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*J Immunol* 2003; 171:5423-5429; doi: 10.4049/jimmunol.171.10.5423
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Autocrine/Paracrine IL-15 That Is Required for Type I IFN-Mediated Dendritic Cell Expression of MHC Class I-Related Chain A and B Is Impaired in Hepatitis C Virus Infection

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We previously reported that monocyte-derived dendritic cells activate resting NK cells by expressing MHC class I-related chain A and B (MICA/B), ligands for NKG2D, in response to IFN-α, but the MICA/B expression was severely impaired in patients with chronic hepatitis C virus (HCV) infection. In the present study, we examined induction of MICA/B on DCs by various innate cytokines and found that DCs from either healthy donors or HCV-infected individuals, upon IL-15 stimulation, express MICA/B and can activate NK cells, which is solely dependent on MICA/B-NKG2D interaction. Of interest is the finding that IL-15- and type I IFN-mediated induction of MICA/B in healthy donors is completely inhibited when DCs are incubated in the presence of anti-IFN-α/βR or anti-IL-15Rα, respectively, suggesting interdependent roles of these cytokines in MICA/B expression. Indeed, DCs produced IL-15 in response to type I IFN, whereas they directly produced IFN-β, in response to IL-15, which was followed by the production of IFN-α. In HCV-infected individuals, type I IFN-mediated production of IL-15 was virtually absent, but IL-15-mediated production of type I IFN was not compromised, which is consistent with the distinct ability of these cytokines to induce MICA/B in these patients. The present study demonstrates that IL-15 and type I IFN lead to DC expression of MICA/B and subsequent DC activation of NK cells, which is critically dependent on each other’s autocrine/paracrine effect, and suggests that impaired IL-15 production is one of the mechanisms of the aberrant response of DC to type I IFN in HCV-infected patients. The Journal of Immunology, 2003, 171: 5423–5429.

Natural killer cells are the major components of innate immunity for mounting the first line of defense against a variety of pathogens by directly killing infected cells (1). Activation of NK cells also affects subsequent adaptive immune responses by releasing various cytokines, such as IFN-γ (2). Recently, it has been shown that dendritic cells (DCs), another cellular lineage of innate immunity, can activate resting NK cells, although the underlying mechanisms seem to vary with the experimental conditions (3–10). MHC class I-related chain A and B (MICA/B) are ligands for NKG2D, which transduce positive intracellular signals in NK cells. In contrast to classical class I MHC molecules, MICA/B are not expressed by most normal cells, but are up-regulated in many epithelial tumor cells, in cells infected with human CMV, and in stressed cells. Therefore, it has been proposed that MICA/B play an important role in the elimination of transformed cells as well as infected cells by activating immune cells (11–14). We previously reported that DCs are able to express MICA/B upon IFN-α stimulation and gain the ability to activate NK cells, but the MICA/B induction is severely impaired in patients with hepatitis C virus (HCV) infection (15).

HCV causes persistent infection in >70% of infected patients. Whereas some of the patients show a carrier-like state, most develop chronic liver diseases, including chronic hepatitis, liver cirrhosis, and hepatocellular carcinoma, making HCV infection a worldwide health problem (16). Although IFN-α administration is a well-established antiviral therapy for chronic HCV infection, only one-third of the patients respond to this therapy (17). Patients who clear the virus mount a CTL response to a larger number of viral epitopes than those with chronic HCV infection (18). The clearance of HCV has also been correlated with a strong HCV-specific Th cell response (19, 20). In addition, NK cell numbers as well as their activity have been demonstrated to decrease in patients with chronic HCV infection (21–23). Taken together, it has been generally accepted that the perturbation of immune effector cells plays an important role in the establishment and/or the persistence of chronicity in HCV infection as well as resistance to IFN therapy. Although the mechanisms by which the functions of innate and adaptive immune cells are altered in HCV infection are largely unknown, defects in MICA/B expression on DCs may affect the activity of NK cells as well as the subsequent T cell responses, especially where endogenous and/or exogenous IFN-α exerts its biological effects. Therefore, it is of great interest to further assess the mechanisms of MICA/B expression on DCs.

DC functions are generally regulated by various innate cytokines, such as type I IFN, TNF-α, IL-12, IL-15, and IL-18 (24–

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Received for publication April 16, 2003. Accepted for publication September 4, 2003.

The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked advertisement in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

1 This work was supported by a Grant-in-Aid for Research on Hepatitis and BSE from the Ministry of Health Labour and Welfare of Japan, and Scientific Research from the Ministry of Education, Science, and Culture of Japan.

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3 Abbreviations used in this paper: DC, dendritic cell; HCV, hepatitis C virus; HCV-DC, DC recovered from HCV-infected individuals; MICA/B, MHC class I-related chain A and B; N-DC, DC recovered from healthy donors.
28). In the present study, we examined the induction of MICA/B on DC by various cytokines and found that IL-15 is capable of inducing DC expression of MICA/B and leads to DC activation of NK cells even in HCV-infected patients. Of interest is the finding that IL-15 and type I IFN lead to DC production of type I IFN and IL-15, respectively, and their autocrine/paracrine effect is critically required for MICA/B induction. In HCV-infected individuals, type I IFN-mediated production of IL-15 was virtually absent, whereas IL-15-mediated production of type I IFN was not compromised, consistent with the distinct ability of these cytokines to induce MICA/B in these patients. The present study demonstrates the interdependent roles of IL-15 and type I IFN in induction of MICA/B on DC and sheds light on the mechanisms of impaired DC function in HCV-infected patients in response to type I IFN.

Materials and Methods

Subjects in this study
Fifteen healthy volunteers and twenty patients with chronic HCV infection were enrolled in this study after informed consent had been obtained. All HCV-infected patients were confirmed to be positive for both serum anti-HCV Ab and HCV-RNA and did not display any evidence of other types of viral infection or liver diseases.

Generation of monocyte-derived DCs from PBMC
Monocyte-derived DCs were generated from the peripheral venous blood of healthy volunteers and chronic HCV-infected patients, as described previously (15). In brief, PBMCs isolated by Ficoll Hypaque density centrifugation were cultured on a Percoll (Sigma-Aldrich, St. Louis, MO) gradient consisting of three density layers (1.076, 1.059, and 1.045 g/ml). The fraction on the middle layer, which contained highly purified monocytes, was seeded in 24-well culture plates at a density of 5.0 × 10^5/ml. After 45 min of incubation, nonadherent cells were removed, and the adherent cells were cultured in Iscove's modified Eagle's medium (Life Technologies-BRL, Gaithersburg, MD) containing 10% FCS, 10 U/ml penicillin/streptomycin, and 2 mmol/L L-glutamine, and supplemented with GM-CSF (1000 U/ml, kindly provided by Kirin Brewery, Gunma, Japan) and IL-4 (500 U/ml, Strathmann Biotech., Hannover, Germany). At day 6, DCs were stimulated for 24 h using the following agents: IFN-α (Sumitomo Pharmaceutical, Osaka, Japan), IFN-β (Toray Pharmaceutical, Tokyo, Japan), IL-15, IL-12, IL-18, and TNF-α (R&D Systems, Minneapolis, MN).

Flow cytometric analysis of MICA expression on DC
DCs (5 × 10^5) were incubated with anti-MICA/B mAb (6D4) (29) at 4°C for 30 min. Isotype-matched Ig was used instead of 6D4 as a control. The cells were then washed and incubated at 4°C for 30 min in FITC-labeled mouse anti-goat IgG (BD Pharmingen, San Diego, CA) as a second-step Ab. The cells were then washed twice and fixed with 2% paraformaldehyde solution. The cells were analyzed by flow cytometry using a FACSscan system (BD Pharmingen), and data analysis was performed using CellQuest software.

Analysis of mRNA expression in DCs by RT-PCR
Total RNA (1 μg) was extracted using ISOGEN (Nippon Gene, Toyama, Japan), and was added to 80 pmol random primers (Takara Shuzo, Shiga, Japan) and 10 nmol/L of each deoxynucleotide triphosphate, incubated at 65°C for 5 min, and quickly chilled on ice. The mixture was combined with 50 mmol/L Tris-HCl, 75 mmol/L KCl, 10 mmol/L DTT, 3 mmol/L MgCl₂, and 100 U Moloney murine leukemia virus reverse transcriptase (Life Technologies-BRL) 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 sense and antisense primer, 10 mmol/L Tris-HCl, 50 mmol/L KCl, 1.5 mmol/L MgCl₂, 2.5 mmol/L each deoxynucleotide triphosphate, and 2.5 μl of TaqDNA polymerase (Takara Shuzo). The following primers were used: MIC sense, 5'-CACCAACCAAGCTGGGGGAT-3', MICA antisense, 5'-GCGAGGAATTGTGAATCCAC-3'; MICB antisense, 5'-AGCAGTCTGAGTTTGCCCAC-3' (14, 15); IL-15 sense, 5'-TAAACAGAAGCCACTG-3'; IL-15 antisense, 5'-CAAGAATGTGTGAGAACACCAT-3'; IL-15Ra sense, 5'-GCTAAAGGCTACAGGCTTTC-3'; IL-15Ra antisense, 5'-GGTGAAGTCTTCTCCGGAGG-3' (30). The amplification protocol included denaturation (MIC, 35 cycles of 95°C for 30 s; IL-15, 30 cycles of 94°C for 30 s; IL-15Ra, 35 cycles of 94°C for 60 s), followed by the annealing steps (MIC, 56°C for 60 s; IL-15, 55°C for 30 s; IL-15Ra, 60°C for 35 s) and extension (MIC, 90 s by 72°C; IL-15 and IL-15Ra, 60 s by 72°C). As a control for the integrity of mRNA, primers specific for G3PDH sense, 5'-GCCACCCAGAAGCTGGTGATGGC-3', and antisense, 5'-CATGTAGGCCATGAGGTCCACC-3', were used.

DC/NK coculture
NK cells were isolated from PBMCs of allogeneic healthy donors and labeled with enrichment Ab cocktails for NK cells, and then with magnetic colloid, according to the product inserts supplied by StemCell Technologies (Vancouver, BC, Canada). More than 90% of the cells were CD56^+ CD3^+ lymphocytes. Enriched NK cells were cultured in 24-well culture plates (5.0 × 10^5/well) in RPMI 1640 medium supplemented with 10% FCS and 10 U/ml penicillin/streptomycin. DCs stimulated with or without IFN-α or IL-15 were added at a concentration of 1.0 × 10^6/ml in complete medium to the same wells. In some experiments, a transwell insert was also used to prevent direct contact of NK cells and DCs in coculture systems, as previously described (14). As controls, unstimulated NK cells were plated in 24-well culture plates. In some experiments, anti-MICA/B mAb (6D4), anti-NKG2D mAb (ID11) (11, 12), anti-IL-15-neutralizing Ab, anti-IL-15Ra-neutralizing Ab, or anti-type I IFN receptor Ab (R&D Systems) was added at the beginning of the DC/NK coculture.

Cytolytic assay
Target cells (K562) labeled with ^51Cr were incubated in NK/DC cocultures (with or without transwell systems) or single cultures for 4 h at various E:T ratios. The supernatants were obtained after the incubation and subjected to gamma counting. The maximum or spontaneous release was defined as counts from samples incubated with 5% Triton X-100 or medium alone, respectively. Cytolytic activity was calculated with the following formula: % lysis = (experimental release – spontaneous release) × 100/(maximum release – spontaneous release). The spontaneous release in all assays was less than 20% of the maximum release.

Analysis of intracellular IFN-γ expression in NK cells cocultured with DCs
Intracellular IFN-γ expression in NK cells in the presence of DCs was examined using flow cytometry. NK cells (5 × 10^5) cocultured with DCs (1 × 10^5) treated with IFN-α or IL-15 for 24 h in 24-well plates were subjected to intracellular cytokine staining, as described previously (15, 31). In brief, NK cells cultured with DCs were incubated with pretreatment of 10 ng/ml FMA plus 1 μmol/L ionomycin (Sigma-Aldrich) in the presence of 1 μg/ml GolgiPlug (BD Pharmingen) for 4 h at 37°C. 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, and then stained with FITC-labeled anti-IFN-γ mAb (mouse IgG1). The stained cells were analyzed by flow cytometry.

Measurements of cytokine production of DCs
IL-15, IFN-α/β, IL-12p70, IL-6, TNF-α, and IL-1β in the culture supernatant of DCs were determined using a single solid-phase sandwich ELISA using paired specific mAbs and recombinant cytokine standards, according to the manufacturer’s instructions (IFN-α/β, IL-12p70, IL-6, TNF-α, and IL-1β from Endogen, Woburn, MA; IL-15 from BD Pharmingen). The limited thresholds of detection of these ELISA systems are as follows: IL-15, 3.7 pg/ml; IFN-α/β, 17.5 pg/ml; IL-12p70, IL-6, TNF-α, and IL-1β, 8.8 pg/ml.

Statistical analysis
The data are expressed as the mean and SD and compared using ANOVA with Bonferroni’s test. Differences were considered significant when the p value was <0.01.

Results

IL-15 induces DC expression of MICA/B in healthy donors as well as HCV-infected individuals
We tested healthy donors and HCV-infected patients to investigate the induction of MICA/B on DC by various innate cytokines (Fig. 1A). As reported previously, DCs from normal individuals (N-DCs), but not DCs from HCV-infected patients (HCV-DCs), expressed MICA/B in response to IFN-α. IFN-β had similar effects.
on DCs to IFN-α. Whereas TNF-α, IL-12, or IL-18 could not induce MICA/B expression on DCs, IL-15 clearly induced MICA/B, which correlated with the induction of MICA mRNA as well as MICB mRNA (Fig. 1, A and B). Interestingly, IL-15 was able to induce MICA/B expression on N-DCs and HCV-DCs. In contrast to IFN-α, MICA/B induction on HCV-DCs was not compromised in response to IL-15; the result clearly excludes the possibility that HCV-infected patients carry the genetic variation of MICA/B, such as polymorphism and null haplotype, which may affect the levels of MICA/B expression (32).

IL-15-stimulated DCs activate resting NK cells via MICA/B-NKG2D interaction

To examine whether IL-15 endows HCV-DCs with the ability to activate NK cells, allogeneic NK cells derived from peripheral blood of HCV-infected individuals were cocultured for 24 h with IL-15-stimulated HCV-DCs and then subjected to the analysis of cytolytic activity against K562 cells and IFN-γ production. For comparison, NK cells were also cocultured with IFN-α-stimulated HCV-DCs (Fig. 2, A and B). In contrast to IFN-α, IL-15 increased the ability of HCV-DC to activate NK cells (Fig. 2, A and B). It is noteworthy that the same dose of IL-15 used for DC stimulation was not capable of efficiently activating NK cells in terms of K562 cytosis as well as IFN-γ production (Fig. 2C). NK cells cocultured with IL-15-stimulated N-DCs increased cytolytic ability against K562 cells as well as IFN-γ production to extents similar to those cocultured with IFN-α-stimulated N-DCs (data not shown).

We next examined whether MICA/B expressed on IL-15-stimulated DCs are involved in the NK cell activation. When NK cells were separately cultured with HCV-DCs by a transwell system,
they did not show increased cytolytic ability, indicating that direct cell-to-cell contact is essential for IL-15-stimulated HCV-DC activation of NK cells (Fig. 3A). NK cells cocultured with IL-15-stimulated HCV-DCs in the presence of anti-MICA/B mAb did not show increased cytolytic ability (Fig. 3B). Also, increased NK cell production of IFN-γ conferred by IL-15-stimulated DCs was completely abolished when either anti-MICA/B mAb or anti-NKG2D mAb was added during the NK/DC coculture (Fig. 3C). Similar results were obtained when N-DCs were applied as stimulator cells (data not shown). These findings indicated that IL-15-stimulated DC activation of NK cells is solely dependent on MICA/B-NKG2D interaction.

**Impaired production of IL-15 from HCV-DCs in response to type I IFN**

It was previously reported that type I IFN induces IL-15 and IL-15R in human and murine DCs (25, 33). The fact that IL-15, but not type I IFN, induced immunologically relevant MICA/B in HCV-DCs led us to hypothesize that IL-15 may be produced in human DCs in response to type I IFN, but may not in those from HCV-infected patients. We therefore investigated whether there is a difference in IL-15 production in the supernatant of DCs between healthy donors and HCV-infected patients. Both IFN-α and IFN-β clearly induced production of IL-15 in N-DCs. In contrast, they were unable to activate HCV-DCs to produce IL-15, whereas there was little difference in the production of other cytokines, such as IL-12, TNF-α, IL-6, and IL-1β between N-DCs and HCV-DCs (Fig. 4A). RT-PCR analysis also revealed induced expression of IL-15 mRNA, in response to IFNα, in N-DCs, but not in HCV-DCs (Fig. 4B).

IL-15 exerts its biological action by binding a trimeric IL-15R complex consisting of a specific α-chain for IL-15 (IL-15Rα), IL-2R β-chain, and common γ-chain (34). To examine whether IFN-α modulates IL-15Rα expression on human DCs, the mRNA expression for IL-15Rα was evaluated by an RT-PCR analysis (Fig. 4B). As previously reported, the RT-PCR yielded at least two

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**FIGURE 3.** Involvement of MICA/B-NKG2D interaction in NK cell activation by IL-15-stimulated HCV-DCs. A. Freshly isolated allogeneic NK cells were cultured for 24 h with HCV-DCs stimulated with IL-15 (50 ng/ml) directly or separately using a 0.4-μm inserting membrane (transwell). 51Cr release assay was performed to evaluate NK cell-mediated cytolytic activity against the K562 cell line. Single-cultured NK cells were also included as a control. B. NK cells were cocultured with unstimulated HCV-DCs or IL-15-stimulated DCs for 24 h in the presence of either anti-MICA/B mAb or isotype-matched control IgG. The cytotoxicity of NK cells against K562 cells was assessed by 51Cr release assay. C. NK cells were cocultured with IL-15-stimulated HCV-DCs in the presence or absence of anti-MICA/B mAb 6D4 or anti-NKG2D mAb 1D11 for 24 h. Intracellular IFN-γ expression of NK cells was analyzed by flow cytometry. The number of the upper right quadrant represents CD56-positive cells expressing IFN-γ. All experiments were performed at least three times, and representative results are shown.

**FIGURE 4.** Expression of IL-15 and IL-15Rα in DCs by IFN-α. A. Production of IL-12, IL-15, TNF-α, and IL-1β from DCs stimulated by IFN-α. DCs generated from healthy (N-DC) and HCV-infected (HCV-DC) individuals were stimulated with or without IFN-α (1000 U/ml) for 24 h (n=5 for each group). IL-15, IL-12p70, IL-6, TNF-α, and IL-1β were measured in each culture supernatant by ELISA. B. RT-PCR analysis of DC expression of IL-15 and IL-15Rα. N-DCs and HCV-DCs were stimulated with or without IFN-α (1000 U/ml) for 24 h. Total RNAs were isolated from DCs and then subjected to RT-PCR analysis for expression of IL-15 (357 bp) as well as IL-15Rα (778 bp) transcripts. Note that PCR products less than 778 bp represent alternatively spliced forms of IL-15Rα. G3PDH expression serves as a control. Similar results were obtained in three independent experiments, and a representative result is shown.
different products, including 778-bp fragments reflecting the presence of functionally relevant IL-15Rα and shorter bands arising from alternatively spliced forms (35). IL-15Rα mRNA was expressed on DCs either with or without IFN-α, but IFN-α clearly up-regulated IL-15Rα mRNA on N-DCs as well as HCV-DCs. These results indicated that IL-15 expression, but not IL-15Rα expression, is severely impaired in HCV-DCs in response to type I IFN.

**Autocrine/paracrine IL-15 is required for DC expression of MICA/B in response to type I IFN**

To examine the possibility of autocrine IL-15 being involved in MICA/B expression on type I IFN-stimulated DC, we added either anti-IL-15-neutralizing Ab or anti-IL-15Rα-masking Ab to the coculture of IFN-α-stimulated N-DCs and NK cells. As expected, both Ab treatments completely inhibited IFN-α-mediated MICA/B induction on N-DCs (Fig. 5). These results clearly indicated that autocrine IL-15-mediated activation of IL-15Rα is essential for MICA/B expression induced by type I IFN on DC and strongly support the idea that impaired production of IL-15 is responsible for the hyporesponsiveness of HCV-DCs to type I IFN in terms of MICA/B expression as well as the ability to activate NK cells.

**IL-15 activates DCs to produce IFN-α/β, which has the autocrine/paracrine action required for MICA/B expression**

IFN-α and IL-15 have been known to coordinate with each other to elicit distinct immune reactions (33, 34, 36). In addition, a recent report suggested that IL-15 induced IFN-α/β secretion in viral-infected DCs and monocytes (37). In the present study, we examined whether IFN-α/β are produced and play a role in induction of MICA/B on DCs stimulated by IL-15. ELISA data revealed that both IFN-α and IFN-β were clearly produced in the supernatant of DCs either from healthy donors or HCV-infected patients upon exposure to IL-15 (Fig. 6A). When anti-IFN-α/βR Ab was added during the incubation of DCs with IL-15, the production of IFN-α, but not that of IFN-β, was substantially suppressed, suggesting that DCs directly produce IFN-β upon exposure to IL-15, followed by the production of IFN-α in an IFN-α/βR-dependent manner. Furthermore, MICA/B expression on DCs in response to IL-15 is completely suppressed in the presence of anti-IFN-α/βR-masking Ab, as demonstrated by flow cytometry as well as RT-PCR analysis (Fig. 6, B and C). Taken together, the data indicated that autocrine IFN-α/β is required for DC expression of MICA/B in response to IL-15 and is conserved in HCV-infected individuals, which is consistent with the observation that even HCV-DCs can express MICA/B upon stimulation of IL-15.

**Discussion**

Innate immune responses are the hallmark of host defense against viral infections, and several cytokines have distinct roles in regulating innate effector cell functions to efficiently eliminate virulent pathogens (2). Among them, IL-15 plays an important role in vivo development as well as activation of NK cells (38, 39). IL-15 also mediates pleiotropic actions by driving the proliferation and maintenance of Ag-specific memory T lymphocytes as well as the production of Th1-driven cytokines (34, 40, 41). Recent studies have also shown that IL-15 can positively regulate the maturation processes as well as functions of DCs to generate efficient pathogen- or tumor-specific CTL responses (27, 42, 43). Moreover, DCs

**FIGURE 5.** Requirement of autocrine/paracrine IL-15 for IFN-α-mediated DC expression of MICA/B. DCs generated from healthy donors were stimulated with or without 1000 U/ml of IFN-α for 24 h in the presence or absence of anti-IL-15 Ab (30 µg/ml) or anti-IL-15Rα-neutralizing Ab (30 µg/ml), and then analyzed for MICA/B expression by flow cytometry (filled histograms). Open histograms represent the staining with control Ab. The number in each histogram represents the mean fluorescence intensity of MICA/B expression. Similar results were obtained in five independent experiments, and a representative result is shown.

**FIGURE 6.** Autocrine/Paracrine IFN-α/β in IL-15-mediated DC expression of MICA/B. A. Production of IFN-α/β from IL-15-stimulated DCs. DCs generated from healthy (N-DC) and HCV-infected (HCV-DC) individuals were stimulated with or without 50 ng/ml of IL-15 in the presence or absence of anti-IFN-α/βR-neutralizing Ab (30 µg/ml) for 24 h (n = 5 for each group). IFN-α and IFN-β were measured in each culture supernatant by ELISA. nd, Not detected. B. IL-15-mediated induction of MICA/B is dependent on type I IFN signal (RT-PCR analysis). N-DCs and HCV-DCs were treated with 50 ng/ml of IL-15 for 24 h in the presence or absence of anti-IFN-α/βR-neutralizing Ab (30 µg/ml), and then analyzed for MICA/B expression by flow cytometry (filled histogram). Open histograms represent the staining with control Ab. The number in each histogram represents the mean fluorescence intensity of MICA/B expression. C. IL-15-mediated induction of MICA/B is dependent on type I IFN signal (RT-PCR analysis). N-DCs and HCV-DCs were treated with 50 ng/ml of IL-15 for 24 h in the presence or absence of anti-IFN-α/βR-neutralizing Ab (30 µg/ml). Total RNAs were isolated from DCs and then subjected to RT-PCR analysis for expression of MICA as well as MICB transcripts. G3PDH expression serves as a control. Similar results were obtained in three independent experiments, and a representative result is shown.
IL-15 AND TYPE I IFN FOR MICA/B EXPRESSION ON DC

produce IL-15 in response to inflammatory stimuli, thus exerting their immunoregulatory function (33, 36). However, it remains largely obscure whether IL-15 affects the ability of DCs to modulate NK cell function. In the present study, we demonstrated that DCs, upon IL-15 stimulation, express MICA/B and activate resting NK cells, which is solely dependent on MICA/B-NKG2D interaction. Although IL-15 is known to act as a NK-stimulating factor (2, 34), IL-15 itself had only marginal effects on NK cell function compared with IL-15-stimulated DCs (Fig. 2C). This observation raises the possibility that DC-NK interaction may be an important means by which IL-15 modulates NK cell function in vivo.

We presented the evidence that type I IFN-induced DC expression of MICA/B is dependent on autocrine/paracrine IL-15, the lack of which is involved in impaired induction of MICA/B on HCV-DCs. Interestingly, type I IFN-induced up-regulation of IL-15 in IL-15Ra was not impaired in HCV-DCs. The mechanisms of how HCV-DCs differently regulate IL-15 and IL-15Ra induction through type I IFN-mediated signaling events remain unknown. It has been reported that IFN regulatory factor-1 is a critical factor for controlling IL-15 gene expression (44), and that STAT-1-mediated signaling is necessary for induction of IL-15 in vivo (36). In contrast, IL-15Ra promoter is highly activated by IFN regulatory factor-4-mediated regulation in HTLV-I-infected cells (45). Furthermore, it was reported that HCV viral proteins could down-regulate IFN responses via suppression of the STAT-1-mediated pathway in cultured hepatoma cells (46). Taken together, it is possible that HCV infection may selectively interfere with some types of IFN-related signaling events, but not with others, leading to diminished IL-15 production despite intact IL-15Ra up-regulation in HCV-DCs. However, further study is needed to clarify the precise mechanisms of distinct induction of IL-15 and IL-15Ra in IFN-α/β-treated HCV-DCs.

Another interesting finding of the present study is that IL-15 is able to stimulate DC production of IFN-α/β, and that autocrine/paracrine IFN-α/β is required for MICA/B expression in IL-15-stimulated DCs. Mattei et al. (33) previously reported that IL-15 enhanced the ability of murine DCs to stimulate Ag-specific CD8-positive T cell proliferation; the magnitude of IL-15-induced DC activation is reduced in mice deficient for type I IFN receptor, suggesting a role of IFN-α/β in the stimulation of DC. Because there is no MICA/B ortholog in rodents, the mechanisms of IFN-α/β-dependent DC activation by IL-15 should be different between rodents and humans. However, the present study suggests that autocrine/paracrine IFN-α/β-dependent DC expression of MICA/B is an important mechanism by which IL-15 modulates the immunoregulatory function of DCs in humans.

At present, we do not know why DC requires both signaling pathways mediated by IL-15 and type I IFN for expression of MICA/B. One possible explanation is that IFN-α/β may promote IL-15/IL-15R interaction by increasing the levels of IL-15Ra expression (Fig. 5B). If so, the IL-15-mediated signal, but not the IFN-α/β-mediated signal, is directly involved in MICA/B expression in DCs. Alternatively, we cannot exclude the possibility that a distinct IFN-α/β-mediated signal other than that leading to IL-15Ra up-regulation may be essential for MICA/B expression. It has been previously reported that the MICA/B expression is regulated under the control of the putative heat shock promoter element (12, 29), but other regulatory mechanisms remain obscure. In this regard, it seems to be interesting to determine the mechanisms of cytokine-mediated regulation of MICA/B expression.

IFN-α has been established as a standard therapy of chronic HCV infection for its potent antiviral property, but only one-third of the patients respond to this therapy (17). Thus, urgent clarification is necessary of the mechanisms involved in impaired anti-HCV actions by IFN-α. The present study sheds light on the possibility that a defect in IL-15 production from HCV-DCs may be one of the factors causing IFN-α not to induce sufficient anti-HCV responses because HCV-DCs could not activate NK cells in the presence of type I IFN. Because innate immunity plays an important role in bridging acquired immunity to trigger the appropriate antiviral immune responses (2), it is also possible that a defect in NK cell activation induced by IFN-α might lead to aberrant T cell responses and thus insufficient HCV recognition. Furthermore, given the previous report that MICA/B function as costimulatory molecules to stimulate NKG2D-bearing CD8αβ+ T lymphocytes (47), it is also postulated that lack of MICA/B expression on DCs may also affect HCV-specific CTL responses. Altogether, it would be interesting to examine whether the administration of IL-15 can improve the antiviral immune response induced by IFN-α in chronic HCV-infected patients.

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