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Review

Common mechanisms in pediatric acute liver failure

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Acute liver failure (ALF) is a rare but potentially fatal disease in children. The etiology is multifactorial, including infection, autoimmune, and genetic disorders, as well as indeterminate hepatitis, which has a higher requirement for liver transplantation. Activation of the innate and adaptive immune systems leads to hepatocyte-specific injury which is mitigated by T regulatory cell activation. Recovery of the native liver depends on activation of apoptotic and regenerative pathways, including the integrated stress response (ISR; e.g., PERK), p53, and HNF4α. Loss-of-function mutations in these pathways cause recurrent ALF in response to non-hepatotropic viruses. Deeper understanding of these mechanisms will lead to improved diagnosis, management, and outcomes for pediatric ALF.

Highlights

In 2022 an epidemic of acute hepatitis causing ALF, which affected children aged <5 years, has drawn attention to the lack of knowledge regarding the pathogenesis of ALF.

Most children with ALF during this epidemic were positive for human adenovirus and adeno-associated virus 2 (AAV2), neither of which is typically linked to ALF in immunocompetent children.

Children with mutations in genes that form part of the ISR (e.g., *PERK*) can develop ALF in response to non-hepatotropic viruses.

Activity in the innate immune system is potentiated by factors derived from the intestinal microbiome and is dependent on Myc.

Regulation of p53 activity is important for determining the balance of hepatocyte senescence and therefore the ability to regenerate during ALF.

Overview of ALF

Acute liver failure (**ALF**; see Glossary) is a life-threatening condition that is relatively rare in children [1]; however, there has been a recent increase in cases of **acute hepatitis** and ALF of unknown etiology [2].

The exact incidence of ALF in children is unclear, but ALF accounts for 7.7% of all pediatric liver transplants [3]. ALF may result in spontaneous recovery or loss of the native liver, requiring extremely urgent liver transplantation. In a cohort of 769 cases, 423 (55%) survived with their native liver [4]. However, the precise rates of recovery depend mainly on the etiology; for example, native liver survival is below 50% in mitochondrial hepatopathies that often present as neonatal ALF [5].

ALF by definition occurs on the background of a normal liver or where no chronic liver disease is known. Etiologies underlying pediatric ALF are highly dependent on age and full differential diagnosis is covered elsewhere [6–11]. For example, Wilson disease may cause ALF in teenagers whereas mitochondrial hepatopathies typically affect infants. A separate group of causes is responsible for ALF in neonates (e.g., gestational alloimmune liver disease), which is also reviewed elsewhere [12,13].

In the first half of 2022, following the relaxation of pandemic regulations, there was an epidemic of acute hepatitis of unknown origin in children, many of whom progressed to ALF[i]. Cases were first identified in Scotland, then in the rest of the UK, and subsequently elsewhere in Europe and around the world[ii]. Most were found to be positive for adenovirus and adeno-associated virus 2 (AAV2), which do not normally cause ALF, and there was no evidence of direct virus-mediated hepatocytolysis.

Children between 1 and 5 years of age are most affected by the current outbreak of acute hepatitis and ALF of unknown origin. In this age group the differential diagnosis includes genetic/metabolic, autoimmune, viral, and idiopathic conditions. In some the trigger is clearly defined

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(e.g., hepatitis A virus, *NBAS* mutations [14]), and in autoimmune hepatitis (AIH) the trigger is broadly known to be a hepatocyte-specific compound, although the precise antigenic target is not clear [15]. However, in up to 50% of the cases no clear cause is identified (i.e., **indeterminate hepatitis**) [16]. The rare and sporadic incidence of these cases has hindered systematic analyses to pinpoint potential triggers that lead to liver injury.

Despite having different etiologies, all diseases share common mechanisms of injury and hepatocellular regeneration – irrespective of the trigger: a combination of the innate and adaptive immune cells plays a central role [17]. Whether an individual progresses to liver transplantation or spontaneously recovers is dependent on the balance of ongoing liver injury versus the rate of hepatocyte regeneration. At the level of the individual hepatocyte, this may be influenced by the outcome of activation of the **integrated stress response (ISR)** [18], caspase activation [19], NF-κB activity [20], and Fas ligation [21], among other factors.

ALF in children has been brought under the spotlight during the recent epidemic of acute hepatitis. This has highlighted how little is known about the pathogenesis and that, particularly for indeterminate hepatitis, there are no specific therapies. Therefore, in this review we cover the current understanding of mechanisms of ALF in children. Through the identification of common pathways perturbed across multiple etiologies of ALF, we hope to stimulate further research in this field.

Adenovirus-associated hepatitis in children in 2022

From March to June 2022 there was a definite increase in acute hepatitis and ALF in children in the UK. Although the cause is not yet clear, >70% of cases were associated with the presence of adenovirus F41 in whole blood[i]. Adenovirus infection is an unusual cause of acute hepatitis, but has been recognized as a cause of ALF [22] in immunosuppressed patients post-transplant [23]. The epidemic is thought to be related to the lack of natural immunity to common childhood viruses secondary to 'lockdowns' and shielding during the pandemic. Similar cases have been reported in Europe, although it is less clear to what extent this is a true rise in the incidence of ALF [2,24]. It should also be noted that only 20% of cases in Europe (excluding the UK) have been adenovirus-positive, and there is thus a phenotypic difference to the UK series, and no other pathogenic factors have been consistently identified[ii,iii]. Data from outside Europe are also emerging, and one case series of children with adenovirus-associated acute hepatitis was reported in Alabama in late 2021 to early 2022 [25,26], and a mechanistic study illustrates the importance of AAV2 (as discussed further in the following section) [27].

Globally, children (mostly between 1 and 5 years of age) presented with sudden onset of jaundice and malaise 1–4 weeks after gastrointestinal symptoms [28]. At presentation, most had bilirubin >100 μmol/l and alanine transaminase (ALT) of >1000 IU/l[i] (i.e., significantly abnormal liver biochemistry indicative of hepatocyte dysfunction/damage). Those that progressed to ALF often had bilirubin >180 μmol/l and ALT >5000 IU/l. The majority of those with adenovirus-associated hepatitis recovered spontaneously, suggesting that in most cases regenerative capacity overcomes ongoing injury.

Histology from explanted livers from children with acute hepatitis who had undergone liver transplant for ALF shows two important features[i,ii,iii]. First, there is no evidence of viral inclusions within hepatocytes. Similarly, no adenovirus has been identified by PCR from hepatic tissue, despite ongoing positivity in the blood. Second, in addition to the widespread hepatocyte necrosis, there is marked biliary injury with evidence of ductular regeneration. This suggests that adenovirus does not appear to be causal in a similar way to how other hepatotropic viruses cause ALF.

Glossary

Acute hepatitis: inflammation of the liver associated with hepatocyte damage, and without evidence of previous (chronic) liver disease.
Acute liver failure (ALF): loss of hepatocyte function resulting in coagulopathy and/or encephalopathy in a short duration of illness.
Acute liver injury: a general term that encompasses acute hepatitis and ALF.
CALFAN syndrome: low γ-glutamyl transferase, cholestasis, ALF, and neurodegeneration; the syndrome is caused by biallelic mutations in *SCYL1*.
Indeterminate hepatitis: acute hepatitis (with or without liver failure) where the cause is unclear despite extensive etiological investigation.
Integrated stress response (ISR): a common molecular pathway resulting from cellular or organelle (e.g., endoplasmic reticulum or mitochondrial) injury, and which typically involves phosphorylation of eIF2α.
Pyrexic stress: the increased physiological demands placed upon the body in response to fever.
Reactive oxygen species (ROS): a broad term that includes any oxygen molecule that is more reactive than O_2. These include the superoxide radical (O_2^-) and hydrogen peroxide (H_2O_2).
Regulatory T cells (Tregs): $CD4^+CD25^+Foxp3^+$ T cells that secrete anti-inflammatory cytokines (e.g., IL-10).
Senescence: a state of altered cell behavior where cell division is highly unlikely.

A putative role for SARS-CoV-2 infection

It is not clear why a minority progress to severe ALF and require liver transplantation, although coinfection with other viruses and/or alteration of immunity post SARS-CoV-2 infections may be contributing factors [29]. The global increase in ALF incidence following the COVID-19 pandemic has raised suspicion regarding the role of SARS-CoV-2, despite the rare detection of infection in the affected children (~15% in the UK with concurrent infection)[iii]. There was also no evidence of a higher prevalence of antibodies to SARS-CoV-2 in affected children compared to controls[i].

SARS-CoV-2 proteins have been detected in liver cells, including Kupffer cells and more rarely hepatocytes [30], although direct propagation of the virus in hepatocytes remains to be confirmed experimentally [31]. In two cohorts of adults with severe COVID-19 that led to multiorgan failure, potential implications of liver tropism of the virus were revealed using transcription-, proteomic-, and transcription factor-based approaches; the study proposed that the molecular impact of previous SARS-CoV-2 liver tropism may sensitize the liver to future injury from other viruses or environmental causes [30].

Supporting this multiple-hit hypothesis, Brodin and Arditi report that previous adenovirus infection can lead to superantigen-mediated type I immune skewing [excessive production of type 1 T helper cell (Th1) cytokines, particularly of interferon γ (IFN-γ], which led to IFN-γ-mediated hepatocellular injury [32]. There is a precedent for an increase in pediatric ALF following a respiratory virus outbreak because this was reported in the winter of 1921–1922 [33]. The range of symptoms in the rare sporadic cases following the influenza pandemic of 1918 fit well with the current increase in unexplained pediatric acute hepatitis during the COVID-19 pandemic [29].

As of the 8th of July 2022, there have been 1010 probable cases of unexplained severe acute hepatitis in children in 35 countries, including 22 deaths[iv]. This tragic increase of a rare disease provides a window of opportunity to investigate potential similarities between cases to determine the correlates of liver failure in some children.

AAV2–adenovirus coinfection

Recent work has implicated AAVs in the pathogenesis of the hepatitis epidemic. Preprints from two groups have independently identified AAV2 in the blood and hepatic tissue of >90% children with unexplained hepatitis [34,35]. AAV2 was very rarely found in the blood or hepatic tissue of controls, including immunosuppressed individuals. However, there was no evidence of direct lytic activity of AAV2 (or adenovirus) in explanted livers. AAV2, which is from the parvovirus family, requires the presence of another virus to facilitate its replication, usually adenovirus. A peak in adenovirus infections in the community post-pandemic around the time of the epidemic (in the UK) would support this hypothesis[ii]. Although this work is not yet formally published, it provides strong circumstantial evidence that AAV2–adenovirus coinfection may trigger an immune response that results in acute hepatitis.

Role of genetic variants in etiology/susceptibility

The majority of children who present with ALF were previously well. Even in those cases where there is a clear cause (e.g., viral hepatitis), the same viral infection may lead to ALF in some children but not in others. This points to an underlying combination of genetic and environmental factors that influence susceptibility to ALF.

In the past decade a range of monogenic disorders that predispose to ALF have been identified. Understanding the causal genes illustrates common pathways that may exacerbate hepatic

decompensation when challenged with insults that are not usually hepatotoxic (e.g., rhinovirus), or insults that usually cause mild-to-moderate liver injury (e.g., influenza).

ISR and reactive oxygen species

Activation of the ISR and the generation of **reactive oxygen species (ROS)** appear to be key mechanisms (Figure 1), exactly as they are important in the pathogenesis of paracetamol-induced ALF [36]. Infantile liver failure syndromes (IFLS) 1–3 are a group of autosomal recessive disorders where children develop recurrent ALF in response to fever. IFLS2–3 [14,37] and **CALFAN syndrome** [38,39] are caused by mutations in genes coding for proteins involved in intracellular vesicle trafficking (i.e., NBAS, RINT1, and SCYL1). Although the function of NBAS, RINT1, and SCYL1 are not entirely understood, these proteins are localized between the endoplasmic reticulum (ER) and Golgi and are required for anterograde [40] and retrograde transport [41–43]. siRNA knockdown in HeLa cells also suggests that NBAS has a role in nonsense-mediated decay through recruitment of UPF1 to the ER membrane [44]. Fibroblasts from affected patients (with *RINT1* and *SCYL1* mutations) have enlarged or fragmented Golgi, particularly after culture at 40°C to mimic the effect of fever on the cells – noting that patients can develop ALF in response to fever alone [37,38,45]. Furthermore, patient fibroblasts harboring biallelic mutations in *NBAS* or HeLa cells with *NBAS* knockdown demonstrate a signature of ER stress on RNA sequencing [14,44] (although protein-level characterization of ATF4 and CHOP expression – canonical markers of the ISR [46] – has not yet been described). RINT1 loss in the brain is associated with increased CHOP expression [47]. Grp78 expression was not increased in patient fibroblasts with *SCYL1* mutations [38], but the pathway has not yet been characterized in detail or under **pyrexic stress**.

N-acetylcysteine (NAC) is an antioxidant that is a well-established treatment for paracetamol-induced ALF. NAC restores the levels of hepatic glutathione, which (in combination with superoxide dismutase) converts superoxide (O_2^-) and hydrogen peroxide into water [48,49]. In the absence of mitochondrial antioxidants, mitochondrial oxidative stress triggers ASK1 and JNK activation to cause hepatocyte necrosis [50]. Similarly, loss of NBAS may also be associated with increased mitochondrial ROS which are clinically relevant given that treatment with NAC as a source of reduced glutathione (GSH) is potentially of benefit for children with ILFS2 ALF [9,51]. Precisely how disruption of the NBAS complex may lead to increased ROS levels is unclear; however, there is a bidirectional relationship between ER stress and ROS where potential mechanisms include alterations in mitochondria-associated membranes and changes in ER–mitochondrion calcium flux [52–55].

Further evidence for the importance of ER stress in susceptibility to ALF comes from individuals with Wolcott–Rallison syndrome (WRS), which is caused by mutations in *EIF2AK3* [56,57]. This gene encodes PERK, an eIF2α kinase which is a key switch in activating the unfolded protein response that helps to restore ER homeostasis [58]. Loss of the catalytic activity of PERK prevents eIF2α phosphorylation which is required for activation of the ISR [56]. Patients with WRS can develop ALF in response to non-hepatotropic viral infections. Although the precise mechanism is not known, it may involve lack of a normal ER stress response. Liver-specific *Perk* knockout mice have increased apoptosis in response to ER stress [59]. Again, treatment with NAC appears to be beneficial in these patients (although this is based on a small number of case reports), which suggests that ROS accumulation plays an important role. Indeed, phosphorylation of eIF2α is needed as part of the normal response to oxidative stress in β cells [60], and liver biopsy from an affected patient showed lack of electron transport chain complex I activity [61].

Genetic mitochondrial disorders leading to ALF

Mitochondria are the principal site of ROS generation, which occurs by a variety of mechanisms including a change in mitochondrial membrane polarity that results in reverse electron transfer

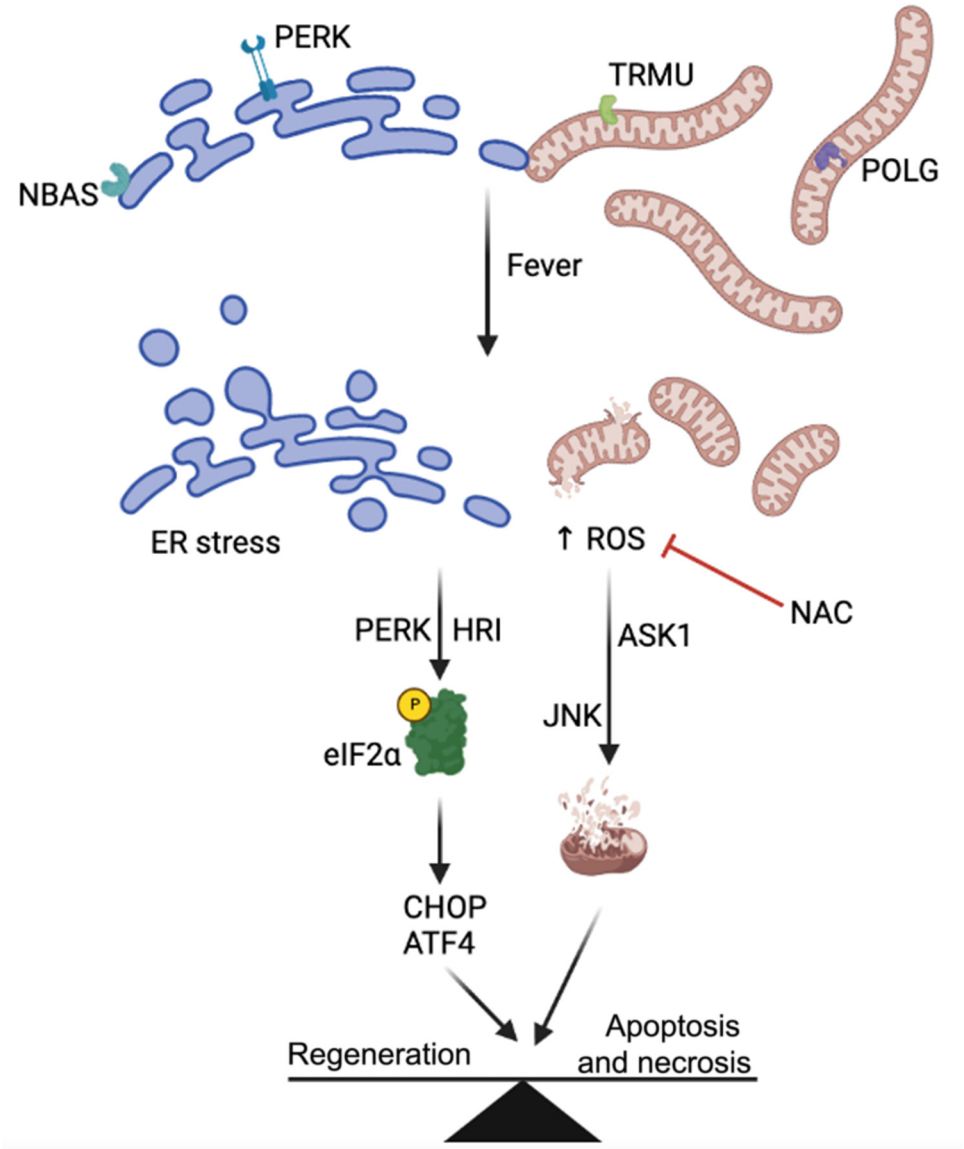

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Figure 1. Monogenic forms of acute liver failure (ALF) cause endoplasmic reticulum (ER; blue, left) stress and mitochondrial dysfunction in response to fever. This figure depicts healthy ER and mitochondria (in a fused network) at the top, annotated with key genes involved in monogenic forms of recurrent ALF. In response to fever, a lack of normal response in *NBAS*, *PERK*, *TRMU*, or *POLG* can lead to ER stress and/or mitochondrial dysfunction (with network fragmentation).This leads to increased levels of reactive oxygen species (ROS) and activation of the integrated stress response (ISR). The ISR is characterized by phosphorylation of eIF2α by the kinases PERK or HRI. This leads to induction of CHOP and ATF, which are key transcription factors. ROS activate ASK1 and JNK pathways, which can lead to mitochondrial disruption and apoptosis. This can be mitigated by use of *N*-acetylcysteine (NAC), which increases the levels of glutathione (an antioxidant). The intensity and chronicity of stress will determine whether an apoptotic or regenerative response is mounted. Abbreviations: ASK1, apoptosis signal-regulating kinase 1; eIF2α, eukaryotic translation initiation factor 2A; HRI, heme-regulated inhibitor; JNK, c-Jun N-terminal kinase; NBAS, neuroblastoma-amplified sequence; PERK, protein kinase R-like endoplasmic reticulum kinase; P, phosphorylation; POLG, DNA polymerase γ; TRMU, tRNA mitochondrial 2-thiouridylase.

through complex III [62]. Mitochondrial dysfunction through almost any means [e.g., respiratory chain complex loss, mitochondrial DNA (mtDNA) damage, and changes in calcium flux] can result in the generation of mitochondrial ROS [63].

Direct human genetic evidence for the importance of mitochondria in ALF comes from several groups of disorders. Transient infantile liver failure syndrome is caused by biallelic mutations in *TRMU* [64], which is required for mitochondrial protein translation. There are also a wide range of mitochondrial cytopathies caused by mutations in mitochondrial chain components or mtDNA [5]. These most frequently present with neonatal or infantile liver failure, but have also been associated with hepatic decompensation during intercurrent illness (e.g., *DGUOK* mutations [65]).

One further group of disorders predisposing for recurrent episodes of liver failure are cytosolic aminoacyl-tRNA synthetase (cARS) deficiencies [66–68], and its most prominent member – infantile liver failure syndrome type 1 – is due to variants in *LARS1* [69]. These disorders are characterized by recurrent episodes of severe liver dysfunction during febrile episodes with further multisystemic involvement [70]. Dysfunction leads to disturbed activation and transfer of amino acids to their cognate tRNAs, and also into the nascent polypeptide at the ribosome, thus hampering functional protein translation. It has been hypothesized that misfolded, defective proteins as a consequence lead to ER stress, which has been shown for glutamyl-tRNA synthetase in HEK293T cells [71]. However, there is broad variation regarding hepatic pathology in different cARS deficiencies, and the cause for this difference is not yet understood.

ISR and cellular survival

Both ER stress and mitochondrial dysfunction result in activation of the ISR (Figure 1), characterized by phosphorylation of eIF2α that causes a generalized cessation of protein translation [72]. The downstream mediators are the transcriptional coactivators CHOP and ATF4 that continue to be expressed due to their upstream inhibitory open reading frames (ORFs) [73]. CHOP and ATF4 have a variety of targets that aim to restore cellular homeostasis, including increased amino acid uptake and cessation of protein translation [46,74]. In addition, the ISR can cause increased expression of prosurvival (e.g., *BIP*) and proapoptotic (e.g., *BIM*, *NOXA*, *PUMA*) genes [75–77]. Similar cellular responses are also triggered by the IRE1–ASK1–JNK pathway, which may be activated by ER stress or mitochondrial injury [78], such as in paracetamol overdose [50]. This cascade also typically results in apoptosis [79]. The overall cellular response is likely to be dependent on the intensity and duration of the stress, as well as on which arms of the ISR are activated [80].

There is currently no evidence that more common (hypomorphic or heterozygous) mutations in the above mentioned genes result in increased risk of ALF. For example, parents of children with ILFS2 do not seem to suffer from raised rates of liver disease [14]. Therefore, genetic predisposition in monogenic disorders highlights the importance of ER/mitochondrial stress and ROS. However, given the rarity of these disorders and the recent increase in acute hepatitis/ALF, variants in these genes are unlikely to be a primary mechanism for the current increase in **acute liver injury**.

Common immune mechanisms

The immune response associated with ALF can be causal (e.g., AIH) or secondary to liver damage. Data over the past few years have identified integral roles for both innate and adaptive arms in ALF across a range of pathologies [17].

The gut–liver axis is central to the pathology of several hepatic conditions [81], and recent work suggests that the microbiome can influence hepatic Kupffer cell activity during acute liver injury

[82]. Potentially harmful factors signal to phagocytes through activation of Toll-like receptors (TLR) using damage-associated molecular patterns (DAMPs), particularly lipopolysaccharides [83,84], that are transmitted via portal blood. Kolodziejczyk *et al.* used single-cell RNA sequencing of mice treated with thioacetamide or paracetamol to identify Myc as a key driver of innate immune cell activation in the liver [85]. Depletion of the microbiome or induction of ALF in germ-free mice resulted in a milder phenotype that has effects similar to inhibition of Myc. Other studies have similarly found that alteration of the microbiome is an effective strategy for mitigating acute liver injury [86].

These findings are of particular relevance to the recent outbreak of indeterminate acute hepatitis because the majority of patients have a 'prodromal' phase of gastrointestinal symptoms 1–4 weeks before presentation[i,ii]. This has led to suggestions that a gut-derived superantigen could be a potential causal factor [32]. This hypothesis seems reasonable given the >60% seropositivity for antibodies against SARS-CoV-2, and the temporal association with the previous wave of Omicron variant SARS-CoV-2[i]. Superantigens are molecules that can cause T cell activation without binding to the antigen cleft of MHC class II [87]. For this to be the trigger of the current acute hepatitis outbreak, it would also require an additional factor that causes hepatocyte-specific immune activity. Superantigens typically result in a polyclonal T cell response, and there are no current reports that a multi-system inflammatory syndrome is associated with the recent outbreak of acute hepatitis[i], unlike that following SARS-CoV-2 infection [88].

An imbalance between immune surveillance, activation, and suppression is the cause of autoimmune disease and may play a more broad role in ALF. Monogenic human data from patients with *AIRE* mutations support a causal role of increased self-reactive lymphocytes [89]. In particular, the balance of type 17 T helper (Th17) cells and $CD4^+CD25^+$ **regulatory T cells (Tregs)** appears to be important across a range of different etiologies [86,90,91].

We previously showed that hepatocytes could regulate Treg numbers in the liver by enclysis – the engulfment and deletion of live Tregs [92]. Enclysis was increased in AIH and reduced in hepatitis B virus infection. The role of other infections in the modulation of enclysis may warrant further investigation [93].

Shifting the hepatic cytokine profile away from 'proinflammatory' IL-17/23, and TNF-α (from Th17 cells) and towards higher IL-10 may be beneficial (Figure 2). However most of the data in this area come from adult models of acute-on-chronic liver failure.

Much of the hepatocyte lysis is mediated by immune-directed killing, typically through expression of Fas-L and release of perforin and granzyme [17]. This may be especially important in indeterminate hepatitis, where a higher density of ($CD103^+$) $CD8^+$ T cell infiltrate has been identified in comparison to other causes of ALF [94,95]. IFN-γ, at least at the transcriptional level, appears to be a key factor in the immune infiltrate [96]. The hepatic immune infiltrate has not yet been characterized in the current outbreak of acute hepatitis, but comparison with previous cases of indeterminate hepatitis may help to identify unique features. In addition, single-cell RNA sequencing of non-parenchymal cells in cases of undiagnosed pediatric ALF would be of use to identify enriched and activated leukocyte subsets.

It is worth noting that germline loss-of-function mutations in perforin may also result in ALF through familial hemophagocytic lymphohistiocytosis (fHLH) [97]. HLH is a disorder of uncontrolled immune hyperactivation that results in widespread organ dysfunction, including ALF.

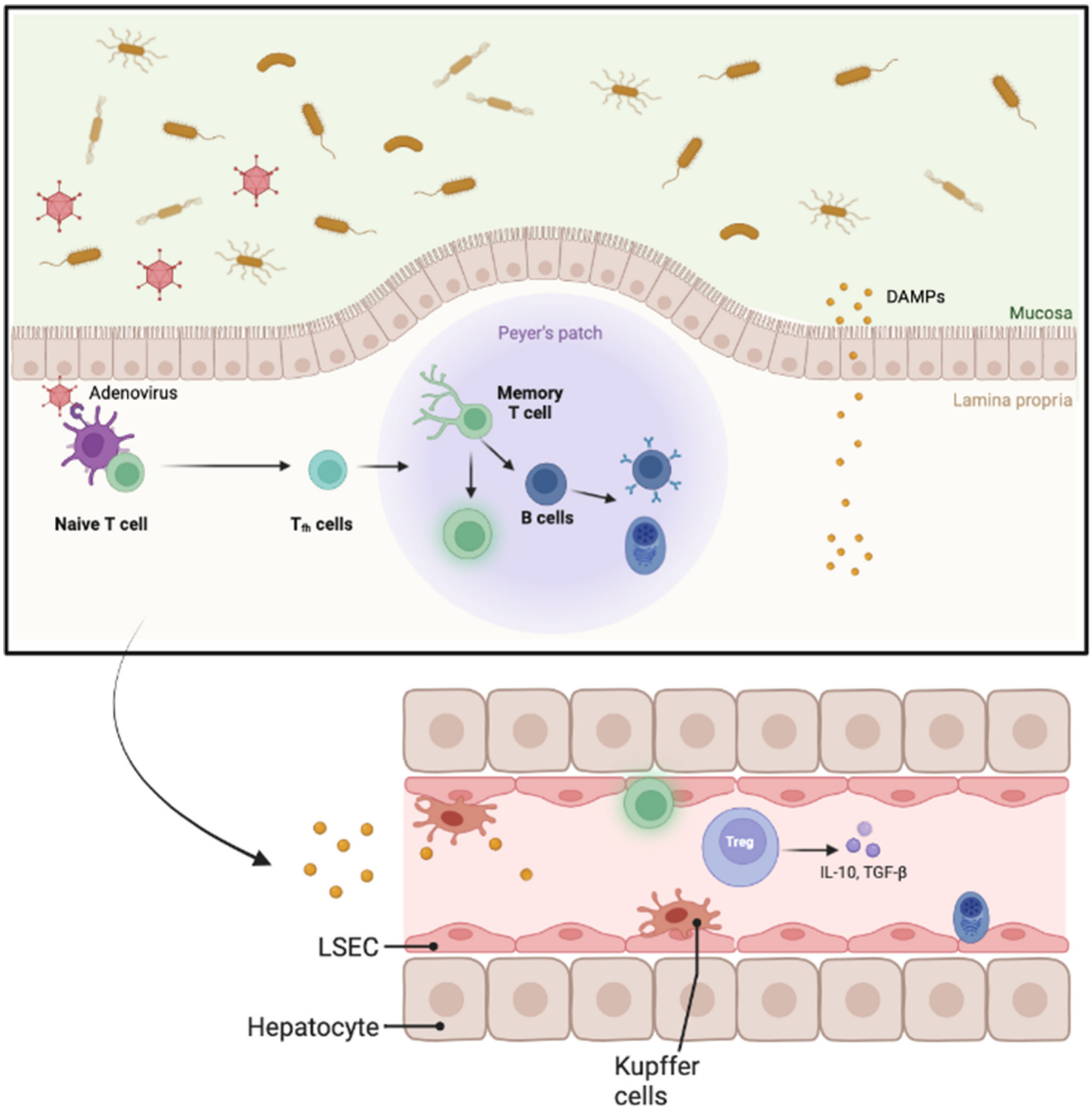

Figure 2. Potential mechanisms for how adenovirus (and other factors) may lead to acute hepatitis. Fragments of adenovirus (red) are presented to naïve T cells by dendritic cells (purple) in the lamina propria of the intestine. These T cells then move to Peyer's patches where they (or memory T cells) activate B cells and/or the T cells undergo further activation themselves. In addition, damage-associated molecular patterns (DAMPs; orange circles) from the intestinal microbiome and virome pass to the liver and activate Kupffer cells. Lymphocytes (B or T cells) activated in mucosa-associated lymphatic tissue can also traffic to the liver and mediate injury. Activity in liver-resident regulatory T cells (T regs) is important for control of immune activation through the secretion of cytokines such as interleukin 10 (IL-10) and transforming growth factor β (TGF-β). Abbreviations: LSEC, liver sinusoidal endothelial cell; T_{fh}, T follicular helper cell.

Therefore, although perforin may cause hepatocyte lysis when released by T cells, it is also required for preventing uncontrolled immune activation.

Viral hepatitis types A–E are a common cause of both chronic and acute hepatitis. Hepatitis B is of particular interest because it is not clear why some individuals develop ALF whereas others have a mild (or asymptomatic) infection [98]. It appears that a combination of host immune and viral factors are important. One small study failed to find any host germline pathogenic mutations that were associated with hepatitis B virus (HBV) ALF [99]. Increased viral genome mutations were associated with HBV ALF, including novel variants [100]. In contrast to indeterminate hepatitis, HBV ALF (in adults) is associated with massive B cell and plasma cell hepatic infiltration [101].

Hepatocyte regeneration

The liver is the archetypal organ for having regenerative capacity. In ALF, particularly in episodes that do not spontaneously resolve (and hence require transplantation), the lack of regeneration is insufficient to account for the degree of hepatocyte damage. Several factors have been identified as key players influencing hepatocyte regeneration (Figure 3).

One approach to regeneration is to understand the factors that promote **senescence**: a quiescent state of altered cell behavior where cell division is highly unlikely. Senescence is of relevance in both acute and chronic liver disease, and is often associated with activation of the ISR [102]. Acute paracetamol toxicity can induce senescence in hepatocytes, in proportion to the severity of liver damage. This is associated with increased p21 and p53 expression, but also appears to be dependent on macrophage-derived TGF-α1 ligand expression [103]. However, a lack of macrophage recruitment due to loss of colony-stimulating factor 1 also impairs liver regeneration [104]. It is not clear whether these mechanisms are equally important in ALF where lymphocyte infiltration is prominent, and studies of infection- or damage-induced senescence following acute injury in children are lacking.

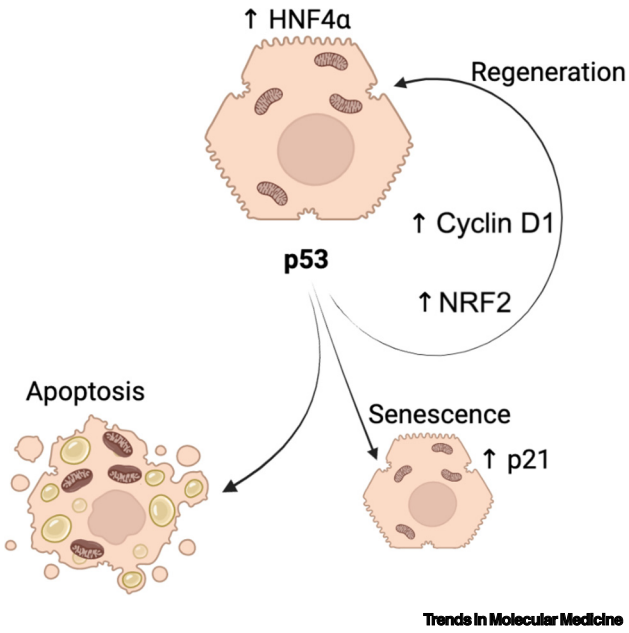

Figure 3. Hepatocyte regeneration requires a coordinated response that facilitates proliferation without triggering apoptosis, both of which are outcomes of p53 (TP53) activity. Expression of p53 in hepatocytes leads to induction of *Nrf2*, which promotes cyclin D1 expression that is necessary for proliferation of hepatocytes. Increased HNF4α is a marker of increased hepatocyte regeneration. Alternatively, hepatocytes may enter a senescent state that will not contribute to liver regrowth. This is also triggered by p53, and therefore can send cells towards apoptosis (bottom left) or senescence – a state of altered cellular function without proliferation that is characterized by increased p21 (CDKN1A) expression (bottom right). Abbreviations: HNF4α, hepatocyte nuclear factor 4α; NRF2, nuclear factor erythroid 2-related factor 2.

Clinician's corner

Acute hepatitis of unknown cause (indeterminate hepatitis) may develop in previously well children and in some cases develop into acute liver failure (ALF), which may require liver transplantation. In the UK, and elsewhere around the world, there has been a recent epidemic of post-pandemic acute hepatitis in which >1000 cases have been identified, drawing attention to the need to understand more about this rare disease.

The post-pandemic epidemic of acute hepatitis in previously healthy children under 5 years of age has been related to their lack of exposure to common childhood viruses because of 'lockdowns' and shielding during the pandemic. About 70% of affected children were positive for adenovirus and AAV2, which are not typically associated with liver injury in immunocompetent patients, and it is presumed that coinfection with no natural immunity caused the acute hepatitis.

Children with rare monogenic disorders may develop recurrent ALF triggered by relatively minor infections and may recover.

The degree of hepatocyte damage is determined by a balance of pro- and anti-inflammatory immune cells (i.e., Tregs).

Survival of the native liver is dependent on hepatocyte regeneration, and α-fetoprotein may be a biomarker of hepatocyte regeneration. Understanding these mechanisms will allow the development of non-invasive methods to diagnose and monitor pediatric ALF.

However, it is known that p53 activity must be carefully controlled. In paracetamol toxicity, p53 (TP53) helps to mount an antioxidative stress response through induction of NRF2 [105–107]. This illustrates the tight balance exerted by systems involved in the stress response: sufficient activity is needed for normal cell proliferation and the adaptive response, whereas excessive activity can induce senescence or apoptosis. Many of the targets involved in growth/senescence are also targets of CHOP and ATF4, the downstream mediators of the ISR [80]. It has been demonstrated, at least *in vitro*, that manipulation of the activation of the ISR can result in either proliferative or apoptotic responses [77,108,109].

Hepatocyte proliferation alone does not appear to be sufficient to restore liver function [110]. Hepatocytes must exit from the cell cycle before they can restore their full functional capacity, and the expression of HNF4α is required for this in the partial hepatectomy model [111]. HNF4α acts in reciprocal action with cyclin D1 – high cyclin D1 (and low HNF4a) is needed for proliferation, and elevated HNF4α imparts mature hepatocyte metabolic function [112]. Post-transcriptional *O*-GlcNAcylation of HNF4α may be important for its normal function in this role [113].

Limitations

We have sought to identify common important mechanisms in ALF and use this to illustrate factors that may be of relevance in indeterminate hepatitis/ALF in children. However, almost all the mechanistic experiments here come from toxic models (e.g., paracetamol- or thioacetamide-induced liver injury), and drug-induced liver injury accounts for up to 5% of pediatric ALF [114]. In addition, much of the human data supporting the basic science studies come from adults where, other than in paracetamol-induced injury, acute-on-chronic liver failure is most common. Lastly, a diverse range of treatment options are available (e.g., antivirals [115], steroids, immunoglobulins) which may reveal features about the underlying mechanisms, but a discussion of treatment is beyond the scope of this review.

Concluding remarks

There has been a recent epidemic of ALF due to AAV2 coinfection with adenovirus, through precisely why this leads of acute hepatitis is unclear (see Outstanding questions). This epidemic has prompted a re-examination of what is understood about ALF in children. Rare monogenic predisposition to ALF demonstrates the importance of the ISR, which also regulates regeneration and hepatocyte senescence. From an immune perspective, etiologies show B cell- or T cell-predominant responses, and gut–-liver signaling is probably important in many cases. Further work will be necessary to establish whether manipulation of these cells (or Treg cells) can be used therapeutically. There is a need to establish biomarkers for regeneration to help to identify children at risk of deterioration.

Declaration of interests

The authors declare no conflicts of interest.

Resources

[i]www.gov.uk/government/publications/acute-hepatitis-technical-briefing/investigation-into-acute-hepatitis-of-unknown-aetiology-in-children-in-england-case-update

[ii]www.ecdc.europa.eu/en/hepatitis/joint-weekly-hepatitis-unknown-origin-children-surveillance-bulletin

[iii]www.gov.uk/government/publications/acute-hepatitis-technical-briefing

[iv]www.who.int/emergencies/disease-outbreak-news/item/2022-DON400

Outstanding Questions

Indeterminate hepatitis leading to ALF is the most common single indication for liver transplantation, although the etiology is unknown. The recent epidemic of adenovirus and AAV2 hepatitis has indicated the importance of prior immunity and the role of susceptibility in the development of ALF, but the underlying mechanisms need to be established. There is no evidence of direct virus-mediated hepatocyte lysis from explanted livers of these children, which suggests hepatocyte-specific priming of immune cells by AAV2 and/or adenovirus. It is not known how (co)infection with either of these viruses might lead to liver-specific injury.

Not all children with indeterminate or other forms of hepatitis require transplant and, at the cellular level, this is determined by the balance of hepatocyte regenerative capacity and apoptotic rate. It is not clear what influences the relative activation of different arms of the apoptotic/proliferative response.

Treg cell activation can cause generalized (or targeted) suppressed immune activity; this is being trialed in chronic forms of autoimmune disease. It is not known whether Treg activity can be manipulated acutely to suppress excessive inflammation, such as in the case of indeterminate hepatitis.

Monogenic forms of recurrent ALF have implicated several genes through loss-of-function mutations. It remains to be studied whether more common variants in these genes also cause/predispose to a liver-related phenotype.

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