Role of NKT Cells in the Digestive System.

I. Invariant NKT cells and liver diseases: is there strength in numbers?

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tion regarding the functional role of the innate immune T cell, invariant natural killer T (iNKT) cells, in the pathophysiology of liver diseases continues to emerge. Results from animal studies suggest that iNKT cells can have divergent roles by specifically promoting the development of proinflammatory or anti-inflammatory responses in liver diseases. In this themes article, I discuss the critical evidence from animal models that demonstrate a vital role for iNKT cells in the pathophysiology of liver diseases with emphasis on viral, autoimmune, and toxin-induced liver diseases. Furthermore, I discuss the controversial issues (including iNKT cell apoptosis) that typify some of these studies. Finally, I highlight areas that require additional investigation.

invariant natural killer T cells; hepatitis; liver; innate immunity; Th1/Th2 cytokines; apoptosis

Invariant NKT cells are usually defined as unconventional T cells that share features of both conventional T cells (i.e., CD4+ and CD8+ T cells) as well as natural killer (NK) cells and are an important component of the innate immune system. iNKT cells were initially identified about 20 years ago (6); however, their role in regulating the immune response remained incompletely understood in part due to the lack of specific cell surface markers for identifying these cells. In addition, synthetic and/or physiological ligand(s) that activate these cells remained elusive. Prior to 1997, fewer than 20 papers were published on iNKT cells, whereas in the last decade more than 1,100 manuscripts have been published. A major reason for this level of activity has been the identification of specific cell surface markers and ligand(s) for these cells, which for the first time provided investigators with the means to identify and determine the function of iNKT cells in immune responses. Specifically, in 1997, a synthetic glycolipid (originally derived from a marine sponge), known as α-galactosylceramide (α-GalCer) was found to be a potent and specific activator of both mouse and human iNKT cells, demonstrating the striking conservation of this immune recognition system (11). Although several potential physiological ligands for iNKT cells have been proposed (12, 15), the specific endogenous ligand(s) is presently unknown (19).

In recent years, the popular definition that iNKT cells are lymphocytes that distinctly express both the T cell receptor (TCR)α/β and NK cell receptor (such as NK1.1) is now viewed as being inaccurate and potentially flawed for the following reasons: this definition was initially based on the notion that 1) all NK1.1/TCRα/β double positive cells are iNKT cells and 2) all iNKT cells express NK1.1. However, many studies have reported that the iNKT cells in some mouse strains (including BALB/c and 129) do not express NK1.1 (6, 14). Furthermore, some conventional T cells (i.e., CD4+ and CD8+ T cells) spontaneously express NK1.1 upon activation (6). Currently, the common consensus is that classical iNKT cells are innate immune T cells that 1) express an invariant Vα14Jβ11 TCR α-chain predominantly paired with β chain biased toward Vβ8.2, Vβ7, or Vβ2 in mice (Vα24Jα18 TCR α-chain paired with Vβ11 in humans); 2) recognize pathogen-derived or self-derived glycolipid antigens presented by the MHC class I-like protein, CD1d (an apparent contrast to conventional T cells, which are activated by peptide antigens presented by MHC class I or II); 3) are reactive to the synthetic ligand α-GalCer; and 4) can be identified flow cytometrically by specific cell surface markers, α-GalCer-CD1d tetramer (and potentially by the newly characterized α-GalCer-CD1d tetramer analog, PBS57-CD1d tetramer).

The discovery of α-GalCer as a synthetic ligand for iNKT cells has played an extraordinary role in characterizing both the mechanism of iNKT cell activation, as well as the function of these cells in various disease states. The established dogma is that α-GalCer bound to CD1d activates the TCR on iNKT cells in mice and humans (6, 14). In parallel, many studies have used α-GalCer to demonstrate that activated iNKT cells (via Th1 and Th2 cytokine release) suppress autoimmune, inflammatory, and infectious disease responses by dampening the recruitment of neutrophil, NK cells, and conventional T cells to sites of tissue injury (6, 22). Furthermore, iNKT cells, as part of the innate immune system, are activated very early in the course of an immune response, and these cells subsequently activate several cell types, including NK cells, neutrophils, macrophages, dendritic cells, and conventional T cells (6, 22). Thus iNKT cells “link” the innate and adaptive arms of the immune response (6, 22). It is noteworthy that 1) the T cell mitogen [i.e., concanavalin (Con) A] and 2) anti-CD3 mAb, both acting via TCR and IL-12 (acting via IL-12 receptor), have been shown to directly activate iNKT cells, resulting in Th1 and/or Th2 cytokine production (1, 4).

Compelling evidence from many studies using the αGalCer-CD1d tetramer has determined that the liver contains the highest frequency of resident iNKT cells, accounting for 10–30% of murine liver lymphocytes, whereas the frequency of resident iNKT cells in the thymus, lung, colon, bone marrow, spleen, lymph node, and blood is low (i.e., <3%) (1, 14). Of

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note, iNKT cells, like other immune cells (such as conventional T cells, NK, and dendritic cell), is localized in the liver sinusoids. The tissue distribution of iNKT cells in humans has not been as extensively studied, although they are clearly lower (<5%) than in mouse liver. The reason for the low-level expression of iNKT cells in human liver is unknown, but the abundance of CD1d on murine hepatocytes and the low expression of CD1d on human hepatocytes could be an underlying factor. The significance of varied iNKT cell frequencies in mouse and human liver is not known. Nonetheless, according to Matsuda et al. (14), “human and mice iNKT cells react to the same glycolipid antigen (α-GalCer), which is presented by CD1d; therefore, studies that characterize crucial components of mouse iNKT cell biology will also prove important for human iNKT cells.” Due to the abundance of these cells in the mouse liver, this immune organ is now widely and routinely used to explore, characterize, and understand better the role of iNKT cells in immune responses (i.e., in nonpathological settings).

The last couple of years have witnessed an explosion in knowledge regarding the role of iNKT cells in the pathogenesis of liver diseases. iNKT cells have been implicated in the pathophysiology of almost all liver diseases examined to date (discussed below). In fact, it has been proposed that the enrichment of the liver with iNKT cells may underlie the critical pathophysiological role of these cells in many liver diseases, an indication, perhaps, that there could be strength in numbers? In this brief themes review article, I will provide a current opinion on the role of iNKT cells in liver diseases (with emphasis on data obtained from animal studies) and highlight areas that require additional investigation.

**Divergent Role of iNKT Cells in Liver Diseases**

Liver diseases are broadly divided into three main categories, namely viral (including hepatitis B and C infections), toxin (e.g., alcohol and drug), and autoimmune liver diseases. Globally, liver diseases jointly affect at least 500 million people. Although genetic factors have been associated with the development of liver diseases, the concept that the cellular immune response plays a critical role in the etiology and pathophysiology of liver diseases is now widely accepted. For example, there is ample evidence demonstrating the relevance of iNKT cells in the pathophysiology of liver diseases (summarized in Table 1). A unique feature of iNKT cells is the ability of these cells to secrete both Th1 cytokines (such as IFN-γ) and Th2 cytokines (including IL-4) on activation; as a result, activated iNKT cells can have disparate and, in some cases, paradoxical functions in liver diseases. Specifically, iNKT cells can exert beneficial or detrimental effects during liver injury (initiated by toxin, autoimmunity, or viruses) by selectively promoting (through Th1 or Th2 cytokine release) the development of either proinflammatory or anti-inflammatory responses. With emphasis on results obtained from animal studies, a current understanding of the role of iNKT cells in liver diseases will be discussed under the two subheadings *Proinflammatory effects of iNKT cells and Anti-inflammatory effects of iNKT cells.*

**Proinflammatory effects of iNKT cells.** There is convincing evidence from preclinical studies that iNKT cells exert proinflammatory/pathogenic effects during liver injury, mediated by toxins (i.e., drugs and alcohol) and autoimmunity (depicted in Table 1).

**Toxin-induced hepatitis.** Administration of ethanol (an alcohol) or acetaminophen (a drug) into mice provides the best characterized experimental models to evaluate the role of iNKT cells in the pathophysiology of toxin-induced liver diseases. Several lines of evidence support the notion that iNKT cells play a critical proinflammatory role in liver injury initiated by the toxins ethanol and acetaminophen. First, murine liver injury initiated by ethanol or acetaminophen administration is associated with increased hepatic recruitment of iNKT cells (13, 16). In addition, increased iNKT cell infiltrates are observed in the peripheral blood of patients that abuse alcohol. Secondly, early liver injury mediated by ethanol administration is delayed in iNKT cell-deficient mice [i.e., Jα18 knockout (KO) mice] compared with wild-type (WT) mice that received a similar treatment (16). Thirdly, antibody depletion of iNKT cells protected mice from acetaminophen-induced liver injury (13), and a similar observation was observed in mice lacking iNKT cells (13).

<table>
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iNKT, invariant natural killer T; α-GalCer, α-galactosylceramide; Con A, concanavalin A; AICD, activation-induced cell death; WT, wild type; KO, knockout; mCMV, murine cytomegalovirus; LCMV, lymphocytic choriomeningitis; TCR, T cell receptor.

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As discussed previously, the conventional view is that iNKT cells secrete an array of Th1 cytokines (including IFN-γ and TNF-α) and Th2 cytokines (such as IL-4, IL-13, and IL-10) on activation, an effect that regulates the immune response. In agreement with this notion, the key cytokine released by activated hepatic iNKT cells during ethanol-induced hepatitis has been shown to be TNF-α (16). Thus a potential mechanism by which activated hepatic iNKT cells could contribute to the pathology of liver injury mediated by ethanol administration is via TNF-α secretion. Interestingly, during ethanol-induced liver injury, hepatic iNKT cells are not directly activated by ethanol, but this toxin increases the susceptibility of hepatocytes to the cytotoxic effects of mediators (i.e., TNF-α, as well as Fas) that are expressed by the activated hepatic iNKT cells during the hepatic inflammatory response (16). Moreover, the ability of cytokines (such as TNF-α, IL-4, and IFN-γ) and the death receptor, Fas, to promote hepatocyte cell death is well documented. In view of their observation, Minagawa et al. (16) speculated that the following scenario may underlie hepatic iNKT cell activation during ethanol-induced liver injury: 1) ethanol induces fatty liver to produce glycolipids, which provide ligands for CD1d on antigen-presenting cells, this in turn induces the activation of hepatic iNKT cells; 2) alternatively, an increase of inflammatory cytokines by activated resident hepatic cells (such as hepatocytes, kupffer cells, or endothelial cells) after alcohol consumption could be responsible for the activation of hepatic iNKT cells.

In the case of acetaminophen-induced liver damage, iNKT cells can exert proinflammatory effects in this model of toxin-induced liver injury, however, only in specific conjunction with hepatic NK cells (13). Specifically, Liu et al. (13) reported that selective or sole depletion of hepatic NK or iNKT cells does not ameliorate acetaminophen-induced liver injury, but the depletion of both hepatic NK and iNKT cells severely impaired liver injury caused by acetaminophen administration. Although Liu et al. (13) did not determine whether acetaminophen can directly activate hepatic iNKT cells, the ability of both cell types to exert proinflammatory effects during acetaminophen-induced hepatitis was largely attributed to the induction of the Th1 cytokine, IFN-γ, by these cells (13). Besides, IFN-γ KO mice are resistant to liver damage mediated by acetaminophen administration (13). Furthermore, the depletion of hepatic NK and iNKT cells also suppressed hepatic chemokine expression during acetaminophen-induced hepatitis (13). Therefore, it was postulated that hepatic NK and iNKT cells could potentially induce chemokine production from resident liver macrophages (i.e., Kupffer cells) whereby IFN-γ released by hepatic NK and iNKT cells subsequently activates Kupffer cells to produce chemokines. Chemokines may recruit additional leukocytes that sustain/propagate/mediate the hepatic injury. Moreover, the role of chemokines in promoting leukocyte recruitment to sites of tissue injury is well recognized (1).

Autoimmune hepatitis. Autoimmune diseases such as multiple sclerosis and diabetes have well-established animal models. However, establishing a reliable and reproducible animal model(s) for autoimmune hepatitis (AIH) that is antigen specific has proved much more difficult. A standard animal model of AIH does not exist, but many of the important insights regarding the role of the cellular immune response in the pathophysiology of AIH has been studied using the murine model of T cell-mediated hepatitis induced by Con A. Although the Con A hepatitis model is not truly regarded as a model of autoimmunity because there is no antigen specificity, this well-characterized murine model of experimental T cell-mediated hepatitis is known to mimic many aspects of human AIH. Many studies have convincingly implicated iNKT cells in the pathology of AIH induced by Con A. First, the administration of Con A into mice caused the activation of hepatic iNKT cells (as shown by high levels of IL-4 and low levels of IFN-γ), and the subsequent apoptosis of these cells by a mechanism known as activation-induced cell death (AICD) (1, 21). Secondly, depletion of hepatic iNKT cells in Con A-treated mice or the administration of Con A into iNKT cell-deficient mice (i.e., CD1d KO or Jα18 KO mice) all severely impaired liver damage mediated by Con A (10, 21). Additionally, the adoptive transfer of iNKT cells isolated from healthy WT mice into healthy CD1d KO or Jα18 KO mice restored hepatic injury mediated by Con A (10, 21). The precise mechanisms by which hepatic iNKT cells contribute to the pathogenesis of Con A-induced hepatitis was documented in a series of elegant studies by Kaneko et al. (10). Specifically, Kaneko et al. (10) demonstrated that Con A activates hepatic resident iNKT cells to produce IL-4; IL-4, in turn, acts on iNKT cells in an autocrine fashion to induce FasL expression on these cells, which subsequently promotes hepatocyte cell death (i.e., cytotoxicity), possibly by interacting with Fas-expressing hepatocytes. In agreement with this concept is the observation that Fas, FasL, and IL-4 exert proinflammatory effects during Con A-induced hepatitis since mice deficient in any of the aforementioned mediators are resistant to Con A-induced liver damage (10).

α-GalCer, a synthetic (i.e., nonphysiological) glycolipid ligand for iNKT cells, has been heralded as a potential therapeutic agent to treat an array of autoimmune diseases and cancer due to its ability to potentiate and specifically activate both mouse and human iNKT cells. With this has come an expectation that may never be met since the fate of iNKT cells following the administration of α-GalCer into mice remains an “enigma.” The two sides to this story are highlighted. The first side argues convincingly that α-GalCer is hepatotoxic since the administration of α-GalCer into mice results in the activation and apoptosis of hepatic iNKT cells by AICD and associated liver damage (2, 17, 18). Furthermore, the hepatotoxicity effect of α-GalCer was found to be mediated by TNF-α produced by the activated hepatic iNKT cells (2). In fact, it was proposed that TNF-α (via an autocrine-dependent mechanism) increases FasL expression on iNKT cells, and these cells, in turn, promote liver damage by interacting with Fas-expressing hepatocytes (2). It is noteworthy that liver injury induced by α-GalCer is believed to potentially mimic some aspects of AIH since according to Biburger and Tieg (2), “α-GalCer could be considered as a surrogate antigen for natural autoantigens that may be presented to iNKT cells by CD1d.” The other side to this story, which is equally convincing, demonstrates that the administration of α-GalCer does not cause AICD of hepatic iNKT cells and ensuing hepatitis (6, 25). Moreover, it was established that the disappearance of hepatic iNKT cells following treatment with α-GalCer was not due to AICD but due to the downregulation of the TCR on these cells (6, 25). The contrasting effects of α-GalCer are intriguing. However, my colleagues and I have observed that α-GalCer does not cause AICD of iNKT cells in vitro (1), an indication perhaps that the
ability of α-GalCer to cause AICD of iNKT cells and the subsequent hepatotoxicity may be a feature that is specific and unique to an in vivo setting (i.e., hepatic milieu). There are a number of unanswered questions: I am puzzled as to why α-GalCer, which has been obtained from the same source, would give contrasting results since the aforementioned studies used α-GalCer obtained from the same supplier. This would seem to suggest that the apparent conflicting or dichotomous effects of α-GalCer in the liver may be due to differences in the quality and batch of α-GalCer used in these studies. It is not known if endotoxin contamination could be an underlying issue. Alternatively, the vehicle/diluent for the α-GalCer could be responsible for the hepatotoxic effects of α-GalCer since some of these studies have used DMSO (9), which in itself is hepatotoxic (unpublished observation). However, some studies have reported that hepatotoxic effects of α-GalCer become apparent when a dose of 2 μg or higher is used (18). These are issues that warrant further study.

Anti-inflammatory effects of iNKT cells. In addition to being able to exert a pathogenic effect during toxin and autoimmune liver diseases (discussed above), iNKT cells also have the potential to exert a hepatoprotective effect during liver diseases (as shown in some experimental models of viral liver disease) by promoting the development of anti-inflammatory responses (Table 1). Hepatitis B virus (HBV) and Hepatitis C virus (HCV) infections affect over 300 million people worldwide. The animal model for HBV infection (i.e., the HBV transgenic mouse) is well characterized (9). However, prospective murine and primate models for HCV infection are not widely available. Therefore, the potential contribution of iNKT cells to the progression or resolution of HCV infection has been at best indirectly deduced from animal models [such as murine cytomegalovirus (mCMV) and lymphocytic choriomeningitis (LCMV) models] that may mimic some of the features of HCV infection. Therefore, I will specifically discuss the role of iNKT cells in the pathophysiology of experimental models of viral liver disease induced by HBV, mCMV, and LCMV infections.

Since its development two decades ago, the HBV transgenic mouse has been widely and routinely used to assess the role of the cellular immune response (including iNKT cells) in the pathophysiology of HBV infection (9). The evidence implicating hepatic iNKT cells in the pathophysiology of HBV infection are as follows: first, Kakimi et al. (9) propose that hepatic iNKT cells die by apoptosis due to a decrease in the population of these cells during HBV infection, but apoptosis was not specifically determined. Second, activation of hepatic iNKT cells with α-GalCer during HBV infection enhanced liver damage relative to that seen in mice that received vehicle (9). Third, the activation of hepatic iNKT cells with α-GalCer during HBV infection results in increased hepatic production of cytokines including IFN-α, IFN-β, and IFN-γ; these cytokines, in turn, suppress the replication of HBV without ameliorating liver damage (9). Fourth, a follow-up study from a different group demonstrated that deletion of hepatic NK cells ameliorates liver damage in these mice (23). Taken together, these studies demonstrate that activated iNKT cells curtail viral replication but also activate hepatic NK cells to promote liver injury. Therefore, the cross talk between hepatic iNKT and NK cells during HBV infection is potentially detrimental.

As discussed above, the specific role of iNKT cells in the progression or resolution of HCV infection is presently unknown due to the fact that prospective murine and primate models for HCV infection are not widely available. Notwithstanding, the LCMV and mCMV animal models are being used as surrogate models to assess the potential contribution of iNKT cells in the pathophysiology of HCV infection. LCMV administration into mice has been reported to induce the production of IFN-α/β; these cytokines cause the apoptotic death of hepatic iNKT cells (7). Although the specific role of iNKT cells in liver damage mediated by LCMV treatment was not determined, Hobbs et al. (7) reported that viral clearance in iNKT cell KO mice was similar to that observed in WT mice after LCMV treatment. This suggests that hepatic iNKT cells are unlikely to contribute to natural control of the LCMV infection. Similarly, it has been reported that hepatic iNKT cells do not contribute to natural control of the mCMV infection since viral clearance in iNKT cell KO mice was similar to that observed in WT mice post-mCMV treatment (24). However, the activation of iNKT cells with α-GalCer improved viral clearance by activating hepatic NK cells (24). Unfortunately, the effect of this treatment on liver damage was not determined. It is noteworthy that results from the clinical setting regarding the role of iNKT cells in the pathophysiology of HCV infection remain a source of confusion since some observational studies report a high expression of human iNKT cells in the liver of patients with chronic HCV, whereas other studies have documented a decrease in the expression of these cells during HCV infections.
cells. Although some progress has been made, it is my contention more mechanistic studies that assess the functional role of iNKT cells in the pathophysiology of viral liver diseases represent a very fertile ground for future investigation.

**Homing and Survival of Hepatic iNKT Cells**

It remains unknown why the liver is highly enriched in iNKT cells relative to other organ systems. The role of chemokine receptors in the recruitment/sequestration of leukocytes (including conventional T cells, neutrophils, and NK cells) during the hepatic inflammatory responses is well defined. In line with this concept, chemokine receptors may also play an important role in the homing and/or survival of hepatic iNKT cells for the following reasons. First, both healthy CXCR3 KO and CXCR6 KO mice are inherently deficient in hepatic iNKT cells (5, 8). Second, my colleagues and I have recently reported that healthy CCR5 KO mice have normal expression of hepatic iNKT cells compared with WT mice; however, during hepatic injury mediated by Con A, hepatic iNKT cells in CCR5 KO mice exhibit reduced cell death and thus promote increased survival of these cells (1). Overall, these data suggest that, in normal healthy mice, CXCR3 and CXCR6 could potentially play critical roles in the homing/sequestration of hepatic iNKT cells, whereas in normal inflamed (i.e., unhealthy) mice, CCR5 suppresses the survival of hepatic iNKT cells. Chemokine receptors are routinely used to characterize conventional T cells by denoting these cells as Th1- or Th2-expressing cells on the basis of their chemokine receptor expression; for example, CCR5, CXCR3, and CXCR6 all promote Th1 type responses (1, 5, 8). Therefore, conventional T cells expressing any of these receptors will be denoted as a Th1-expressing cell. Nonetheless, it is very unlikely that these or other chemokine receptors will or can be used to ascribe iNKT cells to a Th1 or Th2 type profile since iNKT cells (unlike conventional T cells) cannot be polarized because resting iNKT cells contain preformed IL-4 and IFN-γ mRNA (14). However, the α-GalCer analogs, C-glycoside and OCH, can activate iNKT cells (via TCR) and specifically skew these cells to selectively exert Th1 (via IFN-γ release) and Th2 (via IL-4 release) responses, respectively (25).

**Conclusions and Future Directions**

Studies from preclinical models (as described in this review) have provided valuable insights on the functional role of iNKT cells in the pathophysiology of liver diseases mediated by toxins, autoimmunity, and viruses. From these studies, it is also clear that contribution of iNKT cells to the pathophysiology of liver diseases is dependent on a number of factors: 1) the hepatotoxin mediating the liver injury, 2) cytokines produced in the liver by these hepatotoxic agents, and 3) the ability of activated iNKT cells to promote or suppress hepatic NK cell activation. The aforementioned studies also suggest that hepatic iNKT cells can be activated either directly (Fig. 1A) or indirectly (Fig. 1B) by hepatotoxic agents to exert beneficial or detrimental effects during liver diseases by selectively promoting the development of either proinflammatory or anti-inflammatory responses. Although this remains a contentious issue, it is apparent that activated hepatic iNKT cells may contribute to liver injury predominantly via two mechanisms, 1) AICD or 2) expansion/recruitment (see Table 1). Given the mounting evidence, it is my contention that AICD of hepatic iNKT cells may be a feature that is stimulus-dependent and unique to liver diseases. In summary, the differences in the fate and function (i.e., AICD vs. expansion/recruitment) of hepatic iNKT cells during liver diseases could potentially be attributed to the following: first and foremost, the quality of the stimulating signal, which could affect TCR/ligand interaction and ultimately the function of iNKT cells; second, the efficacy of the CD1d-expressing cell types in the presentation of antigen to hepatic iNKT cells since a recent study reports that Kupffer cells and not dendritic cells are the key antigen-presenting cell for hepatic iNKT cells (20); and third, the efficacy of iNKT cell subsets may also potentially dictate the function of iNKT cells during the liver diseases. For example, differences in the efficacy of the iNKT cell subsets, CD4+ T cell expressing iNKT cells vs. CD4(−) T cells expressing iNKT cells, have been reported (6). Furthermore, not much is known about an iNKT cell phenotype which does not express the invariant Vα14/β18 TCR α-chain; interestingly, this iNKT cell phenotype is exclusively expressed in the liver, is unreactive to α-GalCer, and cannot be detected by the α-GalCer-CD1d tetramer (6). In view of the abundant evidence implicating iNKT cells in the pathophysiology of liver diseases, I do believe that the enrichment of the liver with iNKT cells (compared with other organ system) plays a significant role in the function of these cells during liver diseases. Therefore, we cannot completely rule out the possibility that there could be strength in numbers!

A number of issues need further exploration: First, several critical gaps exist in our understanding of the role of iNKT cells in the pathophysiology of viral liver diseases. As discussed above, iNKT cells suppress viral replication during HBV infection but also activate NK cells to cause liver damage. However, the hepatitis B transgenic mouse is a chronic model in which the HBV antigen expressed on hepatocytes is present throughout (i.e., from birth). Therefore, immune responses in this model would very likely differ from those observed when viral antigens are expressed as a result of infection, so I feel that it is inappropriate to assume that activated iNKT cells in the transgenic mice would behave in a similar manner to that observed in a model where the viral antigen is expressed in response to an infection. Second, understanding the role of iNKT cells in the pathophysiology of liver diseases could potentially be attributed to the following: first and foremost, the quality of the stimulating signal, which could affect TCR/ligand interaction and ultimately the function of iNKT cells; second, the efficacy of the CD1d-expressing cell types in the presentation of antigen to hepatic iNKT cells since a recent study reports that Kupffer cells and not dendritic cells are the key antigen-presenting cell for hepatic iNKT cells (20); and third, the efficacy of iNKT cell subsets may also potentially dictate the function of iNKT cells during the liver diseases. For example, differences in the efficacy of the iNKT cell subsets, CD4+ T cell expressing iNKT cells vs. CD4(−) T cells expressing iNKT cells, have been reported (6). Furthermore, not much is known about an iNKT cell phenotype which does not express the invariant Vα14/β18 TCR α-chain; interestingly, this iNKT cell phenotype is exclusively expressed in the liver, is unreactive to α-GalCer, and cannot be detected by the α-GalCer-CD1d tetramer (6). In view of the abundant evidence implicating iNKT cells in the pathophysiology of liver diseases, I do believe that the enrichment of the liver with iNKT cells (compared with other organ system) plays a significant role in the function of these cells during liver diseases. Therefore, we cannot completely rule out the possibility that there could be strength in numbers!

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Some relevant manuscripts in this field were not cited due to the space restriction of this theme article.

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