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Histologic Findings in Protocol Biopsies Performed in Stable Renal Allografts Under Different Immunosuppressive Schedules

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ABSTRACT

Protocol biopsies performed in stable renal allografts show different degrees of acute and chronic lesions. Histologic findings in protocol biopsies have been related to graft outcome. We evaluated histologic lesions observed in protocol biopsies performed in patients under different immunosuppression therapies. From June 1988 a protocol biopsy was performed at approximately 4 months in patients who fulfilled the following criteria: serum creatinine <300 μmol/L; stable renal function; and proteinuria <1 g/d. Histologic lesions were graded according to 1997 Banff criteria. For the present study we considered the following groups according to immunosuppressive schedule: (i) induction therapy with polyclonal or monoclonal antilymphocytic antibodies associated with cyclosporine and prednisone (n = 201); (ii) cyclosporine, mycophenolate mofetil, and prednisone (n = 127); and (iii) tacrolimus, mycophenolate mofetil, and prednisone (n = 51). On protocol biopsy patients treated with tacrolimus displayed a lower acute score (0.61 ± 1.01 vs 1.24 ± 1.23 in group I, 1.28 ± 1.41 in group II; $P < .0001$) and a higher proportion of normal biopsies (57.1% vs 41.9% in group I, 45.1% in group II; $P = .016$). A similar proportion of chronic lesions (chronic score of group I: 1.30 ± 1.56; group II: 1.34 ± 1.80; group III: 1.51 ± 0.95; P = NS) was observed in the three groups. Protocol biopsies displayed fewer acute lesions in patients treated with tacrolimus. This result suggests that the efficacy of new immunosuppression schedules can be evaluated using the protocol biopsy as a surrogate marker of graft outcome.

DURING the last few years, a protocol biopsy program has been started in some centers to determine the presence of acute and chronic lesions in stable well-functioning renal allografts. The information provided by different centers has allowed for an assessment of the evolution of acute and chronic lesions after transplantation. Thus, although acute lesions tend to reach their maximum during the initial months after transplantation, the incidence of chronic lesions is low during the first month, progressively increasing thereafter.[1–3]

Until now, few data have been available on the utility of protocol biopsies to manage immunosuppressive treatment.

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Table 1. Clinical Characteristics of Patients

Variable	Ab + CsA + P	CsA + MMF + P	FK + MMF + P	*P*
Donor age (y)	33 ± 16	40 ± 16*	38 ± 15*	.001
Donor gender (M/F)	143/58	94/33	40/13	NS
Patient age (y)	42 ± 13	47 ± 13*	46 ± 14	.001
Patient gender (M/F)	124/77	83/44	35/18	NS
Transplant (first/second)	170/31	101/26	45/8	NS
PRA (%)	13 ± 26	7 ± 19*	3 ± 12*	.002
HCV (+/−)	53/147	19/107	2/50	.001
DR mismatches	0.6 ± 0.6	0.6 ± 0.6	0.7 ± 0.5	NS
Cold ischemia time (h)	24 ± 6	22 ± 6*	20 ± 5*	.001
DGF (no/yes)	158/43	106/21	42/11	NS
AR (no/yes)	164/37	106/21	44/9	NS
Creatinine (μmol/L)	149 ± 51	135 ± 45*	131 ± 35*	.001
Proteinuria (g/d)	0.35 ± 0.22	0.26 ± 0.22*	0.26 ± 0.18*	.001
Cholesterol (mmol/L)	5.9 ± 1.1	5.6 ± 1.0*	5.3 ± 1.0*	.001

Key: PRA, panel-reactive antibodies; HVC, hepatitis virus C antibodies; DGF, delayed graft function; AR, acute rejection before protocol biopsy.
*Statistical difference by Scheffe test in comparison to the first group.

Probably the most relevant information is that concerning the treatment of early subclinical rejection episodes with high-dose steroids, which has been reported to yield better renal function at 2 years.[4] In the present study, we analyzed histologic findings in protocol biopsies performed in patients receiving different immunosuppression schedules.

PATIENTS AND METHODS

From June 1988 a prospective study of protocol renal allograft biopsies was conducted at our center. A protocol renal allograft biopsy was performed at approximately the fourth month of follow up in patients who gave their informed consent and fulfilled the following criteria: (i) serum creatinine <300 μmol/L; (ii) proteinuria <1 g/d; and (iii) stable renal function, defined as a variability of serum creatinine of <15% during 2 weeks before and after biopsy.

The following variables were evaluated in each patient at the time of transplantation: age and gender of donor and recipient; presence of hepatitis C virus antibodies; last panel-reactive antibodies; number of HLA mismatches; and cold ischemia time. The occurrence of delayed graft function and acute rejection episodes were recorded. At the time of protocol biopsies and during follow up, serum creatinine, proteinuria, total serum cholesterol, cyclosporine (CsA), or tacrolimus dose and levels were recorded.

To achieve a sufficient sample size for the study we considered the following combinations of immunosuppressive drugs: (i) concomitant induction therapy with polyclonal or monoclonal antilymphocytic antibodies; CsA, and prednisone (P) (n = 201); (ii) a triple regimen with CsA, mycophenolate mofetil (MMF), and P (n = 127); and (iii) a triple regimen with tacrolimus, MMF, and P (n = 53). Patients receiving any other immunosuppressive regimen were not considered.

Biopsies were processed for routine light microscopy as previously described.[3] Renal lesions were graded and diagnosed according to the 1997 Banff criteria[5] by two observers in the absence of any clinical information. Only biopsies containing at least one arterial section and one glomerular profile were considered.

Results are expressed as mean values ± standard deviation. Comparison between groups was performed by means of chi-square test for categoric data and analysis of variance in the cases of continuous variables (Scheffé test for individual comparisons). All *P* values were two-tailed, and $P < .05$ was considered statistically significant.

RESULTS

Between June 1988 and June 2002, we performed 498 protocol biopsies at 4 months, among which the present analysis includes 381 biopsies from 378 patients.

The demographic characteristics of patients are shown in Table 1. Patients from groups II and III were older and received kidneys from older donors. Furthermore, these patients showed lower levels of last panel-reactive antibodies and a lower prevalence of hepatitis C virus antibodies. These patient characteristics did not seem to be associated with any modification in clinical events after transplantation, because all groups displayed similar prevalences of delayed graft function and acute rejection episodes (Table 1). At the time of biopsy, patients from group I had a higher serum creatinine and more proteinuria than patients in the other two groups (Table 1). Finally, patients treated with tacrolimus displayed a lower total serum cholesterol at the time of biopsy than patients treated with cyclosporine (Table 1).

From a histologic perspective, fewer patients in group I than groups II and III underwent biopsies that included a sufficient sample for histologic analysis (86.6% vs 96.1% and 92.4%, respectively, $P = .01$). Patients treated with tacrolimus displayed a lower acute score (0.61 ± 1.01 vs 1.24 ± 1.23 in group I, 1.28 ± 1.41 in group II; $P < .0001$) and a higher proportion of normal biopsies (57.1% vs 41.9% in group I, 45.1% in group II; $P = .016$). However, the three groups showed a similar proportion of chronic lesions (chronic score of group I: 1.30 ± 1.56; group II: 1.34 ± 1.80; group III: 1.51 ± 0.95; P = NS).

DISCUSSION

The introduction of cyclosporine in the 1980s for the immunosuppressive treatment of renal transplants reduced

the incidence and severity of acute rejection and improved early graft survival but not long-term results.[6] The introduction of mycophenolate mofetil during the 1990s was associated not only with a further decrease in acute rejection episodes, but also with an increased renal half-life.[7] Unfortunately, it has been necessary to analyze a large number of patients to show this benefit. For this reason, different clinical variables, such as acute rejection or renal function, have been examined as surrogate markers of long-term outcome, but no single variable has been shown to be sufficiently powered to be employed for this purpose.[8]

During the last decade protocol biopsies performed in well-functioning renal allografts have been used to describe the natural evolution of acute and chronic lesions. This natural evolution may be summarized as a rather high incidence of acute lesions in the initial period after transplantation, because the incidence of subclinical rejection is up to 30% during the first 3 months. Furthermore, there is a progressive increase in chronic lesions from 10% at 1 month to 30% to 40% at 3 to 6 months and 60% to 70% at 2 years.[1–4] Unfortunately, various centers have reported different incidences of acute and chronic lesions, suggesting that patient characteristics and transplant management may influence the histologic findings in protocol biopsyies. In any case, all studies have shown that the presence of acute and chronic lesions in stable grafts is associated with long-term outcome, suggesting that protocol biopsies may provide a surrogate of graft survival.

Despite these considerations, protocol biopsies have not been extensively used to assess the efficacy of different immunosuppression schedules. A prospective study showed that triple therapy with cyclosporine, azathioprine, and prednisone was associated with fewer chronic lesions than any combination of only two drugs.[1] Moreover, the incidence of chronic allograft nephropathy at 2 years was not different among patients treated with tacrolimus or cyclosporine.[9]

In the present study we analyzed the influence of immunosuppression on acute and chronic lesions at 4 months. Our evaluation was retrospective but was based on an intention-to-treat analysis. To achieve a sufficient sample size per group, we considered only three therapeutic groups. Patients treated with concomitant induction therapy with either polyclonal or monoclonal antibodies were grouped, because we have previously shown a similar clinical evolution and similar histology in these cases.[10] The clinical characteristics of the recipients and the donors were significantly different, because the majority of patients treated with antilymphocyte antibodies were transplanted between 1988 and 1994, those receiving mycophenolate mofetil between 1993 and 2002, and those with tacrolimus between 1998 and 2002. Taking these limitations into consideration, we observed that patients treated with tacrolimus displayed less severe acute lesions and a higher proportion of normal biopsies, but a similar degree of chronic lesions. Our result support the concept that, although cyclosporine and tacrolimus are both inhibitors of calcineurin and interleukin-2 transcription, they may have differential effects to modulate acute inflammation in stable grafts.[11]

In conclusion, protocol biopsies at 4 months displayed fewer acute lesions in patients treated with tacrolimus. This result supports that the efficacy of new immunosuppressive schedules may be evaluated using a protocol biopsy as a surrogate marker of graft outcome.

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