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expert reviews in molecular medicine

Fulminant viral hepatitis: molecular and cellular basis, and clinical implications

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Fulminant hepatic failure is defined by the sudden onset of severe liver injury accompanied by hepatic encephalopathy in an individual who previously had no evidence of liver disease. This disease causes multiple organ failure and is associated with a high mortality. The most frequently recognised cause of fulminant or subfulminant hepatic failure is viral hepatitis. Data are now emerging to support the hypothesis that, irrespective of the aetiology of fulminant hepatic failure, the host's immune response (including production of proinflammatory cytokines and mediators) contributes to microcirculatory disturbances that result in hypoxic injury and cell death (apoptosis). Impairment of the scavenger function of the reticuloendothelial cell system further contributes to reduced hepatic blood flow and ischaemic necrosis. An increased understanding of the molecular pathogenesis of fulminant hepatic failure now enables new molecular therapeutic modalities to be tested. Given the complexity of this multi-dimensional disorder, the challenge is to provide a rational basis for treatment. This might include enhancement or suppression of immune responsiveness by manipulation of endogenous cytokine synthesis or by cytokine administration and, at the same time, use of strategies to increase hepatic regeneration.

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Viral hepatitis remains a major public health problem and the most common cause of liver disease worldwide. Annually, 300 000 cases of acute viral hepatitis (acute inflammation of the liver occurring within 2–6 weeks of infection) occur in the USA, fortunately only rarely resulting in fulminant hepatitis. Fulminant hepatic failure (FHF) is a clinical syndrome characterised by massive necrosis of liver cells and severe impairment of liver function, and differs from acute viral hepatitis in that, in addition to the liver necrosis, patients show disturbances in mental function – that is, hepatic encephalopathy (see below). FHF is associated with high mortality, with more than half of the patients who develop this syndrome dying without emergency liver transplantation (Refs 1, 2). The poor outcome reflects the severity of liver damage, the lack of liver regeneration and the multiple organ failure that results from systemic release of proinflammatory cytokines (Refs 2, 3, 4). Clinically, patients with FHF develop signs of multiple organ failure, including hepatic encephalopathy, and renal, cardiac and pulmonary failure, and they often die of infection secondary to the systemic inflammatory response syndrome (Ref. 5). Hepatitis viruses, drugs and shock can cause FHF, with viruses and drug-induced liver injury accounting for most cases of FHF. Regardless of the causal agent, the morphological changes of massive hepatic cell death are similar. Virus-induced liver damage generally results from a complex and prolonged interplay between virus replication and host defence.

The inability to propagate human hepatitis viruses in culture and the lack of suitable animal models have impeded determination of the pathological mechanisms accounting for FHF. However, insights into the pathogenesis of viral FHF have been forthcoming from animal models of FHF induced by murine hepatitis virus strain 3 (MHV-3; Ref. 6), transgenic models of hepatitis B virus (HBV) infection (Ref. 7) and clinical cases of FHF (Ref. 5). The evidence generated to date strongly suggests that FHF is caused by the release of inflammatory cytokines, resulting in massive liver necrosis with failure of compensatory hepatic regeneration. This article begins by briefly reviewing current knowledge of viral aetiologies of FHF and viral factors involved in the pathology of FHF, and then focuses on the host response in the process of virus-induced FHF. It ends by discussing the implications for future therapy of FHF.

Aetiology of fulminant viral hepatitis

The most frequently recognised cause of fulminant or subfulminant hepatic failure is viral hepatitis (Ref. 8), although in many cases the aetiology of FHF is indeterminate. The causes of FHF vary geographically. In developed countries, acute hepatitis induced by HBV, either alone or in association with viral co-infection, is the most common identifiable cause of fulminant viral hepatitis. However, the highest overall incidence of fatal hepatitis has been reported among cases of sporadic acute non-A non-B hepatitis (Refs 9, 10), and the disease is rarely associated with infection by hepatitis C or E viruses (HCV, HEV) (Refs 11, 12). Interestingly, reactivation of viral replication in carriers of HBV can also lead to fulminant hepatitis. This has been observed in both cancer and transplant patients following cessation of immunosuppressive therapy. For instance, discontinuation of even low-dose methotrexate (7.5–10 mg orally weekly) can reactivate HBV infection and lead to fulminant hepatitis (Ref. 13).

Non-hepatitis viruses, such as herpes simplex virus, cytomegaloviruses, adenoviruses, Epstein–Barr virus (EBV) and varicella, have also been shown to cause fulminant hepatitis, especially in immunocompromised individuals (Ref. 14). For instance, herpetic fulminant hepatitis is frequently associated with burns, cancer, pregnancy or renal transplantation – all conditions in which the host immune defences are diminished (Ref. 15). EBV-induced fulminant hepatitis has also been reported in a patient after liver transplantation (Ref. 16). Furthermore, human parvovirus B19 virion and mRNA encoding its nucleocapsid (core) protein have been detected in livers from patients with fulminant hepatitis. Viruses that have been shown to cause FHF are listed in Table 1.

Clinical features of virus-induced FHF

FHF is defined by the sudden onset of severe liver injury accompanied by hepatic encephalopathy in an individual who previously had no evidence of liver disease (Ref. 17). This disease causes multiple organ failure with an extremely high mortality rate (Ref. 18). Hepatic encephalopathy is the hallmark feature of FHF and is caused by liver damage. It is classified by disturbances of

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Table 1. Aetiologies of fulminant viral hepatitis (tab001glt)

Hepatitis viruses	Frequency[a]	Refs
Hepatitis A	31%	93
Hepatitis B	29.2%	93
Hepatitis C	Rare, co-infection[b]	94, 95
Hepatitis D+B	Rare, co-infection[b]	96
Hepatitis E	Rare, co-infection[b]	96
Non-ABCDE	38.8%	93
Hepatitis G	Rare, co-infection[b]	96
Herpes viruses	Rare	97
Yellow fever	Rare	98

[a] Percentage of cases of fuminant viral hepatitis attributed to different viruses.
[b] Co-infection with hepatitis B virus.

cerebral mentation (Ref. 19): grades I and II are associated with mild decreases in the level of consciousness, whereas grades III and IV are more severe [characterised by stupor and incoherence (grade III) to frank coma (grade IV)].

A major complication of FHF is cerebral oedema, which is the leading cause of death, especially early in the course of FHF. The diagnosis of cerebral oedema is difficult, and computerised tomography scans of the head have not proven to be particularly useful. Cerebral oedema often leads to intracranial hypertension and cerebral herniation of the uncus of the brain across the falx cerebrum, resulting in brain death in 25% of cases (Ref. 20).

Other major complications of FHF include: (1) hypoglycaemia, as a result of impaired gluconeogenesis; (2) disturbances in acid–base balance characterised by respiratory alkalosis and metabolic acidosis, as a result of increased lactate production (Ref. 21); and (3) severe coagulopathy owing to decreased synthesis of clotting factors II, V, VII and IX, as well as the presence of disseminated intravascular coagulation arising from both hepatic cell necrosis and infection (Ref. 22).

As a consequence of hepatic necrosis, the immunological competence of the patient is directly compromised. This is thought to arise from a combination of decreased complement synthesis, leukocyte dysfunction and bacterial translocation across leaky capillaries as a result of cytokine release (Ref. 23). Subsequently, patients develop bacterial infections, which are a high cause of mortality. Fungal infections occur in a third of patients with FHF and are often associated with bacterial infections or occur as a consequence of the use of broad-spectrum antibiotics (Ref. 24).

Other organ systems are affected by both the direct release of inflammatory cytokines and the haemodynamic changes that occur as a consequence of their release. Patients have a high incidence of renal, pulmonary and cardiac dysfunction and often succumb to renal failure secondary to the hepatorenal syndrome.

Immunopathogenesis of FHF

The role of viral proteins

Most hepatitis viruses are non-cytopathic, and liver damage in the acute as well as the chronic stages is the result of host immune responses directed at viral- or self-antigens expressed on the surface of infected hepatocytes via the major histocompatibility complex (MHC) (Fig. 1) (Ref. 25). It is believed that the immune response to one or more viral proteins is responsible for both viral clearance and liver injury during infection. In transgenic mice expressing hepatitis B surface antigen (HBsAg), acute necroinflammatory liver disease occurs only following infusion of HBsAg-specific cytotoxic T lymphocytes (CTLs) (Fig. 1) (Ref. 7). However, viral proteins also have other roles in the immunopathogenesis of FHF (Table 2).

Patients with FHF caused by HBV have a high incidence of mutations in the precore region of HBV. The precore region is upstream of the coding region for the core protein and mainly contains promoter elements for the core protein (HBcAg) of HBV. The exact significance of these viral mutations is not known, but it has recently been shown that the mutations could lead to transcription of the HBV x protein (HBx), which is encoded by the smallest open reading frame of HBV. Although investigators have suggested that HBx enhances cell growth by activation of cellular oncogenes, it is now known that HBx inhibits focus formation (viral plaque formation) (Refs 26, 27) and induces hepatic apoptosis in the early stage of HBV infection. HBx causes hepatocytes to be more

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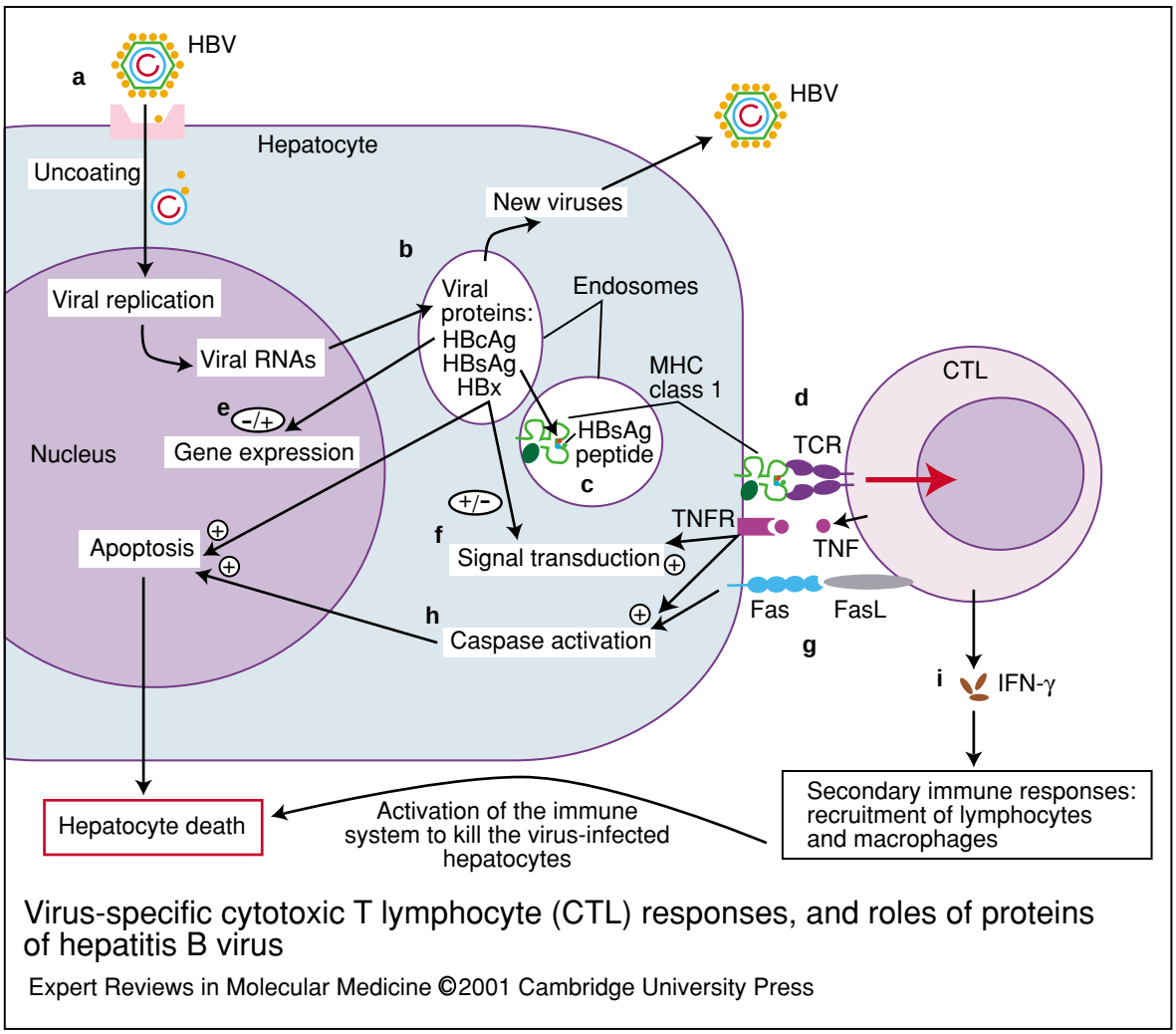

Figure 1. Virus-specific cytotoxic T lymphocyte (CTL) responses, and roles of proteins of hepatitis B virus. (a) After hepatitis B virus (HBV) enters a hepatocyte through an unknown virus receptor, (b) a number of viral proteins are synthesised, including HBc antigen (HBcAg), HBsAg and HBx protein. At the same time, the virus undergoes self-replication. (c) HBsAg peptide is presented at the hepatocyte cell surface by the major histocompatibility complex (MHC) and (d) induces an antigen-specific CTL response through the T-cell receptor (TCR). (e) Core protein (HBcAg) can move into the nucleus of the cell and modulate expression of host genes. (f) HBx protein can interfere with signal transduction and promote the apoptosis pathway. (g) Surface antigen (HBsAg)-specific CTLs send a death signal to hepatocytes through Fas–Fas ligand (FasL) interaction and the binding of tumour necrosis factor (TNF) to its receptor (TNFR). (h) Subsequently, the caspase pathway is activated, which leads to hepatocyte apoptosis. (i) In addition, HBsAg-specific CTLs secrete interferon γ (IFN-γ) and activate secondary immunoresponses, which lead to recruitment of lymphocytes and macrophages and activation of the immune system to kill the virus-infected cell **(fig001glt)**.

susceptible to staurosporine, cycloheximide and tumour necrosis factor (TNF), each of which can cause hepatocyte apoptosis (Refs 28, 29). Thus, although HBx is strongly associated with the oncogenicity of HBV, under certain conditions HBx might also contribute to the development of FHF through induction of the apoptotic pathway, discussed in more detail below.

It has also been suggested that the severity of fulminant hepatitis B is closely associated with the number of mutations in the core promoter and precore gene of HBV, although the mechanism underlying this observation is not clear (Ref. 30). Mutations in the promoter region of the viral genome have been shown to enhance the conformational stability of the

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Table 2. Viral proteins and their roles in fulminant hepatic failure (tab002glt)

Virus	Protein	Proposed function	Mechanism	Refs
HBV	Core	Induces hepatocyte apoptosis	Virus-specific recognition by CTLs	25
	Precore			
	Surface			
HBV	Surface	Enhances hepatocyte sensitivity to IFN-γ	Formation of filamentous particles in ER	11
HCV	Core	Enhances hepatocyte sensitivity to TNF	Inhibition of NF-κB transcription factor	36
HBV	HBx	Enhances cell growth	Activation of cellular oncogenes	26
		Inhibits focus formation	Unknown	26, 27
		Induces hepatocyte apoptosis	Sensitisation of hepatocytes to TNF	29
HBV	Precore mutants	Allows escape from tolerance	Increased viral replication	31, 32
		Affects HBx and core expression	Alters promoter activity	33
HBV	Core	Alters cellular function	Modulates gene expression	34
HCV	Core			
MHV-3	Nucleocapsid	Alters cellular function	Induces expression of fgl2 prothrombinase	38, 39

Abbreviations: CTL, cytotoxic T lymphocyte; ER, endoplasmic reticulum; HBV/HCV, hepatitis B/C virus: IFN-γ, interferon γ; MHV-3, murine hepatitis virus strain 3; TNF, tumour necrosis factor.

encapsidation signal in the viral RNA, thereby enhancing HBV replication rate (Refs 31, 32). In addition, the precore protein has been shown to inhibit HBV replication effectively, allowing a growth advantage of the precore 'escape' mutants over the wild-type viruses (Ref. 33).

Viral proteins also contribute to FHF by affecting host cellular functions directly. For instance, the core protein of HCV modulates expression of host cellular genes, such as c-*myc*, c-*fos*, and those encoding p53 and p21, and viral genes, including those of HBV (Fig. 1) (Ref. 34). HBV surface proteins form long branching filamentous particles that accumulate in the endoplasmic reticulum and cause hepatocytes to become hypersensitive to the cytopathic effects of interferon γ (IFN-γ) (Ref. 7). Viral mutants that do not express HBV e antigen (HBeAg), which is part of the nucleocapsid, are believed to be responsible for the loss of tolerance to the wild-type virus (Ref. 4) in HBV carriers, which leads to fulminant hepatitis B (Ref. 35).

Cells expressing the viral core protein in vitro exhibit enhanced sensitivity to TNF by inhibiting activation of the transcription factor NF-κB, which might also contribute to apoptosis-induced liver cell death through the Fas and Fas ligand (FasL) pathway (Refs 36, 37; and see below). Recently, the nucleocapsid protein of MHV-3 has been suggested to induce the expression of unique proteases (fgl2/fibroleukin) that can cleave prothrombin to thrombin, which is crucial in the pathogenesis of murine fulminant hepatitis (Ref. 38). It has been postulated that the HCV and HBV proteins can contribute to human fulminant viral hepatitis in a similar manner by stimulating the expression of the human counterpart of fgl2 prothrombinase (Ref. 39).

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Host response

The role of CTL responses

Direct killing of infected hepatocytes by MHC class I-restricted $CD8^+$ CTLs is a major determinant of viral clearance in acute and fulminant hepatitis (Ref. 40). The CTL response is relatively weak and is narrowly restricted in persistently infected patients. Although a vigorous polyclonal and multispecific CTL response to HBV has been observed in patients with acute or fulminant hepatitis, the CTL-mediated killing itself might not be sufficient to cause the massive hepatocyte necrosis that is the hallmark of FHF (Ref. 41). Indeed, antigen-specific CTLs have been shown to be capable of not only destroying HBV-infected hepatocytes, but also initiating a series of immune responses that lead to the recruitment of other inflammatory cells that can destroy infected hepatocytes (Refs 33, 42). Moreover, antigen-specific CTLs actively secrete IFN-γ, which causes macrophages to produce proinflammatory monokines such as TNF-α and interleukin 1 (IL-1), and this might contribute to the pathogenesis of fulminant hepatitis (Ref. 7).

Macrophage activation

Macrophage activation plays a key role in the massive liver destruction that characterises viral FHF. Infiltrating macrophages and increased numbers of activated Kupffer cells are classical features of viral FHF. Activated macrophages induce tissue damage by various mechanisms, including release of cytokines, production of reactive oxygen species, and lysosomal protein release (Ref. 43). Macrophage release of cytokines leads to the recruitment of other inflammatory cells (particularly neutrophils), which are responsible for many of the systemic effects of inflammation. In the transgenic HBsAg mouse model of FHF, the delayed-type hypersensitivity reaction initiated by IFN-γ-activated macrophages is responsible for the bulk of liver necrosis (Ref. 7). Macrophages have been shown to produce liver injury by releasing free radicals. This leads to oxidative DNA damage that is seen in fulminant hepatitis produced by D-galactosamine (Ref. 44). Furthermore, activated macrophages can produce immune coagulants, including tissue factor (co-receptor for factor VII) and fgl2, both of which have been shown to disrupt the hepatic microcirculation and result in fibrinoid necrosis (Ref. 45).

Effect of cytokines

Viral FHF is accompanied by local and systemic increases in cytokine levels. These cytokines are induced by viral replication (Ref. 46) and are further amplified by the infiltration of mononuclear leukocytes. Systemic increases in IL-1, TNF-α and IL-6 have been detected in patients with viral FHF (Refs 3, 47), and Kupffer cells in the liver have been shown to be the source of these proinflammatory mediators (Ref. 48). Evidence suggests that local production of cytokines such as TNF-α and IFN-γ in T-cell-driven models of FHF (see below) is essential to the development of liver necrosis (Ref. 48). The local release of cytokines also exerts other effects, including interference of cell growth (regeneration), hepatocyte apoptosis, leukocyte infiltration and activation, and upregulation of vascular adhesion molecules.

Liver cell apoptosis

Fulminant hepatitis is defined histopathologically as a massive necroinflammation of liver tissue. The mechanism underlying the liver injury is still unclear, like those of other types of viral hepatitis. As discussed in an earlier section, hepatocyte apoptosis caused by host immune responses against virus-infected cells is believed to play an important role during the process of liver necrosis. Apoptosis, or programmed cell death, is a morphologically distinguished form of cell death. Among all of the genes that have been identified as being involved in the apoptosis process, a family of cysteine proteases known as caspases has been shown to play a critical role in the initial and execution phases of apoptosis (Ref. 49). Activation of these caspases drives the terminal events of programmed cell death, including chromatin condensation, DNA fragmentation, breakdown of the nuclear membrane, externalisation of phosphatidylserine and formation of apoptotic bodies. Hepatocytes undergoing apoptosis are characterised by pyknosis (i.e. shrinkage and condensation of the cell), followed by membrane budding and karryorrhexis (i.e. fragmentation of the nucleus of the cell). The final irreversible process involves breaking up of apoptotic cells (Councilman bodies) into clusters of apoptotic bodies that are then phagocytosed by macrophages or resident Kupffer cells (Ref. 36).

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During HBV infection, virus-specific CTLs recognise HBV antigens preselected by the MHC on infected hepatocytes, which subsequently triggers processes within infected hepatocytes that result in apoptosis. Hepatocyte apoptosis has the potential to destroy the liver, even in the absence of significant inflammatory infiltrates. Although this event might be limited to very few cells, it has been observed that direct interaction between CTLs and target cells results in widely scattered, acidophilic, Councilman bodies in patients with acute viral hepatitis (Ref. 7), and it is now known that apoptosis in the liver can result in fulminant hepatitis (Ref. 50). Although the exact role of apoptosis in FHF, and the mechanism underlying it, still requires definition, Fas and TNF-α appear to play important roles.

Fas and FasL are strongly expressed in many hepatocytes during the development of FHF, and intraperitoneal injection of anti-Fas antibody results in rapid liver destruction in mice (Ref. 51). Further observations suggest that soluble Fas protein prevents hepatic apoptosis and liver destruction, without affecting early infiltration of inflammatory cells (Ref. 37). TNF-α is also critical for inducing hepatocytes to undergo apoptosis. Recently, a significant increase of TNF-α and its receptor, TNFR-1, was reported in patients who died of FHF, and the levels of expression of TNF-α and TNFR-1 strongly correlated with the number of apoptotic hepatocytes (Refs 52, 53). Both FasL- and TNF-α-induced hepatocyte apoptosis have been suggested to be mediated through the Fas-associated death domain (FADD)/caspase-8, which leads to release of mitochondrial cytochrome *c*, activation of caspase-3 and, finally, apoptosis of hepatocytes. Although there is strong evidence suggesting Fas and apoptosis play important roles in FHF, there are examples in which the role of Fas is less clearly defined.

The role of the coagulation pathway

Activation of the coagulation cascade is an integral component of host inflammation (Ref. 54). Tight interactions exist between inflammatory cascades and coagulation: not only is coagulation activated by many bioactive substances, including endotoxin, cytokines, bacterial products and viruses, but experimental evidence in animal models indicates that the coagulation cascade also plays a crucial role in the outcome of septic and inflammatory insults (Ref. 55). Activation of the coagulation system results in the generation of factors that have direct inflammatory effects, such as thrombin, Factor Xa and the fibrinopeptides. These factors eventually result in fibrin deposition, which simultaneously causes microvascular thrombosis, leukocyte accumulation and upregulation of the inflammatory response.

Thrombin appears to be a pivotal mediator in liver injury and, in particular, in acute viral fulminant hepatitis (Ref. 56). The local production of thrombin might explain the fibrin deposition observed during acute liver injury. In addition, in the wound healing process, thrombin is likely to target the sinusoidal endothelium, leading to activation of endothelial cells, secretion of proinflammatory mediators and adhesion of leukocytes, which together potentiate the inflammatory response (Ref. 57). Activation of the coagulation system in the inflamed liver can also occur through the upregulation of coagulation factor receptors. For example, expression of thrombin receptor on mononuclear cells is dramatically increased in clinical viral FHF (Ref. 57). Marked upregulation of thrombin receptor immunostaining was observed in specimens obtained from patients with fulminant hepatitis, in which massive hepatocyte necrosis is associated with activation of tissue-repairing systems.

Furthermore, it is now appreciated that, as part of their inflammatory repertoire, macrophages and endothelial cells produce immune coagulants including tissue factor and a direct prothrombinase (fgl2) that are intimately involved in the pathogenesis of experimental and human fulminant hepatitis (Ref. 6), rejection of xeno- and allo-transplants and the massive tissue necrosis associated with bacterial sepsis (Refs 58, 59, 60). Infusion of neutralising antibodies to these potent coagulants either ameliorates or prevents these inflammatory conditions, supporting their role in the pathogenesis of such diseases.

Models of FHF

There are two major problems that have impeded understanding of the pathogenesis of fulminant hepatitis. First, there is no suitable cell line that allows propagation of human hepatitis viruses in vitro. Second, large-animal models that closely resemble the clinical syndrome of FHF seen in human patients are unavailable. Despite these difficulties, two small-animal (rodent) models of

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virus-induced FHF have provided great insight into the molecular mechanism of virus-induced FHF (Fig. 2). The first is a transgenic HBV model of FHF in which HBV proteins are overexpressed in mice (Ref. 7). The second involves the RNA Coronavirus MHV-3 (Ref. 6), which produces a strain-dependent pattern of FHF in inbred strains of mice (Ref. 45).

Transgenic mouse model of FHF

Transgenic expression of HBsAg in inbred mice has led to a fatal necroinflammatory liver disease model to examine HBV-induced fulminant hepatitis (Ref. 7). Injection of MHC class I-restricted HBsAg-specific spleen cells and cloned HBsAg-specific CTLs into HBsAg-transgenic mice induces an acute necroinflammatory liver disease (Ref. 61). The development of disease is divided into three stages, ranging from single-cell necrosis to massive destruction of most hepatocytes.

In stage one, within 1 h of injection of CTLs, a few hepatocytes undergo apoptosis as a result of direct interaction with the specific CTLs. Histopathological studies show features consistent with acute viral hepatitis, including the presence of scattered, acidophilic Councilman bodies. The second stage, occurring at 12–24 h post-injection of CTLs, is characterised by increased hepatocellular apoptosis and the formation of necroinflammatory foci. Non-HBsAg-specific inflammatory cells, especially radiosensitive mononuclear cells and neutrophils, are pivotal to this injury. The third stage, at 24–72 h post-injection, is characterised by massive liver necrosis, inflammatory cell infiltration and hyperplasia of sinusoidal lining cells (Kupffer cells). This histopathological feature matches the pathogenic liver changes seen in patients who have HBV-induced fulminant hepatitis. Non-HBsAg-specific inflammatory cells, typically of macrophage origin, play a critical role in the massive hepatocellular necrosis. Prior administration of antibodies to IFN-γ reduces cell death by over 97%, demonstrating the importance of this cytokine in the development of fulminant hepatitis, perhaps through activation of macrophages (Ref. 7).

Although the transgenic HBsAg model is an elegant means of dissecting the pathogenic mechanisms of FHF, the model has limitations in that it differs markedly from the clinical situation, in which a replicating virus exists.

MHV-3-induced FHF model

MHV-3 infection in fully susceptible BALB/cJ mice causes FHF characterised by macrophage activation and marked production of proinflammatory mediators, sinusoidal thrombosis and hepatocellular necrosis (Refs 62, 63). Sequential studies have shown that the development of fulminant viral hepatitis always follows the same pattern and is similar to the clinical situation. Following MHV-3 infection, the virus replicates predominantly but not exclusively in the liver, and within 24–48 h evidence of macrophage activation and sinusoidal thrombosis is seen. Infiltrating mononuclear cells and neutrophils are observed in areas of hepatic necrosis.

Figure 3 suggests a mechanism for the pathogenesis of MHV-3-induced FHF. The viral load itself does not appear to be responsible for the development of massive liver necrosis. It has been shown that following MHV-3 infection, macrophages and endothelial cells express the prothrombinase fgl2, which cleaves prothrombin to the active moiety thrombin. This results in deposition of fibrin in the sinusoids, disturbances of hepatic microcirculation and hepatocyte necrosis. Induction of fgl2 in endothelial cells and Kupffer cells is enhanced by $CD3^+CD4^+$ T helper 2 (Th2) cells, whereas $CD3^+CD4^+$ Th1 cells can suppress induction of fgl2, fibrin deposition and hepatic necrosis. Several lines of evidence have implicated expression of this gene product in the pathogenesis of fulminant murine hepatitis. First, activity of the prothrombinase correlates with the severity of the disease (Ref. 64). Second, there is concordance between expression of fgl2 prothrombinase in the liver and fibrin deposition. Third, neutralising antibodies to fgl2 attenuate the pathological and clinical manifestations associated with MHV-3 infection (Ref. 63).

It is now known that the nucleocapsid protein of MHV is responsible for the liver-specific transcription of fgl2. Using a series of nucleocapsid and fgl2 promoter constructs upstream of a luciferase reporter gene, it was demonstrated that only the nucleocapsid from strains of MHV that produce FHF induces transcription of fgl2. Furthermore, several important hepatocyte-specific transcription-factor-binding sites have been tentatively identified in the promoter of fgl2, which might explain the liver-specific nature of MHV-3-induced FHF (Ref. 38).

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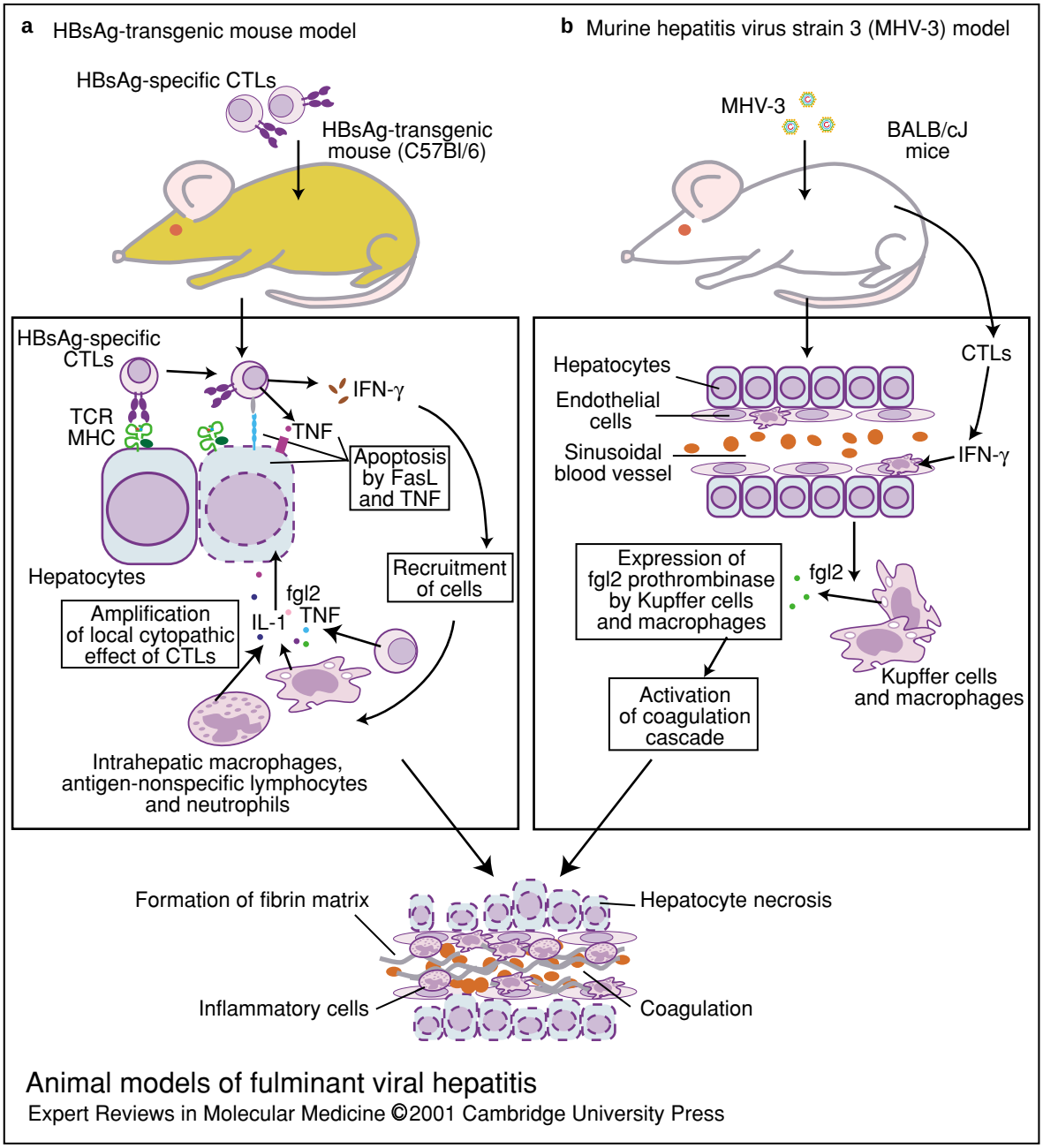

Animal models of fulminant viral hepatitis

Figure 2. Animal models of fulminant viral hepatitis. (a) Transgenic model. Injection of hepatitis B surface antigen (HBsAg)-specific cytotoxic T lymphocytes (CTLs) into HBsAg-transgenic mice can cause fulminant hepatic failure. CTLs first recognise hepatocytes through the T-cell receptor (TCR) and HBsAg peptide presented at the cell surface by the major histocompatibility complex (MHC). This process initiates hepatocyte apoptosis through Fas–Fas ligand (FasL) interaction and tumour necrosis factor (TNF) expression. At the same time, CTLs secrete interferon γ (IFN-γ), leading to recruitment of intrahepatic macrophages and antigen-nonspecific lymphocytes and neutrophils – with subsequent expression of inflammatory cytokines, including fgl2 prothrombinase, TNF and interleukin 1 (IL-1), which amplify the local cytopathic effect of the specific CTL response. (b) Murine hepatitis virus strain 3 (MHV-3) model. Infection of BALB/cJ mice with MHV-3 activates sinusoidal Kupffer cells/macrophages and endothelial cells to express fgl2. Activated T cells produce IFN-γ, which then activates fgl2 expression by both circulating and resident macrophages. The fgl2 prothrombinase then activates the coagulation pathway, which produces fibrin matrix that blocks blood flow and therefore causes hepatocyte necrosis. The massive necrosis finally leads to fulminant hepatic failure **(fig002glt)**.

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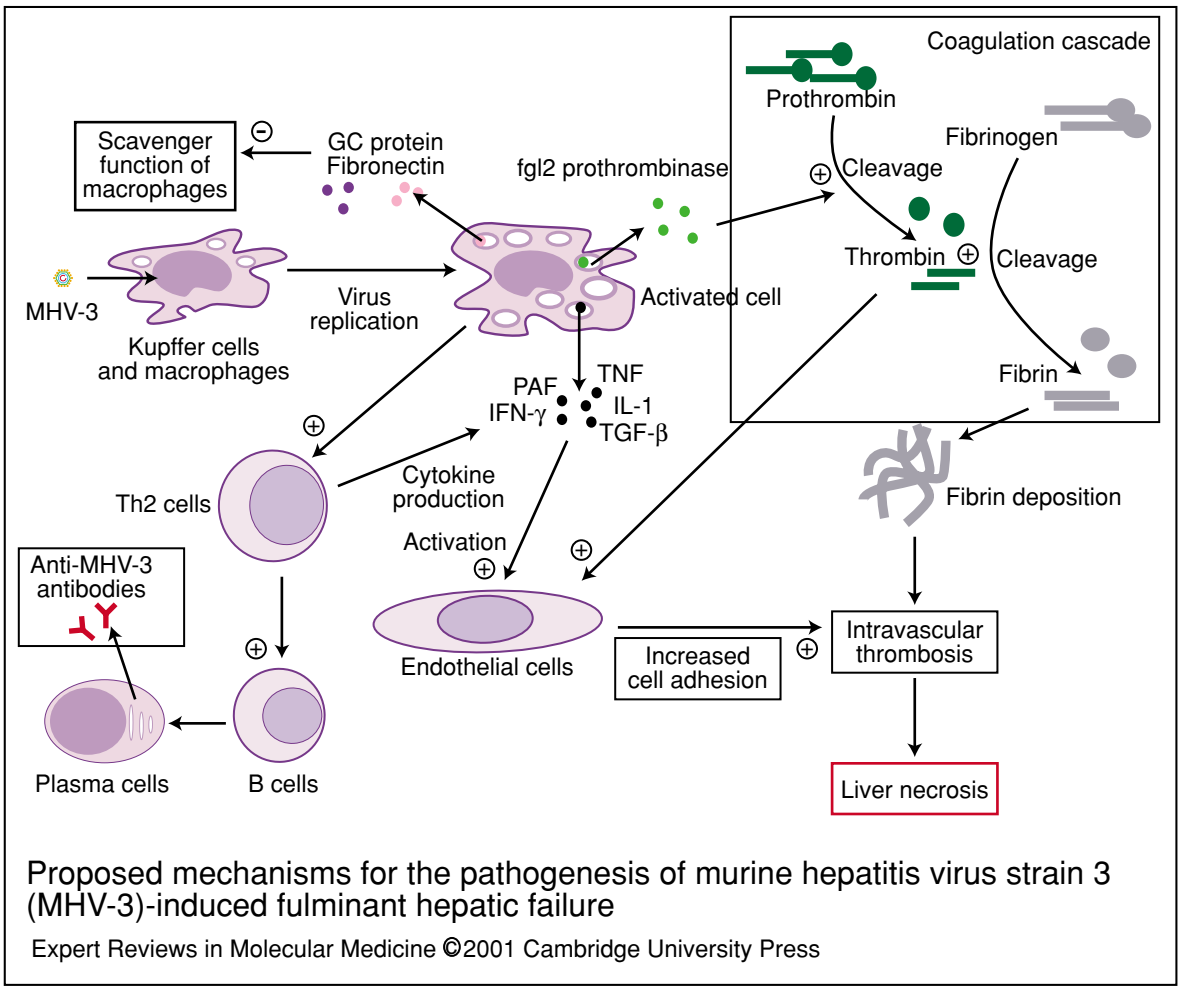

Figure 3. Proposed mechanisms for the pathogenesis of murine hepatitis virus strain 3 (MHV-3)-induced fulminant hepatic failure. Infection by MHV-3 prompts Kupffer cells and macrophages to express fgl2 prothrombinase, which activates the coagulation pathway by cleaving prothrombin to thrombin. Thrombin then cleaves fibrinogen to insoluble fibrin, which contributes to intravascular thrombosis. Virus-infected macrophages induce a T helper 2 (Th2)-cell response that, in combination with the response of activated macrophages, leads to the expression of inflammatory cytokines, including tumour necrosis factor (TNF), platelet-activating factor (PAF), interleukin 1 (IL-1), transforming growth factor β (TGF-β) and interferon γ (IFN-γ), as well as thrombin. These cytokines activate endothelial cells, which in turn enhances cell adhesion and contributes to thrombosis. In addition, activated Th2 cells induce B cells to become plasma cells and produce antibody against MHV-3. Furthermore, MHV-3-infected macrophages produce alpha-2-globulin (GC), which is a component of the extracellular actin-scavenger system, and fibronectin, which decrease the scavenger function of macrophages. Both pathways eventually lead to liver necrosis **(fig003glt)**.

The similarity in the putative promoter elements in human and mouse fgl2 suggests that both the mouse and the human genes are regulated in a similar manner. It is believed that the pathogenesis of the MHV-3-induced fulminant hepatitis model has the same characteristics as the pathogenesis of human viral hepatitis (Ref. 38). The core proteins of HBV and HCV are known to have the ability to modulate host immune function (Ref. 34). Therefore, it is conceivable that HBV and HCV core proteins induce human hepatitis through a similar mechanism to that of MHV-3 (Ref. 34). Indeed, a recent study involving patients with FHF has shown that human fgl2 prothrombinase expression correlated with the development of hepatic necrosis and thrombosis (Ref. 6).

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Clinical implications and future therapies

Virus-induced FHF presents a markedly proinflammatory milieu in which parenchymal destruction is mediated by a combination of lymphocyte- and macrophage-dependent processes. Clinical and experimental data described above suggest that the combination of apoptosis, increased production of oxygen free radicals, and activation of the cytokine network and the coagulation cascade are all involved in liver necrosis and, eventually, FHF (Fig. 4). On the basis of current understanding of the role of inflammatory mediators in vasoconstriction, thrombosis and hepatic necrosis, new forms of treatment have been directed at modifying the early inflammatory events, preserving blood flow to the liver or providing temporary hepatic support in the hope that the liver will regenerate.

Pharmacological approaches

Pharmacological agents that inhibit proinflammatory cytokines, such as

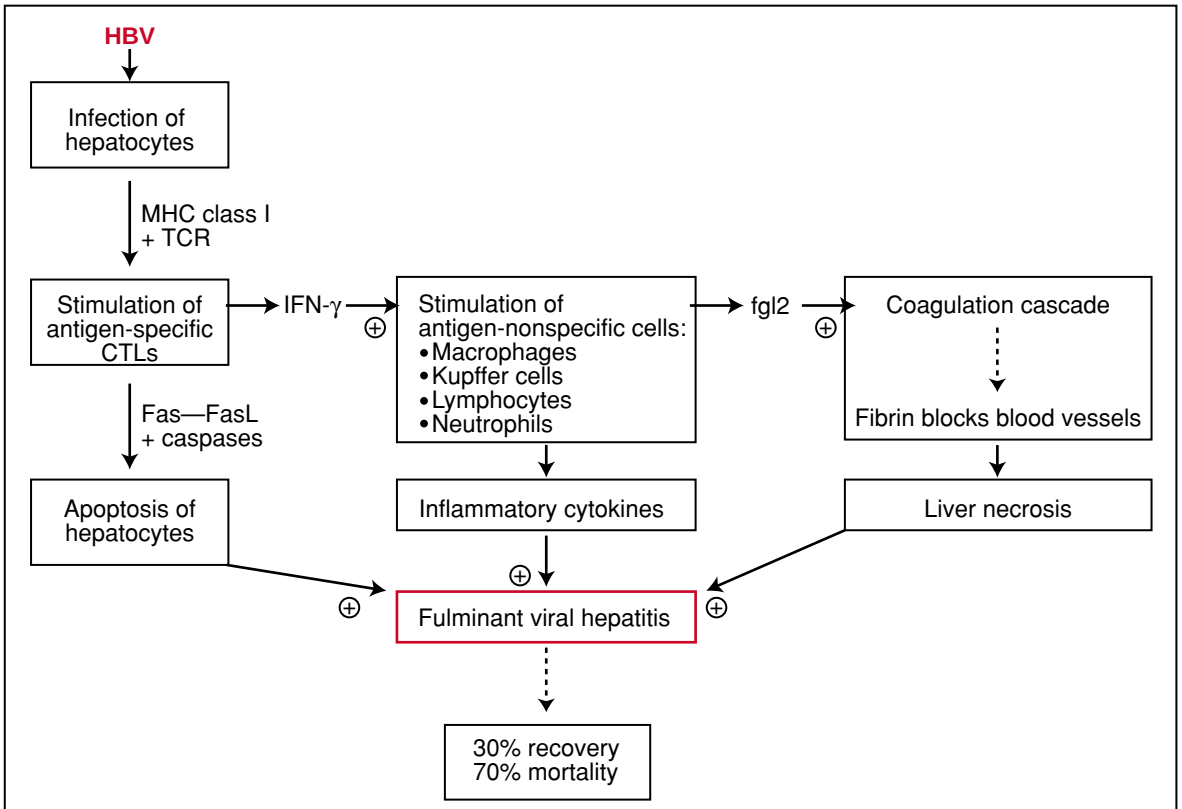

Figure 4. Summary of pathogenesis of virus-induced fulminant hepatic failure. Following infection of hepatocytes by hepatitis B virus (HBV), HBV-derived viral peptides are presented to the T-cell receptor (TCR) on cytotoxic T lymphocytes (CTLs) by the major histocompatibility complex (MHC). CTLs induce hepatocyte apoptosis through Fas–Fas ligand (FasL) interaction. CTLs also recruit antigen-nonspecific lymphocytes and neutrophils and activate macrophages through secretion of interferon γ (IFN-γ). Macrophages and neutrophils amplify the local cytopathic effect of CTLs by secretion of inflammatory cytokines. In addition, activated macrophages/Kupffer cells might produce fgl2 prothrombinase, which activates the coagulation pathway by cleaving prothrombin to thrombin. Thrombin then promotes the formation of fibrin matrix and blocks blood flow in the liver, leading to liver necrosis. All these effects – hepatocyte apoptosis, inflammatory cytokines and coagulation – are important participants in the pathogenesis of fulminant viral hepatitis **(fig004glt)**.

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prostaglandins and *N*-acetyl cysteine, have been investigated as possible treatments for FHF.

Prostaglandins

The use of prostaglandins might be beneficial in patients with FHF because prostaglandins have a vasodilatory action, can inhibit cytokines and have positive effects on the microcirculation, by improving blood flow and inhibiting aggregation of platelets and adherence of white blood cells (Ref. 65). Infusion of prostacyclin (PGI_2) to patients with FHF was shown to increase oxygen delivery and consumption (Ref. 21). Interest in prostaglandin E (PGE) analogues led to a clinical trial in 1989, with promising positive results (Ref. 66). However, a recent randomised, double-blind trial involving 41 patients with FHF treated with either intravenous PGE_1 or placebo failed to show an overall benefit for PGE_1 therapy. Nevertheless, in a recent study, patients with post-operative liver failure were treated with PGE_1 as a continuous hepatic arterial infusion. The rationale for this approach was to achieve high concentrations of the agent within the liver and to avoid systemic distribution of the drug, which is associated with side effects (Ref. 67). The infusion resulted in elevation of total hepatic blood flow and oxygen delivery to the liver with a resultant increase in bile flow and patient recovery. No side effects were observed. Whether this approach is of use in the treatment of FHF awaits further studies.

N-Acetyl cysteine

N-acetyl cysteine is an established antidote following acetaminophen (paracetomol) overdose (Ref. 68). Recently, it has been suggested that even late administration of *N*-acetyl cysteine improves the outcome of acetaminophen-induced FHF (Ref. 68). The increased survival was attributed to an improvement in haemodynamics and oxygen transport by enhancement of tissue oxygen delivery and consumption. However, the beneficial effect might also be related to the inhibition of production of proinflammatory cytokines such as leukotrienes, TNF and oxygen free radicals. Furthermore, the benefit achieved might be secondary to enhanced production of nitric oxide, with inhibition of aggregation and adhesion of platelets. This agent has now been used successfully to treat patients with fulminant viral hepatitis (Ref. 68).

Molecular approaches

Charcoal haemoperfusion and adsorbent columns

Charcoal is an effective adsorbent for a wide range of potentially toxic substances. Although the circulating levels of toxic substances were significantly decreased in patients treated by charcoal haemoperfusion (Ref. 69), an improvement in survival over conventional care was not noted in a subsequent controlled trial (Ref. 19). Recent work has suggested that a variety of circulating cytokines and endotoxin could be effectively removed in vitro using a variety of adsorbent columns (Ref. 70) and, thus, this form of therapy could be important in combination with other modalities.

Hepatocyte columns

A potentially promising approach is the development of a 'bioartificial liver': in essence, a hollow-fibre dialysis cartridge that contains living hepatocytes (Ref. 71). Two such devices are now being evaluated clinically. In the first, hepatocytes derived from a well-differentiated human hepatoblastoma cell line are perfused with the patient's whole blood treated with heparin (to prevent coagulation in vitro) (Ref. 72). In the second, pig hepatocytes are perfused with plasma in a technique similar to plasmapheresis (Ref. 73). Use of both of these devices experimentally in animal models of FHF has resulted in improvements in hepatic synthetic function (albumin and coagulation factors), and metabolism and excretion of ammonia and bilirubin. Furthermore, in clinical trials involving patients with FHF, use of these porcine hepatocyte columns resulted in improvements in hepatic synthesis (increased albumin and coagulation factors), metabolism (ammonia) and excretion (bilirubin), as well as improvement in neurological function of the patients as indicated by an increase in the level of consciousness (reduction in coma scale). Whether these devices can provide sufficient liver function to support a patient with acute liver failure to complete recovery has not yet been determined. To date, the results suggest that at best these devices only bridge patients with FHF to transplantation. This might be explained by the fact that the devices currently used contain only about 200 g of hepatocytes, as compared with 1200 g in the typical adult human liver. In addition, there are no accessory cells such as bile-duct epithelial

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cells, Kupffer cells or endothelial cells, which all contribute substantially to overall hepatic function. Newer devices are being studied that will examine these issues.

Regulation of cytokine production and the coagulation cascade

With the development of new methods to neutralise cytokines, it is now possible to alter the outcome of severe inflammatory diseases by specifically blocking the effects of a particular cytokine (Ref. 74). Recently, it has been shown that carbon-tetrachloride-induced liver injury could be prevented by treatment with an anti-TNFR antibody that neutralises the effects of TNF-α (Ref. 74). Furthermore, the lethality of MHV-3-induced FHF can be prevented by the administration of a monoclonal antibody to the MHV-3-induced prothrombinase fgl2 (Ref. 63). These data provide direct evidence for a role of cytokines in induced FHF and demonstrate a rationale for using antibodies to the cytokine or its receptor in the treatment of these disorders.

Gene therapy

The application of gene therapy to the treatment of hepatic disease has considerable potential. Gene therapy allows not only the replacement of genes, but also the disruption of offending, harmful ones. The gene of interest would be delivered by insertion into vectors that can be administered safely. Several viral vectors are now being examined in both preclinical and clinical studies (Ref. 75). These include retroviral vectors, adenoviral vectors and adeno-associated viral vectors. Although the ideal method for gene insertion has yet to be found, this technology could be utilised to affect a number of processes, including expression of cytokines and hepatic transcription factors that control inflammatory or hepatic-specific genes, as well as apoptosis, hepatic regeneration and delivery of drugs. As an example of the utility of such an approach, recent studies in a rat model of FHF demonstrated that infusion of a chimaeric IL-6 fused to a truncated form of its receptor resulted in reversal of severe hepatocellular injury and increased liver regeneration (Ref. 76).

Inhibition of apoptosis

Caspase inhibitors

Apoptosis has emerged as an important pathway in virus-induced FHF. A clearer understanding of the programmed cell death pathway might provide new approaches to blocking apoptotic cell death and ameliorating FHF. Currently, therapeutics are being developed for various caspase family members, with the most attention being paid to caspase-3, a major contributor to the apoptotic machinery in many cell types (Ref. 77). Preclinical studies have used caspase inhibitors, such as the caspase active-site mimetic peptide ketones, which are molecules with relatively non-selective caspase inhibition properties in animal models of human disease (Ref. 78). This approach has shown remarkable efficacy in preventing disease (Refs 79, 80). For example, in animal models of ischaemia–reperfusion injury of liver, heart, kidney and intestine, inhibition of caspases has demonstrated not only decreased apoptosis, but also improved survival and improved organ function. Importantly, caspase inhibition has shown promise in animal models of infectious disease (Ref. 81). In addition, it seems that the use of a caspase inhibitor in the acute phase of disease will have measurable clinical benefits in addition to a general inhibition of apoptosis. Thus, caspase inhibition could provide a novel therapeutic strategy in treating patients with virus-induced FHF.

Hepatocyte growth factors

A consistent feature of FHF is the lack of hepatocyte regeneration in the face of massive liver necrosis. Thus, a potential treatment for virus-induced FHF could involve compounds that enhance liver regeneration. One such molecule, hepatocyte growth factor (HGF), has recently been suggested as a potential therapeutic agent. HGF has been shown to possess potent hepatotrophic functions, such as enhancement of liver regeneration and inhibition of hepatocyte apoptosis (Refs 82, 83). In addition, HGF has a similar 'trophic' function in kidney (Ref. 84) and lung (Ref. 85). Thus, HGF might act on various tissues as an anti-apoptotic and cytoprotective agent to prevent multiple organ failure, which is a characteristic of FHF. In addition to enhancing hepatic regeneration, administration of HGF abrogates Fas-mediated massive liver apoptosis and FHF in mice. This function most probably results from induction of the expression of Bcl-xL, which is an anti-apoptosis molecule that blocks Fas-mediated apoptosis (Ref. 86). Paradoxically, patients with FHF have extremely high levels of HGF yet lack evidence of hepatocyte

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regeneration. Preliminary data suggest that the receptor for HGF on hepatocytes is lacking, or an inhibitor of HGF binding or function is present (Ref. 87). One approach to overcome this would be to use gene therapy either to increase HGF receptor expression or to permit post-receptor effects to occur.

Transplantation

Hepatocyte transplantation

Hepatocyte transplantation has been used to correct metabolic defects and to provide metabolic support both in experimental animal models of hepatic failure and in human metabolic disorders. The intrasplenic transplantation of differentiated adult hepatocytes in patients with hepatic encephalopathy and multiple organ failure was shown to correct high levels of ammonia in the blood and provide short-term survival until liver transplantation was performed (Ref. 88). Recently, primary human hepatocytes were immortalised utilising retrovirus-mediated transfer of an oncogene that could subsequently be excised by site-specific recombination (Ref. 89). The infusion of these immortalised human hepatocytes prevented lethality in a rat model of FHF (Ref. 89). This approach could potentially be utilised in humans either as a bridge to transplantation or until the native liver regenerated (Ref. 90).

Liver transplantation

For the most severe cases of FHF, transplantation provides the only chance of survival (Ref. 5). During the past decade, liver transplantation has revolutionised the treatment of patients with FHF (Ref. 71). With advances in perioperative management, surgical technique, and immunosuppression, one-year survival rates now exceed 70%. Early identification of patients unlikely to survive without liver transplantation is essential if this therapy is to be successful. Highly predictive prognostic criteria based on clinical, biochemical and histological variables have been developed (Ref. 91). Unfortunately, liver transplantation is limited by the severe shortage of cadaveric organ donors and the brief time available in which to obtain a suitable liver. It is estimated that only 10% of patients with FHF undergo transplantation. The development of live-donor transplantation from relatives, both for adults and for children, has partially alleviated this problem; it allows transplantation to occur quickly and provides the recipient with an excellent-quality organ to ensure that the patient has the best chance to survive.

Another approach has been the use of auxillary liver transplantation, in which part of the recipient's native liver is not removed in the hope that the liver will regenerate, thereby removing the necessity for life-long immunosuppression. This technique has been adopted successfully in several countries and might prove most useful for patients with acetominophen-induced FHF (Ref. 92).

Conclusions

This article has highlighted recent advances in our understanding of the molecular mechanisms contributing to hepatic necrosis in FHF. The importance of the specific viral CTL response, cytokines, coagulation factors and apoptosis in the pathogenesis of FHF has been defined. This increased knowledge has allowed new avenues of therapy to be developed both in experimental and in human FHF. Given the complexity of the disease, the challenge is to provide a rational basis for the management of patients with FHF, using either enhancement or suppression of the immune response by manipulation of endogenous cytokine synthesis or by cytokine administration.

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The American Association for the Study of Liver Diseases organises conferences and an annual postgraduate course.
http://www.aasld.org

The website for the USC Liver Transplant Program and Center for Liver Disease provides details of liver surgery and transplantation techniques.
http://www.livertransplant.org

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