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Bidirectional graft-host hematological traffic in liver transplantation

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Abstract: The highly complex immuno-hematological system of the recipient has to rebalance itself when the liver is replaced with a graft that has its own system. This gives us an opportunity for observation. Here we consider the graft-to-recipient direction with passenger lymphocyte syndrome (PLS) as well as the recipient-to-graft direction with Factor VIII (FVIII) inhibitors, paroxysmal nocturnal hemoglobinuria (PNH) and graft endothelial replacement with liver transplantation. PLS extends beyond the ABO blood groups to any situation where the donor has been sensitized to a recipient antigen. PLS directed against ABO or minor blood group antigens is usually self limiting whereas Rhesus (Rh) PLS persists with life threatening immune hemolysis. Human platelet antigen (HPA) 1A PLS results in life threatening immune thrombocytopenia. Treatments of severe PLS may include reduction in immunosuppression, anti-B-cell therapy, plasmapheresis and splenectomy. Liver transplantation into recipients with FVIII inhibitors has been difficult. Donors with acquired hemophilia may transmit the capacity to make FVIII inhibitors by PLS and should be avoided. Patients with PNH have been transplanted successfully but a considerable cost in the continued use of high dose eculizumab. We speculate that combined bone marrow and liver transplantation would be a better option for recipients with FVIII inhibitors or PNH. Replacement of liver graft endothelium with recipient cells is common and may explain relative transplant tolerance that is believed to occur with liver transplantation.

Keywords: Liver transplantation; passenger lymphocyte syndrome (PLS); human platelet antigen (HPA); paroxysmal nocturnal hemoglobinuria (PNH); endothelium

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Immune hemolysis following transplantation of a blood group O liver into a non-O recipient has been seen since the early days of transplantation when it was reported by Ramsey *et al.* (1). It is thought to be due to the production of antibodies by the donor-derived B lymphocytes in an immune response against the recipient's red blood cell (RBC) antigens (1). This graft-versus-host (GVH) phenomenon has been called the passenger lymphocyte syndrome (PLS) because the donor B lymphocytes have taken passage with the organ into the recipient. The organ donor-recipient match, described above, used to be called a compatible mismatch but is now classified as minor incompatibility, borrowing terminology from stem-cell transplantation. If the combination of donor and recipient was reversed, accelerated or hyperacute rejection could occur and the match is classified a major incompatibly (2,3). Bidirectional incompatibility may occur if a group A liver is transplanted into a B recipient, or the reverse. In North America, allocation of blood group A2 grafts to blood group B recipients is being considered as a way to provide more equitable access to transplantation in which case the match might be considered to have bidirectional minor incompatibility.

ABO PLS has become well known in liver transplantation,

since Romero *et al.*'s original description, where it is considered a relatively benign and short-lived phenomenon (4). PLS is sometimes thought to occur with liver transplantation, rather than with other organs, because the large mass of tissue transplanted, increased the potential for passenger B cells. This is in fact a myth. PLS has been described with transplantation of all minor incompatible organs and stem cells (2,3). Minor incompatible matching is simply a more common allocation in liver than in kidney transplantation. The severity of PLS associated immune hemolysis is probably related to RBC isoagglutinin producing B lymphocytes in the donor before transplantation, and their expansion after it, as measured by the antibody titre in the recipient. Hemolysis is usually self limiting and ceases usually within 6 weeks as the passenger B cells are eliminated (4). Treatment during the period of PLS should include treatment of anemia and if transfusion is required the blood group O (donor type) packed red cells should be used but plasma should be of the recipient's type (2-4).

We encountered an unusual situation where we had to perform a bone marrow transplantation on a young man whose aplastic anemia became evident soon after a liver transplantation for fulminant liver failure (5). The blood group O recipient received a liver from a blood group O donor. He then underwent bone marrow ablation prior to receiving a bone marrow transplant from his human leukocyte antigen (HLA) identical blood group A sister. He made a good clinical recovery but he developed immune hemolysis with increasing titres of Anti-A antibodies. We diagnosed PLS from B-cells in the liver which survived transplantation and bone marrow conditioning. We continued immunosuppression with tacrolimus, azathioprine and steroids. The patient received blood group O packed red cell transfusions as required. PLS faded away about 2 months later and the recipient remains well over 20 years later. This unusual case showed us how resilient the passenger lymphocytes are to cytotoxic therapy but, without persistence, their effect remains self-limited.

Another misconception of PLS is that it only occurs with ABO minor incompatibility. Now we wish to examine other forms of PLS that have significant hematological effects.

Another form of immune hemolysis may arise out of Rhesus (Rh) incompatibility between the donor and the recipient. Several differences between the Rh and ABO system must be noted. While A and B antigens are expressed on a wide variety of tissues, Rh antigens, of which D is the most important, are only present on red cells. Secondly Rh negative individuals do not produce Rh factor such as anti-D unless they are exposed to the antigen, unlike ABO antigen null individuals who constitutively produce antibody to the absent antigen. Therefore, major Rh incompatibility is not relevant in transplantation even if the recipient is sensitized. The biggest concern is that a Rh positive graft might sensitize a Rh negative recipient and have consequences for later transfusion or pregnancy. Covering the patient with passive immunization seems sensible. Interestingly, liver transplantation does not sensitize patients anywhere near as effectively a blood transfusion where tiny aliquots of antigen are known to be highly immunogenic (6). In most cases of Rh minor incompatibility, PLS does not occur because the antigen negative graft is unlikely to have activated plasma cells directed against the Rh antigen. However, PLS has been described in Rh positive recipients of grafts from sensitized Rh negative donors. A common feature of these donors is that they are parous females who were unaware of their sensitization. Rh antigen is highly immunogenic and sensitization persists for life. Immune hemolysis arising from Rh PLS is aggressive and life threatening (7-9). In addition to transfusion with antigen negative red cells, patients may require anti-B-cell therapy and plasmapheresis. They will also require support for the effects of massive intravascular hemolysis with severe hyperbilirubinemia and renal failure. In this special issue of the journal, we report a fatal outcome from anti D antibody PLS immune hemolysis (10). The window for intervention in patients with Rh PLS may close quickly. Interestingly, Rh antibodies are usually immunoglobulin (Ig) G but they do not fix complement. This might be related to the IgG subclass of Rh antibodies (11). The differing effector roles of IgG subclasses is reviewed in another paper in this issue (12). Rh antibodies bind the membrane antigen, marking the red cell for destruction in the spleen. If this is the case, splenectomy might be an effective tool in arresting severe Rh PLS. Almost certainly splenectomy will have to be combined with anti-B-cell therapies as the liver may take over from the absent spleen. One case of severe Rh PLS immune hemolysis has been reported where anti-CD20 monoclonal antibody therapy was successfully followed by splenectomy (13).

Immune hemolysis may be induced by any antibody produced by passenger plasma cells directed against a red cell antigen. While anti-A, anti-B and anti-D antibodies are most commonly described in the literature, case reports of PLS with antibodies to Kpb, Fya, M, Jka and C have been published (1,14-20). In each case, mild or absent immune hemolysis was reported.

PLS may be directed at targets other than RBC antigens. We reported a cluster of PLS causing severe immune

thrombocytopenia in 3 recipients (2 kidney and 1 liver) from the same donor (21). Graft derived antibodies directed against human platelet antigen (HPA) 1A were responsible. Platelet counts dropped below detectable levels. The transfusion requirements of three individuals quickly depleted our supply of HPA 1A negative platelets. One kidney recipient died from uncontrollable hemorrhage. The liver recipient improved immediately after an episode of rejection. The second kidney recipient recovered with a splenectomy. Massive expansion of the passenger donor lymphocytes within the spleen was demonstrated on the specimen. We believe the donor became sensitized from a pregnancy, the last of which was over 20 years prior. Other reports of PLS induced immune thrombocytopenia have been made since then (22,23). Besides Anti-HPA-1a, other anti-platelet antibodies including Anti-GPIIb/IIIa, Anti-GPIb/IX and Anti-PAIgG have been reported to contribute to PLS. If a donor does not express the antigen to which they are sensitized, thrombocytopenia will not be manifest (occult immune thrombocytopenia). Donors with overt immune thrombocytopenia have been associated with high recipient mortality and morbidity to an extent that immune thrombocytopenia should be considered a contraindication to donation (24). Splenectomy or passenger lymphocyte ablation are possible, but untested, remedial strategies.

The liver plays a role in the production of clotting factors, as well as RBC production. Some of the proteins synthesized by the liver include coagulation factors I (fibrinogen), II (prothrombin), V, VII, VIII, IX, X, XI, XIII, as well as protein C, protein S and antithrombin. Polymorphisms of these factors may render them inactive. Liver transplantation provides a new source of these factors and has been shown to restore normal coagulation in hemophilia A and hemophilia B. We had the opportunity to show that liver transplantation could also reverse FXI deficiency, a much rarer condition (25). Patients with inherited hemophilia A can develop antibodies, called inhibitors, to Factor VIII (FVIII) because of exposure to exogenous FVIII. Interestingly, there are no reports of this happening after transplantation of a normal FVIII producing liver into a recipient with hemophilia A. Some patients who do not have inherited hemophilia A develop inhibitors in a condition known as acquired hemophilia. Patients with FVIII inhibitors are at high risk of hemorrhage with surgery. Transplantation represents a particular risk for patients with FVIII inhibitors because the graft endothelium will present antigen to expand FVIII inhibitor production. Ashrani and colleagues described successful liver transplantation for a patient with hemophilia A and intermediate level FVIII inhibitors and suggested that the inhibitors faded with time due to a process of tolerance (26). Hisatake *et al.* described transmission of FVIII inhibitors by PLS from a donor resulting in refractory coagulopathy in the recipient (27). They recommended that liver donation be avoided if high level FVIII inhibitors were present. We performed liver transplantation in a patient with cirrhosis from hepatitis C virus who had hemophilia with high titre FVIII inhibitors. We were confronted almost immediately with massive expansion of the FVIII inhibitor production which appeared to fix complement and cause microangiopathy. Bleeding was uncontrollable until the patient died (28). We recommended that liver transplantation be deferred in similar patients until resilient suppression of FVIII inhibitor was achieved. Horton and colleagues disagreed with us. Using a regime that included FEIBA (factor eight inhibitor bypassing activity) and recombinant activated FVII, they succeeded in transplanting a patient similar to ours. FVIII inhibitor levels increased to 1,000 Bethesda units which persisted for the three months of follow-up that was reported. Late hepatic artery thrombosis occurred with liver abscess formation. The patient was still alive at the time the report was written (29). Presence of antibodies to FVIII in the recipient remains a considerable barrier to liver transplantation. Caution is required to determine if acquired hemophilia is present in a donor as transplantation should be avoided if FVIII inhibitors are present at any level.

Paroxysmal nocturnal hemoglobinuria (PNH) is a rare, acquired hemolytic anemia caused by the absence of a key complement regulatory protein, CD59 which results in intravascular complement-mediated lysis with resulting anemia, hemoglobinuria, and venous thromboses. Bone marrow transplantation may reverse PNH. PNH may result in Budd Chiari syndrome (BCS) requiring liver transplantation. PNH is therefore generally considered a contraindication to liver transplantation but isolated cases of successful liver transplantation have been described with eculizumab, a humanized monoclonal antibody that blocks the activation of the terminal C5 complement (30). We attempted a liver transplantation in a patient with BCS from PNH. The patient was dependant on large doses of eculizumab before transplantation. Recovery was difficult and every intervention required cover with eculizumab. The patient recovered but an incisional hernia repair again required excess eculizumab. Over the first

two-year period after transplantation the patient required twice the dose of eculizumab than before transplantation. Liver transplantation alone does not compensate for host deficiency of CD59.

The origin of endothelial cells is uncertain. Unlike epithelium, replacement cells are not derived locally. Damaged sections of endothelium seem to be replaced by circulating cells from the bone marrow. If not damaged, endothelial cells appear to be long lived. We were able to use the Y chromosome in male-to-female liver transplantation to show replacement of donor endothelium in a transplanted liver with recipient cells that were derived from the bone marrow (31). We had also found that renal transplants which survived vascular rejection were at a very low risk of subsequent rejection episodes (32). We speculated that vascular rejection accelerated endothelial damage, which provides an opportunity endothelial cell replacement. Subsequently Hove and colleagues demonstrated extensive chimerism within vascular endothelium, biliary epithelium and hepatocytes of a liver graft (33). Starzl reviewed our paper and placed it in the context of a continuum of research from the work of Nobel prize winner, Peter Medewar, which implied that endothelial replacement and other chimerism within the graft was a transplantation-mediated phenomenon that could be exploited to reduce or eliminate the life long need for immunosuppression (34).

Summary

All that is required for PLS the antigen positive recipient to have received a graft from a sensitized antigen null donor. PLS against ABO and minor blood group antigens is relatively mild and self limited whereas PLS directed against Rh or HPA 1A is life threatening and persistent. Severe PLS may be treated with transfusion using antigen negative product, plasmapheresis, red cell exchange, anti-CD20 monoclonal antibody, reduced immunosuppression and splenectomy as appropriate. Transplantation to recipients with FVIII inhibitors or PNH is possible but costly leaving patients with their original complaint whereas liver transplantation combined with stem cell transplantation could reverse both the hematological and hepatic disease processes as well as reduce the requirement for immunosuppression. If such a combination is considered, stem cell transplantation should precede the liver for several reasons including the apparent resilience of passenger lymphocytes to conditioning regimes. Liver transplantation is usually followed by recipient repopulation of the graft endothelium which may contribute to reduced liver immunogenicity. Graft-host traffic after liver transplantation is extensive and has significant effects on recipient physiology.

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Footnote

Conflicts of Interest: The authors have no conflicts of interest to declare.

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