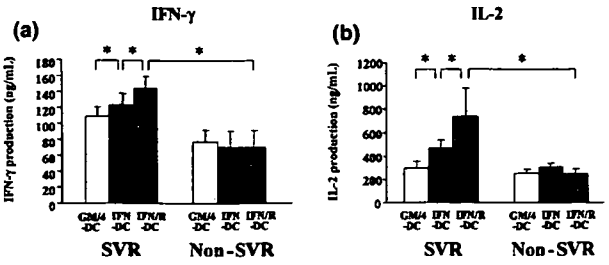

Fig. 5 IFN/R-DC from CHC patients in the SVR group induce more potent Th1 response compared with IFN-DC or GM/4-DC. IFN/R-DC, IFN-DC and GM/4-DC were generated and were cultured with allogeneic naïve $CD4^+$ $CD45RO^-$ T cells for 6 days as described in Materials and methods. On day 4 of the co-culture, half of the supernatants were collected for assessment of IL-2 release from the cells. After 6 days, the cultured cells were stimulated with phorbol myristate acetate and ionomycin for 24 h. IFN-γ and IL-2 concentrations in the supernatants were determined by ELISA. The levels of IFN-γ (a) and IL-2 (b) were compared among them in the SVR and the non-SVR group. The results were expressed as mean ± SEM from five SVR and nine non-SVR patients. Open bars, GM/4-DC; close bars, IFN-DC; gray bars, IFN/R-DC; SVR, sustained virological response. *$P < 0.05$ by Mann–Whitney *U*-test.

In this study, we first intended to elucidate the role of IFN-α in the DC differentiation and its subsequent impact on the ability of DC to stimulate T cells. We added IFN-α from the beginning of DC generation from monocytes in the presence of both GM-CSF and IL-4. Here, we demonstrate that IFN-α is a unique DC differentiation factor in the setting of MoDC generation driven by GM-CSF and IL-4, as it gave rise to MoDC capable of preferentially priming Th1 cells. Of particular interest is the finding that IFN-DC from HCV-infected patients are less able to induce a Th1 response than the healthy counterparts, as evidenced by the analysis of IFN-γ and IL-2 production (Fig. 2a–c). Our results suggest that the IFN-α-induced alterations of DC involving in priming Th1 response are (1) an upregulation of CD86, and (2) a decrease in IL-10 production. However, in CHC patients, such IFN-α-driven alterations in MoDC occur to a lesser degree, thus resulting in impaired DC-primed Th1 response.

As for possible mechanisms of such hyporesponsiveness of patients' DC to IFN-α, the expression of IFN-α receptor on monocyte and DC may be lower in HCV infection. However, this is unlikely as FACS analyses revealed no significant difference in the expression of IFN-α receptor 1 on monocytes or MoDC between the patients and healthy donors (data not shown). Thus, as reported in hepatocytes, signal transduction in DC after binding of IFN-α to its receptor might be hampered by HCV-associated proteins, although the precise pathways linking IFN-α with CD86 or IL-10 remain unclear [33–35]. One of the mechanisms of DC impairment in the ability to prime Th1 in response to IFN-α may be direct HCV infection to monocytes or DC, as reported elsewhere [36,37].

It is well known that DC-derived IL-12 and IL-10 may be involved in Th1 and Th2 polarization, respectively. Thus, the lesser amount of IL-12p70 from the patient' DC may be related to the lesser degree of DC-primed Th1 response in CHC patients than those in donors (Fig. 3a). What remains unknown is how the reduced IL-10 production of DC leads to the enhanced ability of DC to induce a Th1 response. IL-10 is an important key player in the pathogenesis of HCV infection, being induced by various HCV antigens [38]. Moreover, DC functions can be modulated by autocrine IL-10, which is implicated in the enhanced ability to induce Th1 response [39]. The blocking experiments using anti-IL-10 neutralizing Ab including those of our present study revealed that the inhibition of endogenous IL-10 in DC/T cell co-culture enables an increase of the Th1 response [39,40], which may be associated with the relatively enhanced activity of co-existing IL-12p70. Such a reciprocal IL-12 increase and subsequent Th1 augmentation has been observed in DC in which the IL-10 gene had been knocked down by small interference RNA [41]. However, in the present study, the IL-12 levels did not differ between the samples treated with anti-IL-10 Ab and those without it (data not shown). Thus, other DC-derived Th1-inducing cytokines, including IL-27 and IL-23 [42], may be involved in the IFN-DC-induced Th1 response, the possibility of which needs to be further evaluated.

Ribavirin has broad-spectrum activities against both DNA and RNA viruses, however, its mechanism of action for the treatment of HCV is not fully understood. Given that ribavirin has little direct activity against HCV [43–45], a number of studies have shown that ribavirin can modulate immune response by altering the Th1/Th2 bias [14,15,46]. With regard to DC, it has been previously reported that ribavirin alters cytokine production from DC [30]. However, it remains unclear whether or not ribavirin could affect Th1-driving capacity of DC. In the present study, when we analysed the patients as a whole, no additive effect was obtained with ribavirin in phenotypes and functions of DC generated with or without IFN-α. However, when the analyses had been done separately in the SVR patients and non-SVR ones, IFN/R-DC from the SVR group induced more potent Th1 response compared with IFN-DC or GM/4-DC, of which difference was not observed in the non-SVR group. In addition, the levels of IFN-γ and IL-2 released from IFN/R-DC-primed T cells were significantly higher in the SVR group than those in the non-SVR group. It is thus speculated that such better *in vitro* DC response to IFN-α and ribavirin is associated with better *in vivo* virological response in the combination therapy, as the enhancement of HCV-specific Th1 response is necessary for the clearance of HCV by IFN-α and ribavirin combination therapy. As described above, one of the mechanisms of the impairment in IFN-α-stimulated DC in HCV infection is an insufficient alteration of CD86 expression and IL-10 production. However, the addition of ribavirin to IFN-α failed to improve CD86 expression and reduce IL-10

production from patient' DC in the current study, suggesting that other factors may be involved in the mechanisms of ribavirin. In the present study, IL-2 produced in IFN/R-DC and T-cell co-culture was higher than those in IFN-DC culture in the SVR group. Although IL-2 is not a primary Th1-driving factor, it supports Th1 differentiation by promoting T-cell response or survival. Thus, it is plausible that a combination of IFN-α and ribavirin may increase DC-primed IL-2 secretion from CD4 T cells, resulting in enhanced IFN-γ production by T cells.

In summary, in chronic HCV infection, IFN-DC is less able to prime CD4 T cells to produce IFN-γ and IL-2 compared with those in healthy subjects. We also showed the possibility that ribavirin may restore the impaired responsiveness of DC to IFN-α *in vitro* in some HCV-infected patients. Further prospective analyses in large number of patients are warranted to elucidate if a combination of IFN-α and ribavirin directly improves DC function to stimulate Th1 response, thus contributing to HCV eradication from the treated patients.

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Innate immunity in hepatitis C virus infection: Interplay among dendritic cells, natural killer cells and natural killer T cells

Tatsuya Kanto[1,2] and Norio Hayashi[1]

[1]*Department of Gastroenterology and Hepatology and* [2]*Department of Dendritic Cell Biology and Clinical Applications, Osaka University Graduate School of Medicine, Osaka, Japan*

Sequential activation of innate and adaptive immune response is crucial for virus elimination. We thus sought to clarify the role of innate immune system in the pathogenesis of hepatitis C virus (HCV) infection. Dendritic cells (DC) sense virus infection via toll-like receptors (TLR) or retinoic acid inducible gene-I (RIG-I), resulting in the secretion of type-I interferons (IFN) and inflammatory cytokines. Blood DC consist of two subsets; myeloid DC (MDC) and plasmacytoid DC (PDC). In MDC from HCV-infected patients, regardless of higher expression of TLR2, TLR4 and RIG-I compared to the controls, the levels of TLR/RIG-I-mediated IFN-β or TNF-α induction are lower than those in uninfected donors. These results suggest that the signal transduction in the downstream of TLR/RIG-I in MDC is profoundly impaired in HCV infection. In response to IFN-α, DC are able to express MHC class-I related chain A/B (MICA/B) and activate natural killer (NK) cells following ligation of NKG2D. Interestingly, DC from HCV-infected patients are unresponsive to exogenous IFN-α to enhance MICA/B expression and fail to activate NK cells. Alternatively, NK cells from HCV-infected patients downregulate DC functions in the presence of human leukocyte antigen E-expressing hepatocytes by secreting interleukin (IL)-10 and transforming growth factor-β1. Such functional alteration of NK cells in HCV infection is ascribed to the enhanced expression of inhibitory receptor NKG2A/CD94 compared to the healthy counterparts. Invariant NKT cells activated by CD1d-positive DC secrete both T-helper (Th)1 and Th2 cytokines, serving as immune regulators. The frequency of NKT cells in chronic HCV infection does not differ from those in healthy donors. Activated NKT cells produce higher levels of IL-13 but comparable levels of IFN-γ with those from healthy subjects, showing that NKT cells are biased to Th2-type in chronic HCV infection. In conclusion, cross-talks among DC, NK cells and NKT cells are critical in shaping subsequent adaptive immune response against HCV.

Key words: α-galactosyl-ceramide, IL-β, MICA/B, NKG2A, TLR

INTRODUCTION

HEPATITIS C VIRUS (HCV) is one of major causes of chronic liver disease worldwide. HCV is hepatotrophic, but not directly cytopathic and elicits progressive liver injuries resulting in end-stage liver disease unless effectively eradicated.[1] Epidemiological studies have revealed that more than 80% of acutely HCV-infected patients fail to eradicate the virus and they subsequently develop chronic hepatitis.[1] It has been proposed that the ability of infected hosts to mount vigorous and sustained cellular immune reactions to HCV is necessary for control in primary infection.[2] Once HCV survives the initial interaction with the host immune system, it uses several means to nullify the selective immunological pressure during the later phases of infection. First, the virus alters its antigenic epitopes recognized by T cells and neutralizing antibodies to escape immune surveillance. Second, HCV also subverts immune functions in an antigen-specific manner, from innate to adaptive immunity.[3]

Cumulative reports have shown that innate immune system dictates the direction and magnitude of subsequent adaptive immune response. It is generally accepted that HCV-specific $CD8^+$ T cells are responsible for HCV elimination by inducing hepatocyte apoptosis.[2] Innate immune cells, including natural killer (NK) cells and

Correspondence: Tatsuya Kanto M.D., PhD, Department of Gastroenterology and Hepatology, Osaka University Graduate School of Medicine, 2-2 Yamada-oka, Suita, Osaka 565-0871, Japan. Email: kantot@gh.med.osaka-u.ac.jp

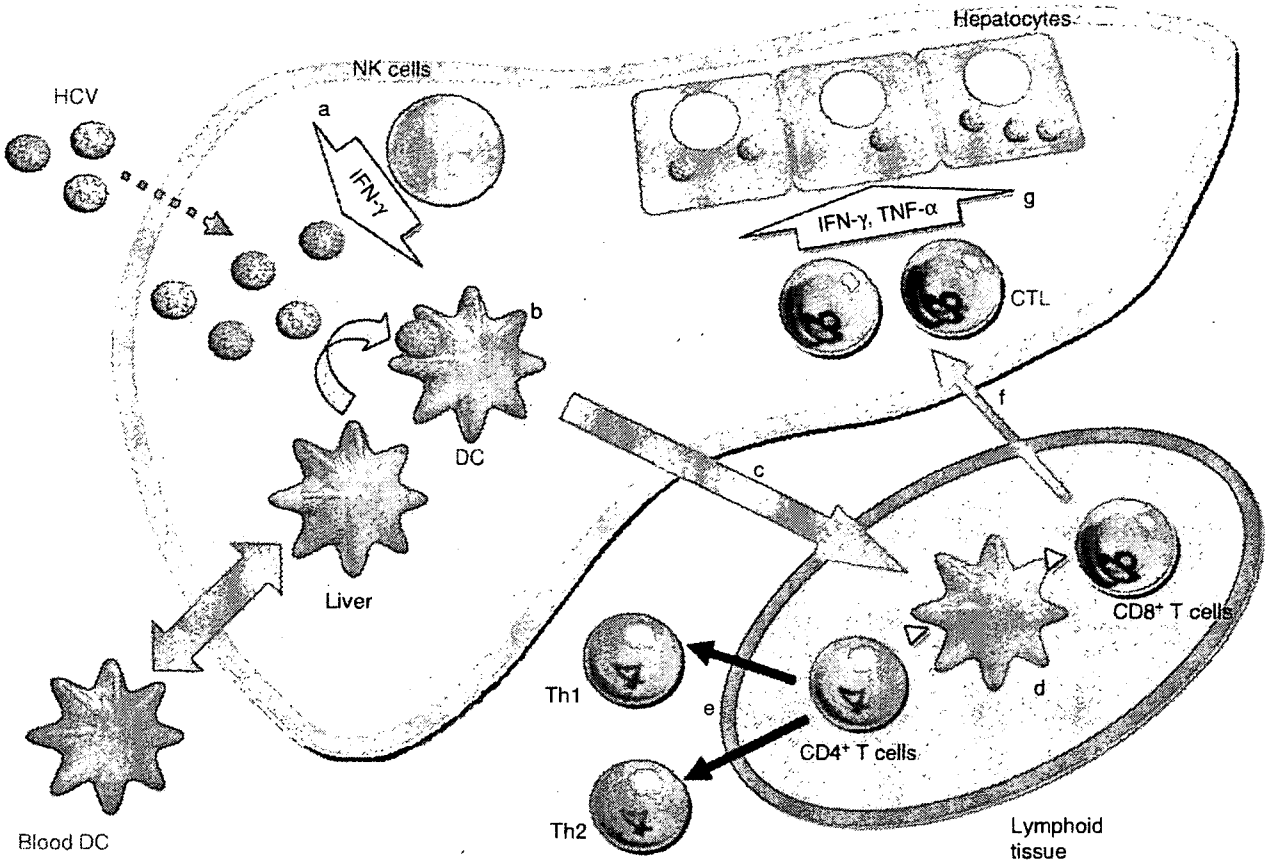

Figure 1 Key players in immune reactions in viral hepatitis. CTL, cytotoxic T lymphocyte; DC, dendritic cell; HCV, hepatitis C virus; NK, natural killer cell; Th, helper T cell. (1–7), see text.

NKT cells, may contribute to HCV eradication after primary infection; however, their roles in a chronically infected state remain elusive. Because dendritic cells (DC) orchestrate anti-HCV immune response by linking innate and adaptive arms of the immune system,[4] functional impairment of DC leads to failure of NK cells, NKT cells, $CD4^+$ and $CD8^+$ T cells. Infiltration of disabled $CD8^+$ T cells to the infected liver may result in weak liver inflammation that is not sufficient for HCV eradication.[5]

In this paper, we discuss the current understandings of the roles of innate immunity in the pathogenesis of HCV infection, especially focused on interferons (IFN), DC, NK cells and NKT cells.

KEY PLAYERS IN IMMUNE RESPONSES TO VIRAL HEPATITIS

AFTER HCV INFECTS the liver, viral replication continues and viral particles are continuously released into the circulation. The first lines of defense are provided by NK and NKT cells, of which populations are relatively increased in the liver compared to the periphery. These cells are activated in the liver, where expression of IFN-α and IFN-inducible genes are extremely high during the early phase of hepatitis virus infection.[6] Activated NK and NKT cells secrete IFN-γ, which inhibits replication of HCV through a non-cytolytic mechanism (Fig. 1a).[7]

Dendritic cells or resident macrophages in the liver are capable of taking up viral antigens, and processing and presenting them to other immune cells (Fig. 1b).[4] Because DC express distinct sets of toll-like receptors (TLR),[8] it is likely that some viral components stimulate DC through cytosolic ligation of TLR. DC develop a mature phenotype and migrate to lymphoid tissues (Fig. 1c), where they stimulate effectors, including T cells and B cells (Fig. 1d). Following the encounter of DC with other cells, DC secrete various cytokines (interleukin [IL]-12, tumor necrosis factor [TNF]-α, IFN-α and IL-10) instructing or regulating the functions of the adja-

cent cells.[4] In addition to these cytokines, DC express various costimulatory molecules and ligands to enhance or limit the functions of immune and infected cells. The existence of functionally and ontogenetically distinct DC subsets has been reported; that is, myeloid DC (MDC) and plasmacytoid DC (PDC).[9] MDC predominantly produce IL-12 or TNF-α following proinflammatory stimuli, while PDC release a considerable amount of IFN-α upon virus infection depending on the immune stimulus; both cytokines in actuality can be made by both cells. Helper T cells have an immunoregulatory function mediated by the secretion of cytokines that support either cytotoxic T lymphocyte (CTL) generation (T-helper [Th]1 with secretion of IL-2, IFN-γ and TNF-α) or B-cell function and antibody production (Th2 with secretion of IL-4, IL-5, IL-10 and IL-13) (Fig. 1e). DC ontogeny and DC-derived cytokines are crucially associated with the polarization of helper T-cell subsets.

It is generally accepted that adaptive immunity performs a critical role during the clinical courses of hepatitis. The involvement of antigen-specific $CD4^+$ T cells in HCV eradication has been well described during both acute or chronic infection.[10] However, there is little evidence that $CD4^+$ T cells mediate direct liver cell injury in HCV infection. Thus, it is likely that $CD4^+$ T cells play a critical role in facilitating other antiviral immune mechanisms, such as enhancing $CD8^+$ effector function. The antigen-primed CTL recruit to the liver (Fig. 1f) and constitute the critical element in the eradication of virus-infected cells (Fig. 1g).

INNATE IMMUNITY IN HCV INFECTION

Toll-like receptors and retinoic acid inducible gene-I as sensors for virus infection

GENE EXPRESSION ANALYSES in HCV-infected liver revealed that HCV triggers expression of type I IFN and IFN-induced genes during primary infection regardless of the outcomes.[6] However, the HCV viral load does not decrease in the early phase, suggesting that HCV impedes the execution of antiviral machineries. Several HCV-derived proteins are involved in the suppression on the signaling pathways inducing antiviral proteins, such as interferon regulatory factor (IRF)-3,[11] nuclear factor (NF)-κB and double-stranded RNA-dependent protein kinases (PKR).[12] Mammalian TLR sense some pathogen-associated molecular patterns embedded in virus components and then induce inflammatory cytokines or type-I IFN, resulting in the augmentation of antivirus immune reactions.[6] Retinoic acid inducible gene-I (RIG-I) is a cytosolic molecule that senses double-stranded RNA (dsRNA) of virus replicative intermediate, which subsequently activates IRF-3 and NF-κB pathways.[13] By using the HCV subgenomic replicon system, it has been demonstrated that HCV NS3/4 A proteins influence the functions of adaptor molecules mediating TLR-dependent and RIG-I-dependent pathways, resulting in an impairment of the induction of IFN-β as well as subsequent IFN-stimulated genes.[14,15] However, it is yet to be proven whether the results obtained from HCV replicon are applicable or not for HCV-infected individuals.

To investigate the roles of TLR/RIG-I in HCV infection, we compared their expressions and functions in MDC and PDC between patients and donors. In MDC from HCV-infected patients, TLR2, TLR4 and RIG-I expression were significantly higher than those in healthy counterparts. Of particular interest, regardless of the higher expressions, specific agonists for these sensors stimulated patients' MDC to induce lesser amounts of IFN-β and TNF-α compared to donor MDC (Miyazaki *et al.*, 2007, unpublished data). These results show that the signal transduction via these receptors is strongly impeded in HCV infection. Inconsistent with the findings of MDC, we previously reported that TLR2 expression on monocyte-derived DC (MoDC) in chronic hepatitis C is lower than those in healthy donors.[16] Because MoDC is an *in vitro*-generated DC mimic, the opposite results of TLR2 in HCV infection might be explained by impaired ability of MoDC to mature in response to cytokines, as reported elsewhere.[17] Further investigation is needed to clarify which TLR or RIG-I is predominantly utilized by HCV to evoke immune reactions.

Blood DC subsets

Impaired antigen presentation by DC might be involved in the failure of the maintenance of sustained HCV-specific T-cell response. MoDC generated from hepatitis C patients have an impaired ability to stimulate allogeneic $CD4^+$ T cells.[18,19] Functional impairment of DC diminished when HCV had been eradicated from patients, revealing the evidence of HCV-induced DC disability.[18] In addition to *in vitro*-generated DC, the alterations in number and function of circulating blood DC have been reported in HCV infection.[20]

Direct HCV infection of DC might be one of the plausible mechanisms of DC dysfunction in chronic hepatitis C. The HCV genome has been reported to be isolated

from MoDC or blood DC.[18] However, these results need to be interpreted carefully, because contamination with free virus in blood cannot be ruled out when amplifying polymerase chain reaction techniques are used. To exclude this possibility, HCV pseudovirus has been developed to investigate the cell tropisms of HCV as well as to determine putative HCV entry receptors to cells. By using this, MDC, but not PDC, displayed susceptibility to HCV pseudovirus possessing chimeric HCV E1/E2 proteins.[21]

Several criticisms have been raised recently about DC dysfunction in the setting of chronic HCV infection,[22] failing to demonstrate any DC defects which may have to do with differences in the populations studied. Cohort studies on chimpanzees following HCV infection showed that functional impairment of DC was observed in some cases but was not a prerequisite of persistent infection.[23] Further study needs to be done to clarify whether DC are indeed disabled in the setting of human chronic hepatitis C and furthermore whether this contributes to the development of HCV persistence or if it is simply a consequence of active HCV infection.

Natural killer cells

Natural killer cells express various functional receptors; the one group that transduces inhibitory signals (killer inhibitory receptors [KIR], CD94, NKG2A) and the other performs activating signals (NKG2D). The function of NK cells is dynamically regulated *in vivo* by the balance between expressions of counteracting receptors and their association with relevant ligands.[24] First, we compared the expressions of NK cell receptors between HCV-infected patients and healthy donors. As for inhibitory receptors, KIR expressions are not different between the groups; however, CD94 and NKG2A expressions are higher in patients than controls.[25] In contrast, activating receptor NKG2D expression is comparable between the groups (Fig. 2). It is yet to be determined how the expression of the NK cell receptor is regulated. In our experience, HCV pseudovirus did not enter purified NK cells, suggesting that NK cells are not susceptible to direct HCV infection (Kaimori A *et al.*, 2004, unpublished data). Thus, some soluble factors and/or direct binding of HCV particles to NK cells might be the cause of NK receptor dysregulation.

Dendritic cells play a decisive role in shaping innate immunity by interacting with NK cells. DC have two means to stimulate NK cells via the production of cytokines (IL-12, IL-18 or IFN-α) and through the expression of NK-activating ligands. In response to IFN-α, DC are able to express major histocompatibility complex (MHC) class-I related chain A/B (MICA/B) and activate NK cells following ligation of the NK receptor, NKG2D.[26] Interestingly, DC from HCV-infected patients are unresponsive to exogenous IFN-α to enhance MICA/B expression and fail to activate NK cells.[26] It is tempting to speculate that the impairment of DC in NK cell activation is responsible for the failure of HCV control in the early phase of primary HCV infection, where HCV continues to replicate in spite of high-level IFN-α expression in the liver. Alternatively, NK cells from HCV-infected patients downregulate DC functions in the presence of hepatocytes by secreting suppressive cytokines, IL-10 and transforming growth factor-β1.[25] Such functional alteration of NK cells in HCV infection was ascribed to the enhanced expression of inhibitory receptor NKG2A/CD94 compared to the healthy counterparts.[25] Further study is necessary to determine if the NK-mediated DC suppression is instrumental or not in acute HCV infection.

Natural killer T cells

Natural killer T cells are a unique lymphocyte subset coexpressing T-cell receptors (TCR) and NK cell markers.[27] The NKT cell population is highly heterogeneous; invariant (or classical) NKT (iNKT) cells express an invariant TCR, composed of Vα24-JαQ preferentially paired with Vβ11 in humans,[27] whereas non-invariant NKT cells express diverse TCR. Invariant NKT cells recognize glycolipid antigens presented on CD1d expressed by DC.[47] Although endogenous ligands of iNKT cells are little known, α-galactosyl-ceramide (αGalCer) has been used as a surrogate for natural ligands. It has been demonstrated that phenotypic as well as functional subsets exist for iNKT cells, which are $CD4^+$, $CD4^-CD8^-$ double negative (DN) and $CD8^+$ ones. The $CD4^+$ and DN iNKT cells produce both Th1 (IFN-γ) and Th2 cytokines (IL-4, IL-5, IL-13). The $CD4^+$ iNKT cells secrete more Th2 cytokines than DN, while $CD8^+$ subsets predominantly secrete Th1 cytokines.[28]

Although iNKT cells comprise a small portion of hematopoietic cells, they regulate various immune responses by secreting Th1 as well as Th2 cytokines in clinical settings. For chronic HCV infection, some controversial reports have been published about the frequency of iNKT cells,[29,30] however, their functional roles in HCV-infected patients are largely unknown. We thus compared the frequency and the cytokine producing capacity of iNKT cells in peripheral blood between chronic hepatitis C patients and healthy individuals. Furthermore, to analyze the functions of activated iNKT cells, we expanded iNKT cells by the stimulation with

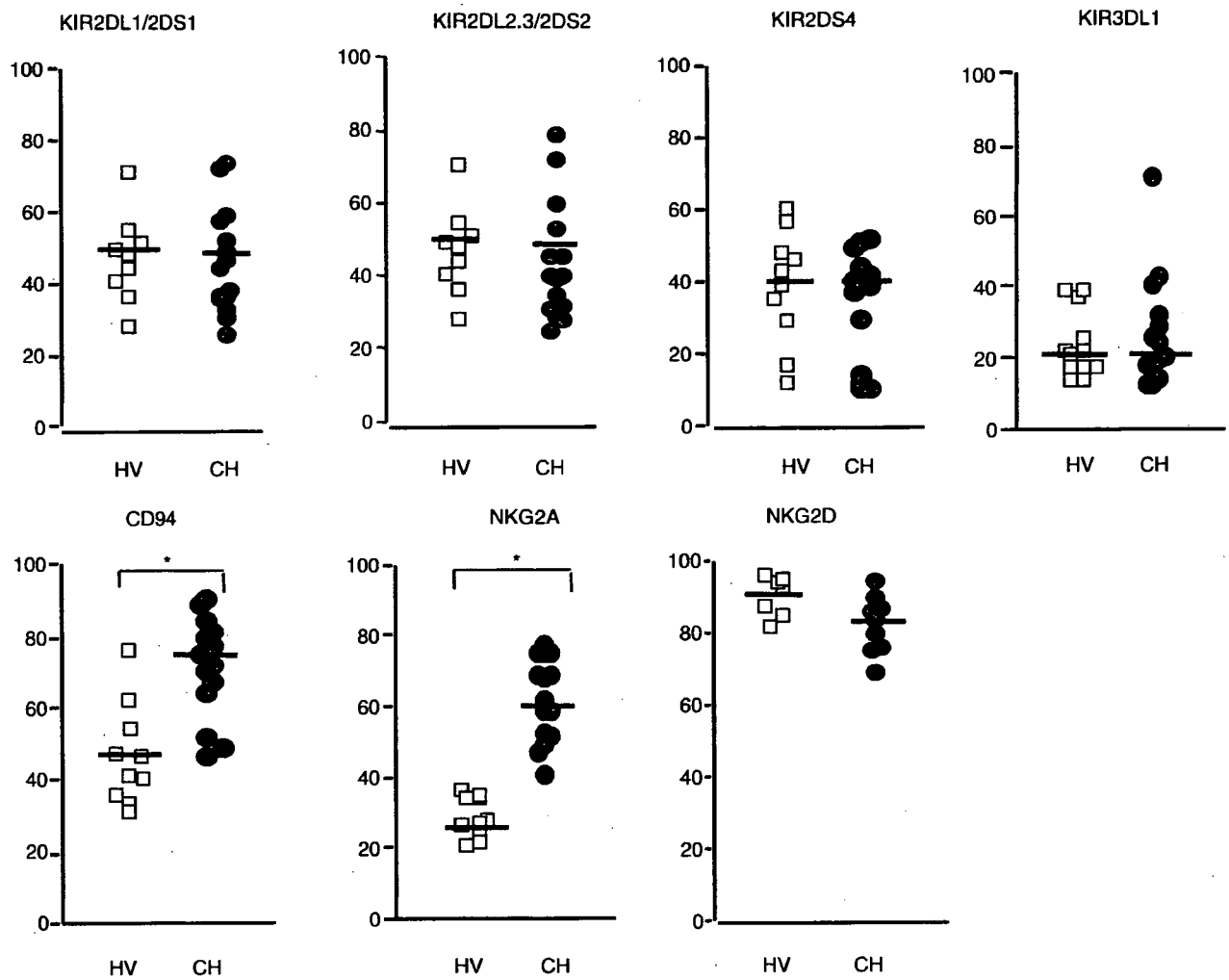

Figure 2 Expressions of NK receptors on NK cells from chronic hepatitis C patients and healthy subjects. Percentages of NK cell that express various NK receptors were determined by flow cytometry. HV, healthy volunteers; CH, chronic hepatitis C patients. Horizontal bars represent the median. $^{*}P < 0.05$ by Mann–Whitney *U*-test.

αGalCer-loaded DC. We demonstrated that the number and functions of iNKT cells from HCV-infected patients are comparable with those from healthy subjects at the steady state (Fig. 3).[31] By contrast, activated iNKT cells from patients released more Th2 cytokines, most significantly IL-13, than those from the controls (Fig. 4).[31] Recently, other groups have reported that IL-4 and IL-13 from fresh iNKT cells were increased in liver cirrhosis caused by hepatitis B virus or HCV, implying that these cells are pro-fibrogenic to the liver.[32] If this is the case, our findings suggest that iNKT cells in chronic HCV infection are pro-fibrogenic per se even in the pre-cirrhotic stage. The reason why iNKT cells in HCV infection are Th2-biased needs to be further investigated.

ADAPTIVE IMMUNITY IN HCV INFECTION

MANY REPORTS HAVE been published on the importance of $CD4^+$ T-cell response in the clearance and control of HCV. In chronic hepatitis C patients, HCV-specific $CD4^+$ T cells were functionally impaired and their activity was not sustained,[33] which was in clear contrast with resolved cases. Inoculation studies of infectious HCV to recovered chimpanzees demonstrated that $CD4^+$ T-cell help was indispensable for the development of effective $CD8^+$ T cell response to protect from HCV persistence.[34]

With regard to HCV-specific $CD8^+$ T cells observed during the chronic stages of disease, conflicting results

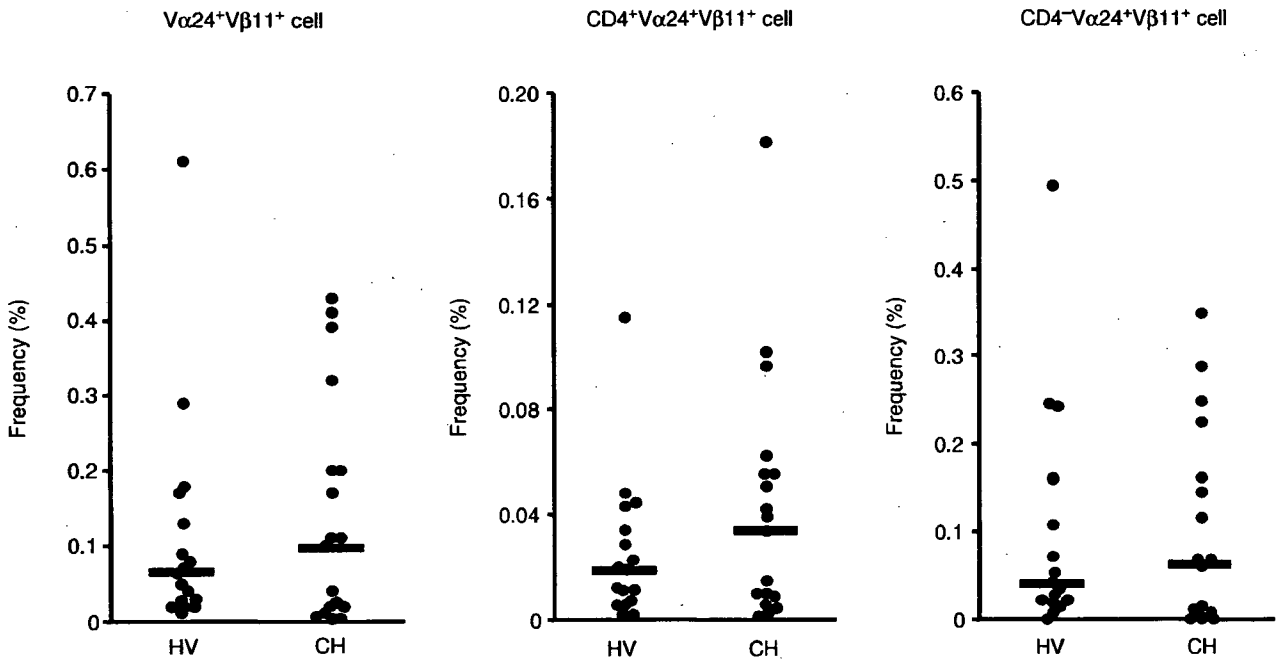

Figure 3 Frequency of peripheral invariant NKT cell subsets in healthy subjects and chronic hepatitis C patients. The frequencies of total invariant NKT (iNKT) cells ($V\alpha24^+V\beta11^+$ cells), $CD4^+$ and $CD4^-$ iNKT cells in PBMC were determined by flow cytometry. HV, healthy volunteers; CH, chronic hepatitis C patients. Horizontal bars represent the median.

have been reported for their roles in HCV replication and liver inflammation. Several investigators have shown that the HCV-specific CTL response is inversely correlated with viral load, suggesting its inhibitory capacity on HCV replication.[35] However, others did not find a significant relationship between these parameters.[36] HCV-specific $CD8^+$ T cells in chronic hepatitis C patients possess lesser capacity to proliferate and produce less IFN-γ in response to HCV antigens. Because $CD8^+$ T cells are reported to be involved in HCV-induced liver inflammation, inefficient $CD8^+$ T cells may evoke only milder hepatocyte injury, which level is not sufficient for HCV eradication.[5]

Several plausible mechanisms have been proposed for T-cell functional failure observed in chronic HCV infection:[3] (i) HCV escape mutation; (ii) primary T-cell failure or T-cell exhaustion; (iii) impaired antigen presentation; (iv) suppression by HCV proteins; (v) impaired T-cell maturation; (vi) suppression by regulatory T cells; and (vii) tolerogenic environment in the liver.

PERSPECTIVES

ANTIVIRAL AGENTS, PEGYLATED (PEG)-IFN-α and ribavirin, have been widely used for the treatment of chronic HCV infection in order to prevent the development to liver cirrhosis and hepatocellular carcinoma.[1] In addition to providing direct inhibition of viral replication, these agents modulate antiviral immune responses, which greatly contribute to the successful therapeutic response. The questions remain unsolved whether an impaired immune system in chronic HCV infection is restored or not by successful HCV eradication after antiviral therapy. Controversial results have been reported about the durability of treatment-induced recovery in HCV-specific immune response,[37,38] which seems to be clearly distinct from that observed in spontaneous HCV resolvers. Protease inhibitors against HCV NS3/4A are now ready to use in clinics. Because they possess potent ability to suppress HCV replication, they are quite promising as an alternative approach for non-responders in PEG-IFN-α/ribavirin therapy. In addition to that, it is anticipated that protease inhibitors are able to restore innate immunity by disarming NS3/4A-mediated suppression on TLR/RIG-I-dependent or -independent pathways. Therefore, extensive immunological studies on the patients treated with protease inhibitors are needed to elucidate if the therapeutic modulation of innate immunity could shape HCV-specific adaptive immunity or not. The next steps in evolving innovative approaches to establish HCV-specific immunotherapy are to determine the

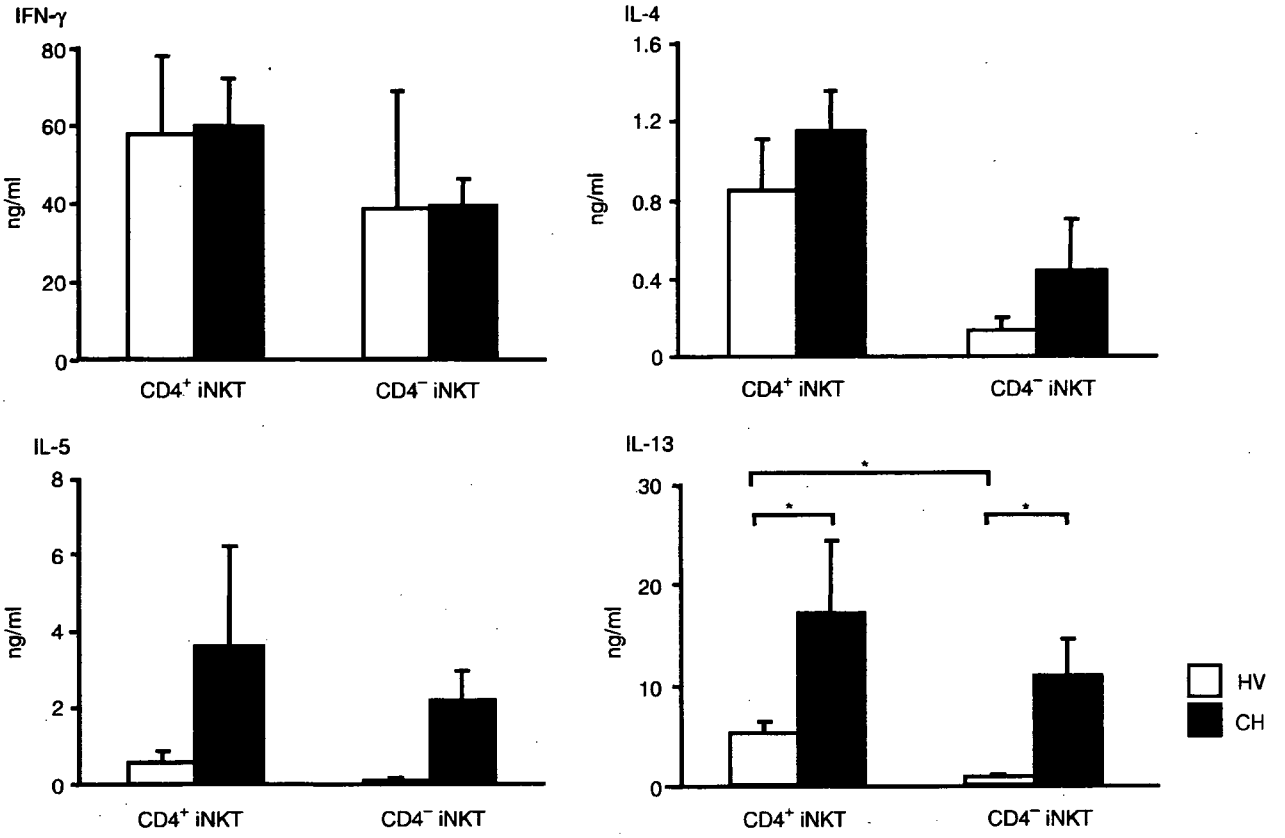

Figure 4 Cytokine production from expanded invariant NKT cells stimulated with α-galactosyl-ceramide (αGalCer)-loaded monocyte-derived DC. Invariant NKT (iNKT) cells were expanded by culture with αGalCer-pulsed autologous monocyte-derived DC (MoDC) and subsequent cell sorting. Activated iNKT cells were stimulated with αGalCer-pulsed allogeneic MoDC for 24 h and the supernatants were collected for cytokine enzyme-linked immunosorbent assay. HV, healthy volunteers; CH, chronic hepatitis C patients. Bars represent mean ± SE of five different subjects. $^*P < 0.05$ by Mann–Whitney *U*-test.

means to direct the magnitude, breadth, quality and duration of antigen-specific immune responses in a desired way. Active modulation of innate immunity may be one of the strategies to gain access to the goal.

CONFLICT OF INTEREST

NO CONFLICT OF interest has been declared by T Kanto and N Hayashi.

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