Stepwise minimization of the immunosuppressive therapy in pediatric liver transplantation
A conceptual approach towards operational tolerance (*)

Raymond Reding, Jérémie Gras, Christophe Bourdeaux, Grégoire Wieers, Quang Dinh Truong, Dominique Latinne, Etienne Sokal, Magda Janssen , Jan Lerut, Jean-Bernard Otte, Jean de Ville de Goyet

Pediatric Liver Transplant Program, Saint-Luc University Clinics, Université catholique de Louvain, Brussels, Belgium.

Abstract

The evolution of immunosuppression in pediatric liver transplantation has been characterized by a steady reduction of the immunosuppressive load, including removal of anti-lymphocyte antibodies, with the aim to reduce the incidence of EBV-related post-transplant lymphoproliferative disorders. Acute rejection rates were studied retrospectively over two decades of pediatric liver transplantation, according to the successive immunoprophylactic regimens. 318 primary pediatric liver transplant recipients, included between 1984 and 2004 in successive prospective trials, were analyzed, with respect to the impact of the immunosuppressive protocol on acute rejection occurence. A progressive decrease of rejection incidences was observed, which corresponded to reduced immunosuppressive load and to transplant eras. Such trend might be related to changing approaches towards acute rejection histology and therapy by transplant clinicians, but also to the stepwise minimization of immunosuppressive protocols, putatively enhancing graft acceptance. We hypothesize that the recent population of liver transplant recipients with low immunosuppression might be more suitable for progressive immunosuppression withdrawal trial, with the aim to reach ultimately operational tolerance. (Acta gastroenterol. belg., **2005**, 68, **320-322**).

Abbreviations

AR : Acute Rejection
IS : Immunosuppression
LT : Liver Transplantation
PTLD : Post-Transplant Lymphoproliferative Disorder

The first human hepatic allograft in 1963 and the 1983 NIH consensus conference identifying liver transplantation (LT) as a valid therapeutic option constitute two significant landmarks in the development of hepatic replacement (1,2). Since 20 years, LT has become an efficient strategy for the management of acute or chronic liver insufficiency, in adults and children (3,4). Further surgical refinements were proposed in the late eighties, including the introduction of technical alternatives allowing the use of segments of adult liver into young pediatric recipients. At the same time, much efforts were done to explore and validate new immunosuppressants (anti-lymphocyte monoclonal antibodies, new anti-calcineurin inhibitors, new anti-proliferative drugs,…), or combination of immunosuppressants in order to minimize the rejection incidence as well as the adverse effects of these therapies. This evolution was essentially biphasic, consisting first in a progressive reinforcement of cyclosporine A baseline immunosuppression using azathioprine and poly- or monoclonal anti-lymphocyte antibodies, and, later on, aiming at a progressive minimization of the immunosuppression (IS) load administered to prevent rejection in the post-transplant period. In pediatric LT, a steady reduction of the IS was also observed since 15 years, including the progressive removal of anti-lymphocyte antibodies, with the particular aim to reduce the incidence of EBV-related post-transplant lymphoproliferative disorders (PLTD). This work proposes a 20-year retrospective "macroanalysis" of acute rejection (AR) rates according to the transplant eras and immunoprophylactic protocols.

Patients and Methods

Between March 1984 and November 2004, a total of 318 pediatric recipients of a primary LT were retrospectively analyzed, all of them being included in pilot or randomized trials studying prospectively the AR incidences according to the type of immunoprophylaxis administered, most of these results being published separately elsewhere (5-7) (Fig. 1). Among these children, 153 were transplanted within the Pediatric Liver Transplant Program at Saint-Luc University Clinics, Brussels (8), whereas the remaining 165, transplanted in nine other centres, were included in the 1997-1999 European, randomized multicentre study comparing cyclosporine A microemulsion and tacrolimus as primary calcineurin inhibitor (9). Since 2001, two steroid-free IS protocols, under tacrolimus-basiliximab or tacrolimus monotherapy, were evaluated at our centre within pilot studies, the comparative demographic data of both latter series being given in Table 1 (9). The peri- and postoperative management of pediatric LT recipients, including surgical techniques and detailed immunosuppressive protocols, operative at our program have been exten-

(*) Presented at the Symposium "20 years of Liver Transplantation at Saint-Luc University Clinics" (April 23, 2004, Brussels, Belgium).

Correspondence : Prof. R. Reding, M.D., Ph.D., Dept. of Surgery, Saint-Luc University Clinics, 10 Hippocrate avenue, B-1200 Brussels, Belgium.
E-mail : reding@chex.ucl.ac.be.

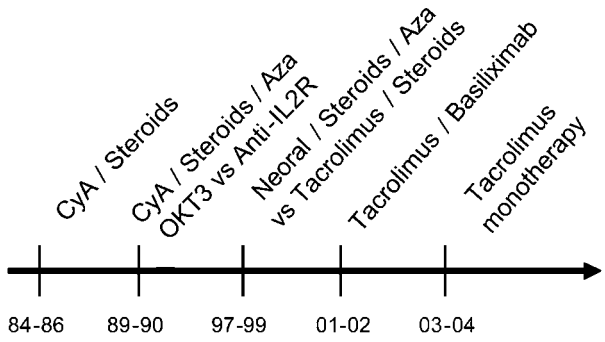

Fig. 1. — Evolution of immunoprophylactic protocols in pediatric liver transplantion at Saint-Luc University Clinics, between 1984 and 2004.

sively described in the literature (10-15). Throughout the 20-year interval, the diagnosis of acute rejection relied on clinical, biochemical and mandatory histological criteria (16).

Results

The overall results of this retrospective study are given in Table 2. In brief, the one-year incidences of AR and steroid-resistant AR progressively decreased during the interval, which corresponded to the successive transplant eras (Fig. 1) as well as to the steady reduction of the IS load administered to prevent post-LT AR (Table 2). The more recent clinical results regarding the two steroid-free IS protocols used in Brussels since 2001 are given in Table 1 : one-year actuarial rejection-free survival rates were 75% in the tacrolimus-basiliximab regimen, versus 67% in the tacrolimus monotherapy group (NS). The incidence of PTLD was also followed along the years, ranging between 10-15% in all protocols, except in the recent series under tacrolimus monotherapy with an incidence at 0% at intermediate analysis.

Discussion

When considering IS in pediatric LT, two major trends have been observed along the last 10-15 years : (i) a stepwise reduction of the total IS load, with, particularly, the removal of T-cell depleting anti-lymphocyte antibodies, incriminated in the development of PTLD in children EBV-negative at the time of LT (14) ; (ii) the introduction of tacrolimus as the baseline calcineurin inhibitor, with a demonstrated efficacy in the prevention of AR episodes as shown in the European multicentre trial in 185 children (9) ; this allowed the possibility to design steroid-free IS protocols, as recently published by our centre (7). The results of the macroanalysis presented in this paper clearly show a decrease in the AR incidences along the last 15-year experience, which seemed to be associated with the stepwise reduction of the IS load. However, such observation should be interpreted with caution. The reduced rejection rate is most probably related at least in part to the changing approaches of transplant clinicians towards AR histology and therapy : an histological picture suggesting AR at day 7 post-LT should certainly not be considered as a clinically-significant rejection episode in every instance (17). Moreover, it may be hypothesized that a lighter IS may putatively enhance graft acceptance, as suggested in rodents where steroids inhibit the development of tolerance in the LT model (18). In this context, the proposal of a novel approach towards IS in LT, referred to as the *dualistic pathway paradigm*, may provide conceptual tools to explain the beneficial effects of a minimized IS as tolerogenic strategy (19). Another interesting finding of the present work was that tacrolimus monotherapy was apparently not associated with a significantly increased incidence of AR, whereas, at interim analysis, the incidence of PTLD was found to be reduced when compared to previous protocols with or without steroids. Obviously, these observations will require further analysis after longer follow-up.

Two strategic approaches are currently proposed in order to provide some form of tolerogenic IS in LT : (i) some authors, particularly from the Pittsburgh group, recommend an heavy IS induction allowing profound and prolonged lymphocyte depletion (20) ; however, as far as pediatric LT recipients are concerned, such T cell depleting strategy may contribute to increase again the PTLD risk in this population mostly EBV-negative at the time of transplantation ; (ii) the second approach is

Table 1. — **Demography and results of two steroid-free, tacrolimus-based immunosuppressive regimen in pediatric liver transplantation**

	Tacrolimus/Basiliximab (n = 20)	Tacrolimus monotherapy (n = 20)
Study interval	Feb 2001-Feb 2002	Sept 2003-Nov 2004
Median age (range)	2.5 years (0.6-13.6)	1.2 years (0.6-15.9)
LRD/PMD	9/11	9/11
1 year patient survival	100%	95%
1 year rejection-free survival	75%	67%
PTLD rate	15%	0%*

(Basiliximab : chimeric mouse-human anti-CD25 monoclonal antibody ; LRD : living-related donor ; PMD : post-mortem donor ; PTLD : post-transplant lymphoproliferative disorder).
* At intermediate analysis.

Table 2. — **Impact of immunosuppressive regimens and transplant eras on the incidence of acute rejection and steroid-resistant acute rejection at one year post-transplant, in 318 pediatric liver allograft recipients**

Immunoprophylactic protocols	n patients	Interval	Acute rejection rate	Steroid-resistant acute rejection rate
CyA/steroids	40	1984-1986	65%	na
CyA/steroids/Aza	15	1989-1990	96%	82%
CyA/steroids/Aza/OKT3	21	1989-1990	81%	49%
CyA/steroids/Aza :LO-Tact-1	17	1989-1990	91%	40%
Neoral/steroids/Aza*	92	1997-1999	60%	30%
Tacrolimus/steroids*	93	1997-1999	45%	6%
Tacrolimus/Basiliximab	20	2001-2002	25%	0%
Tacrolimus monotherapy	20	2003-2004	30%	10%

(CyA : cyclosporine ; na : not available ; Aza : azathioprine : LO-tact-1 : rat anti-CD25 monoclonal antibody ; Basiliximab : chimeric mouse-human anti-CD25 monoclonal antibody).

* Patients included in the European multicentre randomized trial comparing CyA microemulsion (Neoral®) and tacrolimus as baseline calcineurin inhibitor (7).

illustrated in this work and supported conceptually by the recently published dualistic pathway paradigm : a minimal IS might preserve the putative active tolerogenic mechanisms operative immediately following LT. In this context, we hypothesize that the recent series of pediatric LT recipients transplanted under minimized IS regimens may be more suitable for progressive IS withdrawal trial, with the aim to reach operational tolerance (21).

Acknowledgment

This work was supported in part by a grant from the Fondation de la Recherche Scientifique Médicale, Brussels, Belgium (FRSM N° 3.4567.05).

References

1. STARZL T.E., MARCHIORO T.L., ROWLANDS D.T. *et al.* Immunosuprression after experimental and clinical homotransplantation of the liver. *Ann. Surg.*, 1964, **160** : 411-439.
2. National Institutes of Health Consensus Conference Statement. Liver Transplantation-June 20-23, 1983. *Hepatology*, 1984, **4** : 107S-110S.
3. STARZL T.E., DEMETRIS A.J., VAN THIEL D.H. Liver transplantation (first of two parts). *N. Engl. J. Med.*, 1989, **321** : 1014-1022.
4. ALAGILLE D. History of pediatric liver transplantation in Europe. *Acta Gastroenterol. Belg.*, 2004, **67** : 172-5.
5. READING R., VRAUX H., DE VILLE DE GOYET J. *et al.* Monoclonal antibodies in prophylactic immunosuppression after liver transplantation. A randomized controlled trial comparing OKT3 and anti-IL2 receptor LO-Tact-1. *Transplantation*, 1993, **55** : 534-541.
6. READING R., FEYAERTS A., VRAUX H. *et al.* Prophylactic immunosuppression with anti-IL-2 receptor monoclonal antibody LO-Tact-1 versus OKT3 in liver allografting. A two year follow-up study. *Transplantation*, 1996, **61** : 1406-1409.
7. READING R., GRAS J., SOKAL E. *et al.* Steroid-free liver transplantation in children. *Lancet*, 2003, **362** : 2068-2070.
8. READING R., BOURDEAUX C., GRAS J., EVRARD V., BUTS J.P., CARLIER M. *et al.* The paediatric liver transplantation program at the Université catholique de Louvain *Acta Gastroenterol. Belg.*, 2004, 67, 176-178.
9. KELLY D., JARA P., RODECK B. *et al.* The first European, prospective, randomised, multicentre study of tacrolimus and steroids compared with ciclosporin microemulsion, steroids and azathioprine in paediatric liver transplantation. *Lancet*, 2004, **364** : 1054-1061.
10. OTTE J.B., DE VILLE DE GOYET J., READING R. *et al.* Pediatric liver transplantation : From the full-size liver graft to reduced, split, and living related liver transplantation. *Pediatr. Surg. Int.*, 1998, **13** : 308-318.
11. EVRARD V., OTTE J.B., SOKAL E. *et al.* Impact of surgical and immunological parameters in pediatric liver transplantation. A multivariate analysis in 500 consecutive recipients of primary grafts. *Ann. Surg.*, 2004, **239** : 272-280.
12. READING R., DE VILLE DE GOYET J., DELBEKE I. *et al.* Pediatric liver transplantation with cadaveric or living related donors : Comparative results in 90 elective recipients of primary grafts. *J. Pediatr.*, 1999, **134** : 280-286.
13. SMETS F., BODEUS M., GOUBAU P. *et al.* Characteristics of Epstein-Barr virus primary infection in pediatric liver transplant recipients. *J. Hepatol.*, 2000, **32** : 100-104.
14. SOKAL E., CARAGIOZOGLOU T., LAMY M.E. *et al.* Epstein-Barr virus serology and Epstein-Barr virus-associated lymphoproliferative disorders in pediatric liver transplant recipients. *Transplantatio*n, 1993, **56** : 1394-1398.
15. VO THI DIEM H., SOKAL E., JANSSEN M. *et al.* Steroid withdrawal after pediatric liver transplantation. A long-term follow-up study in 109 recipients. *Transplantation*, 2003, **75** : 1664-1670.
16. SNOVER D.C., SIBLEY R.K., FREESE D.K. *et al.* Orthotopic liver Transplantation : A pathological study of 63 serial liver biopsies from 17 patients with special reference to the diagnostic features and natural history of rejection. *Hepatology*, 1984, **4** : 1212-1222.
17. DOUSSET B., HUBSCHER S.G., PADBURY R.T. *et al.* Acute liver allograft rejection. Is treatment always necessary ? *Transplantation*, 1993, **55** : 529-534.
18. WANG C., SUN J., SHEIL A.G. *et al.* A short course of methylprednisolone immunosuppression inhibits both rejection and spontaneous acceptance of rat liver allografts. *Transplantation*, 2001, **72** : 44-51.
19. READING R., DAVIES H.F.S. Revisiting liver transplant immunology : From the concept of immune engagement to the dualistic pathway paradigm. *Liver Transpl.*, 2004, 1081-1086.
20. STARZL T.E., MURASE N., ABU-ELMAGD K. *et al.* Tolerogenic immunosuppression for organ transplantation. *Lancet*, 2003, **361** : 1502-1510.
21. LI Y., KOSHIBA T., YOSHIZAWA A. *et al.* Analyses of peripheral blood mononuclear cells in operational tolerance after pediatric living donor liver transplantation. *Am. J. Transplant.*, 2004, **4** : 2118-2125.