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Role of inflammation in chemical-induced hepatotoxicity☆

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Abstract

The liver, which is the major organ responsible for the metabolism of drugs and toxic chemicals, is also the primary target organ for many toxic chemicals. Increasing evidence has indicated that inflammatory processes are intimately involved in chemical-induced hepatotoxic processes, and like other inflammatory diseases, such as autoimmunity, are responsible for producing mediators that can effect liver damage or repair. This review will summarize our current understanding of how inflammatory processes influence hepatic pathology and repair following exposure to established hepatotoxic chemicals including carbon tetrachloride, an industrial chemical, and acetaminophen, a widely used analgesic.

Keywords: Inflammatory cytokines; Immunotoxicology; Chemical-induced hepatotoxicity

1. Introduction

The vulnerability of the liver to chemical injury is as much a function of its anatomical proximity to the blood supply and digestive tract as to its ability to biotransform and concentrate xenobiotics. Xenobiotics in the blood pass through the portal vein and hepatic artery, and then drain through the central and the hepatic veins into the vena cava. The main hepatic duct joins the cystic duct from the gall bladder to form the common bile duct, which drains into the duodenum. Although hepatocytes compromise the bulk of the liver, approximately 35% of liver cells reside in the hepatic sinusoids and represent endothelial cells, Ito cells and, predominantly, Kupffer cells. Kupffer cells, which are responsible for antigen presentation and clearance of particulates from portal

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circulation, are central to hepatic inflammatory processes.

Increasing evidence suggests that inflammation plays a role in many classical chemical toxicities including, among others, hepatotoxicity induced by carbon tetrachloride or acetaminophen, pulmonary toxicity from asbestos and silica, and even neurotoxicity from organotins (Luster et al., 1999; Schook et al., 1994). The overarching hypothesis that links these toxicities is illustrated in Fig. 1 and can be summarized as follows: initial toxic injury produces focal tissue damage and necrosis in a target organ. As a result of this damage, tissue fixed macrophages, along with adjacent endothelial cells and epithelial cells, are activated and secrete inflammatory products. These products include the proinflammatory cytokine tumour necrosis factor (TNF)α, which is a central regulator that aids in tissue repair by stimulating apoptosis and cell proliferation as well as exacerbating cell damage by initiating an overly aggressive inflammatory process. The latter can result by the ability of TNFα to cause the release of reactive oxygen or indirectly through the recruitment and activation of neutrophils and monocytes into the damaged site. The inability of the system to neutralize the excessive release of reactive oxygen species and the nitrogen-centered radical, nitric oxide, are responsible for cell damage or activating genes responsible for cell proliferation. Support for this pathway, and the fact that TNFα is a central mediator, stems from several observations: (1) elevated levels of inflammatory mediators, including TNFα, chemokines and reactive oxygen and nitrogen species, are found in target organs following exposure to many toxic agents; (2) inhibitors of this pathway (see Fig. 1), such as antioxidants, cytokine antagonists and macrophage poisons, prevent many of the pathophysiological or repair processes from occurring; and (3) direct administration of these mediators, such as TNFα, in experimental animals, mimics many of the pathophysiological responses observed in the chemical response (Corsini et al., 1997; Czaja et al., 1994; Iimuro et al., 1997; Kayama et al., 1995; Laskin et al., 1995; Maier et al., 1995; Piguet et al., 1990a,b; Roth et al., 1997).

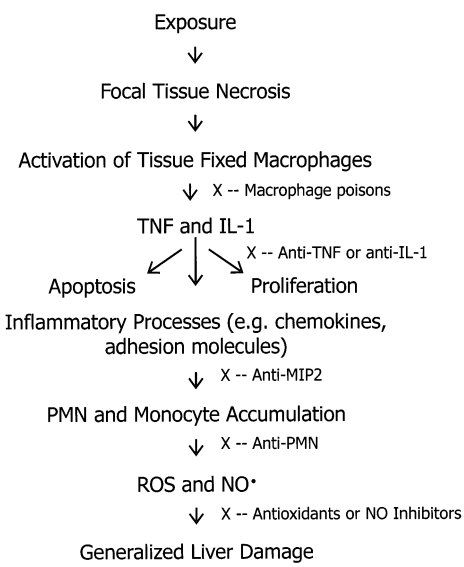

Fig. 1. Hypothesis of chemical-induced liver injury. Increasing evidence has suggested that inflammation plays a major role in chemical-induced hepatotoxicity and repair. Initial damage is thought to produce focal tissue necrosis, resulting in activation of tissue fixed macrophages. These activated macrophages secrete primary cytokines, including IL-1 and TNFα, which may induce apoptosis, stimulate cell growth or initiate inflammatory processes. The inflammatory process ultimately results in the recruitment of activated neutrophils and the release of toxic products such as reactive oxygen species (ROS) and nitric oxide. The strongest evidence for this hypothesis originates from the use of different classes of inhibitors.

2. Results and discussion

Work in our laboratory has focused on the role of TNFα in this process as it is a central regulator for many bioactive molecules including those responsible for chronic inflammation, induction of acute phase proteins, cell proliferation and cytotoxicity. Evidence that proinflammatory cytokines are involved in hepatotoxic responses was first provided from studies (Blazka et al., 1995) that demonstrated neutralizing antibodies to TNFα or interleukin (IL)-1 partially prevented liver damage in mice initiated by hepatotoxic doses of acetaminophen. These studies were stimulated by observations that hepatocytes treated with acetaminophen release factors that activate Kupf-

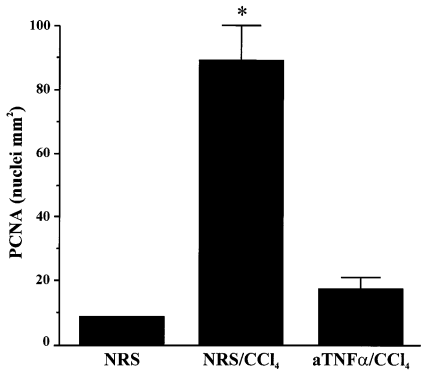

Fig. 2. PCNA immunostaining. Mice were administered either normal rabbit serum (NRS), CCl_4 (0.1 ml/kg body weight) or CCl_4 plus neutralizing antibodies to TNFα (aTNF) (Bruccoleri et al., 1997). Livers were collected 48 h later and the number of nuclei in the S-phase of the cell cycle (per square millimeter) determined using standard methodology (Bruccoleri et al., 1997).

fer cells, which in turn are associated with areas of the liver that subsequently become necrotic (Laskin, et al., 1995). In later studies, we demonstrated that TNFα in the liver is also associated with carbon tetrachloride (CCl_4) exposure in mice. Surprisingly, however, TNFα neutralization was not associated with decreased hepatotoxicity, but rather delayed normal repair processes (Bruccoleri et al., 1997). Subsequent studies using this model for chemical liver injury indicated that TNFα was involved in liver repair through its ability to support hepatocyte proliferation following chemical injury. This is illustrated in Fig. 2 using proliferating cell nuclear antigen (PCNA) staining, a measurement of hepatocyte proliferation. Forty-eight hours following exposure of mice to 0.1 ml/kg body weight CCl_4, a marked increase in the percent of cells in S-phase was detected in the livers of treated mice compared with controls. Nuclear PCNA staining was reduced nearly to control levels in mice pretreated with neutralizing antibodies to TNFα, indicating its importance in hepatocyte proliferation. As with chemical-induced hepatic damage, it had been demonstrated that TNFα is expressed in the liver of rats following partial hepatectomy (Satoh and Yamazaki, 1992) and administration of neutralizing antibodies to TNFα before partial hepatectomy significantly impairs liver regeneration (Akerman et al., 1992). In this respect, the ability of TNFα to serve as a mitogen for human and rodent hepatocytes has recently been demonstrated in vitro (Satoh and Yamazaki, 1992). Thus, TNFα is both mitogenic for hepatocytes, being implicated in liver repair following chemical damage and in regeneration following partial hepatectomy, as well as capable of causing cell damage by inducing an over-zealous inflammatory response.

Cell proliferation is a complex and tightly controlled process that is modulated by cell-to-cell contact and bioactive macromolecules such as hepatocyte growth factor, transforming growth factor (TGF)-α and epidermal growth factor (Michalopoulos and DeFrances, 1997). Evidence exists that TNFα may influence the expression of several of these growth factors in certain cell lines such as TGFα in pancreatic cells (Kalthoff et al., 1993). To determine whether TNFα could influence the expression of TGFα in the liver, isolated mouse hepatocytes were cultured on a growth factor-depleted extracellular matrix and treated with either TPA, as a positive control, or recombinant murine TNFα for up to 2 h (Gallucci et al., 2000). Reverse transcriptase-polymerase chain reaction (RT-PCR) indicated that a sevenfold increase in TGFα mRNA occurred following treatment with 10 ng/ml TNFα (Fig. 3). Although TNFα also increased the expression of IL-6 in hepatocytes (data not shown) and IL-6 has been implicated as a hepatocyte mitogen (Yamada and Fausto, 1998), studies using IL-6 antagonists, recombinant IL-6 or protein inhibitors clearly demonstrated that TNFα directly stimulates TGFα induction and is independent of IL-6 (Gallucci, et al., 2000). These studies have subsequently been confirmed in vivo following injections of TNFα or CCl_4 in mice where increased hepatic TGFα expression was observed. As TNFα is mitogenic for cultured hepatocytes (Satoh and Yamazaki, 1992), we determined whether its mitogenic activity is ultimately due to TGFα by stimulating hepatocytes with TNFα in the presence of antibodies to TGFα and monitoring ^{3}H-TdR incorporation (Gallucci, et al., 2000). The addition of neutralizing antibodies to TGFα prevented the increase in DNA synthesis, suggest-

ing that the stimulatory effect of TNFα is due primarily to its ability to stimulate TGFα.

To help discern the factors that determine whether TNFα will participate in a proliferative or inflammatory process in the liver, TNFα receptor transgenic mice were studied. Like other cytokines, TNFα signals through binding to specific cell surface membrane receptors, found on most nucleated cells. Two distinct receptors mediate the biological activities of TNFα, one of molecular mass 55 kDa (p55, R1) and the other of 75 kDa (p75, R2). R1 is constitutively expressed and historically has been considered the primary mediator for TNFα responses, while R2 is inducible and provides for ligand passing as well as limited responses such as cytotoxicity. In Fig. 4, the percentage of PCNA-positive nuclear stained cells are shown from livers of transgenic mice in which the R1, R2 or R1/R2 genes have been deleted. All mice, including the wild-type, were administered 0.1 ml/kg body weight of CCl_4 48 h prior to examination. Although the percent of PCNA stained cells were reduced to a slightly greater degree in TNFR1 null mice, compared with the TNFR2, both transgenics demonstrated significant effects. A similar pattern emerged when the vigour of the inflammatory response was measured in these knockout mice following chemical exposure (Simeonova et al., submitted). Taken together, these results indicate that both receptors are involved in the TNFα induction of proliferation and inflammation, although R1 may play a more dominant role.

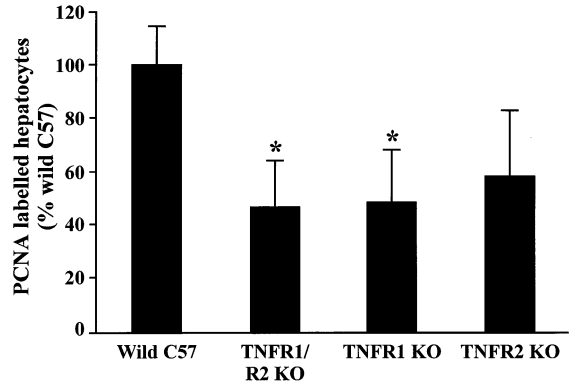

Fig. 4. Wild-type C57 black mice or TNFα receptor deficient mice (TNFR1/R2, TNFR1, TNFR2), originally obtained from Dr L. Schook (Univ. Minnesota), were intraperitoneally injected with CCl_4 as described in Fig. 2 legend. After 48 h, the livers were collected, prepared histologically and stained for PCNA as described previously (Bruccoleri et al., 1997) ($n = 5$, $\pm$S.E.)

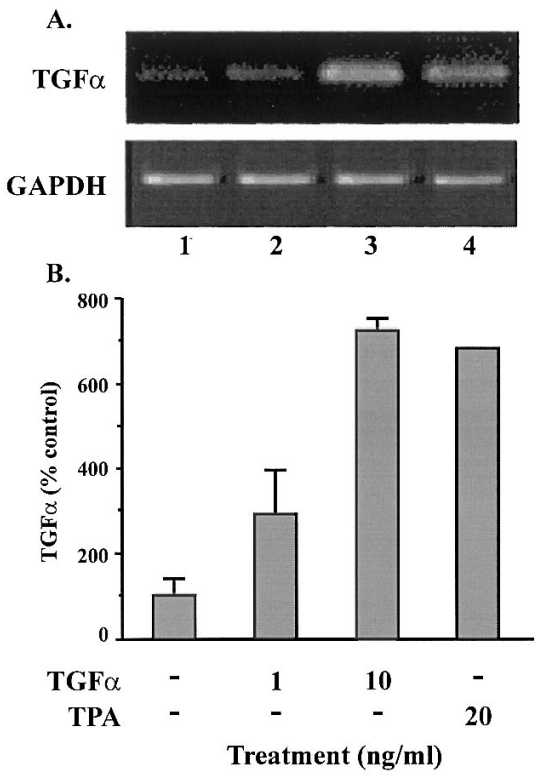

Fig. 3. TGFα expression in isolated murine hepatocytes. Following a 2 h incubation of mouse hepatocytes with murine recombinant TNFα (R&D Systems, Minneapolis, MN) or PMA, RNA was isolated and RT-PCR was performed using mouse commercial TGFα and G3PDH specific primers as described previously (Gallucci et al., 2000). (A) Ethidium bromide stained 1.5% agarose gels representative of three experiments. Lane 1, saline; lane 2, 1 ng/ml TNFα; lane 3, 10 ng/ml TNFα; lane 4, 20 ng/ml TPA. (B) Gels were scanned with a digital image analysis system. The PCR products were quantified and data are expressed as a percent of control values ($n = 3$, $\pm$S.E.).

3. Conclusions

In summary, only recently have toxicologists come to appreciate the role inflammation plays in classic toxicological processes. This relationship can be extremely complex, as inflammation may well be only one facet of a time- and dose-dependent continuum of toxicological and repair processes. Although many mediators are responsible for these processes, pro-inflammatory cytokines have received the most attention as they represent central mediators involved in regulating this process. Not surprisingly, considerable efforts are being undertaken using our newly found understanding of molecular control to develop specific and safe biological and molecular inhibitors of TNFα for potential therapeutic use. The understanding of the molecular basis for inflammatory diseases has also provided additional opportunities to improve human health risk assessment. For example, the identification of functional polymorphisms for many of the genes that code for inflammatory mediators should allow for a better estimate of determining susceptible populations and help improve human risk assessment.

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