Published in final edited form as:

J Hepatol. 2008 September ; 49(3): 478–480. doi:10.1016/j.jhep.2008.06.021.

CHIMERISM AND LIVER TRANSPLANT TOLERANCE

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Abstract

Chimerism and tolerance in a recipient of a deceased-donor liver transplant. Alexander SI, Smith N, Hu M, Verran D, Shun A, Dorney S, Smith A, Webster B, Shaw PJ, Lammi A, Stormon MO.

Complete hematopoietic chimerism and tolerance of a liver allograft from a deceased male donor developed in a 9-year-old girl, with no evidence of graft-versus-host disease 17 months after transplantation. The tolerance was preceded by a period of severe hemolysis, reflecting partial chimerism that was refractory to standard therapies. The hemolysis resolved after the gradual withdrawal of all immunosuppressive therapy.

In the issue of January 2008 of the *New England Journal of Medicine*, an article from Alexander et al. described the unexpected occurrence of liver transplant tolerance with full chimerism in a child, without conditioning or donor cells infusion.

A 9-year-old girl received a fully HLA-mismatched whole liver from a deceased 12-year-old boy after a fulminant hepatitis of probable viral origin. Recipient blood group was O, RhD-negative (ccdee), but donor was O, RhD-positive (ccDEe); both were CMV positive. Standard immunosuppression (tacrolimus plus steroids) was given. Profound lymphopenia ($<1 \times 10^9$ /mL) was present pre- and for over 6 months post-transplant. After 2 months CMV re-activation occurred. After 9 months, recipient shifted to donor's blood group, developing high anti-D and anti-E autoantibodies, severe intravascular hemolysis, anemia, hemoglobinuria and renal failure. Multiple O, RhD-negative transfusions, high-dose prednisone, iv immune globulin, and cyclosporine for tacrolimus, were given. No decline in liver function was seen. After 13 months, bone marrow and peripheral blood showed mainly male chromosomes in myeloid, lymphoid and erythroid cell lineages, suggesting ongoing chimerism. All immunosuppression was weaned, allowing for donor hematopoietic stem cells to fully engraft; in 1 month all alterations normalized.

Five years post-transplant, 4 without immunosuppression, the recipient has normal liver function, with donor HLA at blood testing; no graft-versus-host disease occurred. Loss of

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Special Section Editors: Peter R. Galle, Peter L.M. Jansen, Francesco Negro

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antibody viral memory requiring re-vaccinations, mainly naïve CD45RA+ phenotype for CD4+ lymphocytes, high levels of thymus-derived T-cells, donor-specific unresponsiveness with normal third-party response at MLR, were observed.

Liver transplantation in children with end-stage liver diseases has improved significantly in the last decade, mainly due to the improvement of surgical techniques and anti-rejection treatment and management. Recipient survival is 80–90% at 1 year post-transplant and 75% at 10 years, with graft survival of 70% and 60%, respectively (1).

Immunosuppression-related side-effects are still one of the major concerns in this fragile population, because of an immature system undergoing long-term toxic therapy. Infections, hypertension, nephrotoxicity, metabolic alterations, lymphoproliferative disease and cancer are main causes of morbidity and indirectly of graft rejection (1).

Induction of tolerance is a desirable aspiration in the pediatric setting. Many studies have been performed, mainly in adults, to achieve either 'operational tolerance' or hematopoietic chimerism, with stable and normal graft function and histology, without immunosuppression, and preserved immune-competence. Different immunosuppressive strategies, some including donor bone marrow or hematopoietic stem cells infusion, have been tested, even in young patients, with encouraging but not definitive results (2–4).

Natural occurrence of liver transplant tolerance is a peculiar phenomenon that has been described in both adult and pediatric recipients, in the latter up to 15% of cases, allowing for withdrawal of immunosuppression (5). The immune privileged characteristics of the liver are related to active intra- and extra-hepatic regulated processes, including: lymphocytes and dendritic cells migration to lymph nodes and thymus; release of soluble MHC molecules; clonal deletion or exhaustion of alloreactive T-cells; suppression from CD4+/25+/foxp3+ T-cells; immune anergy from deficient costimulation; and also micro-chimerism (5) (Figure 1).

Pediatric recipients seem to have an increased chance of graft acceptance due to their immature immune system, with a prevalent tolerant Th2 cytokine profile (i.e. IL-10, TGF- β) and few effector or memory cells from infections or antigenic stimuli (6). Early spontaneous transplant tolerance has been previously reported in pediatric liver recipients discontinuing therapy due to life-threatening viral complications (hepatitis C and EBV) (7). Chronic virus infections (i.e. CMV) are able to modify and evade the host immune system by both immunosuppressive and tolerogenic effects, such as anergy/depletion of effector T-cells, altered MHC antigen presentation and diversion to Th2 cytokines (8).

In the case described by Alexander et al. several factors have possibly conditioned the occurrence of full hematopoietic chimerism and liver transplant tolerance.

The fulminant viral hepatitis could have induced a tolerogenic cytokine milieu in the recipient together with the associated profound lymphopenia prior to and after the transplant. The CMV re-activation might have also prolonged such condition, interfering with the mounting of an adequate immune alloresponse. The persistent viral-induced immunodepression could have indirectly acted as an induction or conditioning regimen, favoring the engraftment of donor liver and hematopoietic stem cells, high levels of which are present in a young organ. From the liver a massive migration of hematopoietic stem cells to the bone marrow as well as of leukocytes to the lymphoid organs of the weakened host has probably occurred, mimicking a bone marrow cell transplant setting.

Engraftment of donor hematopoietic stem cells and later immune reconstitution are dynamic events dependent on central and peripheral tolerance. Thymic and peripheral T-cell allo-resistance has to be overcome to allow donor stem cells to colonize recipient bone marrow and

lymphoid tissues. Subsequent generation of donor and recipient tolerant T-cells are responsible for a stable allograft acceptance in the long-term (9).

The immaturity of the recipient's immune system and the intrinsic tolerogenicity of the liver graft might have also contributed, under these circumstances, in generating the appropriate conditions for both donor organ and cells to engraft and survive. The role of steroids, calcineurin inhibitors and anti-viral agents is difficult to evaluate, but their immunosuppressive effect might have favored such events as early post-transplant. In conclusion, organ and bone marrow cells alloengraftment are the result of dynamic reactions between the recipient and donor immune systems leading to a variable reciprocal clonal exhaustion/deletion and immune ignorance, which can result in transplant tolerance without immunosuppression. Natural, induced, or even casual factors may facilitate the occurrence of such event, especially in liver transplants (10).

Different strategies, some already in clinical trials, might increase the probability of chimerism or operational tolerance in transplantation: pharmacological or irradiative lympho- or myelo-depletion; tolerogenic immunosuppressants; anti-inflammatory and costimulatory blockade agents; or immuno-modulatory donor cells infusion. A cautious balance between benefits and risk should precede their use, especially in pediatrics.

Acknowledgements

RO1 DK050759-04; RO1 DK 056953-05; and U42 RRO16603-08

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Legend: HLA = Human Leukocyte Antigen; GvH = Graft versus Host; HvG = Host versus Graft; Ab = Antibody

Figure 1.