

Treatment with Immunoglobulin Improves Outcome for Pediatric Liver Transplant Recipients

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Immunoglobulin mitigates autoimmune disease and facilitates acceptance of ABO-incompatible transplanted organs. To test the hypothesis that treatment with immunoglobulin is associated with improved graft survival and a decreased rate of allograft rejection, a cohort study of primary liver transplant recipients in the Studies of Pediatric Liver Transplantation registry was performed. The outcomes of 336 pediatric liver transplant recipients who received immunoglobulin within 7 days of liver transplantation were compared with the outcomes of 1612 recipients who did not receive immunoglobulin. The outcome measures were patient survival, death-free graft survival, and allograft rejection. The Kaplan-Meier probability of patient survival was not different between patients treated with immunoglobulin and patients who did not receive immunoglobulin. Death-free graft survival was increased in patients treated with immunoglobulin (hazard ratio of death-free survival = 0.57, $P = 0.014$). The probability of allograft rejection at 3 months was 31% for patients treated with immunoglobulin versus 40% for patients who did not receive immunoglobulin (hazard ratio = 0.81, $P = 0.02$). The proportion of patients with 2 or more episodes of allograft rejection was lower in patients treated with immunoglobulin (13.1% with immunoglobulin versus 19.2% with no immunoglobulin, $P = 0.009$). Treatment with immunoglobulin was associated with a decreased risk for allograft rejection, whereas use of cyclosporine as the initial immunosuppression and transplantation before 2002 were independently associated with an increased risk of allograft rejection in pediatric liver transplantation recipients. A trend toward a decreased rate of retransplantation was detected in the population that received treatment with immunoglobulin. *Liver Transpl* 15: 1564-1569, 2009. © 2009 AASLD.

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Intravenous immunoglobulin (IVIG), prepared from pools of human plasma, was introduced in the 1950s as replacement therapy for patients with antibody deficiency. Recent clinical and experimental evidence indicates that immunoglobulin treatment modulates the immune response.¹ Immunoglobulin has been increasingly used to treat autoimmune and systemic inflammatory diseases.²⁻⁶ Immunoglobulin administration has been used to prevent rejection at transplantation, treat steroid-resistant acute rejection after kidney transplantation, and, together with plasmapheresis and anti-lymphocyte antibodies to facilitate acceptance of ABO-incompatible kidney transplants.⁷⁻¹¹

For long-term survivors of liver transplantation, increased serum lipid levels, elevated blood pressure, altered glucose metabolism, cardiac dysfunction, decreased renal function, and increased risk of cancer occur as a result of treatment with calcineurin inhibitors.^{12,13} In contrast, side effects of treatment with immunoglobulin are far less common and are usually limited to chills, fever, headache, tachycardia, and rash with faster infusion rates and more uncommonly anaphylaxis or transmitted infections. If inclusion of immunoglobulin in immunosuppressive therapy after transplantation were to permit a dose reduction of calcineurin inhibitors, then the adverse

Abbreviations: anti-HBs, antibody to hepatitis B surface antigen; CMV, cytomegalovirus; IG, immunoglobulin; IVIG, intravenous immunoglobulin; PELD, Pediatric End-Stage Liver Disease; PTLN, posttransplantation lymphoproliferative disease; SD, standard deviation; SE, standard error; SPLIT, Studies of Pediatric Liver Transplantation.

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TABLE 1. Patient Characteristics

Characteristics	No IG (n = 1612)	IG (n = 336)	P Value
Age (% < 1 year)	557 (34.6)	99 (29.5)	0.07
Race (% white)	944 (58.6)	196 (58.3)	0.86
Sex (% female)	877 (54.4)	169(50.3)	0.17
Primary diagnosis			
Acute liver failure	239 (14.8)	48 (14.3)	0.80
Biliary atresia	673 (41.8)	141 (42.0)	0.94
PELD score at transplant (mean ± SE)	14.3 ± 0.4	14.0 ± 0.9	0.80
Donor type (% deceased donor)	1343 (83.7)	283 (84.2)	0.80
Organ type (% whole organ)	810 (50.5)	181 (53.9)	0.26
ABO-incompatible allografts	40 (2.5%)	3 (0.9%)	0.10

Abbreviations: IG, immunoglobulin; PELD, Pediatric End-Stage Liver Disease; SE, standard error.

effects of long-term immunosuppression might be decreased.

On the basis of this observation that immunoglobulin is immunomodulatory and promotes graft acceptance, a registry of pediatric liver transplant recipients was queried to determine if outcome was improved in patients who received immunoglobulin in the peritransplant period. The study was observational and subject to those limitations, but if differences were detected, then the observations would serve as a foundation for prospective studies of treatment with immunoglobulin.

PATIENTS AND METHODS

Study Population

One thousand nine hundred forty-eight children enrolled in the Studies of Pediatric Liver Transplantation (SPLIT) registry who underwent primary liver transplantation between 1996 and 2005 were included in the study. The registry includes 42 centers from the United States and Canada. The study was approved by institutional review boards at each of the individual SPLIT centers. Informed consent was obtained from the parents of study participants as required by the local institutional review board.

Study Design and Outcome Measures

A cohort study was performed to compare the outcomes of 336 pediatric liver transplant recipients who received immunoglobulin in the form of immunoglobulin or hyperimmune cytomegalovirus (CMV) immune globulin within 7 days of liver transplantation with the outcomes of 1612 transplant recipients who did not receive immunoglobulin. The design was based on the assumption that if patients received immunoglobulin within the first 7 days after transplantation, it was part of a prophylactic regimen rather than a treatment regimen. The outcome measures were patient survival, death-free graft survival (freedom from retransplantation), and allograft rejection. The frequencies of CMV disease, Epstein-Barr virus disease, and posttransplantation lymphoproliferative disease (PTLD) were also determined.

Independent Variables

The primary variable under study was treatment with IVIG or hyperimmune CMV globulin within the first 7 days after transplantation. We considered the following as factors that might influence outcome independently of treatment with immunoglobulin: primary diagnosis, insurance type as a surrogate for socioeconomic status, race, sex, age at liver transplantation, year of liver transplantation, donor type, organ type, cold and warm ischemia times, primary immunosuppression, use of monoclonal or polyclonal immunomodulatory agents, and the components of the calculated Pediatric End-Stage Liver Disease (PELD) score at transplantation based on data collected in the registry.

Analysis

Chi-square and Wilcoxon tests were used to compare the distribution of patient characteristics between those who received immunoglobulin or hyperimmune CMV immune globulin within 7 days of liver transplantation and those transplant recipients who did not receive immunoglobulin. A log-rank test was employed to evaluate the relationship between the immunoglobulin treatment and time to event outcome measures. The time to event was analyzed with the Kaplan-Meier method. Factors determined to be significant at the *P* < 0.20 level in the univariate analyses were included in a multivariate time-dependent Cox regression model. Model reduction was performed with the backward elimination variable selection method. Variables remaining significant at the *P* < 0.05 level were maintained in the final model. All statistical analyses were performed with the SAS System for Windows, version 8.02 (SAS Institute, Inc.; Cary, NC).

RESULTS

Characteristics of the Study Population

Table 1 summarizes the demographic and clinical characteristics of the study population. Thirty-four percent of patients were less than 1 year old at transplantation. Children with biliary atresia accounted for 42% of the

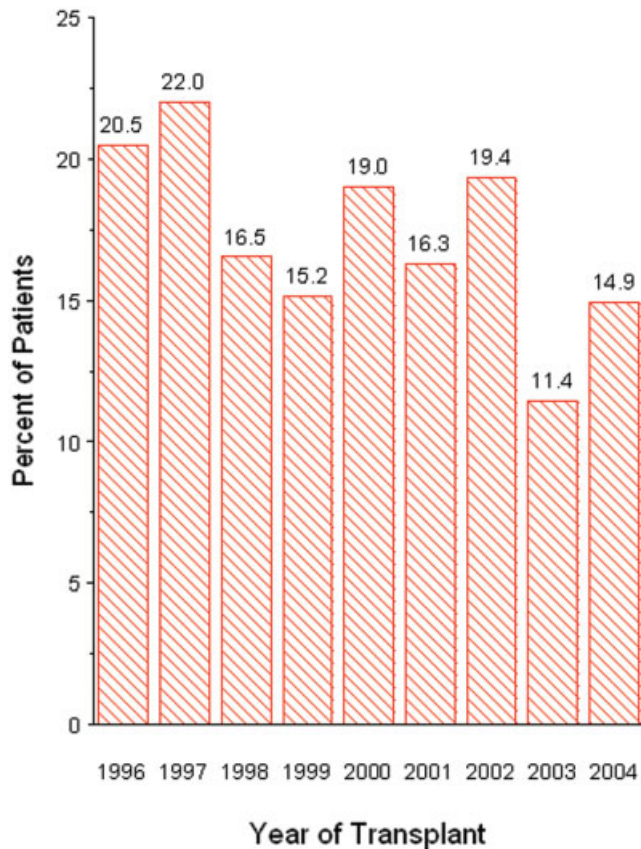


Figure 1. Proportion of patients who received immunoglobulin treatment by the year of transplant.

population, and those with fulminant liver failure accounted for 14.7%. Twenty-nine percent of the cohort received technical variant (reduced-size or split) grafts from deceased donors, whereas 16.2% received allografts from living donors. Age at transplant, primary diagnosis, sex, donor type, graft type, and PELD score did not differ between the groups. The mean calculated PELD score was 14.0 ± 0.9 (mean \pm standard error) for the group that received immunoglobulin versus 14.3 ± 0.4 for the group that did not receive immunoglobulin. We specifically examined the proportion of patients who received ABO-incompatible organs in each group because previous work has suggested that treatment with immunoglobulin, usually with anti-lymphocyte antibodies and/or plasmapheresis, promotes acceptance of ABO-incompatible organs. The percentages of ABO-identical, ABO-compatible, and ABO-incompatible patients were 77.4%, 11.3%, and 0.9%, respectively, for patients who received immunoglobulin versus 75.2%, 15%, and 2.5%, respectively, for the group that did not receive immunoglobulin. The differences did not reach statistical significance. However, the power to detect a difference was only 59%, so a type II error cannot be excluded.

To determine if practice remained constant over the observation period, the proportion of patients who received immunoglobulin as a function of the year of transplantation was determined (Fig. 1). The proportion of patients who received immunoglobulin ranged from

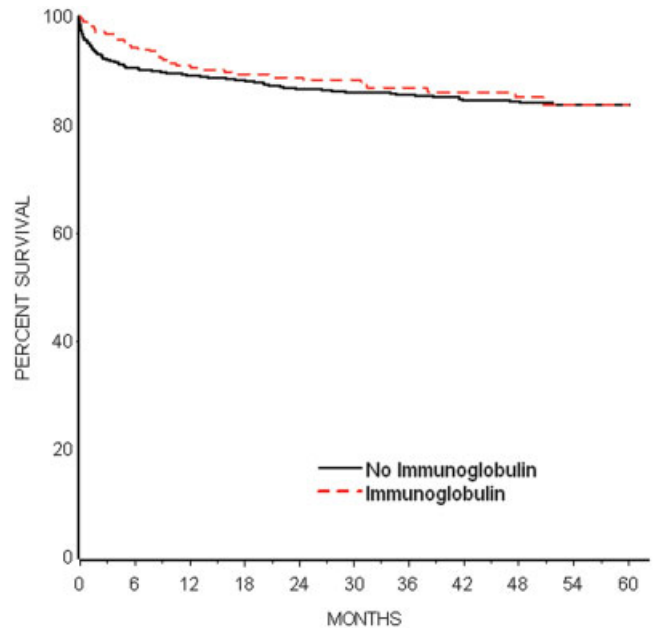


Figure 2. Kaplan-Meier probability of survival with immunoglobulin treatment versus no treatment with immunoglobulin [hazard ratio = 0.97 (confidence interval = 0.71, 1.39), $P = 0.94$].

12% in 2003 to 22% in 1997. The proportions of transplant recipients treated with immunoglobulin were similar before 2002 and after 2002 (43% versus 39%). We used 2002 as a cutoff because it represents the period after implementation of PELD. Patients who received immunoglobulin were more likely to have been treated with monoclonal or polyclonal antibodies ($P < 0.0001$) or tacrolimus ($P = 0.04$).

Patient and Death-Censored Graft Survival

The Kaplan-Meier probability of patient survival was not different between patients treated with immunoglobulin and patients who did not receive immunoglobulin [Fig. 2; hazard ratio = 0.97 (confidence interval = 0.71, 1.39), $P = 0.94$]. The Kaplan-Meier probability of death-censored graft survival, that is, freedom from retransplantation, was increased for patients treated with immunoglobulin compared to patients who did not receive immunoglobulin [Fig. 3; hazard ratio = 0.57 (confidence interval = 0.37, 0.89), $P = 0.014$].

Time-varying Cox proportional hazard analyses were used to develop a model to predict death-censored graft survival. The graft type, donor age, and transplant year were identified as independent predictors in the multivariate analyses. When immunoglobulin treatment was included in the model, the P value of immunoglobulin treatment was 0.0877, which approached but did not reach a P value of 0.05.

Allograft Rejection

The risk of rejection was reduced for patients treated with immunoglobulin [hazard ratio = 0.81 (confidence inter-

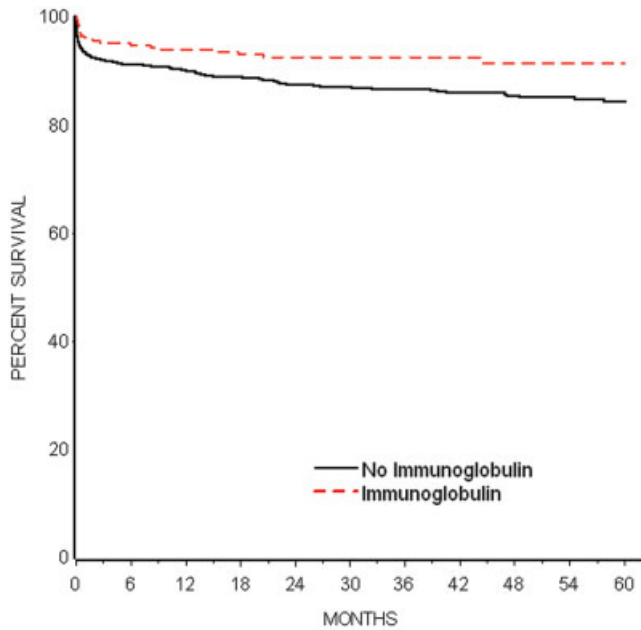


Figure 3. Kaplan-Meier probability of death-censored graft survival with immunoglobulin treatment versus no treatment with immunoglobulin [hazard ratio = 0.57 (confidence interval = 0.37, 0.89), *P* = 0.014].

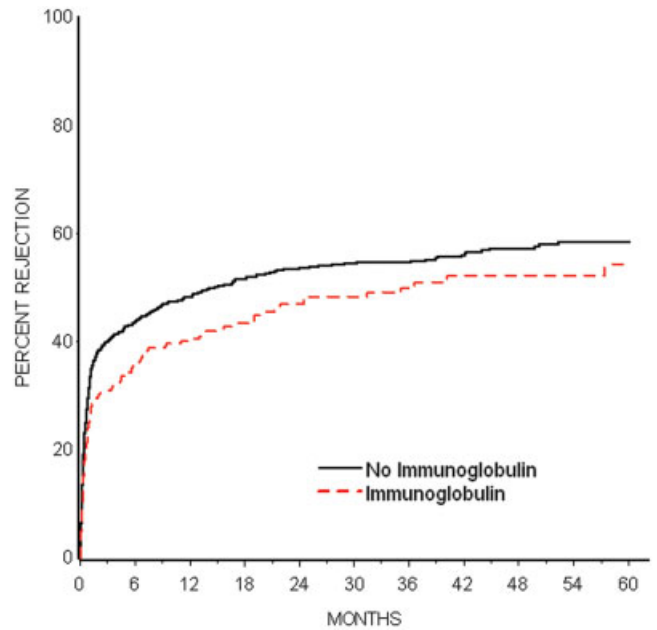


Figure 4. Kaplan-Meier probability of time to first allograft rejection with immunoglobulin treatment versus no treatment with immunoglobulin [hazard ratio = 0.81 (confidence interval = 0.68, 0.97), *P* = 0.02].

val = 0.68, 0.97), *P* value = 0.02]. Thirty-one percent of patients who received immunoglobulin had an episode of allograft rejection within the first 3 months after transplantation versus 40% of patients who did not receive immunoglobulin. The Kaplan-Meier probability of allograft rejection was decreased for patients treated with immunoglobulin compared to patients who did not receive immunoglobulin (Fig. 4). The proportion of patients with 2 or more episodes of allograft rejection was 13.1% for those who received immunoglobulin versus 19.2% for those who did not receive immunoglobulin (*P* = 0.009). The risk of allograft rejection was not affected by the type of immunoglobulin. Forty-four percent of patients receiving CMV hyperimmune globulin had at least 1 episode of rejection versus 40.7% of patients receiving gammaglobulin (CMV hyperimmune globulin versus gammaglobulin: hazard ratio = 1.101, log-rank *P* value = 0.5798).

The final multivariate model was developed with the backward elimination method. The analyses identified treatment with immunoglobulin as an independent predictor of the absence of allograft rejection, whereas use of cyclosporine as initial immunosuppression and transplantation before 2002 were independently associated with an increased risk of allograft rejection (Table 2). *P* values for both the period of transplantation and initial immunosuppression were very small, and the model fit worsened with the removal of either of the factors.

CMV Disease, Epstein-Barr Virus Disease, and PTLD

The incidence of PTLD did not differ between those patients who received immunoglobulin and those who

did not receive immunoglobulin [*n* = 9 (2.7%) in the immunoglobulin group versus *n* = 44 (2.7%) in the no-immunoglobulin group]. The 24-month Kaplan-Meier probability of PTLD-free survival was 97.0% for patients receiving immunoglobulin and 96.8% for patients in the no-immunoglobulin group (*P* = 0.80). Similarly, there was no difference in the incidence of CMV disease in those treated with immunoglobulin (*n* = 34, 10.1%; 24-month Kaplan-Meier probability of CMV-free survival = 87.4%) compared to those who did not receive immunoglobulin (*n* = 131, 8.1%; 24-month Kaplan-Meier probability of CMV-free survival = 89.9%; *P* value = 0.38)

DISCUSSION

In the present work, treatment with IVIG was associated with a trend toward increased death-censored allograft survival and a decreased risk for allograft rejection in pediatric liver transplant recipients. In patients treated with immunoglobulin, the time to the initial episode of allograft rejection was increased, and the probability of 2 or more episodes of allograft rejection was decreased. This study was conducted from data retrieved from multiple centers whose composition reflects the national registry for pediatric liver transplant recipients.

A recent retrospective study did not show benefits of IVIG on post-liver transplant survival or rejection.¹⁴ However, a double-blinded, placebo-controlled study of adult liver transplant recipients showed a survival benefit of 14% for those treated with CMV hyperimmune globulin compared to placebo; this effect was maintained until up to 6 years after transplantation.¹⁵ In

TABLE 2. Multivariate Analysis of Risk Factors for Rejection

Factor	Factor Level	Reference	P Value	Hazard Ratio	95% Confidence Interval	
Age at transplant	6–11 months	<6 months	0.2173	1.210	0.894	1.638
	1–4 years		0.0011	1.620	1.211	2.167
	5–12 years		0.0181	1.452	1.066	1.979
	≥13 years		0.0010	1.743	1.252	2.426
Weight deficit at transplant	0–2 SDs below the mean	Above the mean	0.0589	1.222	0.992	1.504
	>2 SDs below the mean		0.7007	0.965	0.806	1.156
First immunosuppression	Cyclosporine	Tacrolimus	<0.0001	1.518	1.290	1.786
Year of transplant	≥2002	≤2001	<0.0001	0.700	0.592	0.828
IG within 7 days post-transplant	Yes	No	0.0019	0.730	0.599	0.890

NOTE: Overall, the *P* value is 0.0014 for the age at transplant and 0.0392 for the weight deficit at transplant.

Abbreviations: IG, immunoglobulin; SD, standard deviation.

multivariate analyses of the data, CMV hyperimmune globulin was found to be an independent predictor of increased 1-year survival. The improved outcome associated with treatment with CMV hyperimmune globulin exceeded that expected for its antiviral effect. Treatment with antibody to hepatitis B surface antigen (anti-HBs) immunoglobulin and CMV hyperimmune globulin was associated with a decreased incidence of rejection in liver transplant recipients. Associated *in vitro* studies showed that anti-HBs immunoglobulin suppressed functional maturation of and cytokine production by human blood-derived dendritic cells, whereas CMV hyperimmune globulin suppressed functional dendritic cell maturation and alloantigen-stimulated T cell proliferation.¹⁶ In the end, it remains to be determined if this mechanism of action is of significance to liver transplant recipients because antibody-driven allograft rejection is uncommon.

The mechanism of action is likely complex, involving reduction of allospecific antibodies, modulation of T cell responsiveness, and possibly suppression of antigen-presenting cell capacity to stimulate allogeneic T cell proliferation.^{16–19} Immunoglobulin reduces the capacity of mature dendritic cells to secrete interleukin-12 and down-regulates the expression of costimulatory molecules and cytokine secretion from dendritic cells. Immunoglobulin may affect the function of interleukin-2, a cytokine that plays a central role in allograft rejection. Immunoglobulin may block human leukocyte antigen-specific antibodies. *In vitro* studies showed that both immunoglobulin and calcineurin inhibitors decreased T cell proliferation after allogeneic stimulation. Moreover, immunoglobulin-treated dendritic cells showed decreased stimulation of allogeneic T cell proliferation; this effect was not seen with calcineurin inhibitors.²⁰ The impaired T cell stimulatory capacity of

blood dendritic cells occurred as a result of induction of cell death and reduced up-regulation of CD40 and CD80.

As with all observational studies, ours has limitations. The indications for use of immunoglobulin and hyperimmune CMV globulin were not defined, and the indications as well as doses were subject to center preference. In this study, the assumption was made that patients who received immunoglobulin within the first 7 days after transplantation received the infusion as part of a prophylactic regimen rather than a treatment regimen. Nevertheless, the rationale for the use of immunoglobulin was not part of the prospective data collection, and the gap in information may act as an undefined confounder.

Additional factors contributing to posttransplant allograft survival and allograft rejection, including a detailed assessment of calcineurin inhibitor exposure, adherence to the medical regimen, and center-specific target ranges for immunosuppressive drug levels, were not evaluated because these factors are not collected as data elements in the registry. Patients who received immunoglobulin were more likely to have received tacrolimus than cyclosporine; the former is associated with better long-term allograft survival. The use of tacrolimus also was associated with the era of treatment, a significant factor in the final multivariate model. Furthermore, patients who received immunoglobulin were more likely to have received anti-lymphocyte antibodies, although in this study, antibody induction was not an independent predictor of rejection. Moreover, the addition of antibody use to the final multivariate model for rejection did affect the factors. Nevertheless, given the limits of the data set, it was not possible to determine if a specific subgroup of patients was more likely to benefit from treatment with immunoglobulin, and of

course, these data do not shed any light on the mechanism of action of the treatment. Despite these limitations, the improved outcome observed with IVIG treatment warrants further evaluation.

In summary, the observations presented here support previous reports that treatment with immunoglobulin improves outcomes after liver transplantation independently of its antimicrobial effect. Although there is much evidence supporting treatment with immunoglobulin, there are few controlled studies, and it is not known if there is a subset of patients that would particularly benefit from treatment. The consistent observation that immunoglobulin has a favorable immunomodulatory effect is of significant importance. If these results are confirmed, then the number of patients treated to prevent 1 episode of retransplantation is estimated to be 25; this is a very favorable ratio given the favorable risk profile of immunoglobulin infusions compared to that of other immunosuppressive agents. Nevertheless, given the significant cost of immunoglobulin therapy, this report emphasizes the need for prospective, randomized studies to confirm the results of this observational study. Using the preliminary data from this study, we estimated a sample size of 390 patients (185 in each group based on a 2-sided log-rank test with 80% power and 5% type I error) to detect a hazard ratio of 0.730 for a comparison of IVIG use and no IVIG use. If 10% of patients are lost during follow-up, then 390 patients (185 in each group) will be required to conduct a randomized trial to test the hypothesis that use of IVIG leads to a reduction in rejection rates. These estimates bring to light the significant challenges faced by such a clinical trial.

REFERENCES

1. Boros P, Gondolesi G, Bromberg JS. High dose intravenous immunoglobulin treatment: mechanisms of action. *Liver Transpl* 2005;11:1469-1480.
2. Bierling P, Godeau B. Intravenous immunoglobulin for autoimmune thrombocytopenic purpura. *Hum Immunol* 2005;66:387-394.
3. Clynes R. Immune complexes as therapy for autoimmunity. *J Clin Invest* 2005;115:25-27.
4. Krause I, Shoenfeld Y. Intravenous immunoglobulin treatment for fibrosis, atherosclerosis, and malignant conditions. *Methods Mol Med* 2005;109:403-408.
5. Peterlana D, Puccetti A, Simeoni S, Tinazzi E, Corrocher R, Lunardi C. Efficacy of intravenous immunoglobulin in chronic idiopathic pericarditis: report of four cases. *Clin Rheumatol* 2005;24:18-21.
6. Toubi E, Kessel A, Shoenfeld Y. High-dose intravenous immunoglobulins: an option in the treatment of systemic lupus erythematosus. *Hum Immunol* 2005;66:395-402.
7. Jordan SC, Quartel AW, Czer LS, Admon D, Chen G, Fishbein MC, et al. Posttransplant therapy using high-dose human immunoglobulin (intravenous gammaglobulin) to control acute humoral rejection in renal and cardiac allograft recipients and potential mechanism of action. *Transplantation* 1998;66:800-805.
8. Jordan SC, Tyan D, Stablein D, McIntosh M, Rose S, Vo A, et al. Evaluation of intravenous immunoglobulin as an agent to lower allosensitization and improve transplantation in highly sensitized adult patients with end-stage renal disease: report of the NIH IG02 trial. *J Am Soc Nephrol* 2004;15:3256-3262.
9. Jordan SC, Vo A, Bunnapradist S, Toyoda M, Peng A, Puliyaanda D, et al. Intravenous immune globulin treatment inhibits crossmatch positivity and allows for successful transplantation of incompatible organs in living-donor and cadaver recipients. *Transplantation* 2003;76:631-636.
10. Jordan SC, Vo AA, Peng A, Toyoda M, Tyan D. Intravenous gammaglobulin (IVIG): a novel approach to improve transplant rates and outcomes in highly HLA-sensitized patients. *Am J Transplant* 2006;6:459-466.
11. Sivasai KS, Mohanakumar T, Phelan D, Martin S, Anstey ME, Brennan DC. Cytomegalovirus immune globulin intravenous (human) administration modulates immune response to alloantigens in sensitized renal transplant candidates. *Clin Exp Immunol* 2000;119:559-565.
12. Bucuvalas JC, Alonso E. Outcome after liver transplantation: more than just survival rates. *Liver Transpl* 2005;11:7-9.
13. Bucuvalas JC, Campbell KM, Cole CR, Guthery SL. Outcomes after liver transplantation: keep the end in mind. *J Pediatr Gastroenterol Nutr* 2006;43(suppl 1):S41-S48.
14. Nascimbene A, Iannacone M, Brando B, De Gasperi A. Acute thrombocytopenia after liver transplant: role of platelet activation, thrombopoietin deficiency and response to high dose intravenous IgG treatment. *J Hepatol* 2007;47:651-657.
15. Falagas ME, Snyderman DR, Ruthazer R, Griffith J, Werner BG, Freeman R, Rohrer R. Cytomegalovirus immune globulin (CMVIG) prophylaxis is associated with increased survival after orthotopic liver transplantation. The Boston Center for Liver Transplantation CMVIG Study Group. *Clin Transplant* 1997;11(pt 1):432-437.
16. Kwekkeboom J, Tha-In T, Tra WM, Hop W, Boor PP, Mancham S, et al. Hepatitis B immunoglobulins inhibit dendritic cells and T cells and protect against acute rejection after liver transplantation. *Am J Transplant* 2005;5:2393-2402.
17. Bayry J, Lacroix-Desmazes S, Carbonneil C, Misra N, Donkova V, Pashov A, et al. Inhibition of maturation and function of dendritic cells by intravenous immunoglobulin. *Blood* 2003;101:758-765.
18. Bayry J, Lacroix-Desmazes S, Delignat S, Mouthon L, Weill B, Kazatchkine MD, Kaveri SV. Intravenous immunoglobulin abrogates dendritic cell differentiation induced by interferon-alpha present in serum from patients with systemic lupus erythematosus. *Arthritis Rheum* 2003;48:3497-3502.
19. Bayry J, Thirion M, Misra N, Thorenoor N, Delignat S, Lacroix-Desmazes S, et al. Mechanisms of action of intravenous immunoglobulin in autoimmune and inflammatory diseases. *Neurol Sci* 2003;24(suppl 4):S217-S221.
20. Tha-In T, Metselaar HJ, Tilanus HW, Boor PP, Mancham S, Kuipers EJ, et al. Superior immunomodulatory effects of intravenous immunoglobulins on human T-cells and dendritic cells: comparison to calcineurin inhibitors. *Transplantation* 2006;81:1725-1734.