipients of MRL or LPR grafts. However, vascular inflammation present in age matched control LPR, or in LPR grafts in LPR recipients disappeared if the LPR kidney was transplanted into MRL recipients.

Interstitial inflammation was mild (grade 1) in age-matched native LPR kidneys and in LPR iso-grafts. Interestingly, interstitial inflammation was more severe if the LPR recipient received a MRL kidney, but interstitial inflammation also diminished from the LPR grafts in MRL recipients.

#### Flow cytometry

Abnormal B220+ T-lymphocytes were virtually absent in male naive non-transplanted MRL (<0.2%), but continuously increased in the circulation of male LPR mice from week 6 ( $3 \pm 0.5\%$ ) to week 24 ( $87 \pm 12\%$ ). Thus, as expected, both male and female LPR recipients had an elevated CD3+ population compared with MRL animals. Transplantation per se, did not influence CD3+ cell population, or abnormal lymphoproliferation significantly in either sex.

In accordance with the literature, the percentage of CD3+, CD4+ lymphocytes relative to the total lymphocyte count was reduced in both male and female LPR mice. Transplantation of an LPR kidney reduced the percentage of CD3+, CD4+ lymphocytes in male MRL recipients; however, MRL kidneys did not normalize the CD3+, CD4+ lymphocyte count in LPR recipients in either sex (Table 3).

The percentage of CD3+, CD8+ lymphocytes was similar in MRL and LPR mice, and was not influenced by transplantation or sex.

#### Plasma autoantibody

Optical density of anti-Sm, anti-DNA and anti-histone autoantibodies were significantly higher in LPR recipients, whereas MRL plasma antibody levels were similar to non-lupus NMRI mice (Table 4). MRL grafts did not reduce autoantibody production in LPR recipients, however LPR grafts did elevate plasma antibody levels in MRL recipients.

#### Discussion

Summarizing our results, normal (MRL) kidneys do develop nephritis in the LPR surrounding, and LPR grafts became significantly less inflamed in the MRL surrounding. Thus, in the LPR model, LN is not primarily a kidney-specific disease but it

**Table 4** SLE antibody panel. Antibody levels (average optical density: OD  $\pm$  SD) in the plasma of 6-month-old MRL and LPR recipients at graft removal (2 months after transplantation). Reference values from non-lupus prone (NMRI) mice: anti-Sm  $0.01 \pm 0.1$ ; anti-DNA:  $0.1 \pm 0.02$ ; anti-histone:  $0.02 \pm 0.01$

Group	Anti-Sm	Anti-DNA	Anti-histone
fMRL $\rightarrow$ fMRL	$0.02 \pm 0.1$	$0.2 \pm 0.03$	$0.05 \pm 0.02$
fMRL $\rightarrow$ fLPR	$5.6 \pm 0.9^*$	$6.1 \pm 1.6^*$	$3.5 \pm 1.2^*$
mMRL $\rightarrow$ mMRL	$0.03 \pm 0.2$	$0.2 \pm 0.03$	$0.04 \pm 0.01$
mMRL $\rightarrow$ mLPR	$4.9 \pm 1.8^{**}$	$4.6 \pm 0.5^{**}$	$2.7 \pm 0.9^{**}$
mLPR $\rightarrow$ mMRL	$0.06 \pm 0.1^{**}$	$0.5 \pm 0.02^{**}$	$0.1 \pm 0.04^{**}$
mLPR $\rightarrow$ mLPR	$4.6 \pm 0.4^{**}$	$4.8 \pm 0.6^{**}$	$2.8 \pm 1.3^{**}$

\* $p < 0.01$  versus fMRL  $\rightarrow$  fMRL.

\*\* $p < 0.05$  versus mMRL  $\rightarrow$  mMRL.

**Table 3** FACS data. Proportion of different cell types given as percentage of total lymphocyte population gated

Group	CD3+ T-cell (%)	CD3+, B220+, CD4-, CD8- (DN) (%)	CD3+, CD4+ (Th) (%)	CD3+, CD8+ (Tc) (%)
fMRL $\rightarrow$ fMRL	$57.2 \pm 12.0$	$0.1 \pm 0.1$	$40.7 \pm 7.8$	$15.5 \pm 5.0$
fMRL $\rightarrow$ fLPR	$80.8 \pm 3.3$	$46.2 \pm 4.2$	$21.4 \pm 2.8^*$	$11.3 \pm 2.3$
mMRL $\rightarrow$ mMRL	$52.6 \pm 11.9$	$0.1 \pm 0.09$	$36.1 \pm 6.4$	$15.5 \pm 4.8$
mMRL $\rightarrow$ mLPR	$67.0 \pm 8.9$	$24.3 \pm 10.2$	$24.8 \pm 2.7^{**}$	$15.7 \pm 3.1$
mLPR $\rightarrow$ mMRL	$44.7 \pm 13.4$	$0.0 \pm 0.1$	$28.9 \pm 4.5^{**}$	$11.6 \pm 5.9$
mLPR $\rightarrow$ mLPR	$75.3 \pm 5.5$	$22.8 \pm 11.0$	$22.5 \pm 3.0^{**}$	$17.9 \pm 3.1$

\* $p < 0.01$  versus fMRL  $\rightarrow$  fMRL.

\*\* $p < 0.05$  versus mMRL  $\rightarrow$  mMRL.



reoccurs after replacement of the diseased kidney. However, systemic autoimmunity was transplanted to some extent with the kidney, as transplantation of diseased (LPR) kidneys into non-autoimmune MRL recipients were more inflamed at explantation (2 months after grafting) than native MRL kidneys and engraftment of LPR kidneys led to a reduction of CD3+, CD4+ lymphocytes in MRL recipients, accompanied by a slight elevation of lupus autoantibody production.

Our results obtained in male animals were supported by data from the female groups. As expected,<sup>26</sup> we observed a faster progression of disease in female animals. In our study setup, female LPR kidneys could not be used as grafts, because female LPR mice already demonstrated an advanced disease at 4 months of age: the time of transplantation, therefore, kidney transplantation was technically impossible in a number of mice. However, basic pathomechanisms investigated in the present study were similar in the female and male study cohorts, only severity was more intense in females. Thus, we may reasonably suspect that the female LPR transplantation missing from this study cohort would not influence final conclusions of the study.

It has been demonstrated previously by Wada *et al.*<sup>27</sup> that renal parenchymal cells recruit auto-reactive T-cells which elicit renal injury in LPR mice. Thus, it could be assumed that local factors in the kidney determine progression. Our results did not support this hypothesis in the LPR model. However, the same group has demonstrated similar results to ours in the LPR to MRL allograft model. Glomerular and interstitial nephritis developed in MRL grafts transplanted into LPR recipients to a similar degree, as in native LPR mice.<sup>28</sup> Furthermore, this group also suggested that the autoimmune renal destruction in LPR kidneys is induced due to a systemic autoimmune process, rather than local factors in the kidney.<sup>29</sup>

The autoimmunity, including nephritis can be ameliorated in the LPR model by allogeneic bone-marrow transplantation,<sup>30</sup> supporting the hypothesis that autoimmunity is a stem-cell disease in this mouse model.<sup>31</sup> A recent study by Du *et al.* demonstrated that the lack of functional FAS or FAS-ligand on tubular epithelial cells of grafts from LPR or GLD mice protected allografts. Protection was demonstrated by a reduced serum creatinine and better survival of LPR or GLD grafts versus FAS-system-intact grafts.<sup>32</sup> However, this group used a different strain combination: LPR and GLD grafts were derived from C3H donors, and implanted into C57Bl6 recipients. In such a strain

combination more severe rejection can be expected as in our MRL-LPR combination, where bilaterally nephrectomized transplanted animals survived 2 months without any immunosuppression. Although the FAS system may be important in graft rejection, based on our results and the previous study by Wada *et al.*,<sup>13</sup> it does not seem to be important in the progression of autoimmune nephritis.

It has been demonstrated that CD4+/CD8+ ratio and IL-2 production by T-lymphocytes of LPR mice is impaired.<sup>33,34</sup> In the present study, the number of CD3+, CD4+ lymphocytes was reduced in LPR mice. As we observed a decrease in CD3+/CD4+ lymphocytes in MRL recipients of LPR grafts, and this observation is corroborated by the slight elevation of autoantibody production in MRL recipients receiving a LPR graft, we suspect that the disease can be transplanted to some extent with the graft.

## Conclusion

Removing the LPR kidney from the autoimmune surrounding with lymphoproliferation, and circulating immunocomplexes, significantly reduces the inflammation in the kidney. On the other hand, MRL kidneys in LPR mice develop nephritis, similar to LPR kidneys, thus, this model is suitable to study systemic autoimmune processes due to autoimmune lymphoproliferation and the influence of gender, but local renal factors have no major impact on the progression of lupus nephritis in the LPR model.

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