JEM Journal of Experimental Medicine

INSIGHTS

No IL-18BP? Avoid HAV

Michael S. Diamond

Fulminant viral hepatitis occurs in a very small number of infected individuals. Until now, the basis for this phenotype has remained unknown. In this issue of *JEM*, Belkaya et al. (https://doi.org/10.1084/jem.20190669) identify a deletion in the *IL18BP* gene in a severely affected child that results in excessive natural killer cell activation and uncontrolled killing of hepatocytes.

In a small subset of otherwise healthy children or adults, infection with certain hepatotropic viruses (e.g., hepatitis A [HAV], B, or E) can cause acute liver damage, liver failure, a need for transplantation, and death. Although the basis for these relatively rare occurrences has remained unknown, it has been speculated that vulnerable individuals might have a specific genetic basis for uncontrolled virus replication in liver cells and/or an unrestrained immune response that results in massive hepatocyte death and immunopathology. Indeed, rare cases of multiple family members developing fulminant viral hepatitis have been reported (Durst et al., 2001). While this suggests a genetic etiology, this disease is not associated with any of the known primary immunodeficiencies. Belkaya et al. explore this question in the context of explaining why an 11-yr-old child without a prior history of severe viral infections succumbed rapidly to HAV infection with fulminant liver disease.

Belkaya et al. (2019) used a whole-exome sequencing approach that has successfully identified several disease-causing genes and monogenic inborn errors of immunity that resulted in severe viral infections (Casanova, 2015; Meyts et al., 2016). They selected this particular case to identify autosomal recessive genes associated with fulminant viral hepatitis because the girl was born from a consanguineous relationship and her two brothers experienced benign HAV infections. This allowed for filtering and prioritization of homozygous gene variants with low allele frequency. When they searched the sequences for the very rare homozygous nonsynonymous variants present in the patient, but not in her siblings, they identified only six genes meeting these criteria. These genes were prioritized for validation studies based on their known functions in the liver or immunity and the more deleterious nature of the mutation (e.g., loss of protein expression).

Among the candidates, a private (unique to this individual), homozygous 40-nucleotide deletion at an intron-exon boundary in the *IL18BP* gene, which encodes for an IL-18–binding protein, stood out. Both parents and one of the siblings were heterozygous for the mutation, whereas the other sibling did not carry the mutation. IL-18 activity in the context of endotoxin shock is associated with induction of IFN-γ, T cell proliferation, natural killer (NK) cell activation and liver toxicity in mice (Okamura et al., 1995). Secreted IL-18BP neutralizes IL-18 functions and prevents activation of NF-κB signaling and induction of IFN-γ and IL-8 (Novick et al., 1999). Consistent with a key immunomodulatory role, poxviruses encode evasion proteins highly homologous to IL-18BP that attenuate IL-18 activity and interfere with the cytotoxic T and NK cell responses (Xiang and Moss, 1999; Born et al., 2000).

The 40-nucleotide deletion was predicted to impair the splicing of the last exon and affect the Ig-like domain and the C-terminal part of IL-18BP. Through a series of genetic and biochemical experiments, Belkaya et al. (2019) show that the mutation in *IL18BP* disrupted gene splicing and resulted in more rapid degradation of mRNA transcripts and a loss of or abnormal expression of the IL-18BP protein. Subsequently, they evaluated the functional impact of IL-18BP variants in a bioassay in which IL-18BP inhibits the IL-12/IL-18–induced production of IFN-γ by an NK cell line. Protein generated from the patient-encoded mRNA transcripts did not block IL-18 activity, thus confirming that she essentially had a complete functional deficiency of IL-18BP. In contrast, other more common or rare genetic variants in the population found in the homozygous state (e.g., R121Q, Q192H, V23I, and P184L) had no negative impact on IL18-BP function.

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Experiments next focused on defining the expression patterns of IL-18, IL-18R, and IL-18BP in different cell types of the liver. These data, which were obtained largely through the analysis of transformed and tumor cell lines, suggested that IL-18 is produced mostly by macrophages and induces IFN-γ production, particularly by IL-18R responding NK cells. This, in turn, in a

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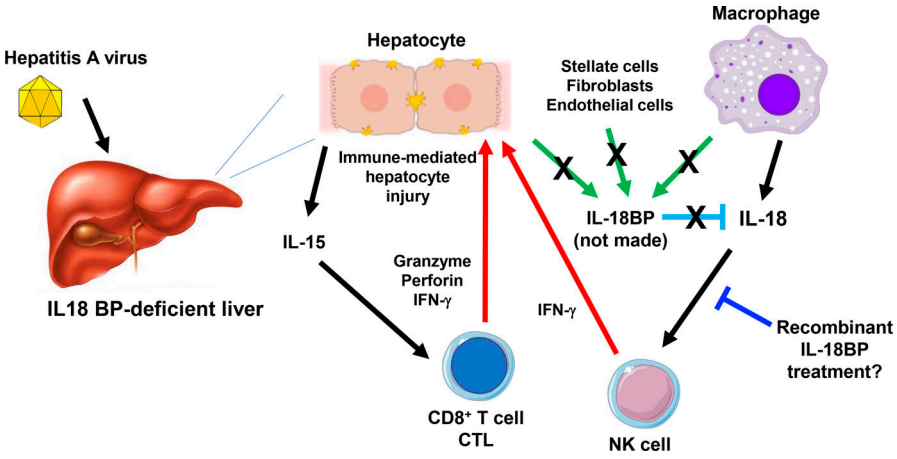

Scheme of HAV-induced fulminant liver injury and the role of IL-18BP. Hepatitis viruses infect hepatocytes in the liver. In response to infection, resident and/or infiltrating macrophages and possibly hepatocytes themselves produce IL-18, which activates NK cells to produce IFN-γ and mature their cytolytic responses. As part of a negative feedback loop, hepatocytes, stellate cells, fibroblasts, and endothelial cells also produce soluble IL-18BP, which neutralizes the activity of IL-18 and prevents excessive liver damage. The child described in the current study had a 40-nucleotide deletion across an exon–intron boundary in her *IL18BP* gene and produced no functional IL-18BP, and thus IL-18 activity was unchecked. Activated NK cells can kill both infected and uninfected hepatocytes, leading to massive liver injury. It is possible that administration of recombinant IL-18BP therapy (e.g., Tadekinig Alfa) would scavenge the free IL-18 in vulnerable hosts and lessen NK cell activation and damage. A major question with therapeutic implications is whether unrestrained IL-18 activity is a common or dominant mechanism for virus-induced fulminant liver disease or whether other immune mediators (e.g., hepatocyte-produced IL-15 and cytotoxic CD8⁺ T cells) account for a significant fraction of severe cases.

feedback loop, triggers IL-18BP secretion from hepatocytes and macrophages. Immunohistochemical analysis of tissue sections from fulminant viral hepatitis patients (including the affected child) showed massive hepatocyte necrosis that was associated with large numbers of infiltrating T and NK cells. IL-18 levels were high in the liver sections of fulminant viral hepatitis cases, with expression observed in both macrophages and hepatocytes. This result is consistent with the elevated levels of IL-18 observed in patients with acute hepatitis and HAV infection (Kim et al., 2018). Finally, the authors performed a series of in vitro studies showing that in the absence of IL-18BP, excessive NK activation by IL-18 results in the killing of human hepatocyte cells. They conclude that the inherited human IL-18BP deficiency in this individual predisposed her to fulminant HAV hepatitis because of a lack of control of the immunopathological actions of IL-18.

This genetic and immunological study provides compelling data for why this individual was vulnerable to severe liver injury after HAV infection. The severity of the phenotype appears to be due to an unchecked host immune and IL-18 response rather than the direct cytolytic activity of the virus, which agrees with postulated immune-mediated mechanisms of HAV-induced disease (Shin and Jeong, 2018). Consistent with this idea, the authors showed in vitro that NK cells kill both HAV-infected and uninfected hepatocytes, and this direct and collateral damage can be reversed by the addition of exogenous IL-18BP.

Based on their findings and prior clinical and animal model data, Belkaya et al. (2019) advance a model in which an uncontrolled IL-18 response drives liver toxicity in the context of fulminant viral hepatitis in humans, even in those without genetic deficiencies in IL-18BP. If true, it would suggest that human IL-18BP could have liver-protective activity and serve as a novel immunomodulatory therapy for the early stages of fulminant viral hepatitis to prevent liver failure. This concept is of great interest because recombinant human IL-18BP (Tadekinig Alfa; AB2 Bio Ltd) currently is being evaluated for clinical use for IL-18–mediated autoinflammatory and autoimmune indications (e.g., hemophagocytic lymphohistiocytosis and Still's disease) and has been suggested as a treatment for preventing acetaminophen-induced hepatotoxicity (Imaeda et al., 2009; Bachmann et al., 2018).

The study establishes a single inborn error of an immune gene that explains the basis of one case of fulminant viral hepatitis in a child who had no prior history of severe viral infections. Given the private nature of this deletion, other as yet undefined genetic variants in IL-18, IL-18R, IL-18BP, or downstream signaling pathways or regulators might explain the majority of cases of severe disease. However, IL-18 is not the only proinflammatory cytokine postulated to cause massive hepatocyte death in the context of viral infection. Indeed, HAV-infected cells can produce high levels of IL-15 that induce T cell receptor–independent activation of memory CD8⁺ T cells, which have innate-like cytotoxic effects without T cell receptor engagement (Kim et al., 2018). Thus, therapeutic strategies that neutralize endogenous IL-18, particularly with recombinant IL-18BP, may be beneficial for some patients with fulminant liver disease caused by HAV but not others. Moreover, it remains uncertain if the pathogenesis sequence is similar in other etiologies of acute viral (e.g., hepatitis B and E viruses) or nonviral hepatitis. Clearly, additional whole-exome or genome sequencing analysis of individuals with fulminant hepatitis of multiple different origins will be informative as to the range of possible applications of IL-18BP treatment. Alternatively, the response rate to exogenous IL-18BP therapy within the structure of a well-designed clinical trial may provide insight into the heterogeneity of causative mechanisms resulting in severe liver disease that occurs in a small minority of affected individuals.

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