

ROLE AND ACTIVATION OF KUPFFER CELLS IN LIVER DISEASE

Rola i aktywacja komórek Kupffera w chorobach wątroby

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SUMMARY

The study of immune-mediated drug-related hepatotoxicity (IDR) is important for medical and pharmaceutical practice. IDR is unrelated to drug action, does not occur in all patients, and is not dose dependent. The mechanism of this phenomenon is not fully understood, but theories suggest a role for Kupffer cells as antigen presenting cells (APCs) in the IDR process.

As APCs, Kupffer cells may influence T-cell tolerance in response to the drug-protein combination. There is evidence that insufficient synthesis of costimulatory molecules by Kupffer cells can lead to induction of T-cell tolerance and avoidance of IDR responses. However, overexpression of costimulatory molecules or disruption of the mechanism of danger signals that activate Kupffer cells can lead to full activation of T cells and induction of IDR.

Another theory suggests that IDR may result from the interaction of the drug with endotoxin, leading to activation of Kupffer cells and secretion of the inflammatory factor TNF- α . This can lead to hypoxia and necrosis of liver cells. The role of Kupffer cells in IDR is an important area of research, and activation or dysfunction of these cells may have a significant impact on the occurrence of immune-mediated adverse drug reactions.

Key words: Kupffer cells, drug-related hepatotoxicity, liver disease.

STRESZCZENIE

Badania nad hepatotoksycznością leków o podłożu immunologicznym (IDR) są istotne dla praktyki lekarskiej i farmaceutycznej. IDR nie jest związany z działaniem leku, nie występuje u wszystkich pacjentów, ani nie zależy od jego dawki. Mechanizm tego zjawiska nie jest w pełni zrozumiany, ale istnieją teorie sugerujące rolę komórek Kupffera jako komórek prezentujących antygen (APC) w procesie IDR. Komórki Kupffera, jako APC, mogą wpływać na tolerancję limfocytów T w odpowiedzi na połączenie lek-białko. Istnieją dowody na to, że niedostateczna synteza cząsteczek kostymulujących przez komórki Kupffera może prowadzić do indukcji tolerancji limfocytów T i unikania reakcji IDR. Jednakże, nadmierna ekspresja cząsteczek kostymulujących lub zaburzenie mechanizmu sygnałów niebezpieczeństwa, które aktywują komórki Kupffera, mogą prowadzić do pełnej aktywacji limfocytów T i wywoływania IDR. Inna teoria sugeruje, że IDR może wynikać z interakcji leku z endotoksyną, co prowadzi do aktywacji komórek Kupffera i wydzielania czynnika zapalnego TNF- α . To z kolei może spowodować hipoksję i martwicę komórek wątroby. Rola komórek Kupffera w IDR jest istotnym obszarem badań, a ich aktywacja lub zaburzenia w funkcjonowaniu tych komórek mogą mieć istotny wpływ na występowanie niepożądanych działań leków o podłożu immunologicznym.

Słowa kluczowe: komórki Kupffera, hepatotoksyczność leków, choroby wątroby.

BACKGROUND

The parenchymal cells of the liver, also known as hepatocytes, are where most of the organ's characteristic metabolic processes take place. Hepatocytes make up 60% of all liver cells. Other types of liver cells located outside the parenchymal tubules, known as non-parenchymal liver cells, include Kupffer cells, sinusoidal endothelial cells, stellate cells and pit cells. Non-parenchymal liver cells account for approximately 35% of the total number of liver cells, occupy only about 6% of the total volume of the organ, and have functions that are fundamentally different from those of hepatocytes [1]. Kupffer cells represent 20% of non-parenchymal liver cells and 80% of tissue macrophages throughout the body. In the liver, they are found in the walls of the sinusoidal vessels, where they are in close contact with blood and migrating lymphocytes. The primary function of Kupffer cells is their ability to eliminate bacteria, endotoxins, damaged cells, immune complexes and toxic substances. As antigen-presenting cells, Kupffer cells oversee the processes of liver tumorigenesis and regeneration. They also play an essential role in innate immunity and in the body's defence through the expression and secretion of soluble mediators of the inflammatory response. Kupffer cells induce apoptosis of infiltrating CD8⁺ T cells in the liver and subsequently phagocytose them. During inflammation and subsequent liver regeneration, they secrete compounds such as nitric oxide, tumour necrosis factor TNF- α , interleukin 1 β and 6, transforming growth factor β and reactive oxygen molecules [1, 2]. The interaction between

Kupffer cells and bacterial lipopolysaccharide can initiate processes leading to liver damage (hepatotoxicity), in particular endotoxemia, alcoholic liver damage, ischaemia/ischemia damage and systemic viral infection [2].

Kupffer cells are activated directly by stimulation of Toll-like receptors. Indirectly, by initiating activation of the complement system in the portal vein or in the general circulation in the presence of high concentrations of lipopolysaccharide [2]. Kupffer cell activation involves a broad spectrum of progressively different changes in the phenotype of these cells, resulting from the interactions of different activators and complement signalling pathways [1–3]. The involvement of Kupffer cells in the pathogenesis of many diseases has made them a target for pharmaceutical research.

STRUCTURE OF KUPFFER CELLS

Kupffer cells, which are tissue macrophages of the liver, take their name from the first discoverer of these non-parenchymatous cells, the pathologist Karl Wilhelm von Kupffer [1]. The walls of the hepatic sinusoidal vessels (sinusoids) are composed of non-parenchymatous cells such as endothelial cells lacking a basement membrane, star cells (also called Ito cells) and extracellular matrix located in the Disse's space (perisinusoidal space) [2–4], and Kupffer cells adjacent to the endothelium on the luminal side of the sinusoid [5, 6]. Kupffer cells make up 80–90% of macrophages in the reticuloendothelial system. They

represent approximately 15% of the total liver cell population [1]. A specific and conserved marker for them is VSIG4, which is found only on Kupffer cells [7, 8]. Although Kupffer cells are considered to be immobile liver macrophages, they have the ability to migrate along sinusoidal walls. Kupffer cells are the first population of macrophages in the body to come into contact with bacteria, endotoxins and microbial degradation products from the gastrointestinal tract that are transported to the liver via the portal vein [1]. Together with natural killer (NK) cells, dendritic cells, soluble compounds such as complement factors and acute phase proteins, Kupffer cells are an important component of innate immunity, the first and rapid response to potentially threatening stimuli [9]. The localization of Kupffer cells in the liver of vertebrates and the fact that they make up a significant portion of the body's macrophages suggests a fundamental role for the liver in the body's immunity [10]. While Kupffer cells are present throughout the liver, there are differences in the population density, cytological characteristics and physiological functions of Kupffer cells in different zones of the liver stroma. Large Kupffer cells are generally distributed in the perihilar zone of the hepatic lobule, so they are the first to contact blood containing pathogens. Kupffer cells of the perihilar zone have higher lysosomal enzyme activity with better phagocytic capacity as smaller Kupffer cells of the intermediate and middle zones of the lobule [1]. Large Kupffer cells show increased synthesis of TNF- α (tumor necrosis factor α , TNF- α), prostaglandin E2 (PGE2) and interleukin (IL)-1 compared to increased production of nitric oxide (NO) by small Kupffer cells [11]. These observations may be particularly important for the local activity of molecules such as NO, or PGE2 [12]. A receptor involved in the uptake of hemoglobin-haptoglobin (Hb-Hp) complexes has been identified on the surface of spleen and liver tissue macrophages, which is identical to the CD163 receptor present on human blood monocytes [12]. Kupffer cells originate from monocytes circulating in the blood. They belong to a system of mononuclear phagocytes derived from bone marrow monocytes, which are transported with blood to various tissues and develop into individual tissue macrophages [10]. Hematopoietic stem cells present in the liver of rodents and humans, attest to the development of myeloid macrophages within this organ. There are conflicting data on the viability of Kupffer cells. Removal of Kupffer cells with clodronate (the drug belongs to the 1st generation biphosphonate group), results in the reappearance of these macrophages in the liver of mice and rats between 14 and 16 days [13]. In bone marrow transplantation in mice, donor Kupffer cells appear in the recipient's liver within 14 to 21 days, while in mice with monocytopenia, the recipient's Kupffer cells can persist for more than 40 days. In liver transplantation, donor Kupffer cells can be completely replaced within 15–30 days in rats, while in humans they can persist for more than a year. The viability of Kupffer cells can vary from a few weeks to a year, which may be related to their limited ability to self-regenerate [14].

FUNCTIONS OF KUPFFER CELLS

Haemoglobin degradation

Aging or malformed red blood cells are removed by class A scavenger receptor type I and II Kupffer cells and splenic tissue macrophages [14, 15]. Erythrocytes lose 20% of their haemoglobin during life, which is rapidly removed from the circulation by Kupffer cells [16]. In addition, the expression of the scavenger receptor CD163 occurs on Kupffer cells, which is involved in the endocytosis of the Hb-Hp complex, thus protecting the tissues from the damage caused by free haemoglobin due to oxidative stress [17]. Following phagocytosis of erythrocytes or uptake of the Hb-Hp complex, haem is degraded by the enzyme haem oxygenase. Two isoforms of heme oxygenase have been identified in the liver: heme oxygenase-1 (the inducible form in Kupffer cells) and heme oxygenase-2 (the constitutive form in hepatocytes) [18].

Expression of heme oxygenase-1 in Kupffer cells and bilirubin secretion increase rapidly after administration of damaged

erythrocytes, whereas reduction of Kupffer cells and inhibition of heme oxygenase-1 reduces bilirubin production. The degradation of haem results in the release of iron, biliverdin and carbon monoxide. The presence of iron ions at low levels protects cells and has an anti-apoptotic effect, most likely through induction of ferritin expression and signalling pathways involving nuclear factor kappa B (NF κ B) [18]. Hepatic haem oxygenase-1 protects the organ from damage associated with sepsis, haemorrhagic shock and ischaemia/ischaemic injury, whereas inhibition of haem oxygenase-1 activity exacerbates this damage [19]. Both heme oxygenase-1 and CD163 receptor expression are increased by exposure to anti-inflammatory stimuli such as IL-10 and glucocorticosteroids [20, 21]. Kupffer cells, CD163 and heme oxygenase-1 form a system that is coherently amplified during inflammation to enhance heme degradation and haemoglobin clearance [22].

Antigen-presenting cells

Kupffer cells, as antigen-presenting cells (APCs) with major histocompatibility complex (MHC) class II protein molecules on their surface, may play a role in the rejection of liver transplants. Animal studies have shown that liver transplants overexpress MHC class II protein molecules, which initiates the process of graft rejection. In humans, transplantation with the recipient's Kupffer cells shortens the so-called graft rejection phase. It is thought that the non-tropical process of small bile duct destruction and liver transplant rejection may be partly related to MHC class I protein molecules found on the surface of donor Kupffer cells [22].

Tumour surveillance cells

The liver is a common site of haematogenous metastasis, particularly in gastric and intestinal cancers (mainly colon and rectal cancers) [23]. Isolated Kupffer cells are cytotoxic to human colon adenocarcinoma cells. Their cytotoxicity is significantly enhanced by stimulation with interferon- γ (INF- γ) and endotoxins. This process is associated with the expression of TNF- α by Kupffer cells, which can be reduced by the use of anti-TNF- α antibodies. Kupffer cells induce Fas antigen (CD95) expression in colon cancer cells and malignant glioma cells, leading to Fas (CD95)-dependent apoptosis [24] and tumour death in the presence of infiltrating lymphocytes and TNF- α [23, 25]. In vivo studies show that the degree of activation or suppression of Kupffer cells influences the number and size of liver metastases induced by portal vein administration of colon cancer cells [26]. Thus, administration of gadolinium chloride, which attenuates and blocks Kupffer cell function, results in increased metastatic size and lesions. In contrast, activation of Kupffer cells with zymosan and *Corynebacterium parvum* bacteria reduces the size of metastases. Microscopic studies have shown that Kupffer cells in the hepatic circulation have the ability to phagocytose tumour cells [27]. Nitric oxide (produced by Kupffer cells after endotoxin stimulation), TNF- α and prostaglandin E2 provide an effective defence mechanism against tumour cells. Indirectly, Kupffer cells can inhibit tumourigenesis by synthesising IL-12, which stimulates the virulence of NK cells. This also explains the anti-tumour effect of octreotide (a cyclic octapeptide that acts similarly to somatostatin) in hepatocellular carcinoma, which has an anti-apoptotic effect on Kupffer cells [28, 29].

Cells that oversee liver regeneration processes

The ability of the liver to regenerate is critical to a positive resection outcome and is particularly important in the context of donor liver donation and survival. The unique ability of the liver to regenerate has been known since ancient times, but the underlying mechanisms have only recently been elucidated. Activation of Kupffer cells is required for optimal liver regeneration, possibly through the release of the cytokines TNF- α and IL-6. These cytokines initiate hepatocyte proliferation in vivo, at least in part, through NF κ B and the transcription factor signal transducer and activator of transcription 3 (STAT-3). Recent research suggests that this sequence of events is initiated by a leukocyte-Kupffer cell interaction mediated by intercellular adhesion molecule 1

(ICAM-1) [30]. It has been shown that the livers of ICAM-1-deficient experimental mice have impaired regeneration after 70% resection of the organ, with reductions in: leukocyte recruitment, tissue TNF- α expression and interleukin levels. The similar reduction in liver regeneration and cytokine synthesis in leukocyte- and Kupffer cell-deficient animals suggests a novel pathway in liver regeneration in which Kupffer cells and leukocytes induce a local inflammatory response leading to Kupffer cell-dependent release of TNF- α and IL-6. The activation of Kupffer cells may be a direct consequence of Kupffer cell-leukocyte interactions. In addition, the local inflammatory response may induce activation of complement components, resulting in the release of potent activators of Kupffer cells. This view is supported by studies indicating the essential role of complement components C3a and C5a in liver regeneration. C3a and C5a deficiency, as well as disruption of C3a and/or C5a receptor signalling after partial liver resection, results in suppression of TNF- α /IL-6-induced stimulation and inhibition of NF κ B/STAT-3 activation [31].

Role in innate immunity and immune tolerance

The rapid clearance of bacteria from the bloodstream has been attributed to tissue macrophages, particularly Kupffer cells. Recent experiments suggest that the actual mechanism is more complex than phagocytosis by Kupffer cells alone. In fact, the rapid clearance of bacteria that have entered the liver via the portal circulation depends on a complex interaction between Kupffer cells and bactericidal neutrophils, which rapidly evolves into a response to infection [19, 32]. Most microorganisms that enter the liver are bound extracellularly to liver macrophages [33]. Experimental data suggest that this process is mediated in part by an interaction between lectins on Kupffer cells and carbohydrates of bacterial origin [34]. In parallel, this binding is complemented by adhesion molecules (i.e. CD11b/CD18 and CD54) that facilitate the adhesion of neutrophils to Kupffer cells, which subsequently internalise and kill microorganisms bound to their surface [35]. Clearing inflammatory tissues of infiltrating neutrophils is necessary for the absorption of the inflammatory infiltrate. Immunohistochemical detection of myeloperoxidase in Kupffer cells in mouse and human liver autopsy sections confirms the role of Kupffer cells in eliminating activated neutrophils by inhibiting the production of toxic metabolites and degradative enzymes. The uptake of apoptotic neutrophils can either enhance or abrogate the receptor-dependent inflammatory response of macrophages. Phagocytosis of apoptotic neutrophils via the α v β 3 integrin/CD36 complex inhibits the synthesis of pro-inflammatory cytokines such as IL-1 β , TNF- α and prostanooids. On the other hand, neutrophil uptake via receptors for the Fc fragment of immunoglobulins induces the synthesis of these inflammatory mediators [36]. The liver is actively involved in immune tolerance processes with the participation of sinusoidal endothelial cells, Kupffer cells and regulatory T cells [37]. The uptake of neutrophils by Kupffer cells may have a profound relationship with the development and expression of adaptive immunity in the liver. The liver plays a key role in counteracting generalised inflammation by eliminating CD8 $^{+}$ T lymphocytes from the circulation [38]. Kupffer cells isolated from an accepted allogeneic liver transplant have a greater ability to induce apoptosis of alloreactive T lymphocytes, while administration of the same Kupffer cells significantly prolongs allograft survival in a model of acute rejection. Macrophages *in vitro* cross-present epitopes derived from ingested apoptotic cells with the participation of MHC class I protein molecules. In the absence of appropriate costimulatory molecules, cross-presentation of antigens by macrophages is likely to lead to tolerance rather than induction of adaptive immunity in non-sensitised T cells. Thus, the uptake of apoptotic neutrophils by Kupffer cells may have a significant effect on the expression of antigen-specific CD4 $^{+}$ and/or CD8 $^{+}$ T cells mediating immunity in the liver, where sensitised T cells are activated and non-sensitised T cells undergo immune tolerance [39].

Mechanisms of Kupffer cell activation

Studies of activation mechanisms use isolated Kupffer cells in primary culture. Activation of Kupffer cells after exposure to

various stimuli is characterised by rapid phenotypic changes. Particles subject to phagocytosis and certain soluble substances have the ability to activate macrophages by binding to specific receptors on their cell membrane. The major activators of Kupffer cells are complement system factors C3a and C5a, β -glucan (a component of the cell wall of bacteria and fungi) and lipopolysaccharide (LPS, the intestinal endotoxin of gram-negative bacteria). Kupffer cells are directly activated by Toll-like receptors (TLRs) [16]. In addition, high levels of LPS can indirectly activate Kupffer cells by initiating activation of the complement system in the portal or general circulation. Activation of the complement system involves degradation of C3 and C5 to C3a and C5a, respectively, which are potent anaphylatoxins that stimulate their specific receptors C3aR and C5aR. Thus, Kupffer cell activation is a broad spectrum of progressively different changes in the phenotype of these cells resulting from the interactions of different activators and complement signalling pathways [40]. Of particular interest is the aspect of endotoxin activation of Kupffer cells involving lipopolysaccharide binding protein (LBP). The main source of LBP synthesis is hepatocytes, although it can also be synthesised locally in the skin and lungs. LBP has a high affinity for lipid A, a component of endotoxin, and catalyses the binding of LPS to the CD14 receptor. The N-terminus of LBP is responsible for binding LPS, while the C-terminus binds to CD14. The truncated form of human LBP consists of 197 amino acids. It has the ability to bind to LPS but does not allow it to react with CD14 [41]. Although LBP is not required for the interaction between LPS and CD14, it significantly reduces the concentration of LPS required to activate Kupffer cells [42]. At low serum concentrations of LPS, LBP can facilitate the transfer of LPS to the CD14 receptor on isolated Kupffer cells *ex vivo*, accelerating binding to the receptor and stimulating greater TNF- α synthesis [43].

In vivo, recombinant mouse LBP reduces the response to LPS (indirectly, TNF- α levels), limits parenchymal tissue necrosis and decreases alanine aminotransferase activity in a model of liver failure induced by LPS and galactosamine administration [44]. LBP concentrations of 3–10 ng/ml and the presence of rCD14 inhibit neutrophil recruitment, whereas LBP concentrations below 3 ng/ml stimulate this process under the same conditions. How LBP can have the opposite effect is unknown, but studies suggest that a number of concentration-dependent interactions between different factors determine the biological effect of this protein [16].

Activation of Kupffer cells may also be associated with ingested drugs such as paracetamol. Isolated Kupffer cells can be activated by paracetamol without the involvement of LPS and/or complement system factors as an alternative pathway for Kupffer cell activation [45]. Overdosage of paracetamol causes centrilobular necrosis of hepatocytes, which can lead to acute liver failure. Depletion of intracellular glutathione and increased production of reactive oxygen and nitrogen species are thought to be critical pathomechanisms in this process. Recent work shows an important role for tyrosine nitration by peroxynitrite, formed by the rapid reaction of NO and superoxide. Surprisingly, acetaminophen toxicity and tyrosine nitration can be significantly reduced by various Kupffer cell inactivators and by ablation of the inducible NO synthase (iNOS) gene, whereas liver damage continues to progress in transgenic mice lacking the NADPH oxidase gene [46]. These findings suggest that Kupffer cell activation and induction of iNOS and subsequent release of NO, but not Kupffer cell-produced superoxide, is a major determinant of peroxynitrite formation and consequent paracetamol toxicity.

The role of Kupffer cells in liver disease

Kupffer cells are the main effector cells against hepatocytes in various forms of liver damage. This occurs through the synthesis of pro-inflammatory cytokines, oxygen radicals, nitric oxide, phospholipases and lysosomal enzymes. Kupffer cells can damage hepatocytes by inducing their apoptosis through signalling via the CD95 receptor (CD95/CD95L). This process is enhanced

by CD8⁺ antigen-positive restrictive T cells and inhibited by CD4⁺, CD25⁺ regulatory T cells. Kupffer cells are the antigen-presenting cells for extrahepatic viruses such as influenza virus, but also for intrahepatic hepatotropic viruses such as hepatitis B virus (HBV) and hepatitis C virus (HCV). Subsequently, antigen-presenting Kupffer cells attract both CD8⁺ T cells and regulatory T cells through chemokine synthesis [47]. T lymphocytes expressing lymphocyte function-associated antigen-1 (LFA-1) are arrested by overexpression of adhesion factors: intercellular adhesion molecule-1 (ICM-1) and vascular cell adhesion molecule-1 (ICAM-1). ICAM-1 and vascular cell adhesion molecule (VCAM), while CD8⁺ T cells can induce apoptosis after direct contact with Kupffer cells. Transforming growth factor β (TGF- β) synthesised by Kupffer cells is involved in the transformation of stellate cells into myofibroblasts, leading to the induction of fibrosis [48, 49]. Finally, the synthesis of glutathione, IL-6 and the chemokine macrophage inflammatory protein 2 (MIP-2) by Kupffer cells may protect hepatocytes from further damage [47]. Of particular interest is the involvement of the liver, and thus Kupffer cells, in infectious diseases of this organ. In studies using a model of sepsis, administration of LPS increases the number of Kupffer cells and reduces the number of *Salmonella* bacteria [50]. Experimentally, in animals with mechanical jaundice, the impaired ability of Kupffer cells to phagocytose and produce superoxide leads to increased susceptibility to infection [51]. In a mouse model of *Listeria monocytogenes* infection, bacterial accumulation in the liver depends on the recognition of bacterial lectins by appropriate receptors on Kupffer cells. Synthesis of inflammatory mediators such as IL-6, IL-12, IL-1 β , TNF- α and nitric oxide by Kupffer cells inhibits microbial proliferation. At the same time, Kupffer cells secrete the chemokines MIP-1 α , MIP-1 β , MCP-1 and MIP-2, which recruit monocytes and neutrophils to the liver, limiting and controlling the course of infection. Inactivation of Kupffer cells results in impaired processes leading to clearance of the infection [52]. Kupffer cells in the glaucomatous zone are the first line of defence against cytomegalovirus and parasites: *Plasmodium bergeri*, *Leishmania*, which invade and multiply within Kupffer cells and then infect other liver cells [2]. The inability of Kupffer cells to clear portal blood of microorganisms and endotoxins may be the reason for the increased incidence of sepsis and septic shock caused by Gram-negative bacteria in the course of acute liver failure. Many studies show that a significant percentage of patients with chronic liver disease have systematic endotoxaemia and high titres of antibodies to enteric bacteria. In contrast, in healthy individuals, endotoxins are only detected in the portal circulation. Influenza-like hepatitis has been associated with the absence of virus in the liver, close contact between Kupffer cells and inflammatory foci, and virus-specific CD8⁺ T lymphocytes. Elimination of Kupffer cells reduces hepatocellular necrosis despite the presence of reactive CD8⁺ cells. Activated T cells are recruited and retained in the liver by interactions of LFA-1 antigen with VCAM and ICAM-1 integrins on the sinusoidal endothelium [48,49]. In this model, Kupffer cells destroy hepatocytes either directly by activating the Fas ligand/CD95-dependent extrinsic apoptotic pathway or indirectly through CD8⁺ T cell interactions with cytokines and other mediators such as phospholipase and nitric oxide. Hepatitis caused by hepatotropic viruses such as HBV, HCV and HEV, as well as infections caused by rubella virus, cytomegalovirus and the virus that causes severe acute respiratory syndrome (SARS), may share a similar mechanism. The only difference is the involvement of Kupffer cells and antigen-presenting dendritic cells, and the presence of virus-specific CD8⁺ T lymphocytes in the portal venous system or sinusoids [53].

The role of Kupffer cells in immune-mediated adverse drug reactions

Data from hepatotoxicity studies contribute to the understanding of immune-mediated adverse drug reactions (IADRs). The place of IADRs in medical and pharmaceutical practice is proving

to be a major concern and the basis for intensive research [54]. IADRs are not related to the action of the drug, do not occur in everyone who takes the drug, and are not dose dependent [55]. The mechanism of this phenomenon is not clear, but it may depend on Kupffer cells which, as APCs, may influence the reduced sensitivity of T lymphocytes to stimuli. Full activation of T lymphocytes occurs via two independent signalling pathways. The first signal is recognition of the major histocompatibility system on APCs by receptors on T lymphocytes [56]. The second signal is the interaction between the costimulatory molecule B7 (ligand) on APCs and the CD28 receptor on T lymphocytes. Induction of T lymphocyte activity by the first pathway alone, without the involvement of the second, is insufficient and leads to immune tolerance [56]. Studies comparing Kupffer cells with dendritic cells confirmed that liver macrophages produce insufficient costimulatory molecules. Thus, a possible mechanism for the induction of T lymphocyte tolerance is the insufficient synthesis of costimulatory molecules by Kupffer cells during antigen presentation [44, 56]. Disruption of this mechanism is critical in many forms of IADR. Kupffer cells, as APCs, can induce T-cell tolerance in response to the drug-protein combination. Studies have been carried out using 2,4-dinitrochlorobenzene (DNCB), which can induce T-cell dependent hypersensitivity (type IV). In contrast, prior administration of a DNCB conjugate with bovine serum albumin leads to the development of immune tolerance to the compound [42]. Tolerance induced by prior administration of the conjugate can be reversed by the use of liposomal clodronate to suppress Kupffer cells. Overexpression of costimulatory molecules or Kupffer cell deficiency results in immune intolerance to the drug-protein combination [54]. In addition, the individual inability of blood proteins to interact with the drug or the administration of another drug with a higher affinity for the protein can effectively prevent this process leading to IADR. The overexpression of costimulatory molecules on Kupffer cells and other APCs is explained by the danger theory, which is based on so-called danger signals released during cell injury [57]. Hydrophobic biological substances called hypos and heat shock proteins have been proposed as endogenous signals [58]. Hypos are physiologically stored in a hydrophilic environment, but their accumulation on hydrophobic surfaces triggers signalling indicating cell damage. The stimulus activates Kupffer cells and other macrophages via TLR receptors [57]. It has been shown that drugs or their tissue-damaging metabolites, as well as drug-associated situations, including infections and surgery, can be responsible for high levels of endogenous danger signals. In summary, danger signals that lead to the overexpression of costimulatory molecules on Kupffer cells may be responsible for IADR. Thus, instead of inducing tolerance, APCs lead to full activation of T cells [59].

A separate aspect of IADR is the inflammatory theory, which does not depend on immune tolerance, but on the interaction of the drug with the endotoxin. The research model is based on the parallel administration of a non-toxic dose of LPS and a therapeutic dose of ranitidine (a histamine H₂ receptor antagonist) [55, 60]. Ranitidine enhances the ability of LPS to activate Kupffer cells. In comparison, the same dose of LPS does not stimulate macrophages in a model without ranitidine. Activated Kupffer cells release TNF- α , which activates the coagulation system, inhibits fibrinolysis and increases synthesis of the macrophage inflammatory protein MIP-2. Fibrin deposition leads to hypoxia and necrosis of liver cells [55]. IADR may also depend on the activation of Kupffer cells through the interaction of LPS with ranitidine (another drug), leading to hepatocyte necrosis via hypoxia.

In conclusion, the role of Kupffer cells in IADR is an important area of research, and the activation or dysfunction of these cells may have a significant impact on the occurrence of immune-mediated adverse drug reactions.

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