Review

The Roles of Innate Immune Cells in Liver Injury and Regeneration

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For predominant abundance with liver-specific Kupffer cells, natural killer (NK) cells, and natural killer T (NKT) cells and their rapid responses to several stimuli, the liver is considered as an organ with innate immune features. In contrast to their roles in the defense of many infectious agents like hepatitis viruses and parasites, hepatic innate immune cells are also involved in the immunopathogenesis of human clinical liver diseases and several murine hepatitis models such as concanavalin A (Con A), lipopolysaccharide (LPS), or polyinosinic-polycytidylic acid (Poly I:C)-induced liver injury. In this review, the destructive roles of NK cells, NKT cells and Kupffer cells in the processes of immune-mediated liver injury and regeneration will be discussed, and some putative mechanisms involving the impairment of liver regeneration caused by activated hepatic innate immune cells are also proposed. ***Cellular & Molecular Immunology*****. 2007;4(4):241-252.**

Key Words: NK cell, NKT cell, Kupffer cell, liver injury, liver regeneration

Liver is an organ with innate immune features

The liver has special double blood supplies, on one hand come from the liver artery, and the portal vein on the other hand. In view of such special anatomical location and function, the liver is continuously exposed in the large load of intestinal antigen that includes pathogens, toxins, tumor cells and harmless dietary antigens (1), making the liver not only bear "internal depurating blood" function, but also remove the foreign matter of intestinal source. These characteristics provide with the possibility that liver is endowed with a unique fast alternative immune response mechanism in response to the liver-specific potential dangers. Innate immune response serves as the first line of defense against invading pathogens, functioning nonspecifically and with no memory. It is now being appreciated that the local immune mechanisms are required to cope with these diverse immunological challenges (2-4). It has been known that the liver is a site for the production of cytokines, complements components and acute phase proteins and contains large numbers of phagocytes, antigen-presenting cells and lymphocytes (5). Structurally and functionally, qualification of liver as a lymphoid organ has been reflected by many studies (6, 7), and therefore a new subspecialty through combination hepatology and immunology termed "hepatoimmunology" has emerged (8). Our previous studies showed that innate immune cells including natural killer (NK) cells, NKT (natural killer T) cells, and Kupffer cells were predominant on the quantity in the diverse liver lymphocytes (Figure 1), compared with peripheral blood and other organs like spleen (9, 10). Injection with polyinosinic-polycytidylic acid (Poly I:C), a stimulant to innate immune, or murine cytomegalovirus (MCMV) infection could rapidly induce NK cell accumulation and activation in the liver (11), followed with high level of interferon γ (IFN-γ) production, which contributed partially to the clearance of virus. According to the high proportion of innate immune cells and rapid responses to the stimuli in the liver, it is supposed that liver is a lymphoid organ with innate immune features. However, it is still not clear why liver is abundant with these innate immune cells.

In broad sense, the innate immune in the liver is a systematic complex, including above-mentioned NK cells, NKT cells, and Kupffer cells, as well as neutrophils, eosinophils, and complement components. In this review, we mainly emphasized the potential influences of NK cells, NKT cells and Kupffer cells on liver injury and liver regeneration, as a repair process of liver injury. Some putative pathways in which hepatic innate immune cells participate leading to impaired liver regeneration are also proposed.

The roles of innate immune cells in liver injury

Up to now, nearly all of innate immune cells in the liver were reportedly to be involved in the diverse liver injury whatever

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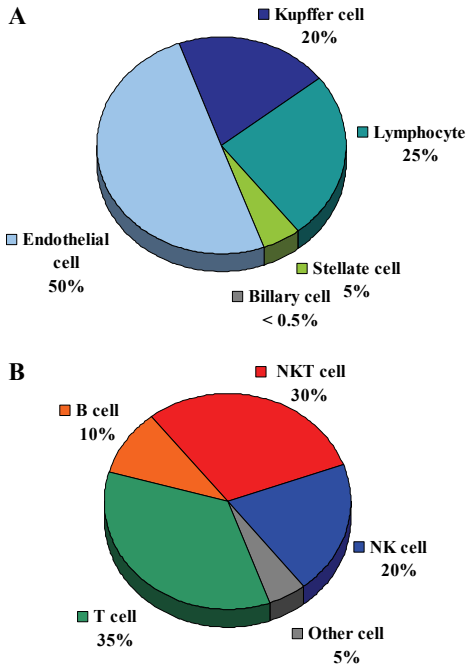

Figure 1. Predominance of innate immune cells in the liver. (A) percentages of hepatic nonparenchymal cells (including kupffer cell, lymphocyte, stellate cell and endothelial cell as well as billary cell); (B) percentages of hepatic lymphocyte subsets (including NKT cell, NK cell, T cell and B cell).

in experimental animal models or in human clinical investigations (12-14). For short of enough human liver samples and the difficulties to manipulate in humans, the accumulating findings regarding human liver injury largely concentrated on the changes of lymphocyte numbers or their phenotypes in the liver diseases and failed to elucidate the exact mechanisms of human liver diseases. These findings had not enough credibility to fully understand the roles of immune cells in liver injury. Several animal models mimicking human liver injury have emerged to be applied for exploring the immunopathogenesis in liver diseases, as the times required. Until now, popularly-used murine models mainly include Concanavalin A (Con A)-induced liver injury (15), lipopolysaccharide (LPS)-induced liver injury, alcohol consumption-induced liver injury as well as recently-reported Poly I:C-induced liver injury (11). Their underlying mechanisms in the pathogenesis of liver injury are summarized in Table 1.

NKT cells

Con A, a plant-derived lectin-like glycan, is always used as a stimulant to T cells. In 1992, Tiegs et al. reported that intravenous injection with Con A could significantly induce massive liver necrosis in mice, simultaneously with lymphocyte infiltration in the livers, high level of apoptotic hepatocytes and elevated serum ALT and AST. The liver injury could be effectively prevented by pretreatment with dexamethasone (DEX), a glucocorticoid as an immunosuppressor, indicating the liver injury was associated with immune response in the liver. The liver injury was confirmed to be $CD4^+$ T cell-mediated by using T cell-deficient mice and T cell depletion with specific antibodies (15). Later, Takeda K and Yoshikatsu K found that CD1d knockout mice, which have deficiency in NKT cells, were resistant to Con A-induced liver injury respectively (16), indicating $CD4^+$ T cells reported in Tiegs's finding might largely refer to $CD4^+$ NKT cells (17). The similar result was also obtained in $V\alpha14^{-/-}$ NKT knockout mice (36). Several apoptosis-related effector molecules, IFN-γ (19), Fas ligand (20), TNF-α (18) were found to take part in the NKT cell-mediated liver injury.

Table 1. Immune cells and liver injury

Liver injury models	Mediated-cells	Effector molecules	References
Con A-induced	T (mainly include NKT cell)	IFN-γ, TNF-α, FasL, IL-4	(15-21)
	Kupffer cell	superoxide anions; chemokines	(22)
	Neutrophil	IFN-γ, reactive oxidants	(23, 24)
	Eosinophil	IL-5, chemokines	(25, 26)
LPS-induced	Macrophage	TNF-α, chemokines	(27-29)
Poly I:C-induced	NK cell	TRAIL	(11, 30)
PEA-induced	NK cell	IFN-γ	(31)
	Kupffer cell	TNF-α, IL-18	(14, 32)
	T cell	TNF-α, perforin	(32)
Alcohol-induced	NKT cell	FasL, TNF-α	(33)
	Neutrophil	chemokines; adhesive molecules	(34)
Carrageenan-induced	NK cell	FasL	(35)
	NKT cell	FasL	(35)

In spite of detailed and ample evidence supporting the roles of NKT cells in the liver injury, there were still several unsolved questions regarding NKT cell function during Con A injection. In particular, we and others found that two to eight hours after Con A injection, $CD3^+NK1.1^+$ NKT cells dramatically went down or disappeared in quantity according to flow cytometric analysis (16). It is out of our knowledge that the reduction or absence of hepatic NKT cells induced by Con A did not influence subsequent pathological changes. There are two possibilities involved in the NKT cell number change happened at the early stage of Con A-induced liver injury. Firstly, NKT cells maybe undertake apoptosis due to activation induced death (AID), which was supported by the fact that NKT cells showed more Fas expression (16) and apoptosis-related Annexin V was elevated following Con A treatment (37). Secondly, NKT cells maybe lose their markers especially NK1.1 due to activation by Con A, which was also found in NKT activation triggered by α-galactosylceramide (αGalcer), a specific activator from sponge (38), or by anti-CD3 plus IL-2 (39), indicating the reduction of NKT cell percentage in liver was under truth, being caused by analytic approach. Further exploration of the puzzle will need to apply other methods for detecting NKT cells such as CD1d-tetramer technique, which can recognize bias NKT cell-specific TCR, Vα14 in mice and Vα24 in humans (40, 41). An alternative way to address the issue is to transfer labeled-NKT cells to recipient mice and dynamically look into the *in vivo* phenotypic changes of the transferred NKT cells.

There is another remarkable issue that unlike the ligands responding to stimulants like LPS and Poly I:C that were used in liver injury models (42, 43), any receptor for Con A still remained under veiling and was not found on the surface of NKT cells. Undoubtedly, it is difficult to understand why Con A preferentially induced liver injury and no significant pathogenesis in other organs. In view of Con A specificity in liver injury, it is indicative that using liver tissue extracts as an experimental target may be an efficient pathway in search of Con A-specific ligand through BIAcore Ligand Assays (44).

There are many reports about how to interfere with NKT cell-mediated hepatitis, and it has been found several cytokines have potential role in preventing the Con A-induced liver injury. IL-6 is a very important and widely-investigated one, which can abolish the liver injury by inhibition of NKT cells (45, 46). In addition, IL-15 is a key cytokine for NKT cell development and differentiation. IL-15 receptor has been found on NKT cells. In recent study, we found IL-15 can also alleviate Con A-induced hepatitis (47). Unlike IL-6, IL-15's protective role is mediated by inhibition of IL-4 and IL-5 secretion by NKT cells, followed by the decreased infiltration of eosinophils cells, which is also considered as an effector cells in Con A-induced liver injury. How IL-15 can decrease IL-4 and IL-5 production by NKT cells remains to explore.

In addition to Con A-induced liver injury, NKT cells are involved in the several kinds of liver injuries like alcohol consumption-induced liver injury (33), LPS-induced liver injury (48) as well as carrageenan (a food additive)-induced hepatotoxicity (35). Although NKT cells could inhibit HBV replication (49), hepatic NKT cells might be an apple of discord in human liver injury. In the liver of HCV patients, CD1d expression in liver was increased and a population of IFN-γ-producing CD1d-restricted NKT cells simultaneously accumulated, suggesting CD1d expression on liver cells is required for NKT cell activation during HCV infection (50). Although with more and more studies concerned of liver immune diseases, it was suggested that NKT cells exactly exerted their destructive roles in liver diseases (51), how NKT cells were activated during microbe infections or exposure to Con A stimulation is still a critical issue regarding NKT cell-mediated liver injury. Antigens presented by CD1d mostly are lipid. Apart from αGalcer, no natural antigens recognized by NKT cells were found from mouse or human. Recently, studies showed that exogenous and endogenous glycolipid antigens from bacteria were found to activate NKT cells during microbial infections (52, 53). The ability of CD1d to present lipid antigens is closely related with chemical features such as sugar head group and ceramide portion (54, 55). It is not clear whether antigens presented by CD1d are natural lipid or altered lipid. Are there possibilities that abnormal lipids due to HCV infection or others pathological changes are altered to be suitable for CD1d presentation?

NK cells

NK cells are always considered as a first-line effector against virus infection. Many groups have described the critical roles of NK cells in the pathogenesis of liver injury model, among which Poly I:C-induced liver injury was more helpful for understanding the roles of NK cells. Poly I:C is a double-stand RNA mimic, which can bind to Toll like receptor 3 (TLR3) expressing on antigen presenting cells (APCs) like DC and Kupffer cells. Poly I:C could significantly induce NK cell accumulation and activation in the liver (11), characterized by elevated CD69 expression. The activated NK cells could subsequently give rise to liver injury, showing local spotty necrosis in the livers and serum slight elevation of ALT and AST, in contrast to massive liver necrosis and high level of ALT and AST induced by Con A. In regard to molecular mechanisms, NK cells expressing TNF-related apoptosis-inducing ligand (TRAIL) showed strong cytotoxicity against primary hepatocytes in the liver injury (30). Although IFN-γ production by hepatic NK cells was increased, interestingly, the liver injury was absolutely independent on IFN-γ. Perforin and FasL, two of important effector molecules of NK cells, were also confirmed to be dispensable in the NK cell-mediated liver injury. Compared with Con A-induced liver injury that has the feature of autoimmune hepatitis, Poly I:C-induced liver injury could be identified to be of virus hepatitis on the base of the pathological characteristics. It had always been thought that for absence of TLR3 on NK cells, Poly I:C could not directly activate NK cells, therefore, it was speculated that Poly I:C-induced NK cell activation and accumulation must rely on TLR3-expressing cells like APC cells. We found that NK cell

activation and subsequent liver injury was absolutely dependent on Kupffer cells-produced interleukin 12 (IL-12), indicating that Poly I:C-induced Kupffer cell activation was an initial event for the liver injury. In other word, NK cell function may be aggravated by a chain of events delivered by Kupffer cells. However, with Toll-like receptors 3 and 9 found on human NK cells, Poly I:C was able to directly trigger NK cells by TLR3, when exposed to DC-derived IL-12. Upon interaction with their ligands TLR3 activated NK cells by inducing both cytolytic activity and cytokine production (56). Moreover, Poly I:C pretreatment could significantly up-regulate TLR3 expression on NK cell surface, and NK cell activation was APC-independent (57). Whether Poly I:C directly triggers NK cells in the liver injury needs to be further investigated.

The roles of NK cells in the pathogenesis of liver injury were also found in other studies like Pseudomonas aeruginosa exotoxin A (PEA)-induced hepatotoxicity (31), LPS-induced liver injury (58) and carrageenan-induced hepatotoxicity (35). Owing to shortage of NK cell-deficient mice, most of supportive data were obtained from NK cell-depleted mice treated with antibody against asialo-GM1 (AsGM1), but since ASGM1 was also found on activated $CD8^+$ T cells and $CD4^+$ T cells, anti-ASGM1 antibody administration could result in the removal of 90% of virus-specific $CD8^+$ T cells and 50-80% of virus-specific $CD4^+$ T cells (59), so the exact roles of NK cells need to further confirm.

Although some available data from SCID mice with only NK cells and perforin deficient mice that have defect in NK cells function supported that NK cells were dispensable in Con A-induced hepatitis, the roles and functions of NK cells have not been investigated in detail, especially why NK cells is not required for the liver injury is enigmatic. As we have known, high level of serum IFN-γ produced by NKT cells was found in Con A-induced hepatitis and IFN-γ was absolutely necessary in the process of the model, which was confirmed in IFN-$\gamma^{-/-}$ mice (60) or IFN-γ-neutralized mice (19). Previous studies also revealed that IFN-γ derived from NKT cells could subsequently activate NK cells in many cases, like αGalcer-activated NKT cells. Resultant activated NK cells also synergized with NKT cells to take part in NKT cell-mediated anti-tumor (61). Hesitatingly, why can not IFN-γ produced in Con A-induced liver injury trigger NK cells to exacerbate the liver injury? Recently, the inhibitory role of IFN-γ on NK cell activation receptor expression was observed by our group (62). We further found that high level of IFN-γ triggered by Con A injection *in vivo* inhibited NK cell function and high level of recombinant IFN-γ also *in vitro* suppress the transcription of NKG2D and effector molecules that mediated cytotoxicity (Dong Z, et al. unpublished work). Consistently, the inhibitory role of IFN-γ was supported by the impaired NK cell cytotoxicity in IFN-γ-transgenic mice (63).

Beyond the destructive effect of activated NK cell in above-motioned models, NK cells are also found to prevent formation of liver fibrosis. During CCL4 induced-liver fibrosis, NK cells have an ability to kill activated stellate cells which have increased NKG2D ligand Rae-1, a stress-inducible molecule, in addition, TRAIL is also responsible for the NK cell cytotoxicity (64). So NK cell role is very complex, and largely dependent on models used.

Kupffer cell is an important innate immune cell for initiation of several hepatitis models. There are several TLRs distributed on the surface of Kupffer cell, with which Kupffer cells sense the TLR ligands such as Poly I:C and LPS to indirectly or directly recruit and trigger other innate immune cells to further amply the downstream event in the liver injury. Moreover, the mutual regulation of TLR on the Kupffer cells by their ligands was recently found (65). When TLR3 ligand pretreatment can alleviate subsequent liver injury induced by TLR4 ligand, LPS, in which the underlying mechanisms is Poly I:C treatment can down-modulate TLR4 expression on Kupffer cells. This is first to describe the crosstalk of TLRs on Kupffer cells.

For there are many review articles collectively pertained to roles of Kupffer cells in liver injury (66, 67), the review do not give unnecessary details to their parts.

In liver injuries, innate immune cells interplay each other or affect other adoptive immune cells. NKT cell-derived IFN-γ could aggravate the liver injury caused by NK cells (68). Recently, we found that if mice were treated with Poly I:C prior to Con A injection, the subsequent liver injury was significantly alleviated under the mechanism that NK cells participate (69). If mice were primed with nontoxic dose of Con A before injection, Poly I:C-induced NK cell-mediated liver injury would be exacerbated, ALT from 200 to about 3,000 U/L and histological manifestation from spotty necrosis to massive necrosis, showing the NK cells could synergize with NKT cells in the liver injury (70). NKT cells are highly considered as a bridge between innate immune and adoptive immune (71), further analysis of role of NKT cells in acquired immunity like specific cytotoxic T lymphocyte (CTL)-mediated liver disorder will be beneficial to comprehend the regulatory role of NKT cells in the liver. In addition, NK cells could facilitate T cell-mediated liver injury to virus infection (72). Therefore, systematic analysis of innate immune cells in the liver and their interactions will be helpful for understanding immunopathogenesis of liver diseases.

In contrast to the roles of innate immune cells in liver injury, how innate lymphocytes repopulate during liver regeneration and how they exert their regulatory mechanism in liver repair such as liver regeneration lack of detailed investigations.

Repopulation of innate immune cells during liver regeneration

As recorded in ancient Greece myth, thousands years ago, Zeus punished Prometheus for his stealing light for humankind and sent an eagle to eat his liver everyday. Interestingly, the damaged liver could be repaired itself in the following day. This may be the oldest record of liver

regeneration (73). It has been known that tissue repair will operate in response to physical, chemical, or biological injuries of parenchymal organs, including trauma, surgery, nonphysiologic temperature, intoxications, infections, or cancer. Tissue regeneration is always one of most common processes, however, in solid organs, only liver can grow to its natural size and perform its normal functions. Now, experimental models for the investigation of liver regeneration available to the research worker mainly induce liver growth through partial hepatectomy (PH, often refer to 70%) or injection of assorted chemical substances, such as tetrahydrochloride, D-galactosamin (74), acetaminophen (75), Con A (76) or ethanol (77). Liver regeneration triggered by PH in rodents has been a commonly used experimental model in studies of liver growth regulation (78). When liver reaches its initial size, regeneration rapidly stops.

Regeneration of the liver is a pathophysiological process, embracing both hypertrophy and hyperplasia. The former refers to increase in cell size or protein content in the pre-replicative phase and the latter refers to increase in cell numbers. How hepatic non-parenchmal cells repopulate is still unknown. Previous studies have revealed that two peaks of non-parenchmal cells number were found at around 12 hours and 36 hours after PH respectively. Among these non-parenchmal cells, mechanisms of repopulation involving Kupffer cells have partially been investigated. Kupffer cells account for about 20% of hepatic non-parenchmal cells. The restoration of Kupffer cells after liver resection or chemical injury contributed mainly to self-renewal and recruitment. Taking account of the cell cycle of hepatocyte proliferation, the peak occurring at 12 hours after PH may contribute to recruitment from peripheral lymphoid tissue or blood, whereas the peak at 36 hours may be due to cell division. Early investigation by Widmann JJ, et al. found the peak mitotic activity of Kupffer cells occurred at about 48 hours after conventional two-thirds PH, indicating that Kupffer cells are capable of dividing locally in the liver. The repopulation of Kupffer cells by local cell division may take the leading key element in the liver regeneration. Although many groups give a brief description about NKT and NK cells accumulation after hepatectomy, the mechanisms of NK and NKT cells repopulation are an untouched filed. Liver is a primitive organ with hematopoiesis at the early fetus and still remains the capability of hematopoiesis in adult individuals (79). Liver is considered as a developmental site for non-classical NKT cells and these NKT cells can be found in the liver of thymus-disrupted mice (80, 81). Hematopoietic stem cells (HSCs) in the liver have the potential ability to differentiate into several kinds of lymphocytes including NK cells (79, 82, 83). These facts raise the possibility that local self-renewal may be a main source of repopulated NK cells and NKT cells. Analysis of NK cells resident in the regenerating liver revealed NK cells were continuously in an appearance with inhibitory features throughout the entire period of liver regeneration (from 3 h to 14 days), manifested by reduced natural cytotoxicity against YAC-1 cell as well as antibody-dependent cytotoxicity against P815 cell (84). There are two possibilities regarding the reason why NK cells are suppressive during liver regeneration. NK cells are truly inhibitory due to cytokines such as TGF-β and HGF releasing by stellate cells (85), or NK cells are under naïve stage or early developmental stage with immature function owing to cell division triggered by PH (86). For little is known about those NKT or NK cell-specific early markers of during development, it is difficult to clearly identify them as immature cells. Future extensive investigations of their functional and phenotypic characteristics will be helpful for understanding their repopulation mechanisms during regeneration. In recent study, CD1d reactive NKT cell migration was impaired in CXCR6 ligand gene-ablated mice (87), implying the chemokine is important for liver NKT cell recruitment or trafficking (88). In addition, sympathetic nerve excitation with surgical operation can act also as promoting factors in NKT cell repopulation (89).

Negative regulation of liver regeneration by innate immune cells

Liver regeneration is a multistep process and can be approximately divided into priming pathways, growth-promoting pathways and growth-inhibitory pathways. Priming factors are responsible for rendering hepatocytes sensitive to growth factors, and growth factors in the growth-promoting pathways make above competent hepatocytes progress through cell cycles G0 to DNA synthesis S phase. Growth inhibitors refer to cytokines, which can suppress hepatocyte proliferation. Many cytokines [IL-6 (90), TNF-α (91)] or circulating factors such as hepatocyte growth factor (HGF) (92) and epidermal growth factor (EGF) (93, 94) or cells [Kupffer cells (95) and Ito cells (96)] are involved in these complex processes. Abnormalities of these factors sometimes lead to decreased or delayed liver regeneration, which has been postulated as one of the major factors for the high mortality among patients with fulminant hepatitis.

In contrast to relatively well-defined understanding of the effects of growth factors, hormone and growth inhibitors in liver regeneration, there are limited investigations focusing on the immune cells in the liver such as lymphocytes and macrophages in the liver regeneration in recent decades. From available data, the relationship between immune responses and liver regeneration seemed to be highly complex. On the one hand, regenerating liver cells could stimulate immune system, as evidenced by the fact that lymphocyte activation was found *in vivo* during liver regeneration after PH. In syngeneic mixed hepatectomized liver cell-lymphocyte culture, the stimulating activity of regenerating liver cells is lost by the pretreatment of them with anti-Ia monoclonal antibody plus complement or the removing Kupffer cells from them, indicating Ia^+ Kupffer cells might play an important role as stimulators in the DNA synthetic responses, on the other hand, activated immune cells during liver regeneration could also restrain regenerating liver cells. Many data from different groups indicated immunosuppressor like FK506 and cyclosporine (CsA) could significantly enhance the liver growth after PH

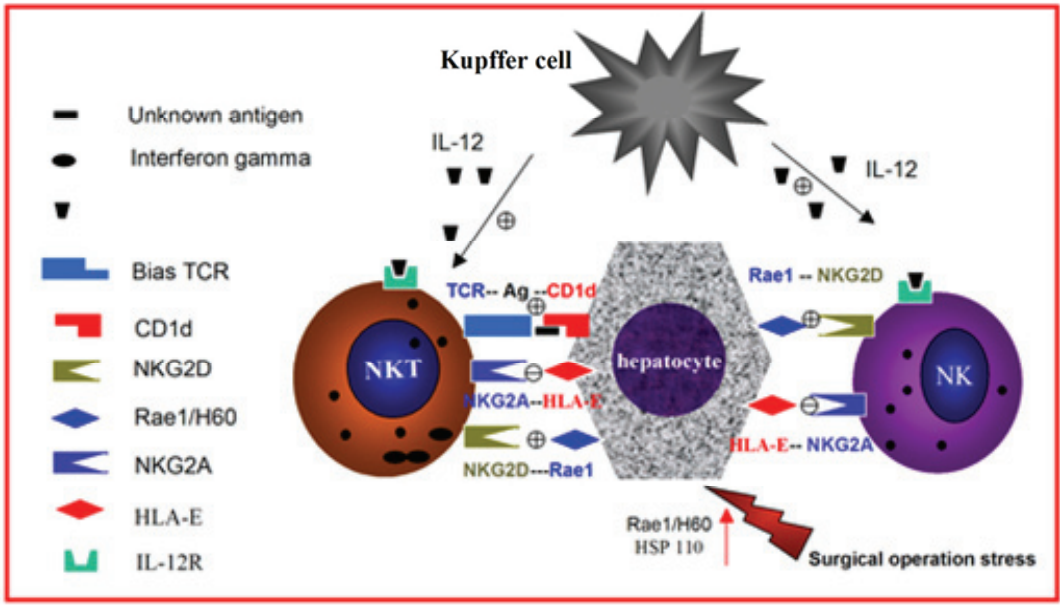

Figure 2. Candidate mechanisms of NK cell and NKT cell activation during liver injury and regeneration. During the occurrence of liver injury and regeneration, NK cell, perhaps NKT cell function is regulated by the balance between the inhibitory signaling and activating signaling. The infiltrated NK cells or NKT cells may be activated or inhibited by the potential pathways. Firstly, NKG2D-mediated activating signaling can trigger hepatic NK cell responses *via* the engagement with NKG2D ligand on hepatocyte, MICA in human, or Rae-1 in mice. Secondly, several inhibitory signalings caused by inhibitory NKG2 family member like NKG2A and KIR through binding with classical or non-classical MHC-I dampens NK cell function. Thirdly, stress-inducible molecules during liver diseases such CD1d, an MHC-I like molecule with an ability to present lipid antigen to NKT cell, activate NKT cells. In general, soluble cytokines including IL-12 are also involved in innate immune cell activation and enrichment in the liver.

(97). In addition, C3a and C5a, two potent inflammatory mediators of innate immune responses, contribute essentially to the early priming stages of liver regeneration (98). Although the interplaying relationship between liver regeneration and immune system seemed obscure, these available results gives a possible clue that immune system especially liver-resident innate lymphocytes maybe take part in the liver regeneration after PH as well as induction of liver injury.

NK cells

NK cells are well-defined effector innate immune cells during liver regeneration. Two decades ago, mice that received IL-2 treatment for long term suffered from deduced liver size and weight (99). Later it has been found that transfer with LAK cells also inhibited liver regeneration in mice (100). These findings were suggestive that NK cells may be a factor of liver size modulation. The roles of NK cells during liver regeneration were revealed until last decade (101, 102). NK cells dynamics and functional characteristics during liver regeneration were described in detail, and the negatively -modulated effect of NK cells on regeneration was confirmed by NK cell depletion with a specific antibody against NK1.1, which distributed on NK cells and NKT cells. Afterward, many suppressors such as FK506 and CsA (103), or growth factors such as ALR and HGF were found to improve liver regeneration through inhibiting NK cell functions (104-106). Liver NK cell dynamics and cytotoxicity during liver regeneration in C3H/He mice were demonstrated to vary depending on age, and the appearance of an increased number of NK cells in the liver seemed to coincide with the slowing of the rapid increase of murine liver weight. These results raise the possibility that liver NK cells might be responsible for regulating hepatocyte growth (102). Recently, Gao B's group reported in detail that in normal condition hepatic NK cells exerted their inhibitory roles during regeneration, and after NK cells were activated by Poly I:C or MCMV infection, the liver regeneration was severely attenuated. IFN-γ produced by NK cells might be an effector molecule in the impaired liver regeneration by activating signal transducer and activator transcript (STAT) 1, which could suppress STAT3 signaling that is a necessary signal pathway in liver regeneration (106, 107). In our lab, we performed PH in severe immunodeficiency disease mice (SCID), which embrace only NK cells and defect in T cells including NKT cells, and found liver regeneration was not attenuated but enhanced to some extent in contrast to wild mice, demonstrating that apart from NK cells, there are other immune cells involved in impaired liver regeneration. About the inhibitory roles of NK cell during liver regeneration, there are many unsolved question about NK cell activation during regeneration. In general, NK cells exert their function by releasing many cytokines such as IFN-γ or direct cytotoxicity against hepatocytes. How NK cells decide to

deal with target cells was dependent on two classes of NK cell receptors, inhibitory and activating. Although some inhibitory and activating NK cell ligands are expressed in normal hepatocyte, distinct characteristics of regenerating hepatocytes especially NK receptor-matched ligands need to identify. In general, stress-induced ligand, MHC class I chain-related (MIC) A, MICB, and UL16-binding proteins are expressed on the proliferating cells (108). For surgical operation is an intense stress for mice, there is possibility that stress-inducible NKG2D ligands are strongly up-regulated or the inhibitory signals delivered by regenerating hepatocytes are decreased leading to the bias to activating signaling and over-activated NK cells during regeneration. Hepatocytes also cover with ligands of NK cell mediated-apoptosis-related effector such as FasL and TRAIL, so evaluation of NK cell receptors and ligands on regenerating hepatocytes will benefit to understanding the inhibitory role of NK cells during liver regeneration (Figure 2).

NKT cells

Casting back previous findings regarding the role of NK cell in liver regeneration, when depletion of NK cells with anti-NK1.1 antibody could accelerate regeneration in normal condition and the promoting role was considered to ascribe NK cells at that time (84). As we have known, anti-NK1.1 antibody treatment could clear both NK and NKT cells. Coincidentally, NKT cell accumulation in the liver at the early stage of regeneration was found and even the number of NKT cell doubled in 12 hours after PH. The NKT cell accumulation in the liver during regeneration was related with sympathetic nerve excitation, and inhibition of the effect with propranolol (PPL), a β-antagonist, could deprive of the NKT cell accumulation (89). In 1999, injection with IL-12 for long term could markedly impaire liver regeneration, but its cellular mechanism remained unknown at that time (109). Ito H, et al. revealed that IL-12-induced impaired liver regeneration was caused by the accumulation of NKT cells and $CD1d^{-/-}$ NKT deficient mice could resist the impaired liver regeneration. IFN-γ-modulated TNF-α production contributed to the impaired liver regeneration (110). So from available data, we can conclude that NKT cells may be a negative modulator in the process of liver regeneration, which was also supported in other NKT cells deficient such $V\alpha14^{-/-}$ mice and SCID mice. Although the inhibitory role of NKT cells during regeneration appeared to be clear, roles of NKT cells in the context of liver diseases such as virus infection remain under investigations. Recently, we used HBV transgenic (HBV-Tg) mice that contained part of genome including Pre-S, S and X protein as a murine model for HBV infection, and on the basis of unpublished data we found liver regeneration in HBV-Tg mice was impaired in contrast to wild-type mice. NKT cells mainly including $CD4^+$ NKT cell and DN NKT cells were recruited or accumulated during the early stage of liver regeneration and the elevated NKT cells produced more IFN-γ and expressed more CD69, which is an early activation marker. Either depletion of NKT cells or neutralization of IFN-γ could significantly improve the liver regeneration in HBV-Tg mice. NKT cell activation was dependent on the increased CD1d expression on the liver cells that was only found in hepatectomized HBV-Tg mice.

Kupffer cells

Kupffer cells can produce many kinds of cytokines, anti-proliferative and pro-proliferative, on which liver regeneration lies. On the basis of these cytokines, Kupffer cells have the potential to exert both stimulatory and inhibitory influences on hepatocyte proliferation (111). As a whole, for short of specific surface marker to distinguish Kupffer cells and specific antibody to deplete them, the data about their exact roles in the liver regeneration seemed to be controversial. The stimulatory role of Kupffer cells was confirmed by the fact that Kupffer cell depletion by dichloromethylene diphosphonate (CI2MDP)-liposomes alters hepatic cytokine expression and delays liver regeneration after PH (95), while their inhibitory roles were proved in murine following selective Kupffer cell depletion by gadolinium chloride ($GdCl_3$) injection (112, 113). The controversial results may be due to the difference of two agents in stimulating Kupffer cells to produce liver-growth cytokines.

Kupffer cells exert their stimulatory effect on liver growth mainly by releasing two pro-proliferative cytokines, IL-6 and TNF-α, which were considered to be the initiators of progression of liver regeneration. IL-6 is a multifunctional proinflammatory cytokine involved in a variety of host defenses and pathologic processes including liver diseases and largely secreted by macrophages like Kupffer cells in the liver. IL-6 acts on the cell by binding the IL-6 receptor chain (IL-6R, also known as gp80), inducing dimerization of the gp130 receptor and subsequent intracellular signaling partners. In the early phase, IL-6 was elevated in the liver after liver excise. Currently, IL-6 has been identified as an absolutely necessary mediator of liver regeneration, with IL-6-disrupted mice displaying markedly abnormal (90). Although there are many liver cells like sinusoidal endothelial cells capable of releasing IL-6 with liver regeneration, it is generally acknowledged its main source was dependent on Kupffer cells (114). Using $ICAM\text{-}1^{-/-}$ mice, Selzner N, et al. found that after PH, ICAM-1 expression in the hepatic sinusoids and leukocyte recruitment triggers a local inflammatory response leading to Kupffer cell - dependent release of TNF-α and IL-6 (115). IL-6 has been demonstrated to activate primarily STAT3. Hepatocyte-specific ablation of STAT3 expression results in impaired regeneration after PH despite intact mitogen activated protein kinase (MAPK) signaling and compensatory activation of STAT1, suggesting that STAT3 is required for cell cycle progression and cell proliferation after PH (116). TNF-α plays multiple roles in the host defense and is mainly produced by macrophages. TNF-α is not dispensable in the early stage of liver regeneration. Mice lacking the TNF receptor I subunit or pretreated with antibody against TNF-α displayed abnormal and decreased liver regeneration in PH model (117). This deficiency could be overcome by extra supply with recombinant IL-6 (91, 118). It is indicative that TNF-α may function as promoting IL-6 release. *In vitro*

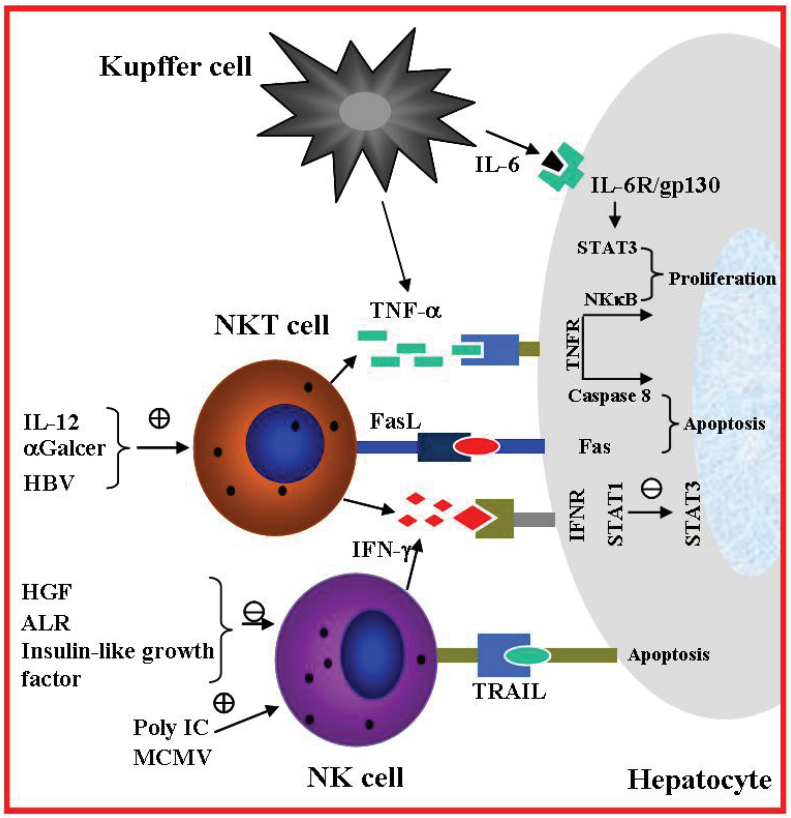

Figure 3. The regulatory effect of innate immune cells on the liver regeneration. Liver is abundant with NK cells, NKT cells and kupffer cells. Kupffer cells play multifunctional roles in the liver regenertion. On the one hand, at the early stage of liver removal, Kupffer cells can produce a large mount of priming cytokines such as IL-6 and TNF-α to promote the initiation of liver growth *via* activating STAT-3 and NF-κB pathway, respectively. On the other hand, Kupffer cells can secrete proinflammtory cytokines mainly TNF-α and TGF-β to inhibit hepatocyte proliferation or induce hepatocyte apoptosis. Liver NK cell can inhibit liver growth in normal condition or upon MCMV viral infection through releasing cytokines. NK cell function is also elaborately suppressed by hepatocyte growth factor (HGF) and augmenter of liver regeneration (ALR). TRAIL-expressing NK cells perhaps exert direct cytotoxicity against regenerating hepatocytes. In the condition of HBV infection or IL-12 repeated administration, NKT cells will accumulate in the liver and then mediated negative regulation of liver growth via a manner similar to NK cells.

experiments also show TNF-α could trigger cultured hepatocytes proliferation. TNF-α could also activate NF-κB and immediate early genes, suggesting that it may function as a priming cytokine that make the resected liver sensitive to subsequent growth factors like HGF.

Kupffer cells exerted their negative regulation on liver repair by release of inhibitory materials. The latter mainly included IL-1α/β, TGF-β and TNF-α (119). In contract to positive effect of TNF-α in the early liver cell proliferation, injection of TNF-α at high doses can inhibit hepatocyte proliferation after early stage of liver regeneration through induction of TGF-β (120).

Several kinds of TLRs are distributed on the surface of Kupffer cells and sense pathogen-associated molecular patterns (PAMPs) like LPS and Poly I:C. TLRs bridge innate and adaptive immunity by activation of APCs (121). Previous studies have shown that TLR4 was crucial for Kupffer cell activation and LPS-liver injury (66, 122). Recently, TLR4 signaling in liver regeneration was explored in CCL4-induced liver injury. Compared with wild-type mice, TLR4-mutant mice failed to restore its normal liver function (123). Moreover, an intracellular adaptor molecule of TLR signaling, myeloid differentiation factor 88 (MyD88), was found to be critical for liver regeneration after PH, indicating that Kupffer cells may influence liver regeneration through TLR signals, including MyD88, which drives transcriptional activation of genes including pro-inflammatory cytokine. Since TLR signaling often interaction with several signaling in liver regeneration, in particular, NF-κB, TLR signaling will become a frontier highlight.

In conclusion, nearly all of innate immune cells play an inhibitory role in the process of liver regeneration except for Kupffer cell's initiating regeneration at the very early stage by secreting IL-6 and TNF-α (Figure 3). The final consequences of liver regeneration rely on the balance of growth-promoting capacity and growth-inhibitory capacity. During growth-promoting pathway, the pro-proliferating power is so strong that the innate immune cell power seems

to be no use, but with the liver reaching its original size and function, the pro-proliferating power is gradually reduced and the innate immune cells limit the liver growth. If these innate immune cells are over-activated on some circumstances like virus infection, they will destroy the normal regeneration by releasing cytokines or direct cytotoxicity. Therefore, the liver growth was elaborately regulated by innate immune system in the liver.

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