The clinical syndrome of acetaminophen-induced liver injury represents the combined result of drug toxicity and a potent innate immune response that follows drug-induced cell death. In this issue of the JCI, Imae and colleagues report that DNA released from dying hepatocytes is a key stimulus of innate immune activation in the acetaminophen-treated mouse liver (see the related article beginning on page 305). They present evidence indicating that hepatocyte DNA promotes immune activation by acting as a danger-associated molecular pattern (DAMP) that stimulates cytokine production in neighboring sinusoidal endothelial cells via TLR9 and the Nalp3 inflammasome.

The analgesic acetaminophen is widely known for its potential to cause severe and sometimes lethal liver injury. When ingested in large amounts, acetaminophen overpowers the normal metabolic pathways of glucuronidation and sulfation and undergoes oxidation to form the highly reactive intermediate N-acetyl-p-benzoquinone-imine (NAPQI). NAPQI is not harmful if it combines rapidly with glutathione; however, when hepatic glutathione stores are depleted, NAPQ1 escapes detoxification, resulting in liver cell death (1). An important but underappreciated aspect of acetaminophen toxicity is that direct, drug-induced harm accounts for only part of the overall syndrome of acetaminophen-induced liver injury. The reason for this is that the initial wave of drug-induced hepatocellular destruction is followed by a robust innate immune response, in which invading inflammatory cells release toxic oxidants and cause a second wave of destruction. The collateral damage inflicted by inflammatory cells can be so severe as to double the degree of tissue injury caused by acetaminophen alone (2).

**DAMPs ramp up drug toxicity**

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**Innate immunity is the result of danger signaling**

Activation of the innate immune system following a noninfectious insult such as drug toxicity arises when dying cells release molecules that serve as “danger signals.” Danger molecules trigger inflammation by engaging pattern recognition receptors such as TLRs (3) and nucleotide-binding domain, leucine-rich repeat–containing proteins (NLRs) (4) and are thus referred to as danger-associated molecular patterns (DAMPs) (5). Through TLRs, DAMPs signal cytokine and chemokine production and upregulate the expression of cell-adhesion molecules. When DAMPs interact with NLRs, they stimulate NLRs to complex with ASC (apoptosis-associated speck-like protein containing a CARD) to form macromolecular complexes called inflammasomes, which activate caspase-1 and stimulate cleavage of the proinflammatory cytokines pro–IL-1β and pro–IL-18 to their mature forms, IL-1β and IL-18 (6). Self molecules that act as DAMPs interact primarily with TLR2, -4, and -9 and an NLR with an N-terminal pyrin domain designated NACHT, LRR, and pyrin domain–containing protein 3 (NALP3; also known as NLRP3). The list of these molecules is rapidly growing (Table 1), emphasizing the importance of endogenous danger signaling to a broad array of medical disorders ranging from gout to systemic lupus erythematosus to Alzheimer disease (7–9). A danger molecule that is believed to play a

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Nonstandard abbreviations used: DAMP, danger-associated molecular pattern; HMGBl, high-mobility group box 1; NALP3, NACHT, LRR, and pyrin domain–containing protein 3; NAPQI, N-acetyl-p-benzoquinone-imine; NLR, nucleotide-binding domain, leucine-richrepeat–containing protein, SEC, sinusoidal endothelial cell.

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central role in inflammatory diseases of the liver is the chromatin-binding protein high-mobility group box 1 (HMGB1). Upon cellular necrosis, HMGB1 is released into the extracellular milieu, where it activates TLR2 and TLR4 and, when complexed with DNA, TLR9 (10, 11). Studies to date indicate that HMGB1 plays an important role in liver injury due to hemorrhagic shock and ischemia/reperfusion (12), but, interestingly, it does not strongly influence acetaminophen toxicity (13).

**Table 1**

<table>
<thead>
<tr>
<th>Self molecules that interact with TLRs and NLRs</th>
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<tr>
<td><strong>Self molecule</strong></td>
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<td>------------------</td>
</tr>
<tr>
<td>TLRs</td>
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<tr>
<td>Heat shock proteins</td>
</tr>
<tr>
<td>Defensins</td>
</tr>
<tr>
<td>NLRs</td>
</tr>
<tr>
<td>Monosodium urate crystals</td>
</tr>
<tr>
<td>Calcium pyrophosphate dihydrate crystals</td>
</tr>
<tr>
<td>Silica crystals</td>
</tr>
<tr>
<td>Alum particles</td>
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<tr>
<td>Amyloid-β particles</td>
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<td>Asbestos particles</td>
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**Danger signaling in acetaminophen hepatotoxicity**

Although the precise nature of the danger signal that activates innate immunity in the acetaminophen-exposed liver is uncertain, there is no question that danger signaling is involved in the process. This is evident from studies in a mouse strain with a mutation in Tlr4, in which liver disease is significantly attenuated following an acetaminophen challenge (14). Recently, acetaminophen toxicity was shown to be reduced in mice that lack the ability to respond to IL-1 (15). This observation is of interest because IL-1 secretion typically requires the combined activity of TLRs and NLRs, the former to stimulate expression of the IL-1 propeptide and the latter to process the propeptide into a mature cytokine. It also suggests a need for two, possibly unique, danger signals in the liver: one to activate a TLR and another to signal NLR assembly into an inflammasome (6).

If the danger signal that augments acetaminophen-induced liver injury is not HMGB1, then another molecule that could accomplish this task is DNA from dying hepatocytes. DNA interacts specifically with TLR9, which, like all nucleic acid-sensing TLRs, is sequestered intracellularly within endosomes. TLR9 was once con-
Considering incapable of binding mammalian DNA because of its affinity for unmethylated CpG motifs characteristic of microbial DNA. DNA from injured mammalian cells, however, has the capacity to activate TLR9 (8, 16), and recently even normal mammalian DNA has been shown to engage this receptor and stimulate an immune response (17). In this issue of the JCI, Imaeda and colleagues (18) demonstrate that DNA is indeed a trigger of the innate immune response that amplifies acetaminophen toxicity. They showed, in a mouse model, that acetaminophen-induced liver injury is dependent upon not only IL-1β, but also IL-18, the two cytokines classically processed by caspase-1 (Figure 1). They determined that acetaminophen-mediated induction of pro–IL-1β and pro–IL-18 mRNA in the liver is TLR9 dependent; in addition, they showed that cleavage of the IL-1β propeptide to the mature cytokine in the acetaminophen-treated liver requires the presence of the Nalp3 inflammasome. Inhibition of either Tlr9 signaling or Nalp3 activity by genetic or pharmacologic means markedly attenuated cytokine gene expression via monosodium urate crystals, which activate Nalp3, but it remains unclear whether this is interpretable as an independent effect of aspirin on caspase-1 activation by the Nalp3 inflammasome (Figure 1).

**Novel concepts and open questions**

Overall, the work of Imaeda and colleagues (18) sheds important light on the role of the inflammasome in drug-induced liver injury. It highlights potential differences between innate immune responses to different stimuli (e.g., drugs versus ischemia/reperfusion) and places SECs in a category with bone marrow–derived cells as orchestrators of innate immune responses to DNA. Still, there remain some unanswered questions. One is whether bone marrow–derived cells, which also contribute to innate immune activation in response to acetaminophen, sense DNA as their danger signal in the same fashion as SECs. Another is whether DNA from hepatocytes treated with acetaminophen has any unique characteristics with respect to Tlr9 activation. Third, one wonders whether immune-mediated collateral damage in acetaminophen toxicity is due entirely to leukocyte invasion, or whether inflammasome-mediated cell death is also involved. Under certain circumstances, NALP3, ASC, and caspase-1 can interact to cause cell death (21). Such a pathway could be operative in SECs during acetaminophen toxicity. If so, this may explain why innate immune activation in the liver is so often accompanied by sinusoidal cell breakdown and parenchymal hemorrhage, which in many animal models of acute liver injury is the fatal event.

Imaeda and coworkers (18) posited that two separate danger signals are required to activate IL-1β and IL-18 in the acetaminophen-treated liver: hepatocyte DNA to induce cytokine gene expression via Tlr9 and another molecule, possibly uric acid or ATP, to activate the Nalp3 inflammasome and promote cytokine cleavage. This theory was logical, based on evidence that few if any compounds stimulate both TLRs and NLRs. Recent work by Muruve et al. (22), however, indicates that mammalian DNA can promote inflammasome formation, albeit in the absence of NALP3. Further research in this area may ultimately lead to a unified theory according to which self DNA activates not only TLR9 but also the NALP3 inflammasome following a cytotoxic insult in vivo, culminating in immune-mediated collateral damage to the affected organ.

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In this issue of the JCI, Semple and colleagues report phenotypic evaluation of patients with a germline mutation in the gene encoding serine/threonine kinase AKT2 (see the related article beginning on page 315). Their findings support the idea that the postreceptor actions of insulin in the liver — suppression of gluconeogenesis and stimulation of lipogenesis — are mediated through divergent pathways that can be uncoupled. The results appear to refine the arrangement of crucial steps along these pathways and show how comprehensive study of the phenotype, “deep phenotyping,” of patients who carry rare mutations might complement other types of experiments to elucidate complex pathways and mechanisms.

“When you hear hoofbeats, think horses, not zebras” is the quintessential maxim of clinical medicine. But in clinical investigation, the “zebras” — rare conditions that recapitulate, often to an extreme, the components of a common disease — can help to understand the “horse” or common complex phenotype. Extending the metaphor, the current pandemic of obesity and insulin resistance is a veritable stampede that threatens to flatten global medical care infrastructures. A multifaceted approach is required to understand the mechanisms underlying this pandemic, ranging from the strategic use of model systems to population studies and clinical trials. Within this methodological spectrum is the evolving discipline of clinical phenomics, which uses objective and systematic acquisition of phenotypic data (i.e., deep phenotyping) of selected informative patients (1). Phenomic evaluation of patients with rare genetic disorders is a potential tool to help solve the puzzle of insulin resistance and its downstream metabolic consequences, such as hyperglycemia, hepatosteatosis and dyslipidemia, elevated triglyceride (TG) levels, and depressed HDL cholesterol levels.

## Insulin-resistant diabetes: a disease of abnormal glucose and lipid metabolism

The complex web of interactions between glucose and lipid metabolism in diabetes has long been appreciated, if incompletely understood mechanistically (2–5). Induced-mutant animal models have steadily advanced our understanding of insulin resistance. For instance, insulin-resistant mice with a liver-specific deletion of the insulin receptor (INSR) develop hyperglycemia but not dyslipidemia (6). This suggests an uncoupling or divergence (see Figure 1) between the post-INSR pathways, with loss of insulin-mediated suppression of gluconeogenesis normally driven by phosphoenolpyruvate carboxykinase and glucose-6-phosphatase, but retention of insulin’s stimulatory effect on hepatic lipogenesis catalyzed by fatty acid synthases (7). Signaling intermediates along the gluconeogenesis limb include serine/threonine kinase AKT2 (also called phosphokinase B) and forkhead box O transcription factor 1 (FOXO1), while intermediates along the de novo hepatic lipogenesis limb include PKCα and SREBP-1c (8, 9). To clarify the basis of such “asymmetric” or partial insulin resistance, deep phenotyping of patients carrying naturally occurring loss-of-function mutations in these intermediate signaling molecules might be instructive.

In their current study in this issue of the JCI, Semple and colleagues (10) have attempted to probe these pathways by studying patients with extremely rare mutations in either INSR or AKT2 genes together with subjects who had either idio-pathic insulin resistance or inherited lipodystrophies. The study took advantage of a valuable archive of phenotypically and molecularly characterized patients who were carefully collected over many years. Among patients with INSR mutations and anti-INSR antibodies, Semple and colleagues first confirmed earlier work that showed severe hyperglycemia and hyperinsulinemia but normal plasma lipids in these patients (11). These findings are