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REVIEW

Current views on rejection pathology in liver transplantation

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Summary

Histological assessments continue to play an important role in the diagnosis and management of liver allograft rejection. The changes occurring in acute and chronic rejection are well recognized and liver biopsy remains the 'gold standard' for diagnosing these two conditions. Recent interest has focused on the diagnosis of late cellular rejection, which may have different histological appearances to early acute rejection and instead has features that overlap with so-called '*de novo* autoimmune hepatitis' and 'idiopathic post-transplant chronic hepatitis'. There is increasing evidence to suggest that 'central perivenulitis' may be an important manifestation of late rejection, although other causes of centrilobular necro-inflammation need to be considered in the differential diagnosis. There are also important areas of overlap between rejection and recurrent hepatitis C infection and the distinction between these two conditions continues to be a problem in the assessment of liver allograft biopsies. Studies using immunohistochemical staining for C4d as a marker for antibody-mediated damage have found evidence of C4d deposition in liver allograft rejection, but the functional significance of these observations is currently uncertain. This review will focus on these difficult and controversial areas in the pathology of rejection, documenting what is currently known and identifying areas where further clarification is required.

Introduction

Since 1997, with the introduction of the Banff classification of liver allograft rejection, most centres have a unified approach to the diagnosis and grading of acute cellular rejection [1]. Although the prevalence of acute rejection (AR) is declining, 20–40% of patients still have one or more episodes requiring treatment with additional immunosuppression [2]. These usually occur during the first 3 months of transplantation and the diagnosis at this time is generally easy. The updated Banff schema published in 2000 is also widely used for the diagnosis and staging of chronic rejection (CR) [3].

Difficulties arise with the diagnosis of late cellular rejection and when rejection presents with 'pure' centrilobular necro-inflammation – isolated central perivenulitis (ICP). It is also unclear whether an indolent subclinical form of rejection exists in the long-term graft. Problems exist around the terminology used to describe inflammatory changes in late post-transplant biopsies that are probably alloimmune-mediated but lack typical histological features of AR or CR. Simplistically, rejection is the immunological attack by the host on the graft, excluding mechanisms which resulted in the native graft disease, and as such could encompass long-term inflammatory changes currently referred to as 'idiopathic' post-transplant chronic hepatitis (IPTH) or '*de novo* autoimmune hepatitis' (DNAIH).

Antibody-mediated rejection (AMR), previously considered to be inconsequential in liver allografts, is beginning to be recognized as a possible cause of early and late graft injury. The histological diagnosis of AMR is difficult, the true incidence is uncertain and the use of C4d immunostaining in the diagnosis is held back by a lack of studies correlating with donor-specific antibodies.

In patients transplanted for hepatitis C, the differentiation of recurrent hepatitis C from rejection, usually late AR, is often difficult. This is attributed to the two conditions having overlapping histological features and frequently occurring simultaneously. In these circumstances, identifying which of the two is the predominant process becomes important.

The aim of this review is to focus on these difficult and controversial areas in the pathology of rejection, documenting what is currently known and identifying areas where further clarification is required.

Acute rejection

Acute rejection typically occurs early, usually within the first month, and has a classic triad of features: portal inflammation, bile duct inflammation and venous inflammation [1,4,5] (Fig. 1). At least two of these three features are required for a diagnosis of AR. Portal inflammatory cells typically include a mixed population of lymphocytes (mostly T cells), 'blast' cells, macrophages, neutrophils and eosinophils in varying proportions. The presence of a mixed inflammatory infiltrate is helpful in distinguishing AR from other graft complications associated with portal inflammation [e.g. viral or autoimmune hepatitis (AIH)], where the infiltrate is usually mainly mononuclear. Minor degrees of bile duct inflammation and portal vein endothelitis can be seen in association with other causes of portal inflammation (e.g. hepatitis C virus infection) [6]. The presence of more severe lesions favours a diagnosis of rejection. These predominantly portal-based features may occur in conjunction with a range of centrilobular necro-inflammatory changes, together termed central perivenulitis (CP) and comprising hepatic venous inflammation with perivenular inflammation and variable degrees of perivenular hepatocyte loss. Perivenular inflammation and hepatocyte necrosis are required to make a diagnosis of severe AR using the Banff criteria [1]. Congestion or haemorrhage may also be present in these areas. Rarely, early AR presents with centrilobular inflammatory changes in the absence of the typical portal triad, termed ICP [7].

Late acute rejection

Late AR, occurring >3 months after transplantation, differs from early AR, both by having 'less classical' histological features, and by occurring at a time when there are more likely to be other graft complications such as recurrent disease. Late AR can be portal and central, but the central component is more common and prominent and more frequently occurs as ICP [8,9] (Fig. 2). Hepatic venous endothelial inflammation is often not present at this stage, but just perivenular inflammation with variable amounts of hepatocyte loss. The portal inflammatory infiltrate is often of lesser intensity, tends to be mainly mononuclear rather than mixed and interface activity is more conspicuous. Inflammation of bile ducts and portal venous endothelium is also less obvious. Late cellular rejection thus has a more hepatitic appearance, with IPTH, 'DNAIH' and a recurrent hepatitis (viral or autoimmune) coming into the differential diagnosis. Late AR is more frequently associated with the development of features heralding the onset of early CR, such as bile duct atrophy, early duct loss or centrilobular fibrosis. While these changes classically follow AR, which fails to respond to treatment, cases presenting late may have a more insidious course [10,11]. Nonprogressive bile duct loss may also result from treated severe AR [12,13].

Central perivenulitis

Central perivenulitis (Figs 1b and 2), as part of rejection, is relatively common, occurring in 40% of patients during

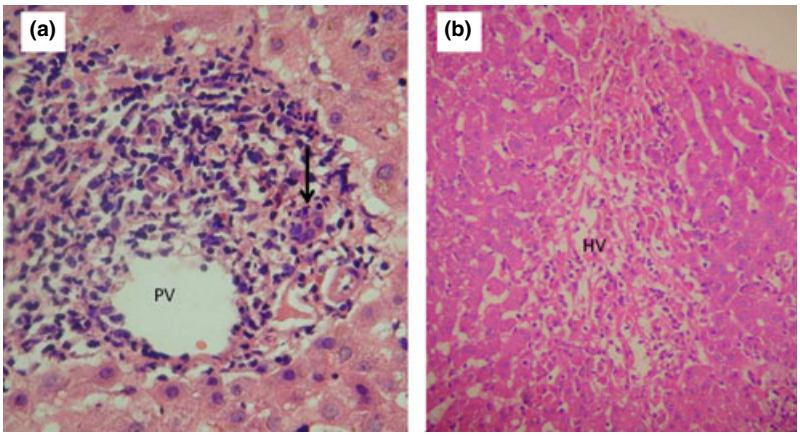

Figure 1 Photomicrographs of typical acute rejection. (a) Portal tract showing the triad of portal inflammation, inflammation of the portal vein (PV) and inflammation of bile ducts (arrow). (b) Central perivenulitis with inflammation of the hepatic vein (HV) and surrounding parenchyma with hepatocyte loss.

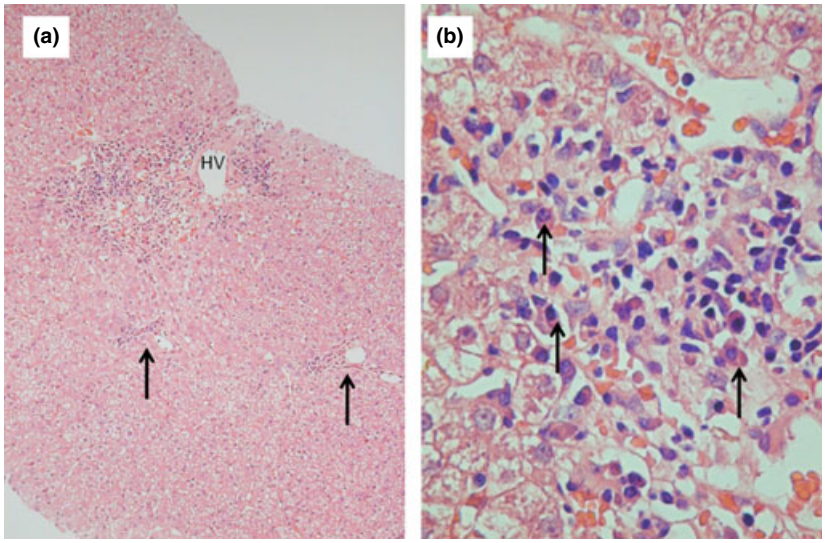

Figure 2 Late acute rejection presenting as isolated central perivenulitis. (a) Portal tracts (arrow) are not inflamed and there is central perivenulitis around the hepatic veins (HV). (b) a high-power view of central perivenulitis with prominent plasma cells (arrows).

their first rejection episode [14]. ICP is generally preceded by at least one episode of CP occurring in conjunction with portal features of rejection [8,9,14,15], occurs in around 30% of protocol biopsies [8,9,16] and is more commonly seen more than 1 year post-transplant [9,17], often with no or mild abnormalities in liver function tests [9]. A grading system for CP proposed by the Banff group [7] appears to correlate with adverse outcomes [9]. Rejection is the usual cause of ICP, but other causes of centrilobular injury such as ischaemia, drug toxicity, viral hepatitis (recurrent or acquired) and AIH (recurrent or acquired) should also be considered [18]. It is usually possible to differentiate between these causes based on time post-transplant, the degree of associated inflammation and the clinical circumstances [7,18]. Recurrent episodes of AR are more likely if CP is present in the first AR episode [14]. CP is less responsive to augmented immunosuppressive therapy than portal ACR [9,19,20] and is often present in cases of ACR, which progress to CR [3,9,15,20–27]. CP usually precedes bile duct loss and prompt augmentation of immunosuppression may prevent progression to irreversible CR [3,20,23]. Inflammation in the graft long term and the development of centrilobular fibrosis are associated with preceding CP [9].

While cell-mediated rejection undoubtedly plays a role in CP, it has been found that the infiltrates are often rich in plasma cells [17,28,29] (Fig. 2b), and may be associated with perivenular sinusoidal C4d deposition [30] and the presence of auto- or allo-antibodies [9,31–35]. Furthermore, CP occurs as part of severe AMR in ABO-incompatible (ABOI) transplants [36]. These observations suggest a possible contributory role of antibody-mediated injury.

Chronic rejection

The prevalence is declining and fewer than 2% of grafts now fail as a consequence of CR [4]. The classical presentation with graft failure during the first 12 months post-transplant is less common and more cases are now presenting later, when they may have an indolent course running over a period of several years [11]. Two main histological features are loss of bile ducts and an obliterative arteriopathy affecting large- and medium-sized arteries. Changes are also commonly present in centrilobular regions of the liver parenchyma [3]. Early CR is characterized by inflammatory and degenerative changes in bile ducts, which have an atrophic or 'dysplastic-like' appearance associated with features of replicative senescence [3,37] (Fig. 3). It is generally accepted that ductopenia should be present in more than 50% of portal tracts in order to make a firm diagnosis of CR. However, duct loss can be patchy in distribution and the assessment of bile duct numbers should be interpreted with caution, particularly in small biopsies with fewer than 10 portal tracts. Unlike other ductopenic diseases, CR is not typically associated with bile ductular reaction or periportal fibrous expansion. However, these changes can be seen in cases where CR presents later (>1 year post-transplant) and is associated with a prolonged course [10].

Arterial lesions are largely confined to large- and medium-sized vessels and are thus rarely seen in needle biopsy specimens. Early lesions are mainly inflammatory and include lymphocytes (mainly T cells) and lipid-laden macrophages. Subsequently, there are increasing numbers of myofibroblasts associated with varying degrees of intimal fibrosis. Small portal tracts may show a reduced

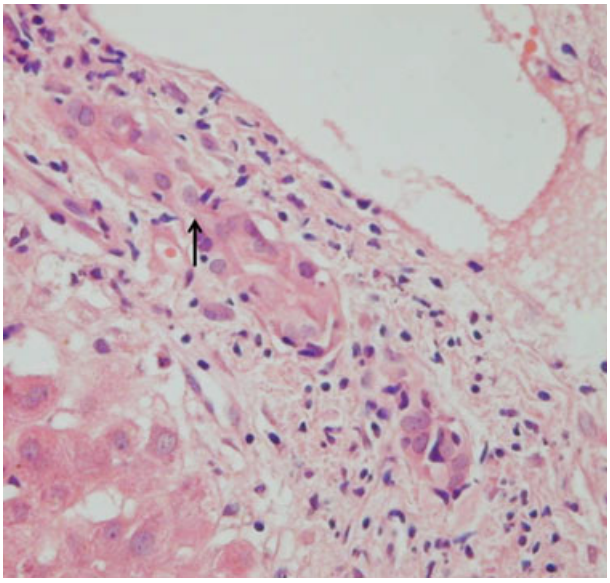

Figure 3 An abnormal bile duct (arrow) in early chronic rejection. There is nuclear pleomorphism and loss of polarity producing a 'dysplastic-like' appearance.

number of small arterial branches and other microvascular channels.

Centrilobular changes include bilirubinostasis, hepatocyte ballooning and hepatocyte loss. Centrilobular inflammation may be present in the early stages ('central perivenulitis'), but often subsides as the disease progresses. In cases with a prolonged course, there is development of centrilobular fibrosis, which may ultimately progress to cirrhosis – this typically has a veno-centric pattern, related to obliteration of hepatic and portal veins [3,10].

Relationship between acute and chronic rejection

The subdivision of rejection into acute and chronic forms is based on three main diagnostic features: time of occurrence (acute – early, chronic – late), response to immunosuppression (acute – reversible, chronic – irreversible) and histological features. While this approach to classifying rejection has proved to be useful clinically, there is an increasing awareness of areas of overlap for each of these features. One example is the recognition of late AR as an entity that has some features that lie between typical early AR and end-stage CR. A summary of the main clinical and histological features of early and late AR and CR and their differential diagnoses is presented in Table 1. For a more detailed discussion of the typical pathological features of liver allograft rejection, the reader is referred elsewhere [1,3–5].

Idiopathic post-transplant hepatitis

The term 'idiopathic' post-transplant hepatitis (IPTH) has been used to describe cases presenting with features of chronic hepatitis that are not readily ascribed to a recognized cause such as viral infection or recurrent autoimmune disease [4,5,7]. IPTH consists of a predominantly mononuclear portal inflammatory infiltrate associated with interface hepatitis and/or parenchymal inflammation including CP with variable hepatocyte loss, without typical features of AR or CR [4,7,38,39] (Fig. 4). The prevalence is difficult to determine because of inconsistent use of terminology for unexplained inflammatory changes in late post-transplant biopsies. Furthermore, IPTH is often subclinical, being more apparent in centres, which perform protocol biopsies, because abnormalities in liver enzymes may be minimal or absent [38,40–42]. Overall, inflammatory changes that could be classified as IPTH have been observed in 10–50% of patients undergoing protocol biopsy >1 year post-transplant [41] and more than 60% of children biopsied >10 years post-transplant [38], making this the commonest diagnosis in annual review biopsies in some centres [43]. In protocol biopsy series, IPTH is documented in 10–30% of adults [40,44,45] and in 22–64% of children [38] compared with 2–11% of children and adults [39,46,47] in indication biopsy series, supporting the concept of this often being subclinical. The prevalence of IPTH increases with time post-transplant [38,42]. There is increasing evidence to suggest that IPTH is an important cause of late graft fibrosis, in some cases progressing to cirrhosis [38,42,46]. Cirrhosis occurs in 10–15% of adults and up to 35% of children at 5–10 years post-transplant [38–40,46,48]. In one study, IPTH was the commonest cause of graft cirrhosis occurring in the absence of disease recurrence [49]. IPTH is more likely to occur in recipients with previous rejection, particularly late AR [39] and CP can progress to a chronic hepatitis with fibrosis [9]. No association was found with blood type compatibility, gender mismatch or HLA matching [39]. Autoantibodies are associated with IPTH in 24–73% of patients [38–40,46]. Treatment with steroids improves the biochemical abnormalities with disappearance of interface activity and may result in a reduction in fibrosis, despite the persistence of autoantibodies in 41% of cases [39]. There also may be a centre bias – IPTH is most frequent in centres which traditionally run patients on low levels of immunosuppression [41], supporting IPTH being a rejection related phenomenon. This may at least partially explain the low incidence in the French series at 10 years post-transplant [11]. Overall, the majority of studies conclude that IPTH is probably a chronic hepatitic form of rejection [38–40,46] as originally alluded to by Kemnitz *et al.* [50].

Table 1. A summary of the main clinical and histological features of early acute rejection, late acute rejection and chronic rejection and their differential diagnoses.

	Early acute rejection	Late acute rejection	Chronic rejection
Clinical features			
Time of presentation	Most episodes occur during 1st month	Any time (after early post-transplant period*†	'Classical' cases progress to graft failure during 1st year – rarely seen now More cases now present >1 year post-transplant
Symptoms and signs	Fever, malaise, jaundice, graft tenderness, reduced bile production	More frequently asymptomatic, particularly during early stages May subsequently develop features similar to early acute rejection	Classical cases present with progressive jaundice Ascites (in cases with hepatic veno-occlusive lesions) Cases presenting later more frequently asymptomatic during early stages. Subsequently develop progressive jaundice.
Biochemical changes	Predominantly cholestatic (rising Alk Phos, gamma GT, bilirubin)	Predominantly hepatitic (rising ALT and AST)	Variable Frequently hepatitic during the early stages, subsequently become progressively cholestatic
Histological findings			
Portal tract inflammation	Mixed inflammatory cell infiltrate (lymphocytes, macrophages, neutrophils, eosinophils)	Inflammatory cells mainly mononuclear (lymphocytes, plasma cells, macrophages)	Early stages may have inflammatory features of early or late AR Inflammation subsides as disease progresses.
Bile ducts	Variable inflammation (mild to severe)	Inflammation rarely more than mild	Variable inflammation (during early stages) Bile duct atypia/senescent changes (during early stages) Progressive duct loss
Portal veins	Variable inflammation (mild to severe)	Inflammation rarely more than mild	Variable inflammation (during early stages) Portal veno-occlusive lesions may develop later
Hepatic arteries	Arteritis may occur in severe cases (rarely seen in needle biopsies)	Arterial lesions rarely seen in needle biopsies	Loss of small arterial branches during early stages (usually precedes duct loss) Obliterative lesions in medium-sized and large arteries (rarely seen in needle biopsies)
Ductular reaction	Variable (extent correlates with severity of cholestasis and bile duct injury)	Rarely more than mild	Typically absent in cases developing graft failure during 1st year May occur in cases presenting later and be associated with features of chronic cholestasis and the development of biliary fibrosis
Interface hepatitis	Rarely more than mild	Variable (may be prominent in cases with 'autoimmune features')	Rarely more than mild
Lobular inflammation	Variable central perivenulitis. Usually associated with hepatic vein endothelitis More diffuse spotty inflammation occurs less frequently	Central perivenulitis more frequent than in early acute rejection Typically occurs with little/no hepatic vein endothelitis	Central perivenulitis common during early stages Inflammation usually subsides later
Centrilobular hepatocyte damage	Ballooning and bilirubinostasis common especially in first few weeks, but largely related to preservation-reperfusion injury	Ballooning and bilirubinostasis uncommon	Ballooning and bilirubinostasis common
	Centrilobular hepatocyte loss (in cases with central perivenulitis)	Centrilobular hepatocyte loss (in cases with central perivenulitis)	Centrilobular hepatocyte loss may persist as inflammation subsides during later stages and progress to centrilobular fibrosis
Hepatic veins	Variable inflammation (mild to severe)	Inflammation rarely more than mild	Variable inflammation (during early stages) Hepatic veno-occlusive lesions may develop later

Table 1. continued

	Early acute rejection	Late acute rejection	Chronic rejection
Fibrosis	Not seen	Variable – typically mild, but may progress with time Pattern may be: • Periportal (in cases associated with interface hepatitis) • Centrilobular (in cases associated with central perivenulitis)	Variable, may progress with time Pattern may be: • Veno-centric (related to obliteration of hepatic and/or portal vein branches) • Periportal/biliary (in cases with duct loss and ductular reaction • Centrilobular (as a consequence of central perivenulitis) In some cases there is progression to bridging fibrosis, rarely leading to cirrhosis
Main differential diagnoses	Preservation/reperfusion injury (produces centrilobular changes of ballooning and bilirubinostasis without inflammation) Biliary obstruction or AMR should be considered in cases with unusually prominent ductular reaction associated with portal oedema‡	Recurrent viral hepatitis (HBV, HCV) Recurrent autoimmune hepatitis De novo autoimmune hepatitis 'Idiopathic' post-transplant hepatitis§	Recurrent PBC Recurent PSC Ischaemic cholangiopathy ¶

*Definitions of 'late' rejection range from >30 days to >12 months post-transplantation.

†Although late rejection typically has different histological features, some cases presenting late have features that are indistinguishable from early acute rejection

‡The diagnosis of acute rejection is rarely a problem during the first month as other causes of graft inflammation are uncommon at this time.

§All of the conditions listed in the differential diagnosis of late acute rejection may be associated with inflammatory changes in portal/periportal and centrilobular regions that overlap with late acute rejection. As discussed in the text, there is increasing evidence to suggest that late acute rejection, *de novo* autoimmune hepatitis and 'idiopathic' post-transplant hepatitis are part of an overlapping spectrum of immune-mediated damage in the liver allograft.

¶Distinction from other causes of graft dysfunction is rarely a problem in cases of chronic rejection presenting during the first few months. Later cases in which duct loss is accompanied by ductular reaction and a 'biliary pattern' of fibrosis may be difficult to distinguish from other diseases associated with chronic cholestatic injury in the liver allograft.

De novo autoimmune hepatitis

A subset of post-transplant chronic hepatitis cases has been labelled as DNAIH based on criteria for diagnosing primary AIH [51] including a prominent plasma cell infiltrate with interface hepatitis (Fig. 5), hypergammaglobulinaemia, raised serum transaminase levels and the presence of 'auto'antibodies. The appropriateness of using these criteria in the post-transplant setting remains uncertain [52]. This entity was first recognized in children [53] and later in adults [54], occurring in 5–10% of paediatric patients [4,55,56] and up to 3.4% of adult patients [4,57]. The higher prevalence in children might be attributable to immunosuppressive drugs interfering with normal T-cell maturation.

Autoantibodies are found in 20–74% of paediatric recipients [32,38,58–60], and 60–70% of adult recipients [61,62], with anti-smooth muscle antibody most common [58,60], and the prevalence increasing with time post-transplant [38,60,62]. As autoantibodies are frequently present in patients with normal liver biochemistry, liver biopsy is required to determine the nature and severity of any associated graft damage [32,58,60]. The autoantibodies found post-transplant may have an atypical staining pattern on rat liver sections, staining the cytoplasm of hepatocytes around hepatic venules [31,33,35,57]. At least a subset of these atypical antibodies has been found to be directed towards glutathione *S*-transferase T1 (GSTT1), in recipients with a GSTT1 mismatch to the positive donor indicating that this is an alloimmune response [35]. The

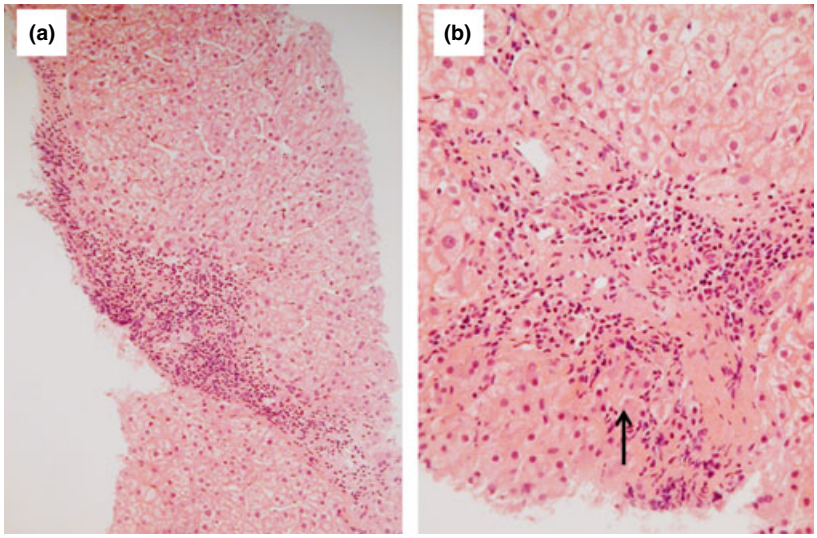

Figure 4 Idiopathic post-transplant chronic hepatitis. (a) There is bridging fibrosis with a moderate chronic inflammatory cell infiltrate in portal/septal areas. (b) Interface hepatitis is seen (arrow) with no obvious inflammation of bile ducts or vessels.

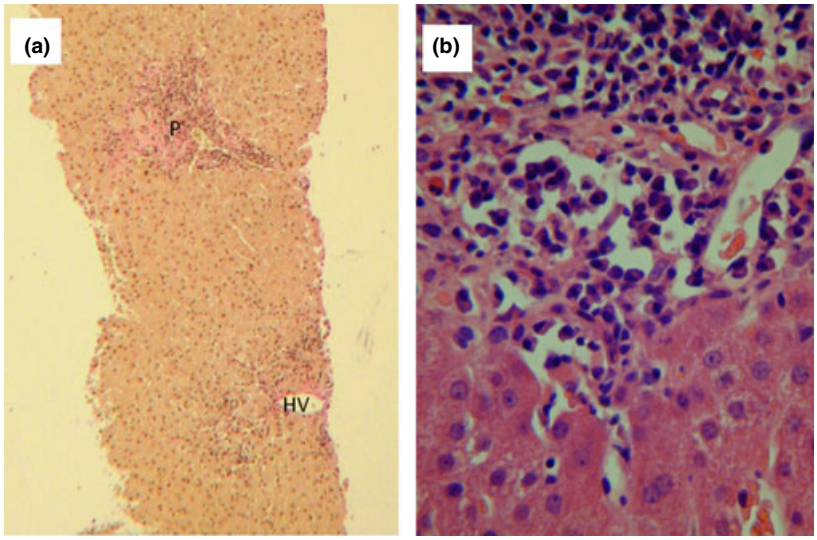

Figure 5 Chronic hepatitis with prominent plasma cells in a patient transplanted for metastatic gastrointestinal stromal tumour. (a) There is inflammation affecting both portal tracts (P) and centrilobular areas. HV, hepatic vein. Haematoxylin–van Gieson stain. (b) The edge of a portal tract containing an almost pure population of plasma cells, which are associated with mild interface hepatitis. The patient was subsequently found to have autoantibodies and a raised IgG and was thus labelled as *de novo* AIH.

glutathione *S*-transferase T1 protein is expressed in hepatocytes, and is lacking in 20% of the Caucasian population and up to 58% of non-Caucasians because of polymorphisms in the gene [33,34]. Compared with AIH in the native liver, lobular inflammatory changes tend to be more prominent in DNAIH [57,63] and may present as ICP [7,9,18]. These observations support the concept that DNAIH may represent an alloimmune response (i.e. a form of rejection), in which immune-mediated injury is directed towards hepatocytes rather than bile ducts or vascular endothelium. Further support for an alloimmune mechanism in DNAIH is the strong correlation with previous rejection history and steroid dependence [28,55,59,64]. In addition, the presence of plasma cell-rich infiltrates and other autoimmune-like histological features is associated with suboptimal immunosuppression [61] or augmentation of the host immune response with the use of pegylated interferon [65–68]. Furthermore, plasma cells form part of the rejection infiltrate, particularly in late biopsies, [17,28,29,61] and autoantibodies can occur with typical AR and CR episodes [32,60,69–71].

Recurrent hepatitis C

Re-infection is universal and begins within a few hours of implanting the new liver. Most cases (>80%) develop graft inflammation related to HCV, but the severity and clinical consequences of graft re-infection are very variable. Histological features are mostly similar to those that are seen in the native liver, with some important differences in the allograft. The disease tends to behave in a more aggressive manner. This may be manifest by more

severe inflammatory activity, which sometimes includes areas of confluent and bridging necrosis (very uncommonly seen with HCV infection in the nontransplant setting). There is also more rapid progression to fibrosis and cirrhosis, i.e. approximately 20–30% of patients are cirrhotic at 5–10 years post-transplant [4]. There may be atypical features, some of which probably reflect the effects of immunosuppression. These include features resembling AIH and cholestatic features resembling so-called fibrosing cholestatic hepatitis, first described as a complication of HBV infection. There are also important interactions with other graft complications, particularly rejection, which may produce complex histological changes that are difficult to interpret.

The distinction between HCV infection and rejection as a cause for graft dysfunction continues to be a major problem clinically [72–74]. Noninvasive methods are not reliable in making the distinction and this therefore remains a common indication for liver biopsy. Unfortunately, the two conditions also have overlapping histological features, making the assessment of liver allograft biopsies difficult. Both conditions are characterized by predominantly portal-based inflammation, which may involve bile ducts and portal venous endothelium. In most cases, the time of occurrence and pattern of inflammation enable the main cause of graft damage to be identified with a reasonable degree of confidence. Most episodes of AR occur during the first 3 months of transplantation, a time at which portal inflammatory changes related to recurrent HCV infections are unlikely to occur. Features favouring a diagnosis of rejection include a mixed population of inflammatory cells with moderate or worse inflammation of bile ducts and/or portal veins. By contrast, the portal inflammatory infiltrate in HCV is mainly mononuclear (sometimes with formation of lymphoid aggregates) and inflammation of bile ducts and vessels is mild. Ductopenia suggests progression to CR and is not a feature seen in HCV infection alone. Lobular inflammatory changes in rejection are mostly perivenular in location and may be associated with varying degrees of hepatocyte necrosis, whereas in hepatitis C, they tend to be more diffuse and spotty and are typically associated with fatty change and acidophil body formation.

Biopsies in which distinction between hepatitis C and cellular rejection is difficult are likely to have changes reflecting a combination of both conditions [7,72] (Fig. 6). In the majority of such cases, rejection-related changes are at most mild in severity; recurrent HCV is best regarded as the primary diagnosis and anti-rejection therapy is not indicated. Increased immunosuppression should only be considered as a treatment option if features of cellular rejection are at least moderate in severity, or if there are features suggesting progression to CR.

As the distinction between recurrent hepatitis C and rejection continues to be difficult in some cases, various groups have looked at immunohistochemical markers to help in the differential diagnosis. These include staining for C4d as a marker of rejection [75–77], for HCV antigens as a marker of HCV infection [78–80] and for the cell-cycle protein mcm-2 to identify the rate of proliferation in portal lymphocytes, which is higher in rejection than HCV [81]. Some of these approaches have helped to identify the main cause of graft damage when histological findings were otherwise inconclusive [78,79,81]. However,

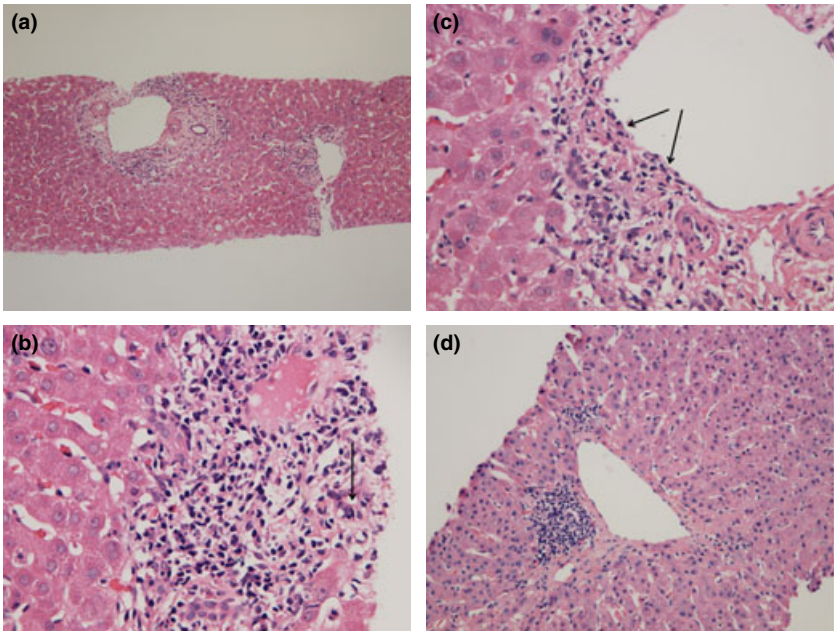

Figure 6 Liver biopsy 3 years post-transplant from an HCV-positive patient who presented with a raised AST (7× normal) showing features in keeping with recurrent HCV and rejection. (a) Features compatible with mild chronic hepatitis C include portal tracts showing mild inflammation with lymphoid aggregate formation, mild interface hepatitis and mild steatosis. Additional features suggesting the presence of co-existent rejection are prominent bile duct inflammation and damage (arrow, b), portal vein endothelitis (arrows, c) and small foci of central perivenulitis (d).

problems with obtaining reproducible staining for HCV antigens in routinely processed tissues and lack of diagnostic specificity of C4d immunostaining [30] limit the utility of these approaches in routine diagnosis.

Recent studies have identified features resembling AIH in patients transplanted for HCV. These have occurred in two main settings. Some have occurred as a complication of antiviral therapy, possibly reflecting an alloimmune response triggered by interferon-induced stimulation of the host immune system [65–68]. Others appear to be unrelated to antiviral therapy and may instead reflect features of concurrent DNAIH [82] or rejection related to suboptimal immunosuppression [61]. In addition to having plasma cell-rich portal and lobular inflammatory infiltrates ('plasma cell hepatitis'), most cases are associated with centrilobular necro-inflammatory features of 'central perivenulitis'. Cases with autoimmune features have a worse outcome than those with 'typical' recurrent HCV.

Antibody-mediated rejection

Antibody-mediated rejection presenting as hyperacute rejection was initially described by Demetris in the precyclosporin era in both ABOI transplants and when preformed lymphocytotoxic antibodies were present [83–85]. The changes were nonspecific initially, resembling a severe preservation reperfusion injury, with intrasinusoidal neutrophil and platelet aggregates and platelets lining vessels. A portal ductular reaction developed with time, often associated with a neutrophil infiltrate. In severe cases, areas of coagulative hepatocyte necrosis progress to large geographic areas of infarction associated with large vessel thrombosis, variably affecting portal and hepatic veins, hepatic arteries and the inferior vena cava [83,84]. ABOI transplantation subsequently ceased, newer immunosuppressants were developed and AMR was no longer considered a problem. More recently, cases of AMR in ABO-compatible (ABOC) liver allografts have been reported [86–90]. ABOI transplants have recommenced in Japan using regimens to lower antibody titres to minimize early graft failure and these have allowed more detailed assessment of histological features correlated with the antibody titres [91,92]. Early/mild AMR is characterized by portal oedema, ductular reaction and a neutrophil-rich inflammatory infiltrate resembling changes seen in biliary obstruction. Portal haemorrhage occurs in more severe cases and is associated with worse graft survival [91]. Periportal coagulative necrosis occurs rarely and is also an adverse prognostic feature [91]. C4d staining of portal capillaries occurs in mild/early AR with stromal staining around portal capillaries and/or bile ducts occurring in the more severe stages [36], possibly an indication of severe microvascular damage with extravasation of serum. Sinusoidal fibrinous sludge and bilirubinostasis develop with time [91]. In failed allografts, there is large bile duct necrosis, sclerosing cholangitis, hepatic artery thrombosis and less commonly submassive necrosis [91].

C4d staining has also been studied in ABOC transplants [75,76,93–98], but the findings are more difficult to interpret because of the lack of correlation of C4d staining with donor-specific antibodies (DSAs). It appears that sinusoidal endothelial C4d staining occurs most commonly (44.5%) (Fig. 7a), followed by portal capillary/venular C4d staining (33.3%) (Fig. 7b) with both patterns occurring together least often (22.2%) [30]. Portal staining can extend into periportal sinusoids [30]. The portal 'biliary' features suspicious of AMR [36,83–85,91] correlate with

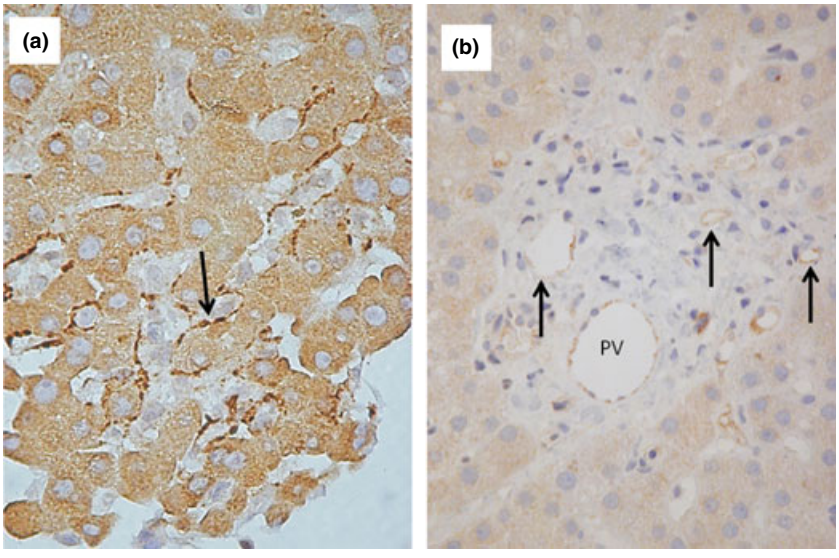

Figure 7 Immunostaining for C4d. (a) Sinusoidal C4d staining pattern. There is dark brown linear C4d staining of sinusoidal endothelial cells (arrow). (b) Portal C4d staining pattern. There is C4d staining of portal microvessels (arrows) and portal vein (PV).

the likelihood of positive C4d staining [30]. Sinusoidal C4d deposition has been found to occur in association with areas of lobular necrosis [30,87,97], and there is some evidence to suggest that CP associated with sinusoidal C4d staining is less responsive to antirejection therapy progressing to CR (El-Maghraby MM. Mechanisms of centrilobular necrosis in chronic liver allograft rejection 2005. PhD Thesis, University of Birmingham, UK). Both preformed DSAs [30,87] and *de novo* DSAs [88,89] have been found to produce these C4d staining patterns.

Antibody-mediated rejection associated with positive C4d staining occurs in conjunction with ACR [30,88,89], particularly if there is a central component to the ACR [94] and independent of ACR [87] where it mimics biliary obstruction with portal oedema and a ductular reaction [87]. C4d staining has been demonstrated in CR [30], a high incidence of anti-tissue antibodies has been found in patients experiencing CR [32,69] and there is an increased incidence of CR in patients with preformed DSAs [99], all of which suggest that AMR plays a role in the development of CR.

Conclusions

There is an overlap of features between late AR, CP, IPTH and DNAIH and the relationship between these entities needs to be further clarified. The contribution of antibody-mediated mechanisms to these processes also requires further assessment. The true extent of AMR is yet to be determined and requires the routine use of C4d staining and simultaneous DSA testing, ideally of both HLA and non-HLA antibodies, including 'autoantibodies'.

There is increasing evidence to suggest that the histological features of rejection change with time. Although we have discussed late AR, IPTH and DNAIH as separate entities, we believe that these three conditions may be part of an overlapping spectrum of immune-mediated damage in late post-transplant biopsies. In some cases, these changes may occur in a subclinical form, possibly related to inadequate immunosuppression, only revealed by the use of protocol biopsy. CP and bile duct loss ('chronic rejection') can occur at each time point within this rejection continuum. The term chronic rejection may be applied more appropriately to the hepatitic form of rejection, encompassing both IPTH and DNAIH, which can result in progressive fibrosis in some cases leading to cirrhosis. The immunological mechanisms involved at each point need clarification as to the role of cell-mediated and antibody-mediated processes.

The differentiation of rejection from recurrent hepatitis C remains difficult because of overlapping histological features with rejection in its various guises, particularly as both often occur together.

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