Published in final edited form as:

Pediatr Transplant. 2008 November ; 12(7): 804–808.

Lymphocyte Subset Reconstitution in Pediatric Liver Recipients Induced with Steroid-Free Rabbit Anti-Human Thymocyte Globulin

Anjan Talukdar, Chethan AshokKumar, Jennifer Farrar, Patrick Wilson, Arun Janakiramanan, Mary Tregaskes, and Rakesh Sindhi

Pediatric Transplantation Laboratory, Hillman Center for Pediatric Transplantation, Children's Hospital of Pgh. And the University of Pittsburgh, Pittsburgh, PA

Abstract

Multiple measurements of lymphocyte subsets in 91 children treated with steroid-free Tacrolimus, and rabbit anti-human thymocyte globulin induction demonstrate early reconstitution of T-cytotoxic cells, and gradual reconstitution of all other subsets, which is complete after 1 year. Rejection-prone children demonstrate significantly higher counts of lymphocytes and all subsets prior to liver transplantation, and may exemplify one basis for enhanced baseline immunocompetence.

Keywords

lymphocyte depleting antibodies; pediatric; liver transplantation; immunosuppression; lymphocyte subsets

Introduction

Induction immunosuppression regimens which eliminate steroids, and minimize calcineurin inhibitor usage are appealing for children who receive liver transplantation (LTx) (1-3). Potentially, steroid-induced impairment of growth and development, and the high incidence of post-transplant lymphoproliferative disorder (PTLD) associated with calcineurin inhibitors can be minimized (4,5). Wider adoption is limited by concerns about the long-term effects of induction with lymphocyte depleting antibodies, on lymphocyte reconstitution in an age-group characterized by a maturing immune system. Among the published reports describing outcomes after anti-thymocyte globulin in pediatric recipients of solid organ allografts (6,9-15), only two single-center reports document serial changes in lymphocyte subsets in pediatric kidney recipients (6,9). No data is available from pediatric LTx.

Methods

Since 2001, over 100 children have received steroid-free Tacrolimus, after induction with a total dose of 5 mg/kg rabbit anti-human thymocyte globulin (rATG, Genzyme, Cambridge, MA.). rATG was preceded by premedications in order to minimize cytokine release

Rakesh Sindhi, MD, 3705 Fifth Avenue (Room 7960), Pittsburgh, PA. 15213, Phone/Fax: 412-692-6110/6116, rakesh.sindhi@chp.edu.

Talukdar A, AshokKumar C, Farrar J, Wilson P, Janakiramanan A, Tregaskes M, Sindhi R. Lymphocyte subset reconstitution in Pediatric liver recipients induced with steroid-free rabbit Anti-human Thymocyte Globulin.

syndrome. These premedications were diphenhydramine 1 mg/kg, acetaminophen 10 mg/kg, and methylprednisolone 1 mg/kg. Target Tacrolimus whole blood concentrations were maintained at 12-15 ng/ml in the first month, and brought down to 8-10 ng/ml after the third month. By the end of month 12, target levels are 5-7 ng/ml. If acute cellular rejection occurs, these targets are delayed by 3-6 months. Multiple measurements (Total n=210, 2.3 time points per patient) of lymphocyte counts, as well as their CD3+ (T-cell), CD3+CD4+ (T-helper cells), CD3+CD8+ (T-cytotoxic cells), CD19+ (B-cell) and CD16+/56+ (NK cell) subsets were recorded for 91 children after approval by the University of Pittsburgh Institutional Review Board. Subjects were grouped as Rejectors (n=47) or Non-rejectors (n=44) based on the presence or absence of biopsy-proven acute cellular rejection during the first 60 days after LTx. Whole blood samples 0.1 milliliter each, were obtained with clinically indicated complete blood count (CBC) with differential, prior to LTx, and post-LTx at 1 month, between 31-100 days, 101-365 days, 12-24 months, and 24-30 months. Subset frequencies were measured by the IMK kit (BD Biosciences, San Jose, CA.), and multiplied by the total lymphocyte count to determine absolute count per cubic milliliter for each subset. For the pre-LTx time-point, Rejectors and Non-Rejectors were compared using two-tailed t-tests for two samples assuming unequal variance for all 91 children. To estimate longitudinal changes, pair-wise comparisons were performed using Pre-LTx subset data from corresponding children as reference.

Results

Rejectors were similar to Non-rejectors with respect to median (range) age [6.4 (0.4 to 21.7) vs. 5.4 (0.1 to 18.4) years, $p=NS$], gender (male: female= 20:27 vs. 21:23, $p=NS$) and race (Caucasian: African-American: Others= 40:5:2 vs. 42:1:1, $p=NS$). Steroids were not used initially in either group. Steroids were initiated to treat rejection, and weaned gradually thereafter among Rejectors. Also, tacrolimus doses were increased to achieve targets similar to the first post-LTx month during episodes of acute cellular rejection. Time to first rejection was (median $\pm$ SEM) 30 $\pm$ 4.4 days (mean=36.1 days). Therefore, whole blood concentrations of tacrolimus (Table 1) were higher among Rejectors, when compared with Non-Rejectors, during 31 to 100 days after LTx ($p=0.01$). Also, during this time period, steroids were initiated for the first time, to treat rejection episodes in all Rejectors. Rejection episodes were graded as mild (n=18), moderate (n=6) and severe (n=0); 23 rejectors were not graded. Rejection episodes were also classified as steroid-sensitive (n=37) and steroid-resistant (n=10). Steroid-resistant rejection episodes were treated with anti-lymphocyte therapy [rATG (n= 5), OKT3 (n= 3) and both (n= 2)]. There were no significant differences among the Rejectors and Non-rejectors with regard to use of other drugs (tacrolimus, ganciclovir, acyclovir, TMP-SMX, amoxicillin and intravenous immunoglobulin; $p=NS$), use of cadaveric vs. living graft (42:5 vs. 35:9, $P=NS$) and primary diagnoses leading to liver failure (table 4). There was no ABO incompatibility and there were no infection ongoing at the time of subset monitoring. Patient and graft survival is 89/91 (97.8%) and 88/91, (96.7%), respectively. Two deaths occurred among 91 children, the first due to recurrent hepatocellular carcinoma and the second due to recurrent disseminated hepatoblastoma. In a third child, re-transplantation was needed for thrombotic graft loss due to a hypercoagulable state. The incidence of EBV-PTLD in this series was 3/91 (3.3%). Median ($\pm$ SEM) follow up was 200 $\pm$ 35 days (R vs NR= 240 $\pm$ 51 vs 174 $\pm$ 46).

With the exception of CD16+/56+ cells, Pre-LTx data for lymphocytes (lymp) and their subsets (Table 2) show significantly greater absolute counts. among Rejectors, when compared with Non-Rejectors. The CD4+/CD8+ ratio was similar between groups.

In paired comparisons, total lymphocytes and all subsets (Table 3 and Figure 1) decreased significantly below pre-LTx levels within the first month after LTx. At 31-100 days, which

corresponded to the period of risk for rejection, all counts appeared to reconstitute to pre-LTx levels, while CD8+ counts increased significantly above pre-LTx level ($p=0.024$). This increase in CD8+ cells was significantly higher among Rejectors when compared with the Non-Rejectors (Fig. 1a). CD4+/CD8+ ratio was reversed during this period for both the outcome groups. CD 19+ cells were also significantly higher (Fig. 1a) during this period among Rejectors ($p=0.011$). Thereafter, CD8+ cell counts declined, but were similar to pre-LTx values throughout the follow-up period. For the total lymphocyte count as well as the remaining subsets, reconstitution to pre-LTx values was sustained after the 1st post-LTx year.

Discussion

An intact immune system is vital for the normal development of anti-infectious and anti-tumor immunity in children. Therefore, after polyclonal depletion, reconstitution of T-, B-, and NK-cells to pre-LTx ranges, which begins within the first three months, but is sustained after the first post-LTx year, is reassuring. In this regard, the two deaths among children transplanted for malignancies have led us to omit induction agents in such patients. The incidence of EBV-PTLD in this series was 3/91 (3.3%) and comparable with the 2-10% incidences reported from our center previously (5). All children with PTLT had experienced early rejection, and had thus been exposed to higher amounts of immunosuppressive drugs early after LTx.

Our observations are noteworthy for the fact that children at risk for rejection had greater numbers of lymphocytes and all subsets, prior to LTx. Although age is known to influence lymphocyte counts in children, the Rejector and Non-rejector groups did not differ in mean age or in gender distribution, thereby making it less likely that our observed differences could be explained by age alone. Also, T-cytotoxic cell counts reconstituted to levels greater than pre-LTx values. Further, this increase in T-cytotoxic cell counts assumed statistical significance when Rejectors were compared with Non-Rejectors, during the period of greatest risk for rejection (31-100 days). These observations suggest that the rejection-prone child may express increased baseline immunocompetence in various ways. Support for this inference is also evident in our prior functional evaluation of sub cohorts of our study population. In these prior studies, children who experienced early rejection demonstrated enhanced resistance of T-cytotoxic cells and B-cells to immunosuppression ($n=25$) (7), and increased and persistent ($n=36$) donor-specific alloreactivity in mixed lymphocyte co cultures (8).

The impact of a relatively slower reconstitution of CD4+ cells, which manifests as a reversed CD4/CD8 ratio during the first 2 years after LTx is unknown. Because no opportunistic infections occurred after the first year, it is possible that any effect of the relatively lower CD4 counts was neutralized by earlier reconstitution of CD8 cells.

Our conclusions remain limited by the absence of longitudinal data on donor specific cellular and humoral alloreactivity in all 91 subjects. However, the numbers of children evaluated, demographic similarities between outcome groups, similarities with respect to use of drugs, and paired comparisons to evaluate time-dependent subset reconstitution strengthen our conclusions by minimizing potential confounders such as non-uniform observations from each patient, and age-dependent variation in normative subset data. At the very least, these observations suggest to us that enhanced baseline immunocompetence in the form of higher numbers of T- and B-cells represent another feature of predisposition to early rejection.

Acknowledgments

NIH-5R01AI49156-05, Unrestricted educational grant-Genzyme, Cambridge, MA and Children's Hospital Research Advisory Committee, Pittsburgh, PA,

References

1. Calne R. Prope tolerance: a step in the search for tolerance in the clinic. *Transplant Proc.* 2000; 32(7):2058–9. [PubMed: 11120065]
2. Swanson SJ, Hale DA, Mannon RB, Kleiner DE, Cendales LC, Chamberlain CE, Polly S, Harlan DM, Kirk AD. Kidney transplantation with rabbit antithymocyte globulininduction and sirolimus monotherapy. *Lancet.* 2002; 360(9346):1662–4. [PubMed: 12457792]
3. Starzl TE, Murase N, Abu-Elmagd K, Gray EA, Shapiro R, Eghtesad B, et al. Tolerogenic immunosuppression for organ transplantation. *Lancet.* 2003; 361(9638):1502–10. [PubMed: 12737859]
4. Balistreri WF, Bucuvalas JC, Ryckman FC. The effect of immunosuppression on growth and development. *Liver Transpl Surg.* 1995; 1(5 Suppl 1):64–73. [PubMed: 9346602]
5. Jain A, Nalesnik M, Reyes J, Pokharna R, Mazariegos G, Green M, Eghtesad B, Marsh W, Caccarelli T, Fontes P, Abu-Elmagd K, Sindhi R, Demetris J, Fung J. Post-Transplant lymphoproliferative disorders in Liver Transplantation (LTx): A Twenty Year experience. *Annals of Surgery.* 2002; 236(4):429–36. discussion 436–7. [PubMed: 12368671]
6. Klaus G, Mostert K, Reckzeh B, Mueller TF. Phenotypic changes in lymphocyte subpopulations in pediatric renal-transplant patients after T-cell depletion. *Transplantation.* 2003; 76(12):1719–24. [PubMed: 14688522]
7. Sindhi R, Magill A, Bentlejewski C, Abdullah A, Janosky J, Zeevi A. Enhanced Donor-specific Alloreactivity occurs independently of immunosuppression in children with early liver rejection. *Am J Transplant.* 2005; 5:96–102. [PubMed: 15636616]
8. Khera N, Janosky J, Zeevi A, Mazariegos G, Marcos A, Sindhi R. Persistent donor-specific alloreactivity may portend delayed liver rejection during drug minimization in children. *Front Biosci.* 2007; 12:660–663. [PubMed: 17127326]
9. Bell L, Girardin C, Sharma A, Goodyer P, Mazer B. Lymphocyte subsets during and after rabbit anti-thymocyte globulin induction in pediatric renal transplantation: sustained T cell depletion. *SourceTransplantation Proceedings.* 1997; 29(7A):6S–9S.
10. Parisi F, Danesi H, Squitieri C, Di Chiara L, Rava L, Di Donato RM. Thymoglobuline use in pediatric heart transplantation. *Journal of Heart & Lung Transplantation.* 2003; 22(5):591–3. [PubMed: 12742424]
11. Bunchman TE. Flow cytometry: its use in pediatric renal transplantation utilizing polyclonal induction. *Cytometry.* 1995; 22(1):16–21. [PubMed: 7587728]
12. Brun P, Sterkers G, Maisin A, Benali K, Baudouin V, Macher M, Loirat C. Flow-cytometry monitoring of antilymphocyte globulin therapy in pediatric renal transplantation. *Transplantation Proceedings.* 1995; 27(2):1744–5. [PubMed: 7725483]
13. Troppmann C, Almond PS, Fasola C, Benedetti E, Gruessner RW, Dunn DL, Gores PF, Payne WD, Matas AJ, Najarian JS. Effect of Minnesota antilymphocyte globulin on T-lymphocytes and their subsets during induction therapy in pediatric renal transplantation. *Transplantation Proceedings.* 1994; 26(1):26–7. [PubMed: 8108968]
14. Lebeck LK, Chang L, Lopez-McCormack C, Chinnock R, Boucek M. Polyclonal antithymocyte serum: immune prophylaxis and rejection therapy in pediatric heart transplantation patients. *Journal of Heart & Lung Transplantation.* 1993; 12(6 Pt 2):S286–92. [PubMed: 8312347]
15. Buchman TE, Kershaw DB, Merion RM, Ham JM, Sedman AB, Walter MB, Kelsch RC. T cell subset analysis by flow cytometry during MALG induction for pediatric renal transplantation. *Transplantation.* 1993; 55(5):1190–3. [PubMed: 8497900]

Figure 1 & 1a.

Changes in lymphocytes and their subsets (Median±SEM) after LTx

Table 1

Tacrolimus whole blood concentration (Median $\pm$ SEM ng/ml) is statistically higher among Rejectors, compared with Non-Rejectors, during 31-100 days after LTx. Tacrolimus was not used pre-LTx.

FK Levels				
	Rejectors	Non rejectors	P-value (Rejectors Vs Non-Rejectors)	n (Rejectors Vs Non-Rejectors)
1-30 days	13.0 $\pm$ 2.2	8.6 $\pm$ 1.5	0.111	23(14 vs. 9)
31-100 days	13.6 $\pm$ 1.0	9.7 $\pm$ 1.0	0.01	38(22 vs. 16)
101-365 days	10.3 $\pm$ 1.5	9.8 $\pm$ 2.0	0.766	35(19 vs. 16)
1-2 yrs	7.7 $\pm$ 1.8	2.6 $\pm$ 2.7	0.361	15(9 vs. 6)

Table 2

Pre-LTx summary data for lymphocytes and their subsets show significantly greater number (Median $\pm$ SEM) among Rejectors, when compared with Non-rejectors. CD16/56+ cells did not show any significant difference.

Pre-LTx counts							
	Total Lymphocytes	CD3+	CD4+	CD8+	CD19+	CD16/56+	CD4+/CD8+
Rejector (n=47)	2716 $\pm$ 496	1622 $\pm$ 319	995 $\pm$ 211	577 $\pm$ 114	688 $\pm$ 170	199 $\pm$ 58	1.7 $\pm$ 0.1
Non-Rejector (n= 44)	1657 $\pm$ 219	1109 $\pm$ 134	724 $\pm$ 93	417 $\pm$ 54	328 $\pm$ 79	127 $\pm$ 47	1.7 $\pm$ 0.1
p-value (Rejector Vs Non-Rejector)	0.006	0.007	0.017	0.008	0.004	0.620	0.418

Table 3

Changes in lymphocytes and their subsets (Median±SEM) after LTx

		Pre vs. Post LTx comparison							
		Lymp	CD3	CD4	CD8	CD19	CD16/56	CD4/CD8	N
Pre	Median±SEM	2272±287	1479±183	875±121	529±67	442±99	154±37	1.7±0.1	91
1-30 Days	Median±SEM	1173±263	706±184	358±99	273±85	251±113	77±41	1.1±0.1	23
	P Value	0.003	0.004	0.001	0.018	0.008	0.037	0.0001	
31-100 Days	Median±SEM	2585±232	1586±243	715±116	751±128	453±126	218±43	0.8±0.1	38
	P Value	0.434	0.994	0.005	0.019	0.482	0.854	2.07E-06	
101-365 Days	Median±SEM	1768±141	1220±101	491±58	552±61	251±50	148±24	0.8±0.2	35
	P Value	0.007	0.012	0.0002	0.563	0.0057	0.028	1.01E-06	
1-2 Years	Median±SEM	1708±369	1265±288	554±175	861±93	335±97	196±34	0.8±0.1	15
	P Value	0.197	0.368	0.03	0.675	0.08	0.512	0.008	
2 Years +	Median±SEM	3129±869	2454±687	1010±344	894±384	241±222	205±152	1.3±0.3	8
	P Value	0.580	0.991	0.472	0.670	0.191	0.652	0.324	

Table 4

Primary diseases leading to liver failure.

Diagnosis	R	NR
Allagilles	2	1
Alpha-1-Antitrypsin Deficiency	1	1
Autoimmune Hepatitis	3	0
Benign Recurrent Intrahepatic Cholestasis	0	1
Biliary Atresia	11	8
Budd-Chiari	0	2
Bylers Disease	2	1
Carolis Disease	1	0
Cirrhosis	1	0
Coagulopathy/Thrombocytopenia	1	0
Congenital Hepatic Fibrosis	1	1
Crigler-Najjar	3	2
Cystic Fibrosis	0	2
End Stage Liver Disease	0	1
Fulminant Hepatic Failure	3	1
Glycogen Storage Disease	1	0
Hepatic Fibrosis	1	0
Hepatoblastoma	0	1
Hepatocellular carcinoma	0	2
Histiocytosis	0	2
Idiopathic Hepatitis	1	0
Intrahepatic Cholestasis	1	1
Methylmalonic acedemia	0	1
MSUD	8	9
OTC	1	1
Paucity of Bile Ducts	0	1
Primary Sclerosing Cholangitis	2	1
Secondary Biliary Cirrhosis	0	1
Tyrosenemia	1	0
unspecified	0	1
Wilson's Disease	2	2