Cytokines in non-genotoxic hepatocarcinogenesis

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Non-genotoxic liver injury

Many toxicants can cause liver injury. Some, such as diethylnitrosamine, are genotoxic and act principally by damaging DNA (1). A second more diverse group cause liver injury but are non-genotoxic (2). Despite differences in the primary target, genotoxic and non-genotoxic hepatotoxins frequently cause tumours in the liver of experimental rats and mice (1,2). This review is concerned with evaluating the hypothesis that cytokines may play a role in mediating the biological effects of liver non-genotoxic carcinogens.

Non-genotoxic liver toxicants constitute a diverse group of chemicals. Some, such as carbon tetrachloride (3), cause acute liver injury followed by regenerative hyperplasia, whereas others cause proliferation without identifiable tissue damage. The former group includes barbiturate drugs such as phenobarbitone (PB) (4), dioxins such as 2,3,7,8-tetrachlorodibenzo-p-dioxin (5,6) and the largest group, the peroxisome proliferators (PPs) (7). The induction of cell proliferation by toxicants that cause liver injury probably underpins their common ability to cause hepatocellular adenomas and carcinomas after prolonged exposure (8).

During chemical-induced carcinogenesis, the tightly controlled balance that exists normally in tissues between cell growth and cell death is disrupted (9–11). Rodent hepatocarcinogenesis in response to PPs is preceded by liver enlargement due to hepatocyte hypertrophy and induction of cell proliferation coupled with suppression of hepatocyte apoptosis (7,12,13). The ability of barbiturates such as PB to stimulate hepatocyte DNA synthesis in vivo has also been demonstrated (14). The capacity of non-genotoxic liver carcinogens to perturb both sides of the growth equation between cell gain and cell loss can be modelled in vitro using hepatocytes prepared from rats or mice. In such models, PPs such as nafenopin can suppress spontaneous apoptosis (15–17) and also that induced by diverse stimuli, including transforming growth factor-β1. Interleukin (IL)-1 can act alongside TNF-α to play a role in inflammation (18) and is implicated in the regulation of hepatic lipid metabolism (19), ligation of Fas or DNA damage (20). Thus, non-genotoxic carcinogens may prevent the removal of damaged or excess cells that would normally be deleted. These cells then remain as targets for further mutagenic stimulation.

TNF-α: role in non-genotoxic liver injury

TNF-α is a pro-inflammatory mediator proximally associated with necrotic injury (21). Initial studies have suggested that TNF-α may mediate the liver injury caused by carbon tetrachloride (22). However, other data suggest that TNF-α does not cause tissue damage per se, but rather the regenerative hyperplasia that follows injury such as surgical resection of the liver (23), treatment with lead nitrate (24) or with carbon tetrachloride (3,25,26). Thus, TNF-α may have a role as a positive mediator of hepatocyte growth.

There are two well-characterized receptors for TNF-α (TNFR), designated TNFR1 (p55) and TNFR2 (p75) (reviewed in ref. 27). These two receptors are widely expressed on different cell types and appear to transduce diverse signals, depending on the tissue and the cellular context of the signal (28–30). Upon activation, TNF-α can recruit a broad spectrum of intracellular signalling molecules, such as receptor-interacting protein (RIP), TNF-α-associated protein 2, Fas-activated death domain (FADD) and TNFR1-associated death domain (TRADD) (29). This in turn leads to activation of two transcription factors, AP1 and NFκB, as well as activation of the Jan N-terminal kinases (29,31). Although these pathways may provide positive growth stimuli, RIP, FADD and TRADD contain death domains that can directly activate the apoptotic protease cascade (32). Recently, a role for TNFR1, but not TNFR2, in mediating liver regeneration has been demonstrated. Transgenic mice lacking TNFR1 were shown to be deficient in liver regeneration following either partial hepatectomy (33) or exposure to carbon tetrachloride (26). These results indicate that signalling from TNF-α can provide a positive growth signal for the liver, driving hepatocyte proliferation.

Although TNF-α is implicated in hepatocyte proliferation following tissue damage, only recently has it been suggested that liver cytokines may mediate the growth perturbation associated with non-genotoxic hepatocarcinogenesis (22,24,25,34–39) (Figure 1). TNF-α is able in vitro to mimic the effects of PPs since it can induce rat or mouse hepatocyte DNA synthesis (39,40) and suppress apoptosis (39). In addition, neutralizing antibodies to TNF-α have been found to attenuate the effects of PPs on apoptosis in vitro (39) and on DNA synthesis both in vitro (39) and in vivo (35).

The major source of cytokines such as TNF-α in the rat liver is believed to be the Kupffer cell (41). Kupffer cells are hepatic macrophages, representing ~10% of the total cell population. Interestingly, Kupffer cells are required for stimulation of mitosis by PPs and PPs stimulate Kupffer cell phagocytosis (34–36,42). Taken together, the available evidence suggests that low levels of TNF-α produced from resident

Abbreviations: FADD, Fas-activated death domain; HBV, hepatitis B virus; IL, interleukin; PB, phenobarbitone; PPs, peroxisome proliferators; PPARα, peroxisome proliferator-activated receptor; RIP, receptor-interacting protein; STAT, signal transducers and activators of transcription; TNF-α, tumour necrosis factor α; TNFR, tumour necrosis factor α receptor; TRADD, TNFR1-associated death domain.
liver macrophages, the Kupffer cells, may mediate the effects of PPs via paracrine signalling mechanisms. In support of this, non-genotoxic carcinogens, such as the PP Wyeth-14,643 and PB, have been shown to activate NFκB (37,43,44), a downstream mediator of TNFR1 activation. In contrast, other investigators have reported that TNF-α can be cytotoxic for cultured hepatocytes (45) and that liver cell proliferation induced by PPs is not associated with increased transcription of TNF-α (46,47). A simple explanation for the former controversy could be the concentrations of TNF-α used, but this appears comparable between the two studies. Instead, these conflicting data may arise from different differences in tissue culture, such as the quantity and quality of serum used. The lack of transcriptional up-regulation of TNF-α by PPs may appear to contradict a role in PP-mediated growth. However, some studies have shown TNF-α mRNA to be widespread under both control and PP-treated conditions, suggesting that this cytokine could be constitutive (48). Thus, TNF-α may be necessary but not sufficient to drive PP-mediated growth changes.

**Evidence for a cytokine signalling network**

As well as a role for TNF-α, there are further candidate cytokines that may mediate hepatic responses to non-genotoxic carcinogens. These include other pro-inflammatory cytokines, such as IL-1 and IL-6. Both forms of IL-1 (IL1-α and IL1-β) act through a single signal transducing membrane receptor, IL-1R1 (49), which shares many downstream signalling pathways with TNFR1 (50). IL-1R1 ligation is able to activate the transcription factor NFκB (51) as well as the JNK family of kinases (52). This suggests that IL-1 may represent a positive growth stimulus for hepatocytes. In support of this, IL-1α and IL-1β have been shown to be produced during hepatic regeneration (53) and IL-1α has been shown to induce hepatocyte mitosis in adult rats in vivo (54). However, IL-1α is reported to inhibit proliferation of cultured mouse hepatocytes (55) and may constitute a negative feedback loop that can arrest hepatocyte proliferation (56). This apparent controversy may result from the time interval between treatment and measurement of response. The direct, acute effect of IL-1 may be to cause growth arrest, but a chronic effect is to induce expression of TNF-α, a positive stimulus in this context.

IL-6 is a pleiotropic cytokine implicated in mediating hepatocyte lipogenesis (19), hepatic acute phase protein synthesis (57,58) and liver regeneration following partial hepatectomy (59). IL-6 acts via ligation of its cell surface receptor, which results in activation of the signal transducers and activators of transcription (STAT) proteins 1 and 3. STAT1 and STAT3 are transcription factors that translocate to the nucleus to initiate gene transcription through specific enhancer elements. In the liver, IL-6 is produced by both Kupffer cells and hepatocytes in response to infection with *Listeria* (57,60). In addition, IL-6 is produced by cultured hepatoma cells, where it induces a typical acute phase response in an autocrine fashion (61). In common with TNF-α and IL-10/β, IL-6 has been shown to induce hepatocyte mitosis (54) and is up-regulated during hepatic regenerative hyperplasia (62). Consistent with this, transgenic mice deficient in IL-6 exhibit defective hepatic regeneration (59). In addition, injection of IL-6 can restore the induction of hepatic DNA synthesis in response to partial hepatectomy in TNFR1 knockout mice (33), suggesting some overlap in signalling pathways. In contrast to this positive role for IL-6, other reports suggest that this cytokine can suppress DNA synthesis induced in cultured mouse hepatocytes by TNF-α (63).

Since many of these cytokines can activate transcription factors such as NFκB and STAT3 that themselves regulate cytokine expression, there is considerable potential for cross-talk and feedback between the different cytokines and their receptors. For example, IL-10, an immunoregulatory cytokine, has been implicated in the suppression of TNF-α expression in hepatocytes, suggesting that IL-10 could be a target for transcriptional regulation by non-genotoxic carcinogens (64). Similarly, Kupffer cell-derived cytokines such as TNF-α can drive expression by cultured hepatocytes of IL-8 (65), a chemotactic cytokine that affects leukocyte infiltration during inflammatory reactions. More recently, it has been suggested that the function of TNF-α in liver regeneration is to prime hepatocytes for stimulation by growth factors (66) such as epidermal growth factor and hepatocyte growth factor. It remains to be determined if TNF-α can prime cells to respond to non-genotoxic carcinogens such as PPs and PB.

The molecular mechanisms through which liver toxicants may cause cytokine production and/or release are presently unclear. However, the PP class of non-genotoxic hepatocarcinogens activate the PP-activated receptor (PPARα) (67), a ligand-activated transcription factor. In 1995, a PPARα null mouse was created (68,69) and found to be refractory to such effects of PPs as peroxisome proliferation, liver enlargement and tumourigenesis (68,70,71). Using a dominant-negative PPARα cloned from human liver, hPPARα-6/29 (72), we have demonstrated that the suppression of apoptosis by PPs in *vitro* was also dependent upon PPARα (73). These data were confirmed by the observation that PPs are no longer able to suppress apoptosis in hepatocytes from the PPARα null mouse.
Cytokines, viral hepatitis and hepatocellular carcinoma

The liver plays a co-ordinating role in the systemic defence mechanisms that are initiated by the host after injury or infection (18). Thus, pro-inflammatory cytokines such as TNF-α, IL-1 and IL-6 may reach the liver from distal sites via the bloodstream or may be synthesized in the liver itself (18). The liver also plays an important role in cytokine clearance (18).

In acute hepatitis B virus (HBV) infection, the immune response to HBV is vigorous, leading to viral clearance. However, in chronic HBV infection, the immune response is relatively weak and may destroy some but not all of the infected cells (83). Hepatocellular carcinoma is a common complication of chronic HBV infection and it has been suggested that clonally integrated viral DNA plays a key role in the carcinogenic process (84). However, it has recently been shown that the development of hepatocellular carcinoma after HBV infection occurs in the absence of viral transactivation and insertional mutagenesis (83). In contrast, the immune response to HBV was vital for tumour development (83). These data suggest that the hepatocarcinogenesis that accompanies chronic hepatitis may result from an immune response to viral infection under conditions where hepatocyte growth regulation may be perturbed by cytokines such as TNF-α, IL-1 and IL-6. In this respect, non-genotoxic and viral hepatocarcinogenesis may share some common features.

Summary and future directions

Some toxicants and viruses have the potential to cause liver injury, frequently leading to hepatocarcinogenesis. This induction of tumours may arise from a perturbation of hepatocyte growth regulation. Evidence from investigations of liver regeneration have suggested that cytokines such as TNF-α and IL-1 may provide a positive signal for liver growth, driving hepatocyte proliferation. Only recently, however, has it been suggested that hepatic cytokines may also mediate the growth perturbation associated with non-genotoxic carcino genesis (Figure 1). Although the published data are limited at present, they support a new, potentially important hypothesis. In future, it will be important to determine whether all non-genotoxic liver toxicants share the ability to cause an up-regulation in cytokine gene transcription, since this may provide the basis of an approach to interrogate the hepatotoxic and hepatocarcinogenic potential of chemicals. In addition, it will be necessary to consider whether there are differences in the potential of non-genotoxic carcinogens to regulate cytokine networks in rodents and humans, since this may provide the molecular basis for species differences in response to non-genotoxic carcinogens.

References


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