Critical Role of MHC Class I-Related Chain A and B Expression on IFN-α-Stimulated Dendritic Cells in NK Cell Activation: Impairment in Chronic Hepatitis C Virus Infection

Masahisa Jinushi, Tetsuo Takehara, Tatsuya Kanto, Tomohide Tatsumi, Veronika Groh, Thomas Spies, Takuya Miyagi, Takahiro Suzuki, Yutaka Sasaki and Norio Hayashi

*J Immunol* 2003; 170:1249-1256; doi: 10.4049/jimmunol.170.3.1249

http://www.jimmunol.org/content/170/3/1249

---

**References**

This article cites 46 articles, 23 of which you can access for free at:

http://www.jimmunol.org/content/170/3/1249.full#ref-list-1

**Subscription**

Information about subscribing to *The Journal of Immunology* is online at:

http://jimmunol.org/subscription

**Permissions**

Submit copyright permission requests at:

http://www.aai.org/About/Publications/JI/copyright.html

**Email Alerts**

Receive free email-alerts when new articles cite this article. Sign up at:

http://jimmunol.org/alerts
Critical Role of MHC Class I-Related Chain A and B Expression on IFN-α-Stimulated Dendritic Cells in NK Cell Activation: Impairment in Chronic Hepatitis C Virus Infection

Masahisa Jinushi,* Tetsuo Takehara,* Tatsuya Kanto,* Tomohide Tatsumi,* Veronica Groh,† Thomas Spies,† Takuya Miyagi,* Takahiro Suzuki,* Yutaka Sasaki,* and Norio Hayashi‡*

Dendritic cells (DCs) augment effector functions of NK cells, but the underlying mechanisms are not fully understood. Here we show in an in vitro coculture system that human monocyte-derived DCs enhance IFN-γ production, CD69 expression, and K562 cytolysis ability of NK cells when DCs are prestimulated with various maturation stimuli such as IFN-α or LPS. Of interest is the finding that NK cell activation mediated by LPS-stimulated DCs was dependent on IL-12 produced in DC/NK coculture, but that IFN-α-stimulated DC-mediated activation was not. Alternatively, MHC class I-related chain A and B (MICA/B), ligands for NKG2D activating receptor, were found to be induced on DCs upon IFN-α stimulation and to be responsible for the NK activation because mAb-mediated masking of MICA/B as well as inhibition of direct cell-to-cell contact using transwell insert completely abolished DC-dependent NK cell activation by IFN-α. Finally, DCs recovered from chronic hepatitis C virus-infected patients showed defects in the induction of MICA/B and impaired ability to activate NK cells in response to IFN-α stimulation. These findings suggested that MICA/B induction on DCs may be one of the mechanisms by which IFN-α activates NK cells; this impairment might affect IFN-α responsiveness in hepatitis C virus infection. *The Journal of Immunology, 2003, 170: 1249–1256.

NK cells are important effector cells involved in innate immunity against tumors and a variety of pathogens (1). They also participate in the induction and regulation of subsequent adaptive immune responses via release of various cytokines and chemokines (2). Dendritic cells (DCs)† represent another cellular lineage involved in the initiation of immune responses. They are highly specialized APCs that can activate resting T cells after undergoing a differentiation step termed maturation (3). Several lines of evidence have established that DCs can also activate resting NK cells (4–7) and augment antitumor immunity by triggering NK cell effector functions in vivo (8). These results suggest that DC-mediated activation of NK cells should play an important role in the initiation and regulation of immune responses. However, the mechanisms by which DCs activate resting NK cells are not fully understood. Indeed, the requirement for direct NK-DC contact (4, 8), soluble factors (5), and DC maturation (6) vary among the studies reported.

It is well known that NK cell activation is regulated by several cytokines such as IL-2, IL-12, IL-18, and IFN-α (9). Whereas IL-2 is secreted from polarized effector Th1 CD4+ T cells, the others are predominantly produced by DCs. Soluble factors such as IL-12 and IL-18 (4, 10) were suggested to be partially involved in DC-mediated activation of NK cells. In contrast, IL-12 was shown to be dispensable under some experimental conditions (5, 7).

Recent advances in research on NK cytolysis have established that NK cell effector functions are regulated by a balance of inhibitory and activating signals transmitted by membrane receptors that recognize ligands on the cell surface of potential target cells (11, 12). NK cells express receptors for MHC class I which, upon ligation, inhibit NK cell-mediated cytolytic activity. NK cells also express several activating receptors, such as NKG2D and a group of natural cytotoxicity receptors including Nkp46, Nkp30, and Nkp44. Whereas viral proteins such as hemagglutinins of influenza viruses were identified as the ligand for Nkp46 and Nkp44 (13, 14), human ligands for NKG2D were recently found to be stress-inducible MHC class I-related chain A and B (MICA/B) (15, 16). MICA/B are predominantly expressed in the gastrointestinal epithelium and tumor cells of epithelial origin (17, 18). Recent reports have demonstrated that Mycobacterium tuberculosis-infected DCs (19) also express MICA/B. These observations led us to investigate the possibility that MICA/B are induced on DCs under certain conditions and function as activator ligands for NK cells. With regard to direct interaction of NK cells and DCs, it should be noted that recent reports have identified Nkp30 (6) and/or Nkp46 (20) as critical cell surface molecules by which activated NK cells kill DCs. In addition, the adhesion molecule LFA-1 was also reported to be involved in NK cytolysis of DCs (21).

In this study, we used an in vitro coculture system to investigate whether MICA/B are induced on DCs and are involved in NK...
activation by DCs after exposure to various maturation stimuli. We found that IFN-α specifically induces MICA/B expression on DCs and thereby endows them with the ability to activate NK cells, as evidenced by an increase in IFN-γ production, CD69 expression, and cytolytic ability against K562 cells. We also found that induction of MICA/B is virtually absent on DCs derived from patients with chronic hepatitis C virus (HCV) infection, suggesting that this impairment may play a certain role in HCV infection.

Materials and Methods

Subjects

Ten healthy volunteers and 15 patients with chronic hepatitis C were enrolled in this study after informed consent had been obtained. Ten of 15 HCV-infected patients had previously received one or two rounds of IFN-α therapy but had not achieved sustained viral eradication, and the others had not received IFN-α therapy. They were histologically diagnosed as having mild or moderate chronic hepatitis and did not display any evidence of other types of liver diseases.

Generation of monocyte-derived DCs from PBMCs

Monocyte-derived DCs were generated from peripheral venous blood of healthy volunteers or chronic hepatitis C patients. PBMCs isolated by ficoll Hypaque density centrifugation were resuspended with 60% Percoll (Sigma-Aldrich, St. Louis, MO) solution, layered 45% and 34% Percoll, and then centrifuged at 2400 × g for 45 min. The light density fraction floating on the middle layer, which contained highly purified monocytes, was seeded in 24-well culture plates at a density of 1.0 × 10⁸/ml (22). After 15 min of incubation at 37°C, nonadherent cells were removed and the adherent cells were cultured in Iscove’s modified Eagle’s medium (Life Technologies, Gaithersburg, MD) containing 10% FCS, 10 U/ml penicillin, 10% FCS, and 10 U/ml streptomycin. They were then washed twice and anti-mouse IgG (Coulter-Immunotech) as a second-step Ab. The cells were washed and incubated at 4°C.

Flow cytometric analysis of DCs

DCs (5 × 10⁵/ml) were washed and resuspended in PBS containing 1% BSA and 0.05% NaN₃ and incubated with a series of mAbs at 4°C for 30 min. The following mAbs were used for immunofluorescent staining: anti-CD86, HLA-DR (BD PharMingen, San Diego, CA), CD83, and DC-lysolipid (BD PharMingen). Each CD83 and CD86 mAb was incubated with the cells for 15 min at 4°C, followed by isotype-matched control IgG. The stained cells were analyzed by flow cytometry. NK cells in the presence of DCs was examined using flow cytometry. NK cells (5 × 10⁵) treated with IFN-γ (1000 U/ml) or LPS (10 μg/ml) for 24 h in 24-well plates were stained with FITC-labeled CD69 and PE-labeled CD56 for 30 min at 4°C. Intracellular staining was performed as described previously (4, 24). In brief, NK cells cultured with DCs were performed with pretreatment of 10 μM PMA plus 1 μM monomycin (Sigma-Aldrich) in the presence of 1 μM Golgi-Plug (BD PharMingen) for 4 h at 37°C. The PMA/monomycin pretreatment in our conditions facilitates detection of intracellular IFN-γ without affecting levels of baseline staining. At the end of the incubation period, NK cells were stained with PE-labeled CD56 mAb for 30 min at 4°C. Cells were then fixed and permeabilized with Cytofix/Cytoperm buffer (BD PharMingen) for 15 min at room temperature. Permeabilized cells were stained with FITC-labeled anti-IFN-γ mAb (mouse IgG1) or isotype-matched control IgG. The stained cells were analyzed by flow cytometry.

Analysis of intracellular IFN-γ and CD69 expression in NK cells cocultured with DCs

Early activation marker CD69 as well as intracellular IFN-γ expression in NK cells in the presence of DCs was examined using flow cytometry. NK cells (5 × 10⁵) cocultured with DCs (1 × 10⁵) treated with IFN-γ (1000 U/ml) or LPS (10 μg/ml) for 24 h in 24-well plates were stained with FITC-labeled CD69 and PE-labeled CD56 for 30 min at 4°C. Intracellular staining was performed as described previously (4, 24). In brief, NK cells cultured with DCs were performed with pretreatment of 10 μg/ml PMA plus 1 μM monomycin (Sigma-Aldrich) in the presence of 1 μM Golgi-Plug (BD PharMingen) for 4 h at 37°C. The PMA/monomycin pretreatment in our conditions facilitates detection of intracellular IFN-γ without affecting levels of baseline staining. At the end of the incubation period, NK cells were stained with PE-labeled CD56 mAb for 30 min at 4°C. Cells were then fixed and permeabilized with Cytofix/Cytoperm buffer (BD PharMingen) for 15 min at room temperature. Permeabilized cells were stained with FITC-labeled anti-IFN-γ mAb (mouse IgG1) or isotype-matched control IgG. The stained cells were analyzed by flow cytometry.

Analysis of MICA and MICB mRNA expression in DCs by RT-PCR

The presence of MICA/B mRNA in DCs was examined by RT-PCR. One microgram of total RNA extracted using ISOGEN (Nippon Gene, Toyama, Japan) was added to 80 pmol of random primers (Takara Shuzo, Shiga, Japan) and 10 mmol/L of each deoxynucleotide triphosphate, incubated at 65°C for 5 min and quickly chilled on ice. The mixture was combined with 50 μl Tris-HCl, 75 mM KCl, 10 mM DTT, 3 mM MgCl₂, and 100 U of Moloney murine leukemia virus reverse transcriptase (Life Technologies) and incubated at 37°C for 50 min. The reaction was terminated by heating to 70°C for 15 min. The resulting cDNA was used in the reaction mixture containing 10 pmol of each upstream MIC sense primer (5′-CACCCAG CAGTGGGGGAT-3′) and downstream MICA antisense primer (5′- GCAGGGATTTGAATCCCGACT-3′) or MICB antisense primer (5′- AGGCATTGCTAGTGGTGGCACC-3′), 10 μl Tris-HCl, 50 mM KCl, 1.5 mM MgCl₂, 2.5 mM each deoxynucleotide triphosphate, and 2.5 U of Taq DNA polymerase (Takara Shuzo). Each primer pair described above was designed to span at least one intron of the corresponding gene. The amplification protocol included a denaturation step at 95°C for 5 min, followed by 35 cycles of 95°C for 60 s for denaturation, 56°C for 60 s for annealing, and 72°C for 90 s for extension. As a control for the integrity of total RNA, primers specific for GP3DH, sense primer (5′-GCAACCCA GAAGACTGGGATGGC-3′) and antisense primer (5′-CATGTAAGGC CATGGATCACCAC-3′) were used.

Measurements of cytokine production of DCs

IFN-γ, IL-12 p70, and IL-18 in the culture supernatant were determined using commercially available ELISA kits according to the manufacturer’s instructions (Endogen, Woburn, MA).

Statistical analysis

Data demonstrated in Fig. 7 were expressed as the mean and SD and compared using two sample t tests with Welch’s correction. Differences were considered significant when the p value was <0.01.

Results

DCs activate NK cells after exposure to various maturation stimuli.

After being cultured in the presence of GM-CSF and IL-4 for 6 days, peripheral monocytes from healthy donors displayed a typical phenotype characterizing immature DCs with low to intermediate expression of CD83, CD86, and HLA-DR and no expression...
when immature DCs were cultured with 1000 U/ml of IFN-α, they obtained maturation phenotypes with high CD83, CD86, HLA-DR, and DC-LAMP. After further stimulation by LPS, poly(I:C), CD40L, or IFN-α for 24 h, they obtained maturation phenotypes with high CD83, CD86, HLA-DR, and DC-LAMP. When immature DCs were cultured with 1000 U/ml of IFN-α for 24 h, their phenotypes were characterized by high CD86, HLA-DR, and DC-LAMP, but low CD83 expression (Fig. 1A). To examine whether DCs can activate NK cells, allogeneic NK cells derived from peripheral blood of healthy human donors were cocultured for 24 h with DCs stimulated with or without maturation stimuli and then subjected to analysis of IFN-γ production, CD69 expression, and cytolytic ability against K562 cells. NK cells cocultured with LPS-, poly(I:C)-, CD40L-, or IFN-α-treated DCs increased cytolytic activity against K562 cells in comparison with NK cells cultured without DCs. In contrast, immature DCs had no effect on the enhancement of K562 cytolyis by NK cells. Although IFN-α is a well-known activator for NK cells, 24-h treatment of NK cells with IFN-α (1000 U/ml) did not augment NK cytolyis against K562 cells in our experimental conditions (Fig. 1B). Also, it should be noted that DCs stimulated with LPS, poly(I:C), CD40L, or IFN-α displayed no cytolitic activity against K562 (data not shown). Flow cytometric analysis showed that IFN-γ production as well as CD69 expression of NK cells clearly increased upon coculture with LPS-, poly(I:C)-, CD40L-, or IFN-α-stimulated DCs, but did not do so efficiently upon coculture with immature DCs (Fig. 1C). These results indicated that DCs gain the ability to activate NK cells after various maturation stimuli.

Expression of MICA/B on IFN-α-stimulated DCs

Expression of MICA/B on DCs after various stimuli for 24 h was determined by flow cytometry (Fig. 2A). Cell surface induction of MICA/B by IFN-α is inhibited by anti-IFN α/β receptor neutralizing Ab. DCs were treated with 1000 U/ml IFN-α for 24 h in the presence or absence of 30 μg/ml anti-IFN α/β receptor neutralizing Ab and then were analyzed for MICA/B expression by flow cytometry. All experiments were performed at least three times and representative results are shown.
MICA/B was not detected on immature DCs. LPS, poly(I:C), and CD40L stimulation did not have any effect on MICA/B expression. In contrast, IFN-α stimulation substantially induced MICA/B expression on DCs. Additional experiments showed that IFN-α induced MICA/B on DCs in a dose- and time-dependent manner (Fig. 2B). RT-PCR analysis revealed that, upon IFN-α treatment, both transcripts of MICA and MICB genes were induced in DCs (Fig. 2C). Furthermore, addition of neutralizing mAb against IFN-α/β receptor abolished the induction of MICA/B on DCs by IFN-α (Fig. 2, C and D), indicating that MICA/B induction by IFN-α is regulated in receptor-mediated signal transduction mediated by IFN-α stimulation.

Cell-to-cell contact through MICA/B is responsible for NK cell activation by IFN-α-stimulated DCs

To examine whether MICA/B specifically induced by IFN-α are involved in NK cell activities, mAb 6D4, which can mask α1 and α2 extracellular domain of MICA/B (17), was incubated during the NK/DC coculture. The incubation of mAb 6D4 during coculture clearly inhibited K562 cytolysis of NK cells stimulated by IFN-α-treated DCs, whereas the same treatment had little effect on cytolytic activity of NK cells stimulated by LPS-treated DCs not expressing MICA/B (Fig. 3A). In addition, IFN-γ production and CD69 expression of NK cells cocultured with IFN-α-stimulated DCs were substantially decreased when mAb 6D4 was incubated in NK/DC coculture, whereas the incubation of mAb 6D4 did not affect NK cells cocultured with LPS-stimulated DCs (Fig. 3B). We also measured IFN-γ production in the supernatant of NK/DC coculture without the pretreatment of PMA/ionomycin. The ELISA result indicated that substantial levels of IFN-γ were produced in NK/DC coculture when DCs were stimulated with IFN-α or LPS. The incubation of mAb 6D4 during the coculture completely suppressed the IFN-γ production in NK/IFN-α-stimulated DC coculture, but it did not in NK/LPS-stimulated DC coculture (Fig. 3C). Taken together, these results indicated that MICA/B induced on DCs by IFN-α are responsible for the activation of NK cells.

To further examine whether direct cell-to-cell contact is necessary for NK cell activation conferred by IFN-α-stimulated DCs, NK cells were cultured with DCs together or separately by a transwell system for 24 h. NK cell cytolytic ability significantly increased when NK cells were cultured together with IFN-α-stimulated DCs, but not when the cells were separated by a 0.4-μm porous membrane. In contrast, LPS-, poly(I:C)-, or CD40L-stimulated DCs enhanced NK cell cytolytic ability, even if the cells were separated in transwells (Fig. 4A and data not shown). Similarly, the levels of CD69 expression and intracellular IFN-γ production of NK cells cultured with IFN-α-stimulated DCs in transwells were apparently lower than those of NK cells cocultured without porous membrane. Coculture in transwells did not affect CD69 expression and intracellular IFN-γ production of NK cells cultured with LPS-, poly(I:C)-, or CD40L-stimulated DCs (Fig. 4B and data not shown). These results indicated that intimate cell-to-cell contact is required for NK cell activation mediated by IFN-α-stimulated DCs, but not for that mediated by LPS-, poly(I:C)-, or CD40L-stimulated DCs.

Involvement of IL-12 in NK cell activation by LPS-stimulated DCs

Because it was previously reported that IL-12 and IL-18 were partially involved in DC-induced NK cell activation under some experimental conditions (4, 10), we measured IL-12 p70 as well as IL-18 in the supernatant of NK/DC coculture by ELISA. IFNα-stimulated DCs did not produce any detectable amounts of IL-12 p70 cocultured with or without NK cells. In contrast, DCs could produce IL-12 p70 in the presence of LPS stimulation; this production was markedly increased when LPS-stimulated DCs were cultured with NK cells for 24 h (Fig. 5A). Although the data are not shown, IL-18 could not be detected under any culture conditions. To examine the role of IL-12 in NK activation, we treated with recombinant IL-12 at the concentration of 1 ng/ml or 10 ng/ml for 24 h, and then subjected to the K562 cytolytic assay. As shown in

FIGURE 3. Induction of MICA/B is responsible for enhanced activation of NK cells by IFN-α-stimulated DCs. NK cells were cultured with DCs treated with 1000 U/ml of IFN-α or 10 μg/ml of LPS for 24 h at ratio of 5:1 in the presence of either anti-MICA/B mAb 6D4 (anti-MICA) or isotype-matched control IgG (IgG). A, The cytotoxicity of NK cells against K562 cells was assessed by 3H release assay. Single-cultured NK cells were also included as a control (NK). B, Intracellular IFN-γ and CD69 expression in NK cells cultured with or without DCs stimulated by IFN-α (upper panel) or LPS (lower panel) were analyzed by flow cytometry. The cultured cells were stained with PE-labeled anti-CD56 and FITC-labeled anti-IFN-γ or anti-CD69. CD56-positive cells were gated and analyzed. Similar results were obtained in three independent experiments and representative results are shown. C, ELISA detection of IFN-γ in NK/DC coculture supernatant. Note that the cells were not prestimulated with PMA/ionomycin.
NK cell mediated cytolytic activity against the K562 cell line. Single-cultured NK cells were also included as a control (NK). A, Intracellular IFN-γ and CD69 expression in NK cells cultured with or without DCs stimulated by IFN-α (upper panel) or LPS (lower panel) were analyzed by flow cytometry. The cultured cells were stained with PE-labeled anti-CD56 and FITC-labeled anti-IFN-γ or anti-CD69. CD56-positive cells were gated and analyzed. Similar results were obtained in three independent experiments and representative results are shown.

**Discussion**

It is well known that DCs, when exposed to infectious agents such as LPS, poly(I:C), IFN-α, or CD40L, undergo a process of differentiation termed maturation, which is characterized by up-regulation of MHC class I and II, adhesion, and costimulatory molecules. DCs can acquire such shared characteristics but also exhibit stimulus-specific responses (28–30). In the present study, we demonstrated that all of the stimuli tested consistently enabled DCs to activate NK cells, as evidenced by increased IFN-γ production, CD69 expression, and cytolytic activity of K562; however, the underlying mechanisms are different. This highlights the need for further studies to elucidate the molecular basis of these differences.
IFN-γ gated and analyzed by flow cytometry. Filled histograms indicate anti-IFN-γ or anti-CD69 stained CD56-positive cells with overlay of the isotype controls (open histograms). Open histograms represent the staining with control Ab. All experiments were performed several times and representative results are shown.

FIGURE 6. Impaired capacity of IFN-α-treated DCs from HCV patients (HCV-DCs) to activate NK cells. A. Impaired ability of IFN-α-stimulated HCV-DCs to enhance NK cytolytic activity against K562. Freshly isolated NK cells from healthy volunteers were cultured with HCV-DCs pretreated with IFN-α or LPS for 24 h. After brief washing, NK-mediated cytolysis of K562 cells was analyzed by standard 51Cr release assay at the indicated E/T ratio. Single-cultured NK cells were also included as a control. B. Impaired ability of IFN-α-stimulated HCV-DCs to stimulate IFN-γ production and CD69 expression in NK cells. Freshly isolated NK cells from healthy volunteers were cultured with HCV-DCs pretreated with IFN-α or LPS for 24 h. The cultured cells were stained with PE-labeled anti-CD56 and FITC-labeled anti-IFN-γ or anti-CD69. CD56-positive cells were gated and analyzed by flow cytometry. Filled histograms indicate anti-IFN-γ or anti-CD69 stained CD56-positive cells with overlay of the isotype controls (open histograms). C. Phenotype of HCV-DCs. Expression of CD83, CD86, HLA-DR, and DC-LAMP as well as MICA/B on immature HCV-DCs (immature) or those stimulated with IFN-α or LPS was evaluated by flow cytometry (filled histograms). Open histograms represent the staining with control Ab. All experiments were performed several times and representative results are shown.

FIGURE 7. Expression of MICA/B on IFN-α-stimulated DCs from healthy donors and chronic hepatitis C patients. The positive cell rate for MICA/B expression on DCs derived from normal volunteers (○; n = 10) as well as patients chronically infected with HCV (∇; n = 15) was determined by flow cytometry. Horizontal bars indicate mean and SD.

IFN-α seems to have somehow different capability in activating DCs from other maturation stimuli.

We demonstrated here that MICA/B, not expressed in immature DCs, are induced upon IFN-α stimulation and that direct cell-to-cell contact through MICA/B is responsible for NK activation by IFN-α-stimulated DCs. This MICA/B-dependent NK cell activation represents a distinct property of IFN-α-stimulated DCs, because other maturation-inducing stimuli tested in this study did not induce MICA/B expression on DCs. It remains unclear why poly(I:C), a known inducer of IFN-α (35), did not up-regulate MICA/B expression, but it may be possible that some soluble factors produced by poly(I:C), but not by IFN-α, may contribute to the suppression of MICA/B. As a mechanism of induction of cell surface MICA/B expression, it has been previously reported that MICA/B expression can increase to high cell surface levels by heat shock and thus may be under control of heat shock promoter element (17, 23). Our present study reveals that IFN-α can up-regulate expression of both transcripts of MICA and MICB through IFN type I receptor, but the precise mechanism of MICA/B induction on DCs should be determined in future study.

It has been generally considered that MICA/B function as activating ligands expressed on potential target cells and work in the effector phase of NK cytotoxicity. However, MICA/B expressed on DCs seem not to be simple target molecules, because mature DCs were relatively resistant to NK-mediated cytolysis (our unpublished data), in agreement with previous reports (36, 37). Therefore, MICA/B induced on DCs may be important for DC-mediated NK cell activation rather than triggering of NK-mediated killing of DCs exposed to IFN-α.

Both IFN-α and IL-12 are predominantly produced by DCs and have been known to be activating factors for NK cells (9). Our present data show that IFN-α itself is not able to efficiently up-regulate NK cytolytic ability and needs DCs as accessory cells (Fig. 1A). In contrast, IL-12 can activate NK cells by itself (Fig. 5B). IFN-α is widely used for clinical treatment of certain tumors and viral infections, and its antitumor and antiviral properties involve, at least partly, the ability to activate NK cells (9, 38). Our finding suggests that MICA/B induction on DCs may be one of the important mechanisms by which IFN-α activates NK cell-mediated immune responses, although further study will be needed to
examine the significance of MICA/B induction on DCs in the in vivo condition. In addition, given the recent findings that pre-DC2 is a main source of IFN-α upon virus infection (39–42), we are currently investigating the possibility that IFN-α derived from pre-DC2 induces its MICA/B expression in an autocrine manner.

The interesting finding of our study is that DCs derived from HCV-infected patients lacked the ability to express MICA/B in response to IFN-α, whereas other surface molecules such as CD86, HLA-DR, and DC-LAMP were normally up-regulated on DCs upon IFN-α stimulation in these patients (Fig. 6C). In addition, IFN-α-stimulated HCV-DCs could not activate NK cells, confirming again the critical role of MICA/B in DC-mediated activation of NK cells. IFN-α is constitutively expressed at low levels under physiological conditions and increases in response to viral infection. IFN-α-based therapy has been established as the most potent therapy for eradicating this virus from chronic HCV patients (43). Although the mechanisms by which IFN-α can eradicate HCV have not been fully elucidated, it has been recently recognized that IFN-α is capable of augmenting immune responses to efficiently eliminate HCV, and lack of this response may lead to insufficient virus eradication and thus critically infectious status (44–46). The present study demonstrates that IFN-α cannot induce DC-mediated NK cell activation in chronic HCV patients. This impairment might contribute to the persistence of this viral infection as well as insufficient outcome for IFN-α therapy. It is noteworthy that DCs derived from chronic hepatitis B patients expressed MICA/B in response to IFN-α stimulation (data not shown), suggesting that impaired MICA/B induction seems to be specific for chronic HCV infection. We have begun investigating whether impaired MICA/B induction on DCs is correlated with the response to IFNα-based therapy with a cohort of patients to further examine the functional relevance of MICA/B expression on DCs in chronic HCV infection.

In conclusion, we have demonstrated that MICA/B are induced on DCs by IFN-α stimulation and are capable of activating NK cells. This novel mechanism of NK activation by DCs might contribute to initiation and regulation of the innate immune response mediated by IFN-α.

References

In conclusion, we have demonstrated that MICA/B are induced on DCs by IFN-α stimulation and are capable of activating NK cells. This novel mechanism of NK activation by DCs might contribute to initiation and regulation of the innate immune response mediated by IFN-α.

References

In conclusion, we have demonstrated that MICA/B are induced on DCs by IFN-α stimulation and are capable of activating NK cells. This novel mechanism of NK activation by DCs might contribute to initiation and regulation of the innate immune response mediated by IFN-α.


