The Impact of Repeated Subclinical Acute Rejection on the Progression of Chronic Allograft Nephropathy

SEIICHIROU SHISHIDO,* HIROSHI ASANUMA,* HIDEO NAKAI,† YOSHIKI Mori,* HIROYUKI SATOH,* ISAMU KAMIMAKI,‡ HIROSHI HATAYA,§ MASAIKO IKEDA,§ MASATAKA HONDA§, and AKIRA HASEGAWA§

*Department of Pediatric Urology and Kidney Transplantation, Tokyo Metropolitan Kiyose Children’s Hospital, Tokyo Japan; †Department of Urology, Dokkyo University School of Medicine, Saitama, Japan; ‡Division of Pediatrics, Clinical Research Department, National Saitama Hospital, Saitama, Japan; §Department of Nephrology, Tokyo Metropolitan Kiyose Children’s Hospital, Tokyo Japan; and ¶Department of Nephrology, Toho University, School of Medicine.

Abstract. Chronic allograft nephropathy (CAN) is due to both immunologic and non-immunologic factors and results in the development of nonspecific pathologic features that may even be present in long-term well-functioning renal allografts. To investigate the natural history of CAN and potential risk factors associated with progression of these histologic lesions, this study evaluated the of histologic alterations of 124 sequential protocol biopsies performed at 2, 3, and 5 yr after transplantation in 46 patients who exhibited histologic evidence of CAN in the 1-yr biopsy. The occurrence of late acute rejection (AR) greater than 4 mo posttransplant was significantly associated with minimal deterioration in renal function after 3 or 6 mo posttransplant and usually occurs in conjunction with proteinuria and arterial hypertension. The exact mechanism responsible for the pathogenesis of CAN is currently unclear, but it has been postulated that both immunologic and non-immunologic factors may play a role (3).

Recent developments in immunosuppressive therapy have significantly reduced the loss of allografts from acute rejection after renal transplantation. However, the long-term attrition of such grafts remains a major clinical problem and is mainly due to chronic allograft nephropathy (CAN) (1,2). CAN is clinically defined as a gradual, though progressive, decline in graft function after 3 or 6 mo posttransplant and usually occurs in conjunction with proteinuria and arterial hypertension. The exact mechanism responsible for the pathogenesis of CAN is currently unclear, but it has been postulated that both immunologic and non-immunologic factors may play a role (3).

CAN is characterized by various histologic features, including (1) fibrous intimal thickening, (2) chronic transplant glomerulopathy, (3) interstitial fibrosis, and (4) tubular atrophy (4–6). However, these histologic changes may be present before the deterioration in allograft function; several recent studies have demonstrated these histologic lesions in stable well-functioning transplants (7–9). In addition, several articles have shown that early pathologic changes detected by protocol biopsy correlate with later graft dysfunction (9–11). However, few studies exist showing an association between chronic pathology present in protocol biopsies performed at later time points and subsequent allograft function. Furthermore, to our knowledge, there is no article that provides any evidence of the impact of chronic active inflammation on the progression of CAN.

In the current study, we histologically evaluated sequential protocol biopsies until 5 yr after transplantation in 95 recipients of living-donor renal allografts to determine the natural history of CAN and to investigate potential risk factors involved in the progression of histologic CAN. Our results clearly demonstrate that low-grade acute rejection superimposed on CAN was significantly correlated with progression of the histologic lesions in subsequent follow-up biopsies. Furthermore, a group of patients who exhibited repeated subclinical acute lesions in sequential follow-up biopsies exhibited a reduced creatinine clearance at 5 yr after transplantation and reduced long-term graft survival. In contrast, a group of patients who exhibited chronic pathology at 1-yr but no subclinical active inflammation in the follow-up biopsies demonstrated comparable long-
term graft function to those patients with normal histology. The results of this study indicate that the persistence of chronic active inflammation may underlie the progression of CAN.

**Methods**

**Patients and Clinical Characteristics**

Between June 1986 and November 1996, 115 pediatric patients underwent their first living donor kidney transplant at our center. At 1 yr after transplantation, a protocol renal allograft biopsy was performed in all patients who fulfilled the following criteria: (a) creatinine clearance greater than 50 ml/min per 1.73 m² calculated by the Shwartz formula (12), (b) proteinuria less than 1 g/d, (c) variability of serum creatinine less than 15% during the 2 wk before and after biopsy. All patients gave full informed consent. Thereafter, sequential follow-up biopsies were performed at 2, 3, and 5 yr after transplantation of allografts that exhibited CAN at 1 yr.

The following clinical data were recorded: age and gender of the recipient and the donor, height and weight of the recipient, etiology of end-stage renal disease, number of HLA mismatches, total ischemia time, and number of acute rejection episodes after surgery. Serum creatinine level, proteinuria, BP, cyclosporine dose and trough level, and growth of the recipient were recorded at the time of biopsy and during subsequent follow-up.

**Immunosuppression**

The immunosuppressive treatment used in our institute has been previously described (13). Briefly, intravenous cyclosporine (CsA) was given postoperatively for 3 d at 70 mg/m² per 12 h, followed by oral CsA at 350 mg/m² per d. Target levels of CsA were 200 to 300 ng/ml (whole blood, FPIA) in the first 2 mo, 150 to 200 ng/ml in the next 2 mo, and 100 to 120 ng/ml thereafter. Azathioprine or mizoribine was given at 1 to 1.5 mg/kg per d or 2 to 3 mg/kg per d, respectively. Methylprednisolone was given at 40 mg/m² per d with taper and switched to alternate-day administration at 6 to 12 mo after transplantation. Fifty patients received anti-lymphocyt globulin (ALG) (500 mg/m² per d for 14 d) as induction therapy.

**Biopsy**

Routine donor biopsies were undertaken 1 h after the release of the vascular cross-clamp. According to protocol, a renal allograft biopsy was performed in all patients with stable allograft function at 1 yr after transplantation, and sequential protocol biopsies were performed at 2, 3, and 5 yr after transplantation in a selected patient population whose initial biopsy exhibited CAN. Two core biopsies were obtained with a spring-loaded 14 gauge Biopty gun (RADIPLAST AB, Uppsala, Sweden) under ultrasound guidance. One core was fixed in 10% formalin, embedded in paraffin, and processed for routine histology; the other was snap frozen. Serial 4-μm-thick sections were stained using routine hematoxylin and eosin, periodic acid-Schiff (PAS), Masson trichrome, and silver methenamine. Biopsies were examined by two renal pathologists and scored in accordance with the Banff 97 classification (4.5).

CAN was divided into three grades, mainly according to the severity of interstitial fibrosis and tubular atrophy. Grade I or mild CAN is characterized by mild interstitial fibrosis (ci1) and tubular atrophy and/or loss (ct1); grade II or moderate CAN is characterized by moderate interstitial fibrosis (ci2) and tubular atrophy and/or loss (ct2); grade III or severe CAN is characterized by severe interstitial fibrosis (ci3) and tubular atrophy and/or loss (ct3). The chronic allograft damage index (CADI) was used to quantify the extent of histologic changes and has previously been demonstrated to correlate well with deteriorating graft function (14,15). The CADI score was calculated as the sum of scores for a) diffuse interstitial inflammation and fibrosis, b) mesangial matrix expansion and glomerulosclerosis, c) intimal proliferation of vessels, and d) tubular atrophy.

Histologic (subclinical) acute rejection was also divided into three types according to Banff 97 schema. Type I is tubulointerstitial rejection without arteritis, further divided into type IA with focal moderate tubulitis and IB with severe tubulitis; type II is vascular rejection by intimal arteritis; type III is severe rejection with transmural arterial changes. Biopsies exhibiting foci of mild tubulitis (one to four mononuclear cells/cross section or ten tubular cells) are categorized as borderline changes. In the present study, however, biopsies with focal infiltrates and a very mild tubulitis were classified as “normal histology” when their severity was considered less than “suspicious.” Furthermore, tubulitis in moderately to severely atrophic tubules reduced in caliber by 50% or more was not graded.

All AR episodes were treated with a course of high-dose methylprednisolone, even in the absence of an elevation in serum creatinine.

**Statistical Analyses**

Results are expressed as the mean ± SD. The Kruskall-Wallis and χ² tests were applied for ordinal and categorical data. Clinical data in two categories were compared either with t test, Mann-Whitney U test, or 2 × 2 contingency tables. Kaplan-Meier analysis was used to calculate graft survival and the Mantel Cox log-rank test was used to compare survival between groups. All P values were two tailed, and a P value of less than 0.05 was considered significant.

**Results**

**Patients**

One hundred fifteen patients gave their consent to participate in this study. Twenty patients lost their grafts within the first year after transplantation and were excluded. Reasons for graft loss included death with a functioning graft (n = 2), acute vascular rejection (n = 3), recurrence of the original disease (n = 2), and noncompliance with treatment (n = 1); ten patients moved to another hospital. Ninety-five patients were finally included in this study; 57 male patients and 38 female patients with a mean (± SD) age at transplant of 11.1 ± 5.8 yr (range, 1.6 to 22 yr). All patients received their first living-donor kidney allograft from their parents (mean age, 39 ± 9 yr). The mean total ischemic time was less than 1 h, and delayed graft function was not observed. The mean period of follow-up was 135 ± 34 mo (range, 72 to 190 mo); during this period of time, 14 grafts were lost at 71 ± 45 mo.

**Histologic Alterations of Biopsies Performed at 1 h**

The morphologic changes in baseline (1 h) biopsies were minimal. Nine biopsies (9.5%) exhibited mild arteriolar sclerosis. Interstitial fibrosis or glomerulosclerosis was quite infrequent.

**Histologic Diagnoses of Biopsies Performed at 1 yr**

All biopsy specimens were satisfactory and enabled a full evaluation regarding acute rejection and CAN scores according to the Banff criteria. Biopsies were diagnosed retrospectively in a blinded manner. The mean number of glomeruli per biopsy was 27.8 ± 16.4, with all biopsies having a total of ten or more
glomeruli. The mean number of small arteries per biopsy was 4.0 ± 2.7, with all biopsies having at least two small arteries.

Forty biopsies (40%) were normal, 9 (9%) exhibited borderline changes, and CAN were found in 46 biopsies (48%). Cases exhibiting CAN were categorized as mild (grade I, n = 30), moderate (grade II, n = 14), and severe (grade III, n = 2). In addition, type I acute rejection (type IA, n = 16; type IB, n = 7) was evident in 23 (50%) of 46 cases with CAN. Twelve cases with CAN were also associated with borderline changes.

The mean CADI score of the 46 biopsies exhibiting CAN was 3.5 ± 1.3. Histologic changes compatible with CsA nephrotoxicity such as hyaline arteriolar lesions were infrequent.

Clinical Variables and Histologic Diagnosis
The characteristics of patients according to the histologic categories of the 1-yr biopsy are summarized in Table 1.

Thirty-eight of 95 patients received ALG as an induction therapy after surgery. Histologic CAN was found in 18 (47.4%) of the 1-yr biopsies in 38 ALG-treated patients and 29 (50.9%) of 57 patients who did not receive ALG. Therefore, the use of ALG had no impact on the prevalence of CAN at 1 yr.

We examined the correlation between previous episodes of acute rejection and the histologic findings at 1 yr. Twenty-four (60%) of forty patients with normal histology, eight (89%) of nine patients with borderline changes, and 20 (43%) of 46 patients with histologic CAN in the 1-yr biopsy had an acute rejection episode within 3 mo of transplantation. There was no difference between the incidence of early AR occurring within 3 mo of surgery and the development of CAN at 1 yr. However, 2 (5%) of 40 with normal histology, 1 (11%) of 9 with borderline changes, and 11 (24%) of 40 patients with histologic CAN in the 1-yr biopsy received treatment for an episode of late AR before the first year protocol biopsy. Statistical analyses indicated that the incidence of late AR episodes in patients with CAN at 1 yr was significantly higher than the incidence in patients with normal histology at 1 yr (P < 0.05).

As shown in Table 1, there was no significant difference in the creatinine clearance of patients with CAN and those with normal histology or borderline changes.

Histologic Diagnoses of Follow-Up Biopsies
To determine the nature of the progression of these histologic lesions, we evaluated 124 follow-up biopsies derived from the 46 patients who exhibited histologic CAN at 1 yr (Tables 2 and 3).

At 2 yr after transplantation, histologic changes compatible with CAN were evident in 37 (86%) of 43 allografts. Cases exhibiting CAN were categorized as grade I (n = 13), grade II (n = 23), and grade III (n = 1). Type I acute rejection (type IA, n = 9; type IB, n = 3) was evident in 12 (32%) of 37 cases. Three grafts were lost due to CAN. Six biopsies exhibited normal histology. The mean CADI of the 43 biopsies was 4.1 ± 1.7. Comparison of the histologic findings at 2 yr with those at 1 yr in individual patients indicated histologic deterioration, defined as an increased CADI score, in 15 of 30 patients with grade I CAN, 4 of 14 patients with grade II CAN (two grafts lost), and 2 of 2 patients with grade III CAN (one graft lost).

At 3 yr after transplantation, histologic CAN was observed in 37 (86%) of 43 allografts: grade I in 12, grade II in 23, and grade III in 2. Type IA acute rejection was evident in 7 (19%) of 36 cases. One graft was lost due to CAN while 5 biopsies were histologically normal. The mean CADI of the 42 biopsies was 4.6 ± 1.5. Comparison of these data with the results of the second year biopsies indicated histologic deterioration in 6 of 13 patients with grade I CAN, 10 of 23 patients with grade II CAN (1 graft lost), and 1 of 1 patient with grade III CAN.

At 5 yr after transplantation, histologic CAN was observed in 32 (80%) of 40 allografts: grade I in 13, grade II in 19, and grade III in 5. Type IA AR was evident in 5 (16%) of 32 cases. Two grafts were lost due to CAN, whereas three biopsies were histologically normal. The mean CADI of the 40 biopsies was 4.1 ± 2.3. Comparison of these data with the results of the third year biopsies indicated histologic deterioration in 3 of 12 patients with grade I CAN, 9 of 23 patients with grade II CAN (one graft lost), and 1 of 2 patients with grade III CAN (one graft lost).

Table 1. Clinical characteristics of patients according to the histologic categories of the 1-yr biopsy

<table>
<thead>
<tr>
<th></th>
<th>Normal</th>
<th>Borderline Changes</th>
<th>CAN</th>
</tr>
</thead>
<tbody>
<tr>
<td>n</td>
<td>40</td>
<td>9</td>
<td>46</td>
</tr>
<tr>
<td>Age (yr)</td>
<td>11.2 ± 6.0</td>
<td>11.4 ± 5.6</td>
<td>10.8 ± 5.3</td>
</tr>
<tr>
<td>Gender (male/female)</td>
<td>22/18</td>
<td>5/4</td>
<td>29/17</td>
</tr>
<tr>
<td>Donor age (yr)</td>
<td>40.1 ± 7.2</td>
<td>37.1 ± 6.1</td>
<td>40.0 ± 5.3</td>
</tr>
<tr>
<td>AR ≤ 3 mo</td>
<td>24 (60%)</td>
<td>8 (89%)</td>
<td>20 (43%)</td>
</tr>
<tr>
<td>AR ≥ 4 mo</td>
<td>2 (5%)</td>
<td>1 (11%)</td>
<td>11 (24%)b</td>
</tr>
<tr>
<td>CsA dose (mg/kg per d)</td>
<td>6.9 ± 2.7</td>
<td>10.8 ± 1.8</td>
<td>7.5 ± 2.3</td>
</tr>
<tr>
<td>CsA trough level (ng/ml)</td>
<td>89 ± 26</td>
<td>103 ± 14</td>
<td>81 ± 20</td>
</tr>
<tr>
<td>Ccr at 1 yr</td>
<td>93 ± 24</td>
<td>89 ± 15</td>
<td>82 ± 22</td>
</tr>
</tbody>
</table>

aData are presented as mean ± SD. CAN, chronic allograft nephropathy; AR, clinical episode of acute rejection; CsA, cyclosporine; Ccr, creatinine clearance (ml/min per 1.73 m²).

bP < 0.05 versus normal histology.
Different (tologic deterioration in these two groups was significantly acute rejection superimposed on CAN. The frequency of histologic deterioration was only evident in 15 (33%) of 45 paired biopsies that did not exhibit follow-up biopsies, whereas histologic deterioration was only evident in 20 paired biopsies that were borderline changes with CAN. Therefore the presence of acute rejection, but not borderline changes, superimposed on CAN correlated significantly with histologic deterioration in the follow-up biopsies (with AR versus without AR, \( P = 0.003 \); with AR versus borderline, \( P = 0.045 \)).

We then analyzed two groups of patients defined by the presence or absence of recurrent subclinical acute rejection superimposed on CAN until 5 yr after transplantation. Group I consisted of 22 patients, out of a total of 46, with histologic CAN at 1 yr but who did not exhibit subclinical rejection at any time point; group II consisted of 24 patients with recurrent subclinical rejection evident in the follow-up protocol biopsies.

As shown in Table 3, a marked histologic deterioration was evident in 20 (83%) of 24 patients of group II. Six grafts with high-grade CAN were lost within 5 yr after transplantation. Moreover, 12 of 15 biopsies (80%) with mild (grade I) CAN at 1 yr revealed progression to high grade of CAN at 2 to 5 yr after transplantation. In contrast, 15 of 22 biopsies (68%) derived from patients in group I showed no histologic progress during the observation period (Table 2).

As show in Table 4, the creatinine clearance (Ccr: ml/min per 1.73 m\(^2\)) at 3 yr and 5 yr after transplantation was significantly lower for patients in group II compared with those in group I (3 yr, 63 ± 19 versus 75 ± 16; 5 yr, 56 ± 15 versus 70 ± 14 [group II versus group I]: \( P < 0.05 \)). One graft was lost in group I as a result of CAN, whereas ten were lost in group II. In contrast, there was no significant difference in the long-term renal function between patients in group I and those who exhibited normal histology at 1-yr biopsy.

Lastly, the actuarial graft survival rate at 5 yr and 10 yr after transplantation was significantly lower in group II compared with group I (5 yr, 100 versus 75%; 10 yr, 93 versus 51% [group II versus group I]; \( P < 0.01 \); Figure 1).

**Discussion**

In this study, we histologically evaluated sequential protocol biopsies until 5 yr after transplantation in 95 recipients of living donor renal allografts. The main findings of the present study can be summarized as follows: (a) at 1 yr after transplantation, 46 (48%) of 95 grafts exhibited histologic changes compatible with CAN; (b) the presence of late acute rejection episodes occurring more than 4 mo after transplantation was significantly associated with the development of histologic CAN at 1 yr, whereas early rejection episodes occurring within 3 mo of transplantation had no impact on the development of CAN; (c) subclinical acute rejection was often observed in association with CAN and was evident in 50%, 32%, 19%, and 16% of cases with CAN at 1, 2, 3, and 5 yr after transplantation, respectively; (d) the superimposition of these acute lesions to CAN was significantly correlated with histologic progression on follow-up biopsies; (e) patients with recurrent subclinical rejection superimposed on CAN in the sequential protocol biopsies had a lower creatinine clearance 5 yr after transplantation and worse long-term graft survival. In contrast, the absence of acute lesions in biopsies with CAN at any time point until 5 yr after transplantation (group I)

---

**Table 2.** Histologic diagnosis of follow-up biopsies derived from 22 patients with histologic CAN at 1 yr but who did not exhibit subclinical rejection at any time point until 5 yr after transplantation (group I)

<table>
<thead>
<tr>
<th>CAN</th>
<th>Grade I</th>
<th>Grade II</th>
<th>Grade III</th>
<th>No CAN</th>
</tr>
</thead>
<tbody>
<tr>
<td>1-yr biopsy</td>
<td>15 (68%)</td>
<td>7 (32%)</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>2-yr biopsy</td>
<td>8 (36%)</td>
<td>8 (36%)</td>
<td>0</td>
<td>6 (27%)</td>
</tr>
<tr>
<td>3-yr biopsy</td>
<td>8 (36%)</td>
<td>9 (41%)</td>
<td>0</td>
<td>5 (23%)</td>
</tr>
<tr>
<td>5-yr biopsy</td>
<td>10 (45%)</td>
<td>8 (36%)</td>
<td>1 (5%)</td>
<td>3 (14%)</td>
</tr>
</tbody>
</table>

---

**Table 3.** Histologic diagnosis of follow-up biopsies derived from 24 patients with recurrent subclinical rejection evident in the follow-up protocol biopsies (group II)

<table>
<thead>
<tr>
<th>CAN</th>
<th>Grade I</th>
<th>Grade II</th>
<th>Grade III</th>
<th>Graft Loss</th>
</tr>
</thead>
<tbody>
<tr>
<td>1-yr biopsy</td>
<td>15 (63%)</td>
<td>7 (29%)</td>
<td>2 (8%)</td>
<td>0</td>
</tr>
<tr>
<td>2-yr biopsy</td>
<td>5 (21%)</td>
<td>15 (63%)</td>
<td>1 (4%)</td>
<td>3 (13%)</td>
</tr>
<tr>
<td>3-yr biopsy</td>
<td>4 (19%)</td>
<td>14 (67%)</td>
<td>2 (10%)</td>
<td>1 (5%)</td>
</tr>
<tr>
<td>5-yr biopsy</td>
<td>3 (15%)</td>
<td>11 (55%)</td>
<td>4 (20%)</td>
<td>2 (10%)</td>
</tr>
</tbody>
</table>

Six (13%), 5 (11%), and 3 (7%) of 46 biopsies with CAN in the 1-yr biopsy exhibited normal histology at 2, 3, and 5 yr after transplantation. Two cases with grade I CAN in the 1-yr biopsy exhibited normal histology at the 2-yr to 5-yr biopsies. In addition, four biopsies with grade I and two with grade II CAN exhibited normal histology in the follow-up biopsies. On the basis of the assumption that CAN does not regress and that the sampling error is normally distributed, 7% (9 of 124) of biopsies may not be properly classified.

The Impact of the Persistence of Subclinical Rejection on the Progression of CAN

First, we investigated whether subclinical inflammation superimposed on CAN correlated with histologic deterioration in the follow-up biopsies. Therefore, the histologic findings of paired biopsy specimens were compared, *i.e.*, 1-yr versus 2-yr, 2-yr versus 3-yr, and 3-yr versus 5-yr specimens. In this study, “histologic deterioration” was defined as an increase in CADI scores. Twenty-eight (70%) of 40 biopsies with acute rejection, 0.45 ± 1.79 in biopsies with borderline changes, and 0.44 ± 1.22 in biopsies without acute rejection. Therefore the presence of acute rejection, but not borderline changes, superimposed on CAN correlated significantly with histologic deterioration in the follow-up biopsies (with AR versus without AR, \( P = 0.003 \); with AR versus borderline, \( P = 0.045 \)).
point was associated with minimal deterioration in renal function or progression of renal lesions during the observation period.

We first evaluated the histologic findings in 95 living donor renal allografts at 1 yr after transplantation. We found that 46 grafts (48%) exhibited histologic changes compatible with CAN at this time, which is consistent with previous reports indicating that CAN is frequently found in protocol allograft biopsies from clinically normal transplant cases (7–9,16). Isoniemi et al. (7) studied the histologic findings in 89 first cadaveric renal allografts 2 yr after transplantation and demonstrated that two thirds of the grafts had fibrosis and tubular atrophy, whereas more than one third of the grafts had glomerulosclerosis and intimal proliferation in vessels. Legendre et al. (9) examined protocol biopsies performed at 2 yr after transplantation and showed that histologic CAN was present in 50% of patients. More recently, Benameno et al. (16) demonstrated that 49% of pediatric recipients with good renal function had histologic CAN evident in protocol biopsies performed 1 yr after transplantation.

Potential risk factors responsible for these histologic lesions have been analyzed in many studies. A previous episode of acute rejection is a major risk factor for the development of CAN (3,8,9,16). Seron et al. (8) reported a strong association between the diagnosis of CAN and the presence of a successfully treated episode of acute rejection before performing the protocol biopsy with similar data being found in the pediatric population (16,17). This analysis confirmed a good association between the occurrence of late acute rejection more than 4 mo after transplantation and the development of histologic CAN at 1 yr. However, early episodes of acute rejection occurring within 3 mo had no impact on the subsequent development of CAN.

Preexisting donor damage (9,16,18), cold ischemic time (15), and CsA nephrotoxicity (9,18) have also been demonstrated to be risk factors for the development of CAN. However, it should be noted that all recipients had a living donor kidney allograft from their young healthy parents in this study. As a result, the cold ischemic time was very short, and delayed graft function was not observed. Baseline (1 h) biopsies confirmed a low prevalence of pathology in the donors. In addition, histologic findings compatible with CsA nephrotoxicity were infrequent at any biopsy time point.

Despite the strong correlation between early rejection episodes and the subsequent development of CAN, there is still controversy as to whether the subclinical rejection detected by protocol biopsy is responsible for its development. Several previous studies reported that a mild tubulitis was observed in a substantial number of stable grafts and was frequently associated with CAN (4,7,8,19). Moreover, some authors suggest that the presence of mild tubulitis is not a significant factor determining graft outcome (4,8). Furthermore, several investigators have proposed that renal interstitial infiltrates may represent a nonpathogenic process, with data from animal model systems of tolerance suggesting that it may even be immunoregulatory in nature (20). However, several recent studies have suggested that subclinical rejection detected by early protocol biopsy is also associated with poor graft outcome. Rush et al. (10,21) reported that 30% of biopsy specimens from stable patients 1 to 3 mo posttransplant exhibited histologic rejection, with such grafts suffering loss of renal function at a later date. More recently, Nickerson et al. (11) elegantly demonstrated that the presence of subclinical rejection and acquired chronic pathology in the 6-mo protocol biopsy were independent risk factors for renal allograft dysfunction at 2 yr. Similar data were

**Table 4. Long-term renal function in three groups of patients**

<table>
<thead>
<tr>
<th></th>
<th>Normal</th>
<th>Group I</th>
<th>Group II</th>
</tr>
</thead>
<tbody>
<tr>
<td>n</td>
<td>40</td>
<td>22</td>
<td>24</td>
</tr>
<tr>
<td>Ccr at 1 yr</td>
<td>93 ± 24</td>
<td>83 ± 22</td>
<td>81 ± 19</td>
</tr>
<tr>
<td>Ccr at 2 yr</td>
<td>89 ± 21</td>
<td>80 ± 20</td>
<td>69 ± 20</td>
</tr>
<tr>
<td>Ccr at 3 yr</td>
<td>81 ± 17</td>
<td>75 ± 16</td>
<td>63 ± 19bc</td>
</tr>
<tr>
<td>Ccr at 5 yr</td>
<td>76 ± 14</td>
<td>70 ± 14</td>
<td>56 ± 15bc</td>
</tr>
</tbody>
</table>

* Normal, patients who exhibited normal histology at 1 year biopsy. Group I, patients with histologic CAN at 1 yr but who did not exhibit subclinical rejection at any time point until 5 yr after transplantation. Group II, patients with recurrent subclinical rejection evident in the follow-up protocol biopsies. Ccr, creatinine clearance (ml/min per 1.73 m²). Data are presented as mean ± SD.

a P < 0.05 versus no CAN.
b P < 0.05 versus group I.

**Figure 1.** Actuarial graft survival in patients who exhibited normal histology at 1-yr biopsy (solid line) and in patients of group I (broken line) and group II (dotted line).
reported by Legendre et al. (9), who revealed that the presence of grade I acute rejection in the 3-mo protocol biopsies was associated with an increased incidence of chronic lesions 2 yr after transplantation. In addition, the recent work by Lipman et al. (22) demonstrated that histologic features of acute rejection are often accompanied by enhanced expression of pro-inflammatory gene transcripts such as cytotoxic T cell effector molecules and interferon-γ, despite the absence of clinically overt graft dysfunction. These data suggest that inflammation in the renal allograft may well be pathogenic even if subclinical in nature.

However, few studies show an association between chronic pathology present in protocol biopsies performed at later time points and subsequent allograft function. To our knowledge, there is no study that provides any evidence of the impact of chronic active inflammation on the progression of chronic renal allograft damage. Our results clearly demonstrate that the superimposition of these acute lesions on CAN is significantly correlated with histologic progression defined as an increased CADI score of the follow-up biopsies. More importantly, a group of patients who exhibited repeated subclinical AR on the sequential follow-up biopsies had a lower creatinine clearance at 5 yr posttransplant and worse long-term graft survival. However, it is very interesting to note that the absence of any components of acute inflammation in association with CAN at any time was associated with minimal deterioration in renal function or progression of renal lesions during the observation period. These data suggest that the presence of histologic CAN at 1-yr may not be inevitably associated with a worse graft outcome unless subclinical active inflammation was found in the follow-up biopsies.

In the present study, a considerable number of episodes of subclinical acute rejection were observed in association with CAN even in the later protocol biopsies. There are a few reports that have examined the histologic features of kidney allografts at time points later than 1 yr after transplantation. Legendre et al. (9) examined 2-yr protocol biopsies from recipients who had not experienced clinical acute or chronic rejection and demonstrated histologic CAN and grade I acute rejection in 48% and 16%, respectively. A study by Solez et al. (14) reported evidence of acute rejection in 8.9% and 9.2% of the 2-yr biopsies in tacrolimus-treated and cyclosporin-treated patients, respectively. Compared with these adult studies, our data may represent a relatively high prevalence of subclinical AR in later protocol biopsies. However, it should be noted that the administration of steroid is reduced at an earlier stage in pediatric recipients to minimize growth inhibition (2). In fact, most recipients were maintained on alternate-day therapy after 6 mo posttransplant; this may be one possible explanation for the higher prevalence of subclinical AR in our series.

The important question is whether treatment of subclinical rejection is beneficial in preventing the progression of CAN. Contrary to the graft dysfunction of most episodes of AR, CAN is generally felt to be unresponsive to an increased dosage of steroids or conventional antibody therapy (23). Rush et al. convincingly demonstrated that the treatment of subclinical rejection diagnosed on protocol biopsies with corticosteroids had a beneficial impact on renal function 2 yr after transplantation (21). In the present study, some patients who displayed repeated active lesions in association with CAN exhibited substantial histologic deterioration at the follow-up biopsy and a worse long-term graft outcome despite the fact that all subclinical acute rejections were actively treated with steroid pulse therapy. These results therefore suggest that short-term treatment with corticosteroids may be insufficient to prevent chronic active inflammation and clinicopathological deterioration. Therefore, we have recently tried to convert the baseline immunosuppressive treatment (e.g., CsA to tacrolimus or mizolbin to MMF [24]) of patients who exhibited recurrent acute rejection in combination with CAN.

In summary, this study indicates the prevalence of CAN in a 1-yr protocol biopsy in pediatric patients who received a living donor kidney allograft. These histologic changes were associated with the occurrence of late acute rejection during the first year. Furthermore, in patients who exhibited CAN at the first-year biopsy, we demonstrated the prevalence of subclinical rejection in association with CAN in periodic follow-up biopsies performed until 5 yr after transplantation. The presence of these histologic lesions significantly correlated with histologic progression defined as an increased CADI score of the follow-up biopsies. Moreover, a group of patients who exhibited repeated subclinical AR on the sequential follow-up biopsies had a lower creatinine clearance at 5 yr after transplantation and worse long-term graft survival. In contrast, the absence of any evidence of AR superimposed on CAN at any time point was associated with minimal deterioration in renal function or progression of renal lesions during the observation period. These results suggest that the persistence of chronic active inflammation may be at least partially responsible for the progression of CAN. If this is the case, then sequential protocol biopsies may provide important information that may be used to modify the natural history of CAN.

Acknowledgments

We thank Dr. Sadao Kawamura, Professor Emeritus, Department of Pathology, Toho University School of Medicine, for his pathologic instruction.

References


