Late acute rejection occurring in liver allograft recipients

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EM YOSHIDA, CR SHACKLETON, SR ERB, et al. Late acute rejection occurring in liver allograft recipients. Can J Gastroenterol 1996;10(6):376-380. To study the effect of immunosuppressive reduction on the incidence and consequence of late acute rejection (LAR) in liver allograft recipients, mean daily prednisone dose, mean cyclosporine A (CsA) trough and nadir levels were retrospectively reviewed for the nearest 12-week period preceding six episodes of LAR in five liver allograft recipients (group 1). Results were compared with those from a cohort of 12 liver allograft recipients who did not develop LAR (group 2). LAR was defined as acute rejection occurring more than 365 days post-transplantation. Median follow-up for both groups was similar (504 days, range 367 to 1050, versus 511 days, range 365 to 666, not significant). Mean trough CsA levels were lower in patients with LAR compared with those without (224±66 ng/mL versus 233±49 ng/mL) but the difference was not statistically significant. In contrast, mean daily prednisone dose (2.5±1.6 mg/day versus 6.5±2.9 mg/day, P=0.007) and CsA nadir values (129±60 ng/mL versus 186±40 ng/mL, P=0.03) were significantly lower in patients who developed LAR compared with those who did not. Five of six episodes (83%) of LAR occurred in patients receiving less than 5 mg/day of prednisone, versus a single LAR episode in only one of 12 patients (8%) receiving prednisone 5 mg/day or more (P=0.004). In all but one instance, LAR responded to pulse methylprednisolone without discernible affect on long term graft function. The authors conclude that liver allograft recipients remain vulnerable to acute rejection beyond the first post-transplant year; and reduction of immunosuppressive therapy, particularly prednisone, below a critical, albeit low dose, threshold increases the risk of LAR.

Key Words: *Acute rejection, Cyclosporine, Liver, Prednisone, Transplantation*

Rejet aigu tardif chez des receveurs d'allogreffes hépatiques

RÉSUMÉ : Afin d'étudier les effets de la réduction de l'immunodépression sur l'incidence et les conséquences du rejet aigu tardif (RAT) chez des receveurs d'allogreffes hépatiques, les taux quotidiens moyens de prednisone et les creux et les pics moyens de cyclosporine A (CsA) ont été rétrospectivement passés en revue pour la période de 12 semaines la plus rapprochée précédant six épisodes de RAT chez cinq receveurs d'allogreffes hépatiques (groupe 1). Les résultats ont été comparés à ceux d'une cohorte de 12 receveurs d'allogreffes hépatiques chez le RAT n'est pas survenu (groupe 2). Le RAT a été défini comme un rejet aigu, survenant plus de 365 jours après la transplantation. Le suivi moyen des deux groupes a été semblable (504 jours, entre 367 et 1050, contre 511 jours, entre 365 et 666, non significatif). Les creux moyens des taux de CsA ont été plus bas chez les patients ayant manifesté un RAT que chez les autres (224 ± 66 ng/mL, contre 233 ± 49 ng/mL) mais la différence ne s'est pas révélée statistiquement significative. Par contre, la dose quotidienne moyenne de prednisone (2,5 ± 1,6 mg/jour, contre 6,5 ± 2,9 mg/jour, P = 0,007) et les pics moyens de CsA (129± 60 ng/mL, contre 186 ± 40 ng/mL, P = 0,03) ont été significativement plus bas chez les patients ayant présenté un RAT que chez les autres. Cinq épisodes de RAT sur six (83 %) sont survenus chez des patients qui recevaient moins de 5 mg/jour de prednisone, contre un seul épisode de RAT sur les 12 patients (8 %) qui recevaient 5 mg/jour de prednisone ou plus (P = 0,004). Dans tous les cas sauf un, le RAT a répondu à de la méthylprednisolone pulsée sans affecter perceptiblement la fonction de la greffe. Les auteurs en concluent que tous les receveurs d'allogreffes hépatiques restent sujets au rejet aigu au-delà de la première année suivant la transplantation; et la réduction du traitement immunosuppreseur, surtout de la prednisolone, sous un seuil posologique critique, bien que faible, augmente les risques de RAT.

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A major long term objective of immunosuppressive therapy is to maintain the allograft recipient on a regimen that balances the risks of opportunistic infection, de novo malignancy and drug toxicity, against the risk of graft rejection. Better understanding of the interplay among the forces of graft rejection, acceptance and their chronology has allowed the tapering of immunosuppressive drug dosing within several allograft systems. However, within any given organ system the schedule of immunosuppressive dose reduction and the tolerable lower limit of maintenance immunosuppression remain largely empiric, vaguely defined and often centre-specific.

The desire to decrease immunosuppressive therapy must be balanced by the need to avoid graft rejection despite that a degree of tolerance may eventually develop in some instances (1). This is underscored by the fact that episodes of late acute rejection (LAR) may still occur long after transplantation. LAR is well described in heart (2,3) and kidney (4-6) allografts. It has been reported to occur as long as 16 years after renal transplant (5). Moreover, LAR occurring within the renal allograft, although often responding to conventional antirejection therapy, may not be innocuous – there is evidence that, in many instances, graft function does not return to pre-LAR baseline levels following treatment (6).

Although the liver may be a more immunopriviledged organ, with greater potential for chimerism or tolerance-induction via its abundant lymphodendritic cell complement (7) compared with the heart or kidney, LAR has also been reported to occur in liver allografts (8). LAR in liver transplantation, however, has not been well characterized, particularly in relation to immunosuppressive drug tapering schedules and the long term affect on graft function. With the aforementioned considerations in mind, we retrospectively reviewed our experience with LAR occurring in liver allograft recipients, with particular reference to differences in the immunosuppressive medication profile between patients who developed LAR and those who did not.

PATIENTS AND METHODS

In the 45-month period ending June 30, 1993, 50 orthotopic liver transplants were performed within the Vancouver Hospital and Health Science Centre. The majority (37) were performed in the preceding 24 months, and 21 patients had been followed for at least 365 days. Four of these 21 patients manifested features of chronic rejection and were excluded from analysis. The present study consisted of a retrospective review of the remaining 17 cases.

LAR was defined as an episode of biopsy-confirmed acute rejection requiring treatment 365 or more days from the date of transplantation. Five patients developed LAR (group 1) and 12 did not (group 2). All patients were first graft recipients and all received induction immunosuppression with OKT3 (5 mg intravenously daily for seven to 10 days) in addition to cyclosporine A (CsA) (Sandimmune; Sandoz), azathioprine and prednisone. These last three agents were prescribed according to the protocol outlined in Table 1.

TABLE 1
Immunosuppressive drug dosing protocol according to time post-transplant

Drug	
Cyclosporine A level (ng/mL)*	
1-3 months	350-450
3-6 months	250-350
6-12 months	200-250
>12 months	150-200
Azathioprine dose (mg/kg/day)	
First week	1.5
After first week	1.0
Methylprednisolone/prednisone dose (mg/day)	
Day zero	1000
Postoperative day 1	500
Postoperative day 2	80
Postoperative day 3	60
Postoperative day 4	40
Postoperative day 5 to 29	20
≥ Postoperative day 30	Taper 5 mg/day/month

**Monoclonal radioimmunoassay on whole blood (Incstar-Cyclo-trac, Minnesota)*

CsA dosing was adjusted on the basis of steady-state (during intravenous therapy) or trough (during oral therapy) levels obtained on whole blood using a monoclonal radioimmunoassay (Incstar-Cyclo-trac, Minnesota). After the first post-transplant month, depending on clinical circumstances and physician preference, maintenance prednisone was tapered by 5 mg/day/month. The dose was reduced below 5 mg/day when treatment for acute rejection had been stopped for three to six months and when a stable pattern of allograft function was present. Acute rejection, early or late, was treated initially with a two- to three-day pulse of methylprednisolone 500 to 1000 mg, followed by a return to oral prednisone starting at 20 mg/day and tapered by 5 mg/day/month depending on clinical circumstances. Acute rejection was considered to be resolved if serum transaminases reverted to the patient's pretreatment baseline level.

Clinic records were reviewed for the study variables obtained within the nearest 12 weeks preceding antirejection therapy (group 1) or last follow-up visit (LFV) (group 2). LFV was defined as the last clinic visit for which the daily dose of prednisone had remained unchanged for 140 days. This period was chosen to ensure a stable clinical immunosuppressive equilibrium and to avoid the possibility that patients in group 2 with prednisone reduction shortly before the end of the study period would develop LAR after the study ended. Median follow-up was calculated from the date of transplant to the date of LAR (group 1) or LFV (group 2). Factors analyzed in the preceding 12 weeks included mean daily prednisone dose, mean CsA trough level (level determined every one to three weeks depending on patient availability and clinical circumstances), nadir CsA trough level (the lowest individual CsA level in the 12-week period) and mean daily dose of azathioprine. Other factors analyzed were time from transplantation to LAR, treatment for acute rejection within the first year, use of OKT3 for acute rejection

TABLE 2
Demographic characteristics of the study group

Characteristic	Group 1	Group 2
Patients/episodes LAR (n)	5/6	0/12
Mean age in years at OLT (range)	36 (17-50)	34 (24-60)
Male:female ratio	1:4	4:8
Median follow-up days (range)	504 (367-1050)	511 (365-666)
Incidence of EAR (%)	2/5 (40)	8/12 (67)
Mean days to EAR (range)	102 (27-176)	43 (8-198)
Use of OKT3 for EAR (%)	0/5 (0)	2/12 (17)
Autoimmune CAH* (%)	3/5 (60)	5/12 (42)

*P was not significant for any of the variables tested. *Proportion of patients with autoimmune chronic active hepatitis (CAH) as the indication for orthotopic liver transplant (OLT). EAR Early acute rejection; LAR Late acute rejection*

TABLE 3
Immunosuppressive profiles

Agent	Group 1	Group 2	P
Prednisone (mean ± SD) (mg/day)	2.5±1.6	6.5±2.9	0.007
CsA mean value (mean ± SD) (ng/mL)	224±66	233±49	NS
CsA nadir value (mean ± SD) (ng/mL)	129±60	186±40	0.03
Azathioprine (mean ± SD) (mg/day)	42±20	56±24	NS

CsA Cyclosporine A; NS Not significant

TABLE 4
Incidence of late acute rejection (LAR) as a function of threshold values for immunosuppression

Parameter	LAR (%)	P
Mean prednisone dose (mg/day)		
<5	5/6 (83)	0.004
≥5	1/12 (8)	
Mean CsA trough level (ng/mL)		
<200	3/6 (50)	NS
≥200	3/12 (25)	
CsA nadir value (ng/mL)		
<150	4/7 (57)	NS
≥150	2/11 (18)	

CsA Cyclosporine A; NS Not significant

within the first year, incidence of steroid resistance and outcome of therapy.

Student's *t* test and Fisher's exact test were used for statistical analysis. The demographic characteristics of the study groups are shown in Table 2.

RESULTS

Five of 21 patients followed for more than 365 days from the time of transplantation suffered six episodes of LAR (23.8%). Mean time to LAR was 589 days (range 367 to 1050 days). The immunosuppressive drug profiles of patients with LAR (group 1) versus those without (group 2) are summarized in Table 3. Mean CsA trough levels (224±66 ng/mL versus 233±49 ng/mL) and daily azathioprine dose (42±20 mg/ day versus 56±24 mg/day) for the two groups in the 12 weeks preceding LAR or LFV were not significantly different. In contrast, patients in group 1 received a significantly lower mean daily dose of prednisone than those in group 2 (2.5±1.6 mg/day versus 6.5±2.9 mg/day, P=0.007) and had a significantly lower nadir value for CsA (129±60 ng/mL versus 186±40 ng/mL, P=0.03).

Results of a threshold value analysis for immunosuppressive parameters are listed in Table 4. Five of six episodes (83%) of LAR occurred in patients receiving less than 5 mg/day of prednisone, versus a single LAR episode in only one of 12 patients (8%) receiving prednisone 5 mg/day or more (P=0.004). Although twice as many patients with a mean CsA trough value less than 200 ng/mL and three times as many patients with a CsA nadir value less than 150 ng/mL developed LAR compared with those with values above these thresholds, the differences were not statistically significant (Table 4). Within group 1, mean time from the nadir value for CsA to LAR onset was 23±22 days (range 10 to 67 days).

Histological severity of rejection was graded mild in five of six episodes; it was moderate in one patient who developed progressive chronic rejection and graft loss due to noncompliance. All other LAR episodes resolved following treatment with pulse methylprednisolone (OKT3 was not required). Following treatment for LAR, no differences in graft function were detected between groups 1 and 2 as determined by serum aspartate aminotransferase (20.5±7.5 IU/L versus 20.5±3.8 IU/L, not significant) and total bilirubin (11.8±1.9 μmol/L versus 18.5±11 μmol/L, not significant) values recorded at the last clinic visit before enrolment in the present study.

DISCUSSION

Early acute rejection is a common experience in liver transplantation, occurring in 60% to 75% of patients in the cyclosporine era (9,10), usually within the first three months. LAR occurs but there are few references in the literature. We defined LAR as acute rejection occurring after one year but other centres have defined LAR as occurring after three months (11) and after six months (8). The incidence of LAR cited in the literature using a six-month definition is 6.4% (12). Our incidence was about 24% in patients with grafts older than one year. Except for one episode, all our episodes of LAR were mild and responded to pulse steroids and an increase in maintenance steroids. In the only study of LAR in liver transplantation (8) that we are aware of, the severity of LAR was not given. In that study, 71% of LAR patients responded to steroid therapy; the rest required OKT3 or FK 506, with 16% (three patients) experiencing persisting rejection. The incidence of LAR in our study was very likely a consequence of an aggressive policy towards steroid withdrawal, which, as a consequence of the findings of the present study, has been modified. We therefore expect that our subsequent incidence of LAR will be comparable with that of other centres.

Analyzing the differences between those who developed LAR and controls, we discovered that the mean dose of prednisone before an LAR episode was significantly lower in group 1 than in the control group (2.5 mg/day versus

6.5 mg/day). Moreover, the incidence of LAR was 10-fold higher (83% versus 8%) in patients receiving less than 5 mg/day of prednisone compared with the control group who received prednisone 5 mg/day or more. This suggests that, at least in a subgroup of long term post-transplant patients, a need for steroid therapy exists and that a threshold, perhaps a low one, exists below which there is an increased risk of LAR. We found a trend towards a greater proportion of LAR episodes with a nadir serum CsA level less than 150 μg/L in the 12 weeks before LAR, compared with the control group (57% versus 18%). Although not statistically significant in our study, it is possible that low maintenance steroid dosing in combination with occasional low CsA levels are synergistic risk factors.

Renal transplant literature also suggests that withdrawal of maintenance glucocorticosteroids may increase the risk of LAR. Steroid withdrawal after six months has been found to produce an increased incidence of early rejection (13). In a small study of LAR in renal grafts it was speculated that some cases of rejection may have been predisposed by a low steroid dose (14). In another study comparing prednisone with no steroids in renal transplant patients treated with CsA, there were more episodes of both early acute rejection and LAR (24%) in the no steroid group compared with the prednisone group (6.8%) (15). This difference was not statistically significant but does suggest a trend favouring prednisone. Unfortunately, in the study of LAR in liver transplantation (8), steroids were not analyzed as a factor and daily doses were not given. However, a recent study of steroid withdrawal after three months in adult liver transplant patients did not find a significant degree of acute rejection (16). In that study, acute rejection occurred in 4.5%, with only one of 154 patients having acute rejection after 12 months. Looking at steroid withdrawal in pediatric liver transplants, Margarit et al (17) found acute rejection in four of 22 patients (18%), but attributed the rejection in two of these patients to subtherapeutic CsA levels. They felt that, in pediatric transplants, the overall benefits of steroid withdrawal outweighed the risks of rejection as long as CsA levels were monitored.

The undesirable side effects of chronic corticosteroid therapy are well known; there is less muscle wasting and fat deposition in regimens without prednisone than with prednisone (mean daily dose in this study was 18 mg) (18). These side effects have led investigators to favour the withdrawal of steroids in renal transplants despite an increased risk of rejection (15,19). It is therefore surprising that two studies of steroid withdrawal in heart transplants did not find significant differences in side effect profiles compared with either steroid-free controls (20) or before and after steroid withdrawal patients (21). This risk:benefit ratio of steroid withdrawal suggests that steroid withdrawal is not uniformly accepted.

Subtherapeutic serum CsA levels increase the risk of LAR in liver transplants (8). In our study, 57% of LAR occurred when there was a subtherapeutic serum CsA level within the preceding 12 weeks. When confronted by a low serum CsA level, compliance with medications is always a question. Only one of our patients admitted to noncompliance; she had the most histopathologically severe degree of LAR and the worst outcome (subsequent chronic rejection leading to graft loss and death). This is in keeping with previous findings that noncompliance of immunosuppression is associated with excessive graft loss and death (22).

Although LAR can occur in liver transplants, and our study indicates that a low corticosteroid dose increases risk together with the known risk associated with subtherapeutic serum CsA levels (8), the question of how serious a problem LAR really is arises. This question is obviously important in deciding the risks versus benefits of maintaining or reducing immunosuppressive medications. There is evidence from the renal transplant literature that both early acute rejection and LAR predict chronic rejection (23). However, in an analysis of late graft loss in liver transplantation, no grafts were lost as a result of LAR (12), and in the present study, in all but one instance LAR was mild in severity, responsive to pulse steroids and did not appear to compromise the ultimate quality of graft function. The one case in our study who did proceed to graft loss was a clear case of patient noncompliance. As well, in studies of steroid withdrawal in kidney transplants, even with a trend towards increased rejection or a statistically significant increased risk of rejection, a benefit regarding graft loss and survival has not been demonstrated (13, 15,19). Because it would be unethical not to treat LAR, even mild episodes, the answer to this question remains unanswered.

Because LAR in liver transplant patients is readily treated with pulse steroids or, in the case of steroid-resistance, OKT3 (11), the risk:benefit ratio may favour steroid reduction for some patients who can be followed on a regular basis. On the other hand, for patients who cannot be followed regularly or may be inaccessible for treatment, the risk of rejection may favour steroid reduction. We suggest that decisions regarding steroid reduction be individualized for each patient.

CONCLUSIONS

Despite the potential for chimerism and tolerance induction (1), a subset of liver allograft recipients remain susceptible to acute rejection beyond the first post-transplant year. Our study, although small and retrospective, appears to demonstrate that in some patients, there is a threshold dose of corticosteroid below which the risk of LAR is increased. Subtherapeutic serum CsA levels have been previously identified as a risk factor for LAR development (8). A subtherapeutic dose of corticosteroid may be an independent risk factor or, in combination with subtherapeutic serum CsA levels, an added risk factor. Finally, the results of the present study suggest that, in the majority of instances, LAR is mild in severity and responsive to pulse steroids without apparent long term deleterious consequences for liver allograft function. It is reasonable to recommend that decisions regarding steroid withdrawal be individualized. However, a more definitive assessment of the risks and benefits of steroid withdrawal in liver allograft recipients must await the results of a randomized prospective trial.

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