Allergic hepatitis induced by drugs
José V. Castell\textsuperscript{a,b} and Marta Castell\textsuperscript{c}

Purpose of review
To examine recent advances in our understanding of how drugs trigger a hypersensitivity reaction in the liver, how tolerance is lost, the mechanisms of damage to hepatocytes and the strategies towards a better assessment of an idiosyncratic drug liver reaction.

Recent findings
Formation and presentation of drug–protein adducts, or a direct interaction with the major histocompatibility complex/T-cell receptor complex is a necessary but not sufficient stimulus to trigger a hypersensitivity reaction. Liver shows considerable tolerogenic potential towards drug adducts. Recent studies highlight allergic hepatitis as a loss of liver tolerance towards drug antigens, the mechanisms of which are beginning to be unravelled. Cell injury caused by the drug itself, a concomitant inflammatory process, or a coincidental stimulus probably represents the additional signal needed to initiate the process.

Summary
Drug-induced liver injury is of concern due to its unpredictable nature and serious clinical implications. Clinically, both hepatocellular injury and cholestasis can occur and most episodes have good clinical prognoses upon drug discontinuation. In few cases damage to the liver cells may continue in the form of an autoimmune hepatitis. The available diagnostic tools to confirm an immune-mediated hepatic injury are still very limited, and rely on the lymphocyte transformation test.

Keywords
drug induced liver injury, hepatocytes, idiosyncratic hepatotoxicity

Introduction
Drug-induced allergic hepatitis is a liver-specific inflammatory reaction caused by hypersensitivity to a particular drug. Although less common than other forms of drug-induced hepatotoxicity, it has more serious clinical implications, the outcome can sometimes be fatal, and it appears to increase in proportion to the number of prescribed drugs. There is convincing experimental evidence to implicate the immune system in the pathogenesis of many drug hypersensitivity reactions. The onset of a hypersensitivity reaction frequently involves covalent binding of the drug to proteins (or more often as a result of its metabolism and bioactivation) to form immunogenic conjugates, followed by antigen uptake, processing, presentation and T-cell proliferation [1\textsuperscript{**},2].

Hepatocytes, because of their capability to metabolize drugs, usually form drug–protein adducts, for which the immune system normally shows tolerance. Hypersensitivity reactions occur when this tolerance is impaired. Additional signals, likely a concomitant inflammatory reaction, may eventually be needed to break this tolerance. The allergic hepatitis induced by drugs is generally a type IV hypersensitivity reaction involving CD4\textsuperscript{+}, CD8\textsuperscript{+} cytotoxic lymphocytes as well natural killer cells. Antibodies directed to the drug are much less common. Antibodies against cellular components may also occur when the sensitization process evolves towards an autoimmune reaction [3,4].

Allergic hepatitis is frequently associated with fever, rash and liver cell infiltration (drug rash with eosinophilia and systemic symptoms (DRESS) syndrome) [5]. Clinically, both hepatocellular injury and cholestasis can occur, and most episodes have good clinical prognoses upon drug discontinuation. In few cases the damage to liver cells may continue, even upon drug withdrawal, in the form of an autoimmune hepatitis. The available diagnostic tools to confirm the involvement of a given drug in an immune-mediated hepatic injury are rather limited, and this is largely due to a still incomplete understanding of the pathogenesis of drug allergy in the liver. Better understanding the molecular and cellular events will definitively help to identify risk factors, and facilitate the prediction and prevention strategies [1\textsuperscript{**},6].

Drugs and hepatocyte injury: two pathways of action
Compounds causing injury to liver are known as hepatotoxins. Some of these compounds will cause toxic
Table 1 Compounds recently claimed to cause allergic or drug-induced autoimmune hepatitis (2002–2006)

<table>
<thead>
<tr>
<th>Drug</th>
<th>Clinical features</th>
<th>Laboratory features</th>
<th>Biopsy</th>
<th>Others</th>
<th>References</th>
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<tbody>
<tr>
<td>Allopurinol, hyperuricemia, goit treatment</td>
<td>Exfoliative dermatitis, hepatitis and interstitial nephritis</td>
<td>Eosinophilia</td>
<td>Diffuse portal tract and lobular inflammation with a prominent eosinophilic infiltrate</td>
<td>After 6 days of therapy with oral allopurinol</td>
<td>[5]</td>
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<tr>
<td>Carbamazepine (anticonvulsivant)</td>
<td>Fever, morbilliform macular rash, induced fulminant liver failure</td>
<td></td>
<td></td>
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<td>[8]</td>
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<tr>
<td>Cetirizine (anti-H1 receptor antagonist)</td>
<td>Weakness, nausea, anorexia, and hyperchronic urine</td>
<td></td>
<td></td>
<td></td>
<td>[9]</td>
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<tr>
<td>Dapsone (leprosy drug)</td>
<td>Jaundice, fever, eosinophilia, Stevens-Johnson syndrome-like</td>
<td></td>
<td></td>
<td></td>
<td>[10]</td>
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<tr>
<td>Halogenated volatile anesthetic drugs</td>
<td>Acute liver failure</td>
<td>Auto IgG4, decreased complement C3a and C5a</td>
<td></td>
<td></td>
<td>[12]</td>
</tr>
<tr>
<td>Infliximab (Anti TNFα therapy)</td>
<td></td>
<td></td>
<td></td>
<td>After the sixth infusion; history of psoriatic arthritis</td>
<td>[13]</td>
</tr>
<tr>
<td>Lamotrigine (anticonvulsivant)</td>
<td>Headache, vomiting, diarhoea, fever. Maculopapular rash, jaundice</td>
<td></td>
<td></td>
<td></td>
<td>[14]</td>
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<tr>
<td>Metamizole analgesic</td>
<td>Generalized exanthema</td>
<td></td>
<td></td>
<td>Positive LTT to metamizole plus three metabolites</td>
<td>[15]</td>
</tr>
<tr>
<td>Mynocicline</td>
<td>Severe jaundice</td>
<td></td>
<td></td>
<td></td>
<td>[16]</td>
</tr>
<tr>
<td>Nevirapine (antiretroviral therapy)</td>
<td>Fever, toxic epidermal necrolysis, acute liver failure</td>
<td>Serum liver enzymes, eosinophilia; ANA</td>
<td></td>
<td>Inflammatory cells infiltrate in portal tract</td>
<td>[17]</td>
</tr>
<tr>
<td>Nevirapine (antiretroviral therapy)</td>
<td>Drug rash with eosinophilia and systemic symptoms syndrome</td>
<td>Serum liver enzymes</td>
<td></td>
<td>No biopsy performed</td>
<td>[18]</td>
</tr>
<tr>
<td>Statins</td>
<td>Liver injury with characteristics of an autoimmune disease</td>
<td>Serum liver enzymes; ANA</td>
<td></td>
<td>Weeks to months after treatment</td>
<td>[19,20]</td>
</tr>
<tr>
<td>Twinrix (inactivated HVA + rHBsAg vaccine)</td>
<td>Severe jaundice</td>
<td>Serum liver enzymes, BR; IgG and ANA</td>
<td></td>
<td>Marked bridging fibrosis, moderate chronic infiltrate</td>
<td>[21]</td>
</tr>
</tbody>
</table>

Characteristic drugs causing immune-mediated reactions in the liver include a wide variety of compounds such as sulphonamides, halogenated anaesthetics, tienilic acid, and dihydralazine, among others (for a comprehensive review, see [22–24]). CRP, C-reactive protein; ALT, alanine aminotransferase; AST, aspartate aminotransferase; GGT, γ-glutamyl transferase; ALP, alkaline phosphatase; BR, bilirubin; ESR, erythrocyte sedimentation rate; TNF, tumour necrosis factor; ASMA, antismooth muscle antibody; HVA, hepatitis virus A; HVB, hepatitis virus B; CMV, cytomegalovirus, EBV, Epstein Barr virus; LTT, lymphocyte transformation test; ANA, antinuclear antibody; IgG, immunoglobulin G; AIH, auto-immune hepatitis.
Mechanisms behind the onset of a hypersensitivity reaction to drugs in the liver

There are several working hypotheses to explain the onset of a drug allergic phenomenon. The hapten hypothesis [3] postulates that drugs, or reactive moieties derived from the drugs, can react with cell proteins forming covalent drug–protein adducts. This can occur in the course of biotransformation reactions by the hepatocyte, when reactive intermediates are formed, which can, in turn, react with cell macromolecules forming stable adducts (haptenization) [25]. The extent of drug covalent binding is a determinant of the onset of an allergic reaction and is dependent on the proportion of the chemical converted into a reactive metabolite, its half life, the reactivity towards functional groups of biomolecules and the ability of the cells to block (neutralize?) these intermediates with endogenous molecules (i.e. glutathione) [26,27,28,29–31].

The next step requires these neoantigens to become accessible to the surveillance of the immune system. The classic pathway assumes that those adducts are captured, internalized and processed by professional antigen presenting cells (APCs) which expose the drug–peptide adducts associated to the major histocompatibility (MHC) II complex (Fig. 1). From then on, APC cells can expose these complexes to CD4+ or CD8+ cells, stimulating them to proliferate [31].

The formation of drug–protein adducts in the liver is not an unusual phenomenon. Indeed, many drugs that form protein adducts to a certain extent rarely cause idiosyncratic drug reactions. Consequently, the formation of adducts is not per se determinant of the process: additional signals seem to be required for triggering a hypersensitivity response. Matzinger et al. [31,32] proposed that it is not the foreignness but rather the ability of a compound to trigger ‘alarm’ signals that determines whether it will induce an immune response. The danger hypothesis foresees that, in addition to recognition of the foreign nature of the drug adducts (signal 1), cell damage caused by the drug or its metabolites and the subsequent ‘danger’ signals (signal 2) upregulate the expression of costimulatory factors required to induce an immune response [32–34]. Interestingly, many drugs that are associated with idiosyncratic reactions first cause mild reversible liver injury in exposed patients (i.e. halothane), while many other patients do not show drug idiosyncratic reactions in a context that is likely to constitute a danger signal (i.e. surgery, inflammation).

To further complicate this picture, Pichler [34] formulated the pharmacological interaction hypothesis to explain the existence of reactive T clones in patients with a history of drug hypersensitivity reaction, which proliferate in the presence of the drug but not with drug metabolites or drug covalent adducts. This was interpreted as compounds being able to interact (reversibly?) with the MHC–T-cell receptor (TCR) complex and inducing an immune response [34].

Most recent views tend to consider the different hypotheses as being complementary rather than exclusive to
each other. In this way, formation and presentation of drug–protein adducts, or direct interaction with the TCR/MHC complex, would be a necessary but not sufficient stimulus to trigger the hypersensitivity reaction. Cell injury caused by the drug itself, a concomitant inflammatory process, or a coincidental stimulus (i.e. a viral infection) may represent the additional signal needed to initiate the process. It is also likely that the different hypersensitivity reactions may involve different combinations of these possibilities. [1**,2].

Frequently, drug immunoallergic responses are initiated or intensified under concomitant inflammatory states, which are believed to alter the production proinflammatory cytokines. Prandota [35**] has reviewed the potential role of cytokine alterations in determining the balance between T helper 1 (Th1) and Th2 cells that may lead to a shift in immune response determining liver cell injury by drugs. In this context it is worth noting that reactions may occur with the combination of an immune stimulus (i.e. virus infection) plus drug intake. If the virus infection is not present, reaction will not occur [1**,34].

### Hypersensitivity versus tolerance

The incidence of immune-mediated drug hepatotoxicity is relatively low. The microenvironment of the liver is believed to favour immune tolerance rather than inflammatory immunity. This tolerogenic property may be attributable to different factors: the liver’s production of regulatory cytokines (e.g. interleukin (IL)-4, IL-6, IL-10, IL-13, IL-15) and other inhibitory factors (e.g. prostaglandins) [36]; or the role of the different immune cells in the hepatic sinusoid towards naïve lymphocytes [37]. The mechanisms of induction and maintenance of tolerance in self-reactive T cells in the periphery are poorly understood. Current knowledge assumes that successful T-cell activation occurs only if in addition to TCR recognition of the antigen (signal 1) there is a costimulatory signal (signal 2); signal 1 in the absence of signal 2 is either ignored or induces tolerance [38**].

Liver sinusoidal endothelial cells are active in the uptake and cross-presentation of oral antigens from portal venous blood and engage in the induction of CD8 T-cell tolerance towards these antigens. In-vitro experiments reveal that naïve T cells are activated by resident sinusoidal endothelial cells but do not differentiate into effector T cells. These T cells show a cytokine profile and a functional phenotype that are compatible with the induction of tolerance [39,40**, 41]. Liver sinusoidal lining cells can take up antigen, process the antigen and present it to T cells but, probably due to the lack of input from T helper cells, the end result is tolerance rather than immunity. This major function of sinusoidal endothelial cells prevents immunological reaction to the wide spectrum of potentially antigenic molecules that are assimilated from the gastrointestinal tract [39].

Besides sinusoidal endothelial cells, other cell populations of the liver, such as dendritic cells, Kupffer cells and perhaps also hepatocytes may contribute to tolerance induction. Recent studies also point to an important role for dendritic cells in the induction of peripheral tolerance. It was proposed [42] that the role of dendritic cells in the
immunity/tolerance decision could be associated simply with dendritic cell maturation states.

Two main hypotheses have been put forward to explain such a dichotomy in behaviour of dendritic cells. The first hypothesis argues that the role of dendritic cells in the immunity/tolerance decision could be associated with dendritic cell maturation states, that is, immature dendritic cells lacking costimulation may induce tolerance. It has been correctly pointed out, however, that immature dendritic cells do not process endocytosed antigens well to form MHC plus peptide complexes on the cell surface. Self-specific T cells, therefore, would not be able to recognize their ligand on immature dendritic cells. Moreover, a maturation signal is necessary to induce migration of immature dendritic cells from peripheral tissue to local lymph nodes. The obvious question is how might immature dendritic cells induce tolerance to self-antigens [37,43]?

A second hypothesis points at dendritic cells having different maturation programs in the absence or presence of 'danger' signals: activated, mature dendritic cells induce T-cell immunity, and resting, nonactivated but fully differentiated mature antigen-presenting dendritic cells can induce tolerance, leading to mature-tolerogenic and mature-immunogenic phenotypes, respectively [42,44,45].

Hepatocytes may also function as antigen-presenting cells. Extension of hepatocellular microvilli through intercellular junctions between sinusoidal endothelial cells can allow direct contact with naïve CD8\(^+\) T cells. Experiments reveal, however, that T-cell activation by hepatocytes leads to premature T-cell death or tolerance rather than to an activated lymphocyte. T cells activated by hepatocellular antigen presentation are phenotypically different from T cells activated in the spleen or lymph nodes. Apoptosis of hepatocyte-activated T cells is suspected to be an example of death by neglect resulting from absence of an effective c-stimulatory signal. Cross-linking of CD28 on hepatocyte-stimulated T cells, which simulates costimulation by B7 molecules from macrophages, abrogated the early apoptosis of T cells, caused an increase in expression of bcl-x(L) and IL-2 in hepatocyte-activated T cells and resulted in sustained T-cell proliferation and cytotoxic activity [38\(^*\)].

Finally, Kupffer cells may also have a role as primary inducers of immunological tolerance against hapten-induced delayed-type hypersensitivity responses. Pretreatment of mice with a protein adduct of dinitrochlorobenzene led to its accumulation in Kupffer cells and to a subsequent tolerance to dinitrochlorobenzene sensitization [46\(^*\)]. Moreover, tolerance is impaired if Kupffer cells are depleted [37]. A role for stellate cells has also been suggested [47].

**Hepatocyte injury in the course of an allergic hepatitis: drug-induced liver autoimmunity**

The immune response to foreign antigens in the liver is generally associated with a strong and sustained CD4 and CD8 T-cell response. Immune-mediated killing of hepatocytes is mainly achieved by cytotoxic T cells. Activated CD8\(^+\) T cells are recruited to or trapped in the liver irrespective of their antigen specificity. Only upon recognition of their cognate antigen, however, do these CD8\(^+\) T cells undergo rapid proliferation. Proliferation presumably occurs directly in the liver in this scenario, as increased numbers of antigen-specific T cells are not detectable in draining lymph nodes during the early days after adoptive transfer. The lytic activity of cytotoxic T lymphocytes (CTLs) can occur by at least two pathways. In the perforin/granzyme-mediated pathway the pore-forming agent perforin, probably in conjunction with granzymes, induces apoptosis in target cells. In the Fas-mediated pathway, engagement of Fas and Fas ligand triggers apoptosis of the CTL-bound target cell by a death domain-initiated caspase cascade. Regardless of the initiating pathway, the downstream events that lead to apoptosis appear to be similar [38\(^*\)].

Natural killer cells and natural killer T (NKT) cells are effector cells in the liver. Natural killer cells are bone marrow-derived mononuclear cells that have markers of both T lymphocytes and macrophages. The cytoplasmic granules contain perforin and granzymes, which are involved in cell membrane attack and induction of apoptosis in target cells. As opposed to target recognition by cytotoxic T lymphocytes, recognition of target cells by natural killer cells is not restricted to MHC-antigen presentation and their major role is the defence of the liver against invading tumour cells acting by the Fas/Fas ligand pathway, resulting in activation of the caspases cascade and apoptosis [38\(^*\)].

NKT cells are considered to be separate from natural killer cells and pit cell populations. In addition to natural killer phenotype, they present surface expression of TCR. The TCR on NKT cells interacts with CD1, as opposed to the MHC-1 or MHC-2 interaction with the TCR on T lymphocytes, and can interact with target cells without restrictions. This liver-resident, locally regenerating pool of rapid response killing cells has a significant role in defending the liver from invading tumour cells [38\(^*\)].

Natural killer and NKT cells are likely to play a role in the progression of drug-induced liver injury by secreting interferon-\(\gamma\) and provoking a concomitant inflammatory response (chemokine production, accumulation of neutrophils, and upregulating Fas ligand expression in the liver), thus contributing to the severity and progression of liver injury downstream of the metabolism of the drug hepatotoxicity [48]. Nevertheless, natural killer and
NKT cells have been reported to dramatically diminish in a case of fulminant drug hepatitis, suggesting that both may be involved in hepatic injury in fulminant hepatic failure [49].

The role of Kupffer cells in drug hepatotoxicity is contradictory and likely to be indirect. These cells can be activated by different stimuli resulting in the release of mediators acting on hepatocytes (tumour necrosis factor α, nitric oxide, reactive oxygen species) [12] that exert important catabolic effects on hepatocytes [50]. Activation of Kupffer cells seems to be one of the early events in acetaminophen toxicity, yet a protective effect has also been described [51].

Autoimmune hepatitis can be one of the consequences of a drug-mediated hypersensitivity reaction, in which damage to liver continues once the use of the drug has been discontinued. The symptoms of drug-induced autoimmune diseases (i.e. systemic lupus erythematosus) or be organ-specific autoimmune reactions (i.e. liver) [4]. If not recognized promptly, they can give rise to chronic hepatitis (resembling viral hepatitis, i.e. α-methyldopa, halothane, hydralazine and other hydrazine-containing drugs, minocycline, nitrofurantoin, and oxyphenisatin) or cholangitis (resembling primary biliary cirrhosis, i.e. chlorpromazine). Several antibiotics, notably penicillins, cephalosporins, and macrolides, may cause severe cholestatic hepatitis, but rarely, if ever, cause self-perpetuating autoimmune liver disease [19].

A conceptual framework for the pathogenesis of autoimmune hepatitis points at environmental agents triggering a cascade of T-cell-mediated events directed at liver antigens in a host genetically predisposed to the disease [52*]. A T-cell-mediated immune response is thought to play a major role in the causation of autoimmune liver damage. In addition to CD4+ T cells, there is growing evidence to suggest a role for CD8+ T cells [53]. The syndrome differs from typical drug hypersensitivity reactions in that drug-specific T cells or antibodies are not involved, and may even not result in immune sensitization to the drug. Certain drugs are already known to induce autoimmune hepatitis (e.g. diclofenac, methylxyludopa, nitrofurantoin, minocycline, clomixin, and interferon) [22] while others (rifampicin, arotastatin/ezetimibe) have recently claimed to cause autoimmune hepatitis [23,24] as well as other systemic manifestations, such as lupus [54]. Many severe forms of drug-induced cholestasis persist after the drug has been discontinued, and a small number of patients who develop drug-associated cholestatic hepatitis develop progressive self-destruction of cholangiocytes [55]. There are no clear mechanisms to explain the phenomenon by which drugs may disrupt immune tolerance to self antigens. CD4+ T lymphocytes expressing the IL-2 receptor chain (CD25+) appear to be central to self-tolerance maintenance, preventing the proliferation and effector function of autoreactive T cells [53].

Autoimmune hepatitis elicited by drugs resemble type 2 which is characterized by auto-antibodies directed mainly to drug-metabolizing enzymes (anti-LKM-1/2/3, CYP2D6, CYP2C9, UGT1A and others) [53*].

Assessment of the allergic nature of an idiosyncratic drug liver reaction

The multifactorial nature of liver drug hypersensitivity and the involvement of unknown metabolites in the generation of antigens has made it considerably difficult to develop suitable laboratory tests to identify the causative drug. Despite recent advances in our knowledge of the mechanisms implicated, the diagnosis of allergic hepatitis remains a difficult task because specific tests are not available [56]. The strategy usually relies on incriminating a drug in the observed liver symptoms, and the exclusion of alternative causes of liver damage. The use of diagnostic algorithms adds consistency to the diagnostic suspicion by providing a framework that emphasizes the features that merit attention in cases of suspected adverse hepatic reactions [57].

Remarkably, no experimental models exist for allergic idiosyncratic hepatotoxicity. Indeed, most of the idiosyncratic hepatotoxins have reached clinical trials or marketing stages without showing any significant evidence of their risk at the preclinical stages. In the preclinical stages, the industry has been using different approaches to screen drugs for covalent binding, reactive metabolites reacting with glutathione. These phenomena, however, are not necessarily predictive of clinical problems. The hope is that these new approaches using animals and cells, as well as the identification of reactive metabolites, will provide clues concerning pathophysiology, which may be relevant to susceptible humans. The pharmaceutical industry is currently exploring the suitability of the omics technologies in an attempt to develop fingerprints of such toxicities. Although this would seem a challenging approach, its potential has not yet been convincingly shown, nor is it certain that it will be [1**,58].

The lymphocyte transformation test, which measures the proliferation of T cells of a suspected sensitized patient when exposed to the causative drug in vitro, is the most consistent test for identifying a drug suspected of causing allergic hepatitis, yet it is not fully reliable. Cell responses are fundamentally dependent on the efficacy of antigen presentation, and the type of reaction elicited may be conditioned by signalling cross-talk between the antigen-presenting cell and the T cell, which may be difficult to reproduce in vitro. This is indeed the major factor limiting reproducibility in this test, which has been
shown to critically depend on the number of lymphocytes and APCs as well the causative drug. Notwithstanding the difficulties outlined above, this test remains the procedure of reference for drug allergic hepatitis [59].

Conclusion
Idiosyncratic liver toxicity is the most common idiosyncratic toxicity leading to drug withdrawal from the market; yet, there are no reliable predictive experimental models to anticipate this type of adverse reaction in humans. Our understanding of the mechanisms of drug antigen generation, presentation and recognition in the liver has considerably increased, as well the mechanism behind the so-called tolerogenic liver effect. This considerable amount of knowledge, however, has not been properly translated into generating advanced cellular methods for accurate diagnosis of drug allergic hepatitis as well to identify drugs with potential risk, at early drug developmental stages.

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References and recommended reading
Papers of particular interest, published within the annual period of review, have been highlighted as:
• of special interest
•• of outstanding interest

Additional references related to this topic can also be found in the Current World Literature section in this issue (pp. 000–000).

2 Watkins PB, Seeff LB. Drug-induced liver injury: summary of a single topic idiosyncratic drug hepatitis. The authors make a comprehensive analysis of the outstanding review of the basic principles governing the phenomenon of drug idiosyncratic drug hepatitis. This paper reviews the location of the metabolic activation of drugs and its relevance to drug hypersensitivity, with reference to the liver.

Relevant paper illustrating the experience gained with previous compounds involving the mechanisms of organ-specific idiosyncratic drug toxicity.
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Excellent review of immunology of the liver, with a description of the different cell types and their role in surveillance and activation mechanisms.
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