

## **CONTINUING PHARMACY EDUCATION SERIES: HEPATOTOXICITY – A REVIEW**

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**Hepatotoxicity** (from hepatic toxicity) implies chemical-driven liver damage. The liver plays a central role in transforming and clearing chemicals and is susceptible to the toxicity from these agents. Certain medicinal agents when taken in overdoses and sometimes even when introduced within therapeutic ranges may injure the organ. Other chemical agents such as those used in laboratories and industries, natural chemicals (e.g. microcystins) and herbal remedies can also induce hepatotoxicity. Chemicals that cause liver injury are called hepatotoxins.

More than 900 drugs have been implicated in causing liver injury and it is the most common reason for a drug to be withdrawn from the market. Chemicals often cause subclinical injury to liver which manifests only as abnormal liver enzyme tests. Drug induced liver injury is responsible for 5% of all hospital admissions and 50% of all acute liver failures.

## **TOXICOLOGY**

### **INTRODUCTION**

Toxicology is a young science that developed only during the last 40 years as concern for consumer and worker health and for the environment increased. Currently, toxicology encompasses mainly activities to determine the potential for adverse effects from chemicals (both natural and synthetic), with the objective of assessing hazard and risk to humans and animals. The following two key issues are embedded in the term toxicology: First, a close structural or functional contact of chemicals, or their conversion products, to tissues or organs and second a quantitative relationship between the triggering agent and the effect. Such a dose-response relationship is important for a causality assessment and to predict the dimension of risk and hazard.

The term "toxicity" is used to describe the nature of adverse effects produced and the conditions required for their induction; i.e. toxicity is the potential for a material to cause harm in biological systems<sup>3</sup>. For pharmacologically active and therapeutic agents undesired effects are described by different terms: side-effects, overdosage, underdosage, loss of effect, intolerance, idiosyncrasy, secondary effects, and adverse drug interactions. The nature and magnitude of a toxic effect of a compound depend on several factors like its physicochemical properties, its pharmacokinetic behavior, the conditions of exposure and the presence of bioprotective mechanisms.

Examples for the expression of a toxic response are inflammation, necrosis, apoptosis, enzyme inhibition, biochemical uncoupling, lethal synthesis, lipid peroxidation, covalent binding, receptor interaction, or immunosuppression.

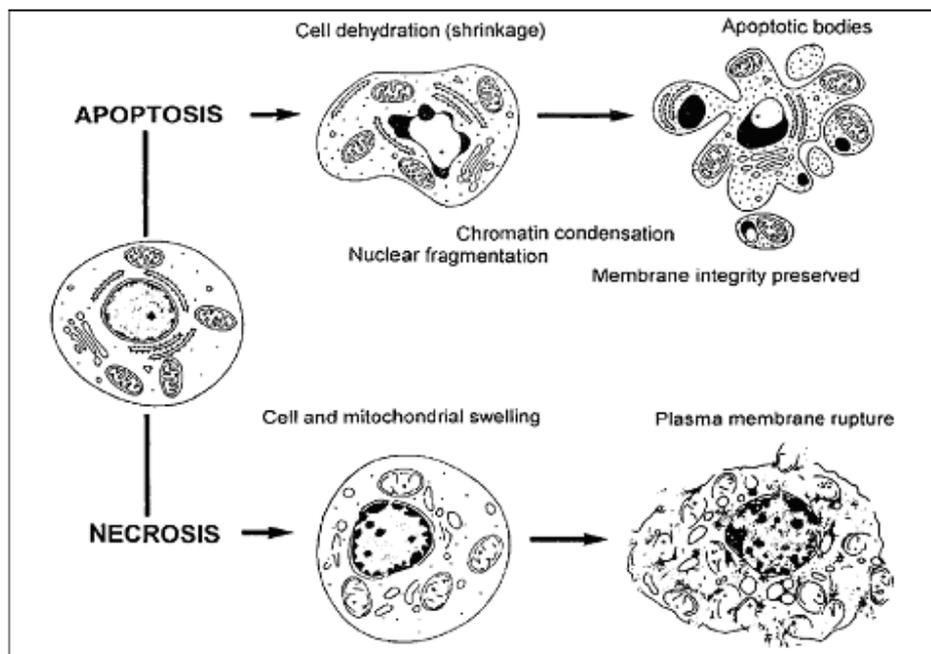
## MECHANISMS OF TOXICITY

### APOPTOSIS AND NECROSIS

In physiological conditions, cell death is mediated by a process called *apoptosis* and is a strictly regulated course of events removing superfluous, aged, or damaged cells. Apoptosis is derived from the ancient Greek and means "the falling of petals from a flower" or "of leaves from a tree in the autumn". However, cells can not only die from apoptosis, but also from another kind of cell death termed necrosis. *Necrosis* is always induced after exposure to (high doses of) external factors or drastic changes in the physiological environment, such as heat, anoxia, loss or increase of ions, ethanol, toxic substances and usually is a pathogenic process (1).

The image of apoptosis is characterized by the shrinkage of the cell and the cell nucleus, condensation of chromatin, DNA fragmentation, and blabbing of the cell membrane with the constriction of apoptotic bodies without loosing the structural integrity and most of the plasma membrane function. Whereas necrosis is represented by swelling of the cell and the cytoplasmic organelles and disruption of the cell membrane leading to inflammation and thus causing further tissue damage (1) (**Figure:1**). In apoptosis, inflammation is usually prevented by the engulfment and lysis of apoptotic bodies by phagocytes, which in addition results in the release of anti-inflammatory cytokines and immune tolerance. Also scar formation usually does not take place. However, if apoptosis is massive or phagocytic cells are lacking, apoptosis can eventually turn into necrosis. Apoptosis can be triggered mainly by two pathways: the intrinsic and the extrinsic pathway<sup>3</sup>. The intrinsic pathway is starting from the mitochondria as consequence of extra- or intracellular stress, the other is triggered by an extracellular ligand activating a death receptor in the cell membrane (**Figure:2**). In both pathways so-called caspases are usually involved. Caspases are cysteine containing aspartic acid-specific proteases and can be divided into initiator caspases (caspases 8, 9 and 10) and downstream or executioner caspases (caspases 3, 6, 7). Upon activation of a death receptor (Fas-, TRAIL- or TNF-receptor) recruiting of adaptor molecules (such as FADD: Fas associated death domain proteins) and

procaspases occurs, forming an "apoptosome", a so-called DISC (death-inducing signaling complex).

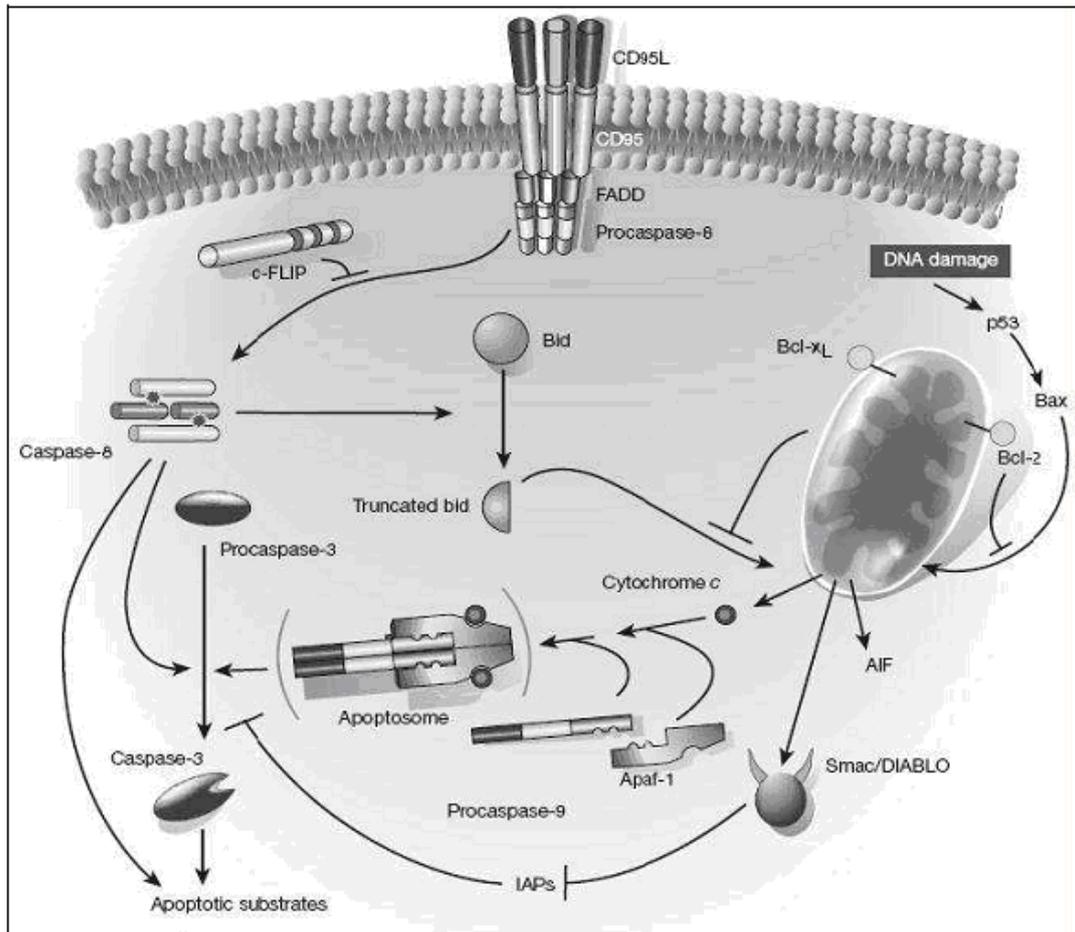


**Fig. 1 Morphological and Biochemical changes during apoptosis and necrosis**

In this complex procaspases are transactivated and subsequently cleaved, resulting in active caspases, which activate downstream caspases. These effector caspases then cleave cellular substrates leading to the phenomena of the apoptotic picture. The intrinsic pathway is initiated by some kind of stress, like oxidative stress or treatment with cytotoxic drugs, and starts with the release of cytochrome  $c^4$  from the mitochondrial intermembrane space into the cytosol. Cytochrome  $c$  aggregates with apoptotic protease activating factor 1 (Apaf-1), procaspase-9 and ATP to form an apoptosome. Upon procaspase-9 activation an effector caspase, e.g. caspase-3, is activated. The latter can trigger the evolvement of the apoptotic morphology, like in the extrinsic pathway. Apoptosis, and especially caspase activation, is an energy-requiring process. In case ATP is decreased apoptosis is blocked and turns into necrosis (2).

In reality, the whole situation is much more complex. The two pathways are not only connected by the convergence at the level of the executioner caspases but can also be at an earlier stage. The activated initiator caspase-8 not only cleaves and activates caspase-3 but also Bid, a proapoptotic member of the Bcl-2 family. Bid acts on mitochondria to trigger the release of cytochrome  $c$  with the above described consequences (2) (*Figure:2*).

The whole course of events can be expressed as a three-step model of apoptosis: a premitochondrial phase during which signal transduction cascades and damage pathways are activated; a mitochondrial phase during which mitochondrial membrane function is lost; and a post-mitochondrial phase during which proteins released from mitochondria elicit the activation of catabolic proteases and nucleases.



**Fig 2. Molecular death-receptor pathway of apoptosis**

The death-receptor pathway (left pathway) is triggered by members of the death-receptor super family (such as CD95 (= Fas) and tumor necrosis factor receptor I). The mitochondrial pathway (right) is used extensively in response to extracellular cues and internal insults such as DNA damage. These diverse response pathways converge on mitochondria, often through the activation of a pro-apoptotic member of the Bcl-2 family. In addition, pro- and anti-apoptotic Bcl-2 family members meet at the surface of mitochondria, where they compete to regulate

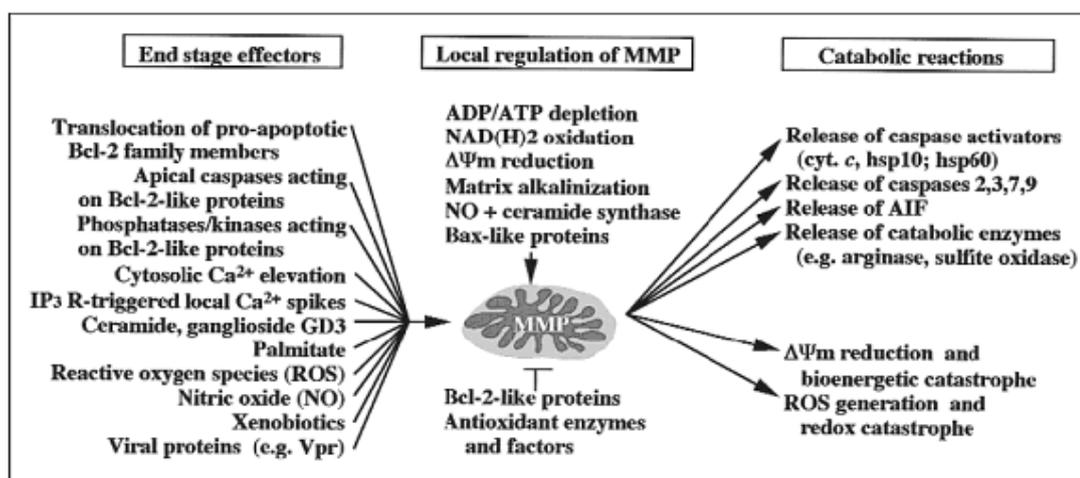
cytochrome *c* exit. If the pro-apoptotic camp wins, an array of molecules is released from the mitochondrial compartment. The death-receptor and mitochondrial pathways also converge at the level of caspase-3 activation. Caspase-3 activation and activity is antagonized by the IAP proteins, which themselves are antagonized by the Smac/DIABLO protein released from mitochondria (2).

### **ROLE OF MITOCHONDRIA**

Mitochondria are key players in the functioning of a cell as their primary role is providing cells with energy in form of ATP, which is produced by the coupling of the ATP synthase with the electron transport chain. Mitochondria consist of two compartments, the matrix, circumvented by the inner membrane, and the intermembrane space, which is delineated by the outer membrane. The inner membrane is folded forming so-called cristae, which clearly enlarge the surface, and is hardly permeable under physiological conditions. This tightness ensures the maintenance of the electrochemical gradient, comprising a membrane potential (negative inside) and a pH gradient (basic inside), which is the basis of the coupling of the respiratory chain to oxidative phosphorylation. The protein complexes of the respiratory chain are embedded in the inner membrane. The outer membrane is much more permeable for low-molecular-weight solutes. Mitochondria consume more than 90 % of the cells oxygen and supply most of our ATP.

A drawback of the production of ATP is the generation of reactive oxygen species (ROS) by the respiratory chain, which is especially pronounced at complexes I and III. Mitochondria are the major source of ROS and as mentioned above play also a pivotal role in apoptosis. They are the central element in the intrinsic apoptosis pathway. Upon the impact of a trigger they release several molecules from the intermembrane space, which initiate the apoptotic answer including Apaf-1, cytochrome *c*, apoptosis inducing factor (AIF). Concomitantly, an opening of the mitochondrial permeability transition (MPT) pore and a dissipation of the mitochondrial membrane potential occurs, both of them early signs of cell death. Mitochondrial apoptogenic factors can be divided into two groups: the caspasedependent substances and the caspase-independent factors (e.g. AIF, high temperature requirement protein A2 (HtrA2/OMI) and endonuclease G).

Induction of the MPT is sufficient to trigger apoptosis or necrosis and the pharmacological inhibition of MPT prevents cell death (3). As a consequence of MPT pore opening, solutes  $< 1500$  Da diffuse across the inner mitochondrial membrane, causing mitochondrial depolarization, uncoupling of oxidative phosphorylation, and large amplitude swelling, which in turn can lead to depletion of ATP and cell death<sup>5</sup>. MPT can be favored by high calcium concentration in the mitochondrial matrix, NAD(P)H oxidation and mitochondrial generation of ROS, whereas magnesium, low pH and cyclosporine A inhibit MPT. (*Figure:3*)

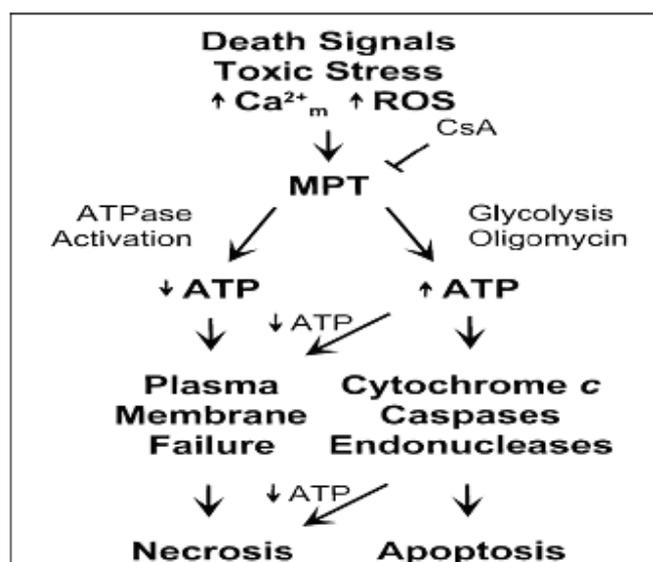


*Fig. 3 Mitochondrial involvement in cell death*

With focus on events proximal to the regulation of mitochondrial membrane permeabilization (MMP = MPT). Mitochondrial membrane effectors may cause MMP, depending on local regulators which sensitize mitochondria to MMP or inhibit MMP. Permeabilization of the outer and/or inner mitochondrial membranes then triggers a series of catabolic reactions that entail cell death, either by apoptosis or by necrosis.

Massive induction of MPT leads, via depletion of ATP, to necrosis, whereas a more subtle, regulated induction of MPT gives time for the activation and action of proteases ending in apoptosis. This could explain why some substances induce apoptosis as well as necrosis, depending on the applied concentration. In this context, a new term can be introduced: necrapoptosis. Necrapoptosis describes death processes which begin with a common stress or death stimulus, share the same pathways, but finally lead to either necrosis or apoptosis, depending on modifying factors like ATP (4) (*Figure:4*).

Once cytochrome *c* is released and mitochondrial membrane potential is disrupted, the cell is committed to die either by apoptosis – through Apaf-1 mediated activation of caspases – or by necrosis – as a result of the collapse of the respiratory function due to increased ROS and insufficient supply of ATP (4). When cells are exposed to environmental stress cell cycle is arrested at G0 or G1 phase and mitochondria are motivated to produce more ATP in order to provide more energy for repairing the damage sustained.

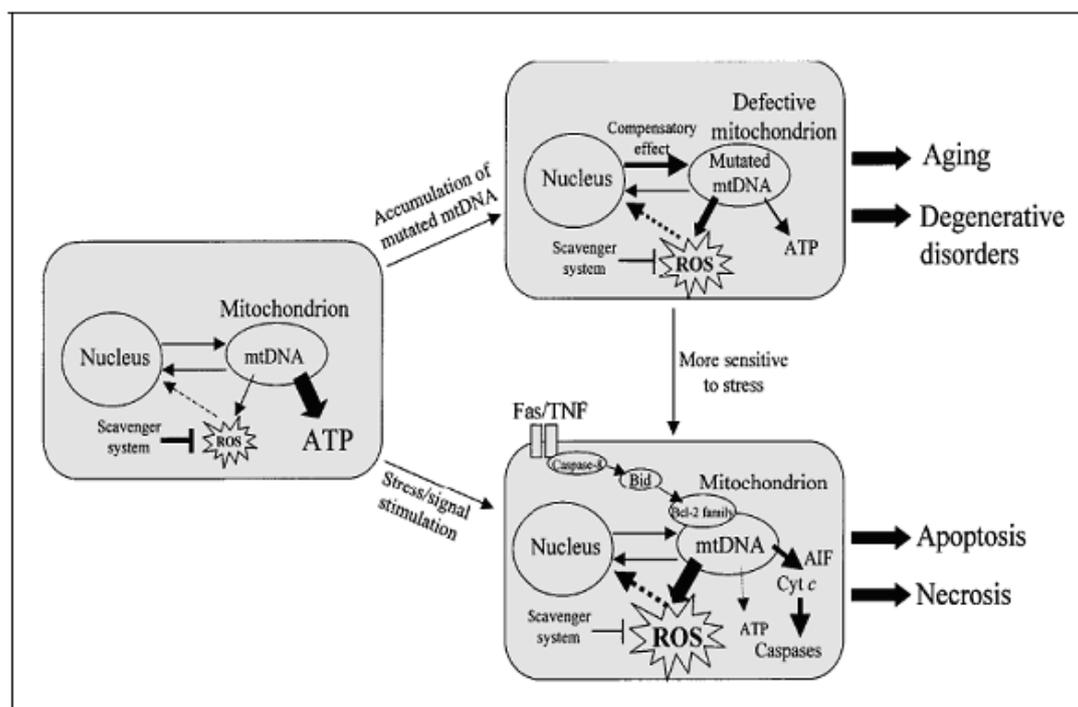


**Fig. 4** Role of ATP in necroptosis

(Scheme showing the role of ATP in necroptosis mediated by the mitochondrial permeability transition. When the MPT occurs abruptly, activation of mitochondrial ATPases causes ATP depletion, which leads to plasma membrane rupture and necrotic cell death. In case MPT progresses relatively slowly ATP levels remain relatively preserved even after onset of the MPT. Under such conditions, cytochrome *c* release activates a cascade of caspases, endonucleases, and other degradative enzymes, causing apoptotic rather than necrotic cell death. At any time, ATP depletion can supervene to cause secondary necrosis (4).

The mtDNA is more susceptible to mutations than nuclear DNA, e.g. caused by ROS, as it is a naked compact DNA molecule without protective histones, is rapidly and frequently replicated without proofreading or efficient DNA repair systems and is located near the major ROS source (7). This leads to an increased percentage of damaged mtDNA (somatic mutations) with increasing age, possibly associated with decreased mitochondrial function and enhanced

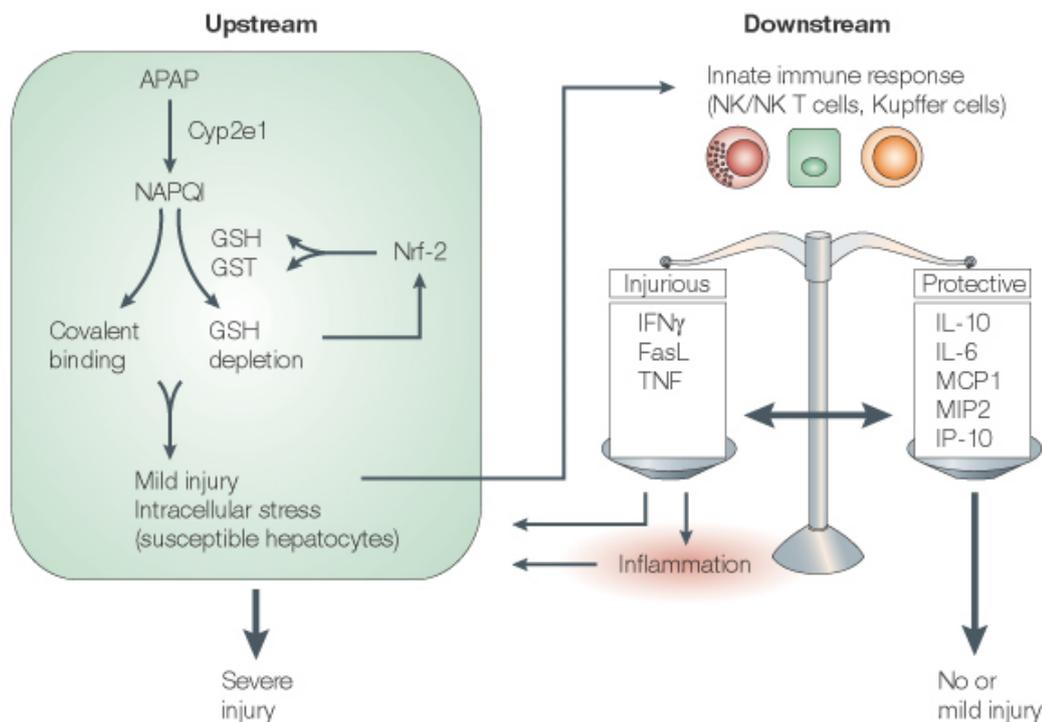
ROS production (*Figure:5*). The resultant age-related decline in oxidative phosphorylation would lead to a reduction of bioenergetic capacity until a certain threshold is undershot and symptoms or senescence occur.



**Fig. 5 Mitochondrial role in determination of cell life and death**

Under normal physiological conditions, mitochondria are the major source of bioenergy and ROS. To cope with the ROS, human cells have developed an efficient scavenger system, which includes antioxidant enzymes and small molecular-weight antioxidants (left panel). Although this system can dispose of ROS and free radicals, a small proportion of them may escape these defense mechanisms and cause damage to cellular constituents including DNA, RNA, proteins, and lipids. The ratio of damaged or mutated mtDNA to wild-type mtDNA can result in bioenergetics and/or redox alteration of tissue cells. Therefore, in a synergistic manner, all mutations and oxidative damage to mtDNA cause a deleterious effect on the respiratory function of mitochondria and lead the affected individual to aging and degenerative disorders (right upper panel). On the other hand, mitochondria can sense and respond to extracellular and intracellular signals and stresses. Once beyond a threshold of stress or challenge (e.g., ROS-

elicited oxidative stress), mitochondria may drive the cell into an irreversible cell death process (6) (right lower panel).



Upstream events in hepatocytes lead to exposure to NAPQI which undergoes covalent binding after preferential depletion of glutathione (GSH). A protective response mediated by the transcription factor NRF2 modulates the toxic threshold. Upstream events promote intracellular stress and mild injury activates the downstream innate immune system, which represents a balance of pro- and anti-inflammatory responses, the interplay of which determines progression to severe injury or no injury. APAP, acetaminophen; FasL, Fas ligand; GSH, glutathione; GST, GSH S-transferase; IFN, interferon; MCP1, monocyte chemoattractant protein 1; MIP2, macrophage inflammatory protein 2; NK, natural killer; TNF, tumour-necrosis factor.

## ROLE OF REACTIVE OXYGEN SPECIES

Another important factor influencing cell functioning is the occurrence of reactive oxygen species. Cells are constantly generating ROS during aerobic metabolism. ROS is an umbrella term for several reactive molecules containing oxygen, including O $_2^{\cdot-}$  (superoxide anion),  $\cdot$ OH (hydroxyl radical), and H $_2$ O $_2$  (hydrogen peroxide). They origin mainly from the

mitochondrial respiratory chain, where about 1 – 5 % of the electrons lose their way and directly interact with oxygen to form  $O_2^{\cdot}$ . Other sources are the microsomal electron transport chain or oxidative enzyme systems like xanthine oxidase, cyclooxygenase, lipoxygenase, cytochrome P450 and NADPH oxidase.

Most of the produced ROS are caught by the endogenous antioxidant system. This system consists of enzymes like catalase, superoxide dismutase, glutathione peroxidase, and the molecule glutathione. Glutathione is a tripeptide (g-Glu-Cys-Gly) occurring in two forms in the cell: reduced (GSH) and oxidized (GSSG) glutathione, with GSH being the predominant form. Depletion of GSH is sufficient to trigger apoptosis in some cell types and is able to make cells more susceptible to other apoptosis inducing stimuli (7).

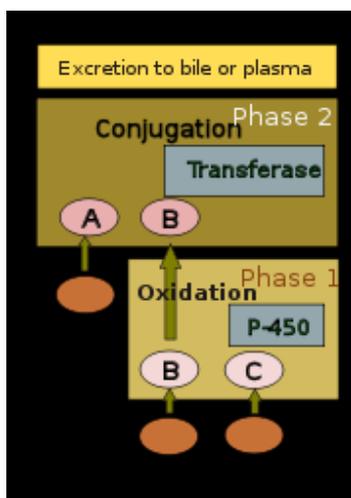
Oxidative stress is induced if the natural antioxidant defense cannot cope anymore with the incidental ROS. The effects that ROS may have on the cell are manifold. First of all, ROS are able to oxidize DNA with subsequent wrong or hampered synthesis of proteins leading to malfunctioning and structural changes of these proteins. Moreover, they may also peroxidize membrane lipids causing dysfunction of the cell membrane. By modifying the DNA bases they are able to impair the DNA repairing mechanisms and through hydrolysis they lead to malondialdehyde production from deoxyribose (8).

## **DRUG METABOLISM IN LIVER**

The human body identifies almost all drugs as foreign substances (i.e. xenobiotics) and subjects them to various chemical processes (i.e. metabolism) to make them suitable for elimination. This involves chemical transformations to (a) reduce fat solubility and (b) to change biological activity. Although almost all tissue in the body have some ability to metabolize chemicals, smooth endoplasmic reticulum in liver is the principal "metabolic clearing house" for both endogenous chemicals (e.g., cholesterol, steroid hormones, fatty acids, and proteins), and exogenous substances (e.g. drugs). The central role played by liver in the clearance and transformation of chemicals also makes it susceptible to drug induced injury.

Drug metabolism is usually divided into two phases: phase 1 and phase 2. Phase 1 reaction is thought to prepare a drug for phase 2. However many compounds can be metabolised by phase 2 directly. Phase 1 reaction involves oxidation, reduction, hydrolysis, hydration and many other rare chemical reactions. These processes tend to increase water solubility of the drug

and can generate metabolites which are more chemically active and potentially toxic. Most of phase 2 reactions take place in cytosol and involve conjugation with endogenous compounds via transferase enzymes. Chemically active phase 1 products are rendered relatively inert and suitable for elimination by this step.



Drug metabolism in liver: transferases are: glutathione, sulfate, acetate, glucuronic acid. P-450 is cytochrome P-450 enzymes. 3 different pathways are depicted for Drugs A, B and C.

A group of enzymes located in the endoplasmic reticulum, known as cytochrome P-450, is the most important family of metabolizing enzymes in the liver. Cytochrome P-450 is the terminal oxidase component of an electron transport chain. It is not a single enzyme, but rather consists of a family of closely related 50 isoforms; six of them metabolize 90% of drugs. There is a tremendous diversity of individual P-450 gene products and this heterogeneity allows the liver to perform oxidation on a vast array of chemicals (including almost all drugs) in phase 1. Three important characteristics of the P450 system have roles in drug induced toxicity:

### 1. Genetic diversity:

Each of the P-450 proteins is unique and accounts to some extent for the variation in drug metabolism between individuals. Genetic variations (polymorphism) in CYP450 metabolism should be considered when patients exhibit unusual sensitivity or resistance to drug effects at normal doses. Such polymorphism is also responsible for variable drug response among patients of differing ethnic backgrounds.

**2. Change in enzyme activity:**

Many substances can influence P-450 enzyme mechanism. Drugs interact with the enzyme family in several ways. Drugs that modify Cytochrome P-450 enzyme are referred to as either inhibitors or inducers. Enzyme inhibitors block the metabolic activity of one or several P-450 enzymes. This effect usually occurs immediately. On the other hand inducers increase P-450 activity by increasing its synthesis. Depending on inducing drug's half life, there is usually a delay before enzyme activity increases.

**3. Competitive inhibition:**

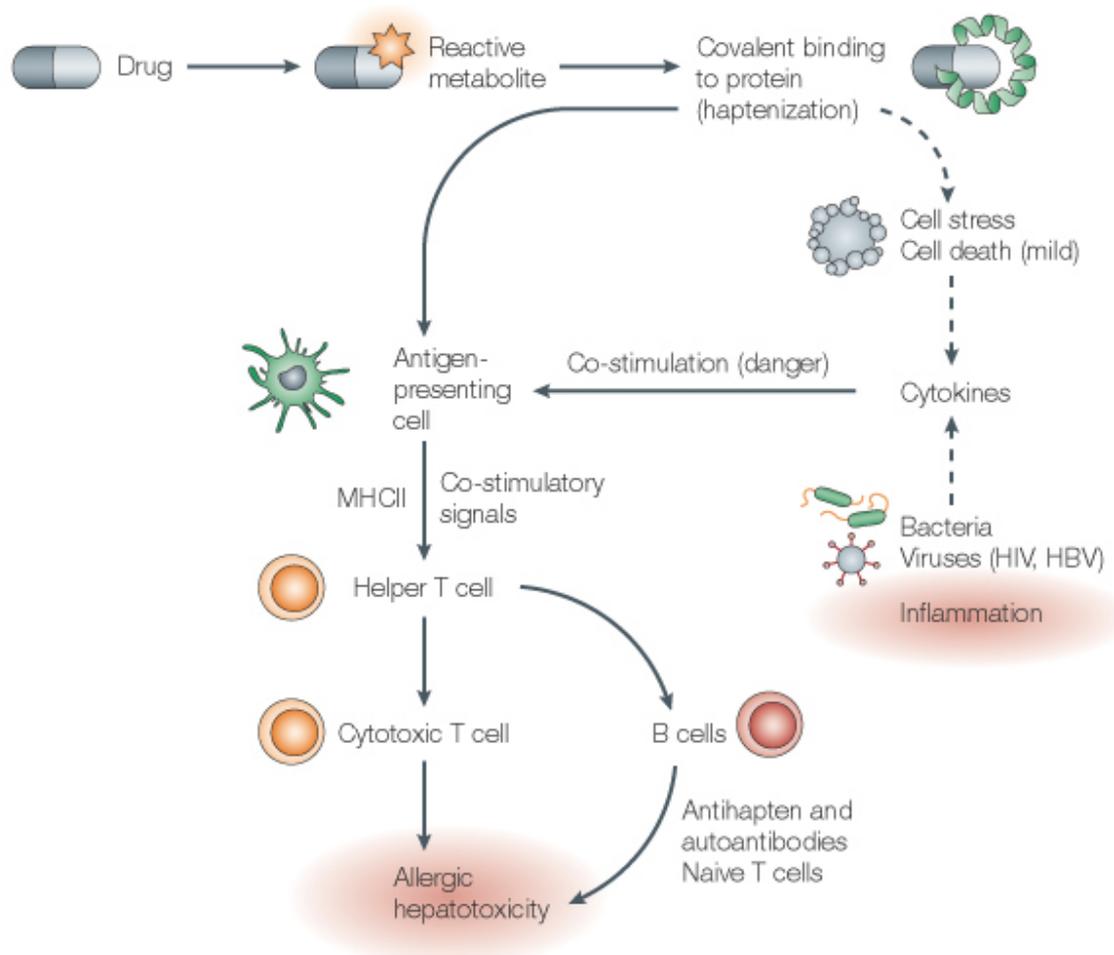
Some drugs may share the same P-450 specificity and thus competitively block their bio transformation. This may lead to accumulation of drugs metabolised by the enzyme. This type of drug interaction may also reduce the rate of generation of toxic substrate.

**DRUG - INDUCED HEPATOTOXICITY****INTRODUCTION**

Drug-induced hepatotoxicity (DIH) is a major health problem that challenges not only health care professionals but also the pharmaceutical industry and drug regulatory agencies. According to the United States Acute Liver Failure Study Group, DIH accounts for more than 50% of acute liver failure, and idiosyncratic liver injury triggered by other drugs (13%) (9). Because of the significant patient morbidity and mortality associated with DIH, the U.S. Food and Drug Administration (FDA) has removed several drugs from the market, including bromfenac, ebrotidine, and troglitazone. Other hepatotoxic drugs, such as risperidone, trovafloxacin, and nefazodone, have been assigned “black box” warnings. DIH is the most common cause for the withdrawal of drugs from the pharmaceutical market (10).

The clinical picture of liver toxicity corresponds to the common liver diseases and can vary from reversible, mild liver enzyme elevation to fatal liver failure, including necrosis, hepatitis, cholestasis, steatosis, veno-occlusive disease, cholangitis, cirrhosis, hepatocellular carcinoma, with acute hepatitis being the most frequent presentation (90 % of cases). Several risk factors are known for the development of hepatotoxicity of drugs, like gender, age, lack of food, adipositas, diabetes mellitus, liver diseases, renal diseases, heart diseases, hyperthyrosis,

psoriasis, rheumatic diseases, AIDS, pregnancy, long-term therapies, dosage, polymedication, alcohol abuse, genetic factors, and others.



Hapten formation leading to major histocompatibility complex class II (MHCII) presentation of haptened peptide by antigen-presenting cells (APCs) along with co-stimulation of APC signalling molecules by mild injury, inflammation or infection promotes helper T-cell activation leading to T-cell responses to the antigen. The cytotoxic T cells are then targeted against hepatocytes that express haptened protein or MHCII presentation of haptened peptides on the cell surface. Antibody to haptened protein or concomitant autoantibodies could theoretically mediate and promote antibody-dependent cell-mediated hepatotoxicity.

Causes for the development of hepatotoxicity of metabolic-toxic origin consist of the following mechanisms: metabolite-mediated toxicity and endothelial lesions, inhibition of biliary secretion, decreased secretion of lipoproteins, inhibition of fatty acid mitochondrial  $\beta$ -oxidation,

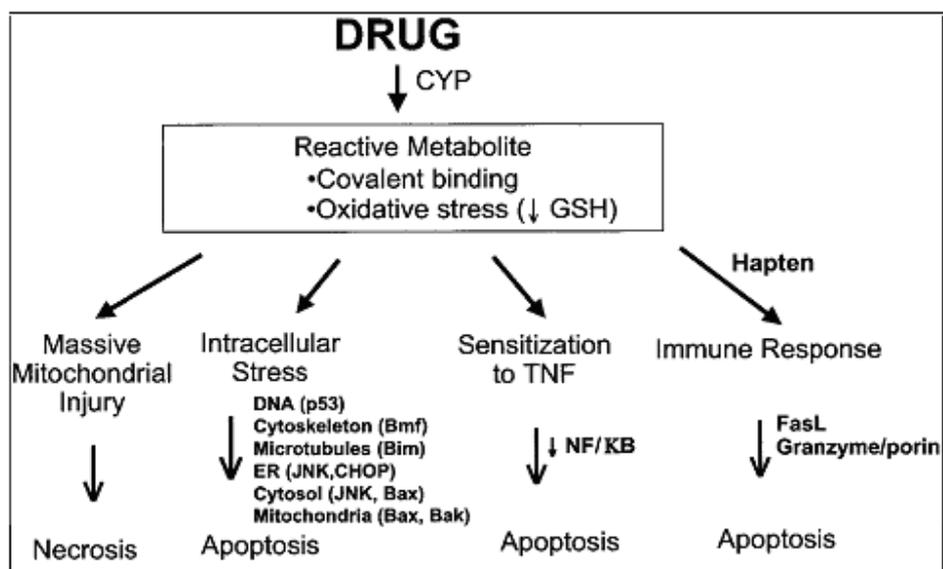
or activation of Ito cells. Activation of Kupffer cells leads to the release of cytotoxic mediators, such as reactive oxygen species, and proinflammatory mediators, such as cytokines and chemokines.

## **MECHANISMS OF DRUG-INDUCED HEPATOTOXICITY**

Although the exact mechanism of DIH remains largely unknown, it appears to involve 2 pathways — direct hepatotoxicity and adverse immune reactions. The pathogenesis of drug-induced liver injury usually involves the participation of a toxic drug or metabolite that either elicits an immune response or directly affects the biochemistry of the cell. In either case, the resultant cell death is the event that leads to the clinical manifestation of hepatitis (11). Metabolism of chemicals takes place largely in the liver, which accounts for the organ's susceptibility to metabolism-dependent, drug induced injury (12). The drug metabolites can be electrophilic chemicals or free radicals that undergo or promote a variety of chemical reactions, such as the depletion of reduced glutathione; covalently binding to proteins, lipids, or nucleic acids; or inducing lipid peroxidation (*figure:6*). All of these have consequent direct effects on organelles such as mitochondria, the endoplasmic reticulum, the cytoskeleton, microtubules, or the nucleus. They may also indirectly influence cellular organelles through the activation and inhibition of signaling kinases, transcription factors, and gene-expression profiles. The resultant intracellular stress leads to cell death caused by either cell shrinkage and nuclear disassembly (apoptosis) or swelling and lysis (necrosis) (13). Hepatocyte death is the main event that leads to liver injury, although sinusoidal endothelial cells (14) or bile duct epithelium (15) may also be target.

Sensitization to liver-specific cytokines can also occur, thereby causing cytokine-induced hepatotoxicity. Alternatively, the reactive metabolite may covalently bind to or alter liver proteins, such as cytochrome P450 enzymes, leading to an immune response and to immune-mediated injury (16). This immune mediated, drug-induced hepatitis is usually characterized by fever, eosinophilia, or other allergic reactions that distinguish it from non-immune-mediated drug-induced hepatitis (17). The mechanism for the induction of the immune-mediated drug reaction is not clear, but it may involve a hapten-like action (18). Generally, low-molecular-weight organic chemicals or drugs are not immunogenic, but they may become so when they are bound to a macromolecule, such as a protein. If a drug metabolite produced by cytochrome P450

is able to act as a hapten, it would covalently bind to a liver protein and, subsequently, alter that protein (19). This altered protein would then be perceived as foreign by the immune system, resulting in an autoimmune attack on normal hepatocellular constituents.



**Fig. 6 Cellular mechanism of drug Hepatotoxicity**

*Bmf, Bim, Bax, and Bak are proapoptotic members of the B cell lymphoma-2 protein family; CHOP, c/EBP homologous protein-1 $\theta$ ; GSH, glutathione; JNK, c-jun-N-terminal kinase; f, inhibition.*

This hypothesis, however, does not explain many aspects of immune-mediated drug-induced hepatitis. For instance, covalent binding (haptentation) is a regular occurrence with drugs, such as halothane, that rarely cause immune-mediated toxicity (20). It is possible that a reactive metabolite may also have to injure or stress liver cells, in addition to modifying a protein, to induce an immune response (21).

Certain drugs exclusively or predominantly induce cholestasis. Several of these, such as sulindac and chlorpromazine, are associated with hypersensitivity-type reactions. The specific immunological targets of these hypersensitivity-type adverse reactions are poorly understood. However, given that the predominant histological features are portal inflammation and biliary injury, they are likely to be related to the bile duct. It is possible that toxic metabolites undergoing canalicular excretion react with macromolecules in the duct cells or undergo further metabolism within these cells, resulting in ductal injury. Drug-induced immune-mediated injury, therefore, is an adverse immune response against the liver and/or bile duct that results in a

disease with clinical features that are hepatic, cholestatic, or a mixture, the mechanisms of which are not clearly understood.

## **DRUG-INDUCED DIRECT HEPATOTOXICITY**

Direct hepatotoxicity is often caused by the direct action of a drug, or more often a reactive metabolite of a drug, against hepatocytes. One classically studied drug used to examine the mechanisms of hepatotoxicity is APAP. APAP is a popular over-the-counter analgesic that is safe at therapeutic doses but at overdose can produce centrilobular hepatic necrosis, which may lead to acute liver failure. APAP is metabolized to a minor electrophilic metabolite, N-acetyl-p-benzoquinoneimine (NAPQI) (22), which during APAP overdose depletes glutathione and initiates covalent binding to cellular proteins. These events lead to the disruption of calcium homeostasis, mitochondrial dysfunction, and oxidative stress (23, 24) and may eventually culminate in cellular damage and death. In most instances of DIH, it appears that hepatocyte damage triggers the activation of other cells, which can initiate an inflammatory reaction and/or an adaptive immune response. These secondary events may overwhelm the capacity of the liver for adaptive repair and regeneration, thereby contributing to the pathogenesis of liver injury.

## **DRUG-INDUCED IMMUNE-MEDIATED HEPATOTOXICITY**

### **INNATE AND ADAPTIVE IMMUNE SYSTEM OF THE LIVER**

The innate immune system provides a first line of defense against microbial infection, but it is not sufficient in eliminating infectious organisms. The lymphocytes (T and B cells) of the adaptive immune system provide a more versatile means of defense and possess “memory” which is the ability to respond more vigorously to repeated exposure to the same microbe. Moreover, cells of the innate immune system play an integral role in the initiation of adaptive immunity by presenting antigens and are crucial in determining the subsequent T-cell- or antibody-mediated immune response. Because of the liver continuous exposure to pathogens, toxins, tumor cells, and harmless dietary antigens, it possesses a range of local immune mechanisms to cope with these challenges. The liver contains large numbers of both innate and adaptive immune cells, including the largest populations of tissue macrophages (KC), NK cells, and NKT cells (26). The liver also possesses a unique combination of intrahepatic lymphocytes, which include not only the conventional CD4<sup>+</sup> and CD8<sup>+</sup> T cells but also high percentages of  $\gamma\delta$

T cells and CD4 – CD8 – T cells. Collectively, the innate and adaptive immune cells contribute to the unique immune responses of the liver, including removal of pathogenic microorganisms, clearance of particles and soluble molecules from circulation, deletion of activated T cells, and induction of tolerance to food antigens derived from the gastrointestinal tract.

KC play an essential role in the phagocytosis and removal of pathogens entering the liver via portal-venous blood. Upon activation, KC produces various cytokines and other mediators, including prostanoids, nitric oxide, and reactive oxygen intermediates. These KC products play prominent roles in promoting and regulating hepatic inflammation, as well as modulating the phenotype of other cells in the liver, such as NK and NKT cells (25, 26). Furthermore, KC represents a major population of antigen-presenting cells (APCs) within the liver. It has been demonstrated that KC can activate T cells in vitro, but they do so less efficiently than peritoneal-exudates macrophages (27). One characterized function of hepatic NK and NKT cells is their cytotoxic capacity against other cells. It has been demonstrated that freshly isolated liver NK cells spontaneously induce the cytotoxicity of various cell lines, whereas NKT cells are cytotoxic in the presence of IL-2 (28). This cytotoxicity is further enhanced by IL-12 and IL-18, which are produced by activated KC. Another function ascribed to NK and NKT cells is their ability to produce high levels of T helper (Th) 1 and Th2 cytokines upon stimulation (29).

NK cells have been shown to represent a major source of IFN- $\gamma$  in many types of liver disease. 30 NKT cells produce either IFN- $\gamma$  or IL-4, or in some cases both cytokines, depending on the differentiation state of the cells and the stimuli (31). It has also been demonstrated that IL-4 produced by NKT cells may be associated with the initiation and regulation of Th2 responses (32).

It has also been reported that liver sinusoidal endothelial cells (LSEC) are capable of selectively suppressing IFN- $\gamma$  producing Th1 cells while concurrently promoting the outgrowth of IL-4-expressing Th2 cells (32). Active suppression of T-cell activation resulting in liver-induced tolerance is also likely to occur within the liver because of its unique anatomy and composition of “tolerogenic” APCs. Within the liver, blood flow slows down through the narrow sinusoids (7-12 $\mu$ m) and is temporarily obstructed by KC, which reside in the sinusoidal lumen. Because of this reduction in blood flow, circulating T cells can interact with LSEC and KC. Consequently, native T cells, which would normally encounter APCs in lymphoid tissues, could

be primed directly by LSEC and/or KC within the liver. Current evidence suggests that LSEC and KC as well as hepatic dendritic cells are important in the induction of tolerance, rather than the activation of T-cell responses. It has been further demonstrated that although LSEC are capable of presenting antigen to T cells, LSEC-activated CD4 + or CD8 + T cells fail to differentiate into Th1 cells or cytotoxic effector cells, respectively (33, 34).

### **ROLE OF INNATE IMMUNITY IN DIH**

Drug-induced stress and/or damage of hepatocytes may trigger activation and inflammatory responses of the innate immune system within the liver. Activated cells of the innate immune system produce a range of inflammatory mediators, including cytokines, chemokines, and reactive oxygen and nitrogen species that contribute to the progression of liver injury. Some of these mediators, such as IFN  $\gamma$ , Fas, or Fas ligand, are directly involved in causing liver damage. On the other hand, the innate immune cells also represent a major source of hepatoprotective factors, as it has been demonstrated that transgenic mice deficient in IL-10, IL-6, or COX-2 are more susceptible to APAP-induced liver injury.

The innate immune cells reported to participate hepatotoxicity include NK and NKT cells, macrophages, and neutrophils. A recent study demonstrated that depletion of NK and NKT cells protected mice from APAP-induced liver injury (35). This protective mechanism seems to involve eliminating the production of IFN- $\gamma$  and various other proinflammatory chemokines as well as decreasing neutrophil accumulation within the liver. APAP hepatotoxicity has also been attributed in part to the activation of KC secondary to hepatocyte damage (36, 37). KC activation results in the release of a wide range of proinflammatory mediators, such as TNF- $\gamma$ , which may directly induce tissue damage, and IL-12 and IL-18, which are important activators of NK and NKT cells. However, other studies suggest that KC may play a protective role in addition to their protoxicant effect, as KC are the predominant source of IL-10 and IL-6, which are important in counteracting inflammatory responses and/or stimulating liver regeneration (38).

### **ROLE OF ADAPTIVE IMMUNE RESPONSE IN DIH**

The clinical features of some cases of DIH strongly suggest an involvement of the adaptive immune system. These clinical characteristics include (1) concurrence of rash, fever, and eosinophilia; (2) delay of the initial reaction (1-8 weeks) or requirement of repeated exposure to the culprit drug; (3) rapid recurrence of toxicity on reexposure to the drug; and (4)

presence of antibodies specific for native or drug-modified hepatic proteins. Drugs suspected to induce these types of reactions include halothane, tienilic acid, dihydralazine, diclofenac, phenytoin, and carbamazepine (19).

Our current understanding of drug-induced adaptive immune responses is largely based on the hapten hypothesis and the p-i concept. The hapten hypothesis proposes those drugs, or more often reactive metabolites of the drugs, act as haptens and covalently bind to endogenous proteins to form immunogenic drug-protein adducts. These immunogenic adducts elicit either antibody or cytotoxic T-cell responses (17). The hapten hypothesis is supported by the detection of antibodies that recognize drug-modified hepatic proteins in the sera of DIH patients. The p-i concept suggests that certain drugs can bind to T-cell receptors, mimicking a ligand and its receptor interaction, and cause T-cell activation in an MHC-dependent fashion. Despite the detection of drug-specific antibodies and T cells, it has been difficult to directly prove the pathogenic role of the adaptive immune system in DIH, in part because of the lack of animal models. An important reason for the difficulty in developing animal models is that the default response of the liver to antigens is immunological tolerance. This tolerogenic response could also explain the low occurrence of this type of DIH in humans. As described in the above section, the anatomy, cellular composition, and microenvironment of the liver favor tolerance rather than pathogenic immunity. Therefore, DIH mediated by adaptive immune reactions against a drug can occur only when the tolerance mechanism is deficient or abrogated in susceptible individuals (17).

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