

# The Liver Allograft, Chronic (Ductopenic) Rejection, and Microchimerism: What Can They Teach Us?

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**T**HICKENING of the most internal layer of the arterial walls in a human allograft (arterial intimal fibromuscular hyperplasia) was first reported in 1955 by the pathologist Gustav Dammin, in collaboration with Hume.<sup>1</sup> Since then, many different terms have been used to describe this complication of allogeneic kidney transplantation: obliterative arteriopathy (OA), transplant atherosclerosis, transplant arteriopathy, and others. In 1963, Porter et al<sup>2</sup> linked this complication to a genetic disparity between the recipient and the allograft, thus showing OA to be a specific subtype of rejection, widely known today as chronic rejection.

Although OA is the principal manifestation of chronic rejection in all organ allografts, other accompanying features include interstitial and periarterial fibrosis and subsequent atrophy or loss of parenchymal cells.<sup>3</sup> Chronic rejection of the liver, which is the focus of this presentation, has been defined by an international panel of experts, sanctioned by the World Congresses of Gastroenterology<sup>4</sup> as: a "... usually irreversible process characterized by two histopathologic features: obliterative vasculopathy and loss of bile ducts. Although these two components usually co-exist, they occasionally may occur independently. The process is elicited by a genetic disparity between the donor and the recipient, but other co-factors may be involved."

## FEATURES OF CHRONIC LIVER ALLOGRAFT REJECTION

The arterial disease in liver allografts most commonly affects medium and large-sized, first and second-order branches of the hepatic artery in the liver hilum.<sup>5-10</sup> The main feature is intimal thickening because of deposition of macrophage and/or myointimal cells that are often stuffed with lipids, combined with more modest myofibroblast proliferation and collagen deposition.<sup>3</sup> Endothelial cell hypertrophy, transmural inflammation of varying severity, medial thinning, and inflammation and sclerosis of the adventitia also are frequently seen. The thickened intima may contain inflammatory cells, like T and B lymphocytes, macrophages, and occasionally, dendritic cells that signal the presence of an ongoing immune reaction.<sup>3,11</sup> Eventually, the intimal thickening causes luminal narrowing and diminished arterial blood flow to the dependent bile ducts. Small arterioles in the portal tracts are usually spared from the intimal changes but may show medial hypertrophy and/or hyalinization.

When the OA in large perihilar arteries is severe, small portal tract arteries, arterioles, and capillaries in the peribiliary arterial plexus may be completely destroyed.<sup>12,13</sup> This results in compromised arterial flow to the dependent

bile ducts, which can already be damaged or destroyed by direct immunologic injury. Moreover, progressive narrowing of the lumen of larger arteries predisposes them to thrombosis, which in turn can cause necrosis and/or stricture of large perihilar bile ducts.

Damage and loss of small interlobular bile ducts is the second hallmark feature of chronic liver allograft rejection.<sup>6-9,14-16</sup> This manifestation is generally considered to be of secondary importance because isolated duct loss can occur for other reasons, such as obstructive cholangiopathy and adverse drug reactions. Bile ducts normally are present in 75% to 80% or more of portal tracts containing a hepatic arterial branch. Theoretically, duct loss could be considered to be present when more than 25% of the portal tracts are devoid of a bile duct. Unfortunately, in routine clinical practice difficulty with reproducing bile duct and/or portal tract counts, and sampling problems associated with a small biopsy of a large organ, necessitates using a more conservative figure of a 50% deficiency to be certain that there is ductopenia.<sup>4</sup>

## RISK FACTORS AND PATHOPHYSIOLOGIC MECHANISMS OF INJURY

Risk factors identified by different groups for the development of chronic rejection in the liver include multiple and/or poorly controlled acute cellular rejection episodes, chronic rejection in a previous failed allograft, a positive pretransplant lymphocytotoxic crossmatch, anti-MHC antibodies that develop after transplantation, cytomegalovirus (CMV) infection, matching at the class II MHC locus and mismatching at the MHC class I locus, non-Caucasian recipient race, chronic hepatitis virus infection, and treatment with  $\alpha$ -interferon.<sup>6,7,11,15,17-24</sup> Hyperlipidemia, hypertension, diabetes, and preservation injury have, to our knowledge, not been associated with chronic rejection of the liver, but are thought to contribute to the development of OA in other solid organ allografts.

Several groups including ours have suggested that the pathogenic mechanisms involved in the development of OA

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include a response to injury, the same hypothesis as that used to explain the development of atherosclerosis in the general population.<sup>3,11,13,25,26</sup> Thus, attempts at classification are based on the nature of the initial insult. In an allograft, the cause of the injury appears to be primarily rooted in an allogeneic immune response. Likely arterial targets of this response include the endothelium, periarterial dendritic cells, and lymphatic capillary endothelium in the adventitia; disruption of the lymphatics could cause arterial wall edema. However, multiple other cofactors, including preservation injury, viral infections (especially CMV), hyperlipidemia, hypercholesterolemia, hypertension, and diabetes certainly could contribute to the insult and make worse the arterial disease. In any event, the arterial injury triggers a cascade of inflammation, growth and repair (explored in detail by Hayry and colleagues in this issue and elsewhere<sup>11</sup>) that eventually results in the characteristic OA described above.

The second major target for injury in chronic rejection, the bile ducts, appears to be subjected to "double jeopardy".<sup>13,26</sup> They are susceptible to direct immunologic injury from invading inflammatory cells and indirectly to ischemic damage because of arterial occlusion and destruction of the peribiliary capillary plexus.<sup>12,13,26</sup> Direct injury via cytotoxic T lymphocytes (CTL) is usually noted histopathologically and is one of the defining features of both acute and chronic rejection. In addition, cytokines locally released by the invading lymphocytes can either directly injure the ducts or recruit neutrophils and macrophages via chemotaxins that indirectly cause damage through release of oxidative products. These same effector mechanisms can also destroy the small portal arterioles<sup>13,26</sup> and fine webbing of capillaries<sup>12</sup> that are the final conduit of arterial blood to the ducts. In addition, this unique portal microvasculature can be plugged and ruined by platelets and neutrophils in sensitized patients,<sup>20,27,28</sup> explaining the association between preformed antibodies and bile duct loss.

Lastly, Donaldson et al<sup>21</sup> have suggested that bile ducts can also contribute to their own destruction by acting as antigen-presenting cells (APC) when their class II MHC antigens are matched with the recipient. In vitro, we have shown that such a process is possible<sup>29</sup>; however, the duct cells are much less efficient antigen presenters than are endothelial cells tested in the same assay.

#### RESISTANCE OF THE LIVER ALLOGRAFT TO CHRONIC REJECTION

Primary allograft survival for the first 2 to 3 years after transplantation usually is worse for liver than for heart or kidney allograft recipients. After this, the survival curves for primary liver allografts begin to flatten out between 3 and 5 years after transplantation whereas those for the heart and kidney show continual attrition, mostly because of chronic rejection.<sup>30</sup> This apparent difference in long-term allograft resistance to chronic rejection gleaned from survival curves also surfaces at individual centers in studies of the struc-

tural integrity and causes of late allograft dysfunction of liver vs other organs.

Pappo et al,<sup>31</sup> who studied the causes of liver allograft dysfunction in recipients who had survived for more than 5 years with the same liver, showed that recurrent viral hepatitis is more often responsible for late liver allograft dysfunction than either acute or chronic (ductopenic) rejection.<sup>31-34</sup> Obstructive cholangiopathy and recurrent alcohol are also surprisingly common.<sup>31</sup> In all studies of long-term survivors,<sup>31-34</sup> rejection (acute and/or chronic) is found in less than 25% of patients with biochemical evidence of liver injury, and less frequently in asymptomatic patients with normal liver injury tests.

Although these studies of long surviving recipients lend credence to the notion that a liver allograft is less susceptible than kidney or heart allografts to chronic rejection, Wight et al (this issue) have offered the alternative explanation that needle biopsy evaluation may underestimate the incidence of chronic rejection. This suggestion was not supported in a recent series of tolerance induction experiments by Murase et al<sup>35</sup> that focused on microchimerism in rats. Experimental evidence was provided showing that liver allografts are more resistant to chronic rejection than hearts. It was shown that a liver allograft can protect another solid organ from the same donor from chronic rejection.<sup>35</sup> In these experiments, a 2-week course of FK 506 was able to induce acceptance for more than 100 days of a liver, heart, kidney, or bone marrow allograft. At first, these observations seemed an apparent contradiction to our recent hypothesis that microchimerism is a necessary precondition for the evolution of donor-specific tolerance.<sup>36-39</sup> All of these drug-free animals accepted at 100 days a challenge heart allograft from the same donor with survival for 100 more days without regard for the kind of primary transplant, and with or without hematolymphoid chimerism detectable by immunocytochemistry. However, examination of the surviving challenge cardiac grafts 100 days posttransplantation showed both multiple Quilty lesions<sup>40</sup> and severe OA in the animals conditioned initially with a heart graft. In contrast, the challenge hearts in rats initially given a liver allograft of the same donor strain were completely protected from chronic rejection and had no Quilty lesions or OA. In these experiments, the presence of hematolymphoid microchimerism in the recipient correlated with resistance to chronic rejection, although the study design precluded exclusion of a role for hepatic parenchymal cells.<sup>35</sup> At the end, we also concluded that we had developed a useful experimental model with which to study chronic rejection in the challenge grafts without interference from immunosuppressive drugs.

We believe that protection from chronic rejection in this model is mediated by the multilineage donor hematolymphoid cells that emigrate from the liver and persist in small numbers in the recipient.<sup>36,37,39,41,42</sup> In essence, the hypothesis holds that a tiny fragment of the donor immune system, which is responsible for immunologic self-definition of the donor, integrates into the larger immunologic network of

the recipient.<sup>39,43</sup> If this is correct, one might expect that other properties of the donor immune system, such as resistance to autoimmunity, could be transferred to the recipient as previously shown for delayed-type hypersensitivity reactions.<sup>36</sup>

Delaney et al<sup>44</sup> recently tested this corollary hypothesis in BN rats which are susceptible to mercuric chloride-induced autoimmunity. LEW rats, which are resistant to HgCl<sub>2</sub>-induced autoimmunity, were used as allogeneic bone marrow donors. In this BN model, rejection of the allogeneic bone marrow is prevented by a 2-week course of FK 506. Although all immunosuppression is then stopped, rare (<1:3,000) donor LEW hemolymphoid cells continue to survive in the BN recipients for more than 100 days. These microchimeric recipients become almost totally resistant to the development of autoimmunity from the HgCl<sub>2</sub> injections.<sup>45</sup>

These experimental observations may be relevant to the observation in long-term liver allograft survivors treated originally for autoimmune chronic active hepatitis and primary biliary cirrhosis (PBC) who have a low incidence and severity of recurrent disease.<sup>46-51</sup> The potent immunosuppression required to prevent rejection has been used to explain these findings, but the same lack of recurrence has been noted in patients weaned from immunosuppression.<sup>52</sup> It may be that the immune stimulation and persistence of an integrated fragment of the donor's immune system analogous to that in the rat experiments is the critical protective factor.

The discovery that the development of sustained microchimerism is an integral component of whole organ graft acceptance has opened many cryptic portals of transplantation immunology and has provided a rational explanation for chronic rejection. This concept will allow new insights about chronic rejection to be developed and subjected to direct experimentation. Our prediction is that immunobiologic manipulation of the recipient, such as the augmentation by donor bone marrow that currently is being evaluated<sup>53</sup> clinically, will dispel the cloud of chronic rejection that has for so long darkened the horizon for kidney and heart allograft recipients.

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