Activation of the Leukemia Plasmacytoid Dendritic Cell Line PMDC05 by Toho-1, a Novel IDO Inhibitor

AKIE YAMAHIRA[1], MIWAKO NARITA[1], MINAMI IWABUCHI[1], TAKAYOSHI UCHIYAMA[1], SHUNPEI IWAYA[1], RIE OHIWA[1], YOSHINORI NISHIZAWA[1], TAKAFUMI SUZUKI[2], YUSAKU YOKOYAMA[2], SHIGEO HASHIMOTO[3], JUN TAKIZAWA[4], HIROHITO SONE[4] and MASUHIRO TAKAHASHI[1]

[1]*Laboratory of Hematology and Oncology, Graduate School of Health Science, Niigata University, Niigata, Japan;*
[2]*Department of Pharmaceutical Sciences, Toho University, Chiba, Japan;*
[3]*Division of Hematology, Nagaoka Red Cross Hospital, Nagaoka, Japan;*
[4]*Division of Hematology, Endocrinology and Metabolism, Department of Internal Medicine, Niigata University, Niigata, Japan*

Abstract. *Background/Aim: Indoleamine-2,3-dioxygenase (IDO) is a rate-limiting enzyme for tryptophan metabolism and plays an immunosuppressive role. Antigen-presenting cells, when activated, increase the expression of IDO, which results in the suppression of subsequent immune reaction. A novel IDO inhibitor, Toho-1, was explored for its applicability to immunotherapy. Materials and Methods: We investigated the effects of Toho-1 on antigen presentation and antigen-specific cytotoxic T-lymphocyte-inducing ability of leukemia plasmacytoid dendritic cell line PMDC05, which was established in our laboratory. Results: While antigen presentation-associated molecules in PMDC05 cells were increased by stimulation with lipopolysaccharide and interferon-γ, IDO mRNA and protein expression were also enhanced. Such treatment of PMDC05 cells in combination with Toho-1 enhanced the antigen-presenting and CTL-inducing ability of PMDC05 cells. Conclusion: These findings suggest the ability of Toho-1 to potentiate antigen-presenting cells and its applicability in immunotherapy of cancer.*

Indoleamine-2,3-dioxygenase (IDO) is a rate-limiting enzyme acting in the early phase of metabolism of tryptophan, which is an essential amino acid and rare among whole amino acids (1). Although there are several pathways in tryptophan metabolism, 95% of tryptophan is converted to kynurenine by the IDO-associated pathway. IDO oxidatively cleaves the double bond between the 2' and 3' carbons of indole ring in tryptophan (2, 3). Starvation of tryptophan by IDO induces cell-cycle arrest of T-lymphocytes (4), giving rise to apoptosis of cells (5). In addition, the metabolic products of tryptophan, such as kynurenine, 3-hydroxykinurenine and 3-hydroxyanthranilic acid, are toxic to T-lymphocytes (6, 7), leading to apoptosis of cells (8), and induce differentiation of naïve T-cells into regulatory T-cells (9). IDO is widely expressed in tumor cells, dendritic cells (DCs) (1), macrophages (4), microglial cells (10), eosinophils (11), fibroblasts (12) and endothelial cells (13). DCs with IDO expression play a role in immunoregulation (1). DCs do not express IDO without activation but begin to express IDO by stimulation with interferon (IFN) or various ligands of Toll-like receptors (1, 14).

1-Methyltryptophan (1-MT), a representative IDO inhibitor, which is a mimetic of tryptophan and has a competitive action against IDO, is in phase I/II clinical trials as a cancer vaccine adjuvant in a single administration or in combination with other cancer therapies (15). Recently methyl-thiohydantoin-tryptophan (MTH-Trp: Necrostatin-1®) was reported to be a more potent IDO inhibitor in comparison to 1-MT (16). Yokoyama *et al.* developed a novel IDO-inhibiting tryptophan derivative, Toho-1 (MTH-benz[e]tryptoline) (Figure 1), with high ability to block IDO activity (17). Although 1-MT, Necrostatin-1 and Toho-1 are all competitive inhibitors of IDO, Toho-1 has been reported to inhibit the metabolism of tryptophan to kynurenine as efficiently as Necrostatin-1 and more potently than 1-MT (17).

In order to potentiate the antigen-presenting ability of PMDC05 cells for application in cellular immunotherapy for tumors and severe viral infections, we investigated the use of Toho-1in activated PMDC05 cells, in which IDO activity is elevated.

Correspondence to: Masuhiro Takahashi, Laboratory of Hematology and Oncology, Graduate School of Health Sciences, 746-2 Asahimachi-dori, Chuo-ku, Niigata 951-8518, Japan. Tel/Fax: +81 252272387, e-mail: matak@clg.niigata-u.ac.jp

Key Words: Novel IDO inhibitor, Toho-1, leukemic plasmacytoid dendritic cell line, PMDC05, antigen-presenting ability, CTL-inducing ability, Necrostatin-1®.

Materials and Methods

Reagents and antibodies. LPS was purchased from Sigma-Aldrich (St. Louis, MO, USA). IFN-γ was donated by Shionogi Pharmaceutical Co. (Osaka, Japan). Phycoerythrin (PE)-labeled monoclonal antibodies to IgG1, CD54, CD58, CD80, CD86, HLA-DR (BD Biosciences, San Jose, CA, USA), CD40 and CD83 (Immunotech, Marseille, France) were used for flow cytometric analysis. Monoclonal antibody to human IDO (IDO-mAb) was purchased from Oriental Yeast Co. (Tokyo, Japan). IgG1κ from murine myeloma (MOPC21) (Sigma-Aldrich) and Alexa Fluor 488-conjugated goat anti-mouse IgG secondary antibody (Molecular Probes, Eugene, OR, USA) were used for staining IDO. Toho-1 was synthesized and generously donated by Dr. Yusaku Yokoyama (Faculty of Pharmaceutical Sciences, Toho University) (17). Necrostatin-1 was purchased from Sigma-Aldrich.

Culture of PMDC05 cells. Leukemia plasmacytoid dendritic cell (pDC) line, named PMDC05, was established from leukemia blasts of an HLA-A*02:06/24:02 patient with pDC leukemia by culture in Iscove's modified Dulbecco's medium (IMDM; Invitrogen, Grand Island, NY, USA) with 10% fetal bovine serum (FBS) in our laboratory (18). Proliferation of PMDC05 cells was maintained without cytokine or feeder cells. The characteristics of PMDC05 cells have been reported previously (19-22).

Stimulation of PMDC05 cells. PMDC05 cells were cultured at a density of 2×10^6/ml in 10% FBS-containing IMDM with or without 0.1 μg/ml LPS and 1,000 U/ml IFN-γ for 24 h. In the study relating to the effects of Toho-1, Toho-1 was added to the culture simultaneously with LPS and IFN-γ at a concentration of 1 μM.

Flow cytometric analysis. For analysis of surface molecules, PMDC05 cells were stained with PE-labeled mAb such as CD1a, CD40, CD80, CD83, CD86 and HLA-DR in the presence of Fc-γ receptor blocking reagent (phosphate buffered saline (PBS) containing 0.5% human γ-globulin and 0.1% NaN_3). For analysis of cytoplasmic IDO protein, PMDC05 cells were fixed with 250 μl BD Cytofix/Cytoperm solution (BD Biosciences) at 4˚C for 20 min after being washed once with PBS. Then PMDC05 cells were washed with 1 ml BD Perm/Wash buffer twice and stained with IDO-mAb (primary antibody) at 4˚C for 20 min in the presence of Fc-γ receptor blocking reagent. MOPC21 was used as an isotype control. Thereafter the cells were washed with BD Perm/Wash buffer twice and stained with Alexa Fluor 488-conjugated goat anti-mouse IgG secondary antibody at 4˚C for 20 min. Stained cells were analyzed with FACSCalibur flow cytometry (BD Biosciences) and data were analyzed with CellQuest Pro software (BD Biosciences).

Allogeneic mixed leukocyte culture (MLC). One hundred thousand normal peripheral blood mononuclear cells (PB-MNCs) were co-cultured in a 96-well flat-bottom microtiter plate (BD Biosciences) with graded numbers of PMDC05 cells, which had been cultured with or without LPS/IFN-γ for six hours in the presence or absence of Toho-1, then washed and irradiated with 60 Gy of ^{137}Cs generated gamma irradiation (PS-3000SB Cs-137, Pony, Osaka, Japan) immediately before MLC, as described previously (18).

CTL induction using WT1 peptide-pulsed PMDC05 cells. $CD8^+$ T-cells were separated from PB-MNCs of a healthy person with HLA-A*24:02 by using fluorescein-isothiocyanate (FITC)-labeled CD8 monoclonal antibody and anti-FITC microbeads (Miltenyi Biotec, Bergisch Bladbach, Germany) according to the manufacturer's instructions. PMDC05 cells were activated with LPS/IFN-γ for 24 hours in medium containing 10 μg/ml mutant-type 9mer WT1 peptide (CYTWNQMNL; NeoMPS, San Diego, CA, USA), which possesses antigenicity in HLA-A*24:02$^+$ person (21). PMDC05 cells were exposed to 1 μM Toho-1 or 40 μM [selected from previous literature (23)) Necrostatin-1 during the activation period. CD8+ T-cells were co-cultured with activated and 30 Gy-irradiated PMDC05 cells at a cell ratio of 2:1 in a 24-well plate containing 1.5 ml of 5% autologous serum-containing RPMI-1640 medium. Interleukin-2 (IL2) (Shionogi, Osaka, Japan) and IL7 (R&D Systems, Minneapolis, MN, USA) were added to the co-culture on day 3 at a final concentration of 50 U/ml and 10 ng/ml, respectively. Two-thirds of the medium with IL2 and IL7 were replenished every two to three days throughout the culture period. The co-culture was re-stimulated with the same WT1 peptide-pulsed PMDC05 cells on day 7, and mutant WT1 peptide/HLA-A*24:02 tetramer analysis was performed on day 7 and day 14 as described previously (21). Briefly, the co-cultured cells were double-stained with FITC-labeled CD8 antibody (BD Biosciences) and PE-labeled WT1 tetramers. Stained cells were analyzed with FACSCalibur flow cytometry and the data was analyzed by CellQest Pro software.

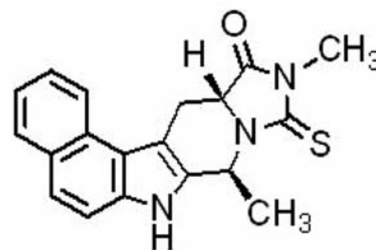

Figure 1. *Structure of a novel IDO inhibitor, Toho-1*.

Reverse transcription/real-time quantitative-polymerase chain reaction (RT/RQ-PCR). Total RNA was extracted from PMDC05 cells, which had been cultured with or without LPS/IFN-γ in the presence or absence of Toho-1, using RNAiso plus (TaKaRa BIO, Otsu, Shiga, Japan). Genomic DNA was eliminated from the total RNA by TURBO DNase free kit (Ambion, Austin, TX, USA). cDNA was synthesized from genomic DNA-eliminated total RNA using High Capacity cDNA Reverse Transcription Kit (Applied Biosystems, Foster City, CA, USA). RQ-PCR was performed by KAPA SYBR FAST qPCR kits (KAPA BIOSYSTEMS, Woburn, MA, USA) using the reverse-transcribed cDNA as the template and the primer sets of IDO and β-actin (internal control) listed in Table I. Threshold cycle (CT) was determined from a crossing point of a real-time PCR amplification curve and a threshold line by the instrument software. For quantification of *IDO* mRNA, the comparative ΔC_T and $\Delta\Delta C_T$ methods were used as described below. $\Delta C_T = C_T$ for *IDO* RNA of sample – CT for β-actin RNA of sample, which means that *IDO* mRNA was normalized with internal control mRNA. The relative quantification of the mRNA was calculated by the formula of $2^{-\Delta CT}$.

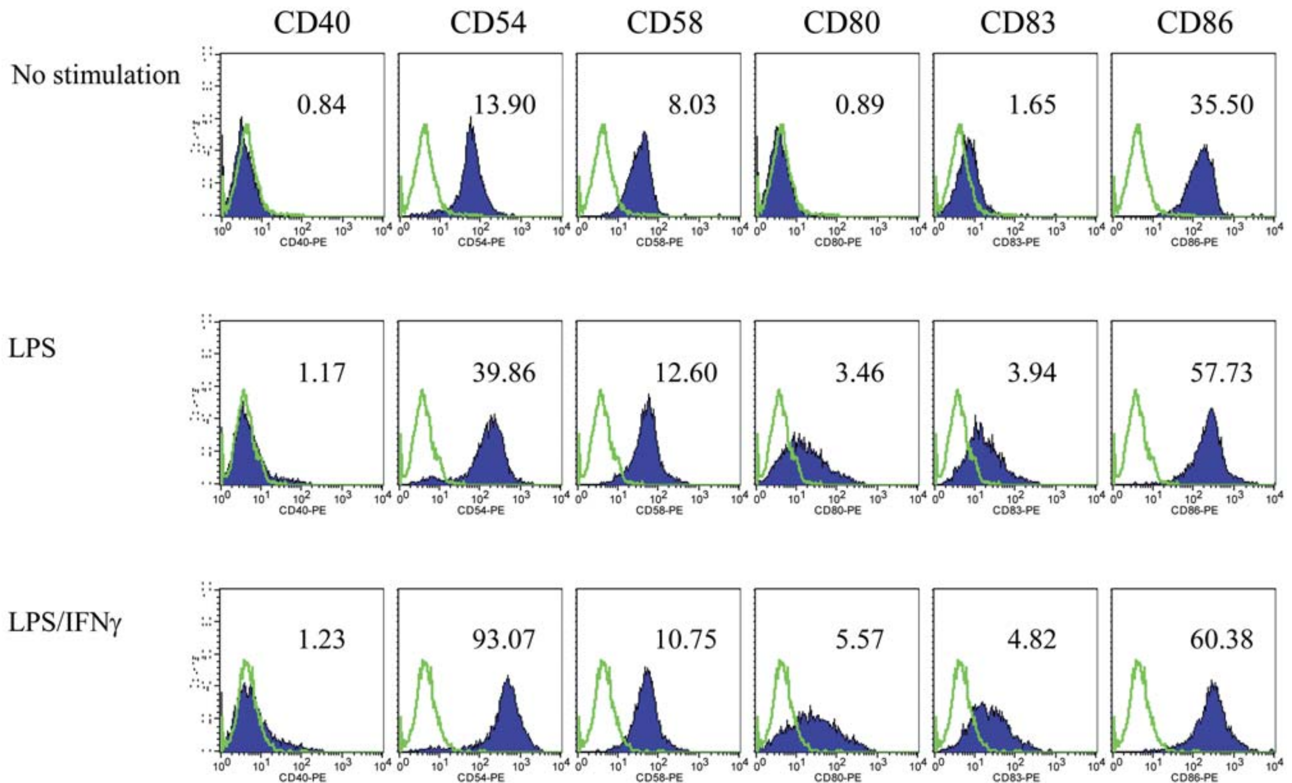

Figure 2. *Enhancement of antigen presentation-associated molecules in PMDC05 cells. Non-stimulated, LPS-stimulated and both LPS and IFN-γ-stimulated PMDC05 cells were analyzed for the expression of antigen presentation-associated molecules by flow cytometry. Values indicated are mean fluorescence intensities (MFI). Representative data of five experiments are shown.*

$\Delta\Delta C_T = \Delta C_T$ of *IDO* RNA for each sample – ΔC_T for RNA of the calibrator sample (namely RNA extracted from PMDC05 cells without any stimulation). This means that *IDO* mRNA of each sample was normalized with that of the calibrator sample.

Statistical analysis. The statistical relevance of differences in ^{3}H-thymidine incorporation in MLC was evaluated with the Student's *t*-test. Differences were considered significant at $p<0.05$.

Results

Enhancement of the expression of antigen presentation-associated molecules by LPS/IFN-γ in PMDC05 cells. PMDC05 cells were stimulated with LPS, IFN-γ or LPS and IFN-γ for 24 h and analyzed for the expression of antigen presentation-associated molecules by using flow cytometry. Stimulation with LPS enhanced the expression of CD54, CD58, CD83, CD86 and HLA-DR, which were already expressed in PMDC05 cells without any stimulation. Moreover, CD80, which was hardly expressed at all in PMDC05 cells without any stimulation, was expressed after stimulation with LPS or LPS with IFN-γ. CD40 expression was slightly increased by stimulation with LPS with IFN-γ. Enhancement of the expressions of these antigen presentation-associated molecules was more prominent by stimulation with both LPS and IFN-γ than with LPS alone (Figures 2 and 3). Concerning the effects of Toho-1 on CD80, CD86 and HLA-DR, the expression of these molecules was slightly increased at a concentration of 1 μM of Toho-1, but was reduced at 10 μM and 20 μM in dose-dependent manner (Figure 3).

Enhancement of the expressions of IDO mRNA and protein by LPS with IFN-γ in PMDC05 cells. PMDC05 cells were stimulated with LPS, IFN-γ , or LPS and IFN-γ for 24 h and analyzed for *IDO* mRNA expression by RT/RQ-PCR. *IDO* mRNA expression in PMDC05 cells was remarkably enhanced by the stimulation with LPS and IFN-γ compared to LPS and to IFN-γ alone (Figure 4). In addition, PMDC05 cells stimulated with IFN-γ alone showed a slight enhancement in the expression of IDO protein and the expression was clearly increased by the stimulation with both LPS and IFN-γ (Figure 5).

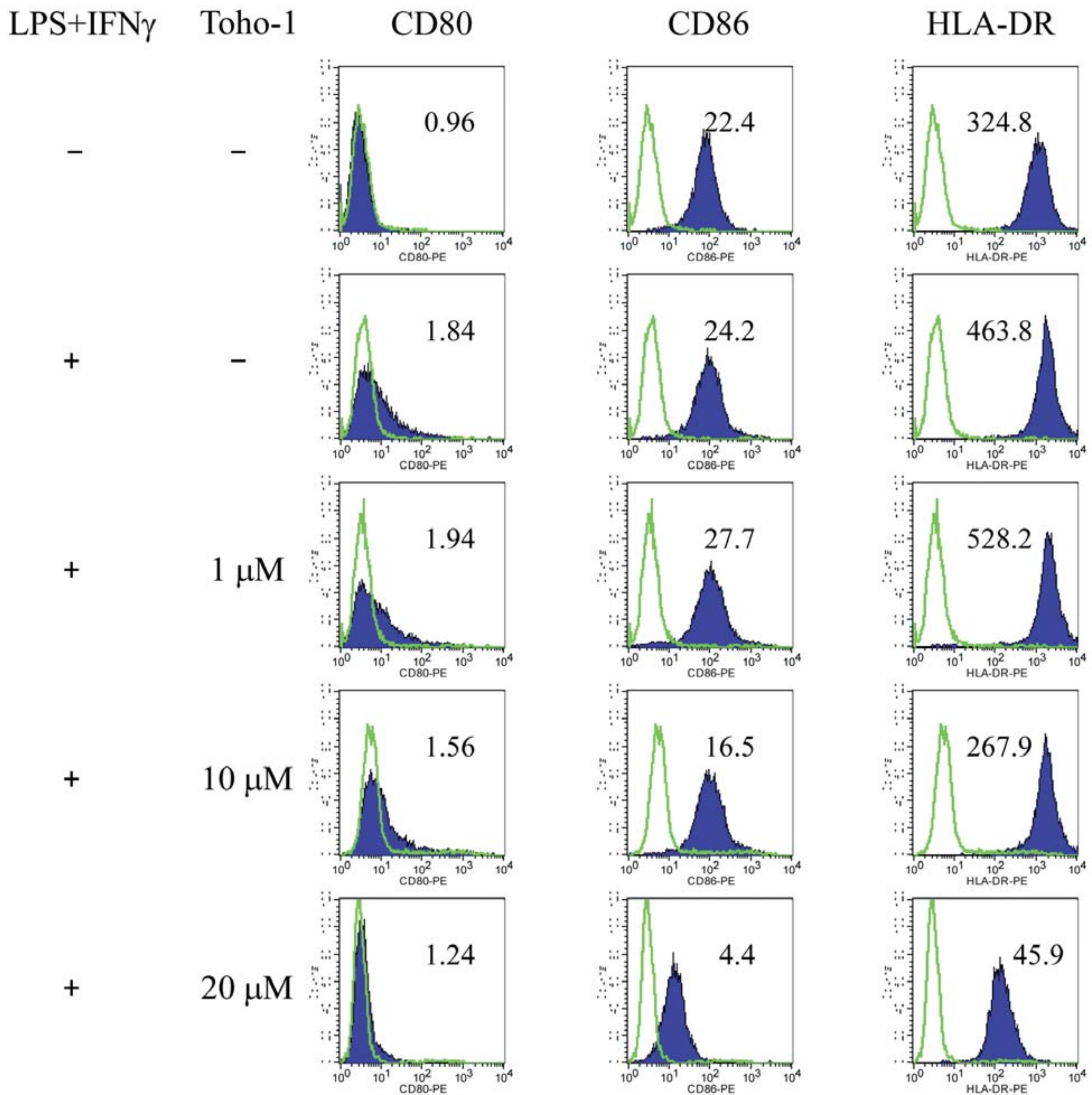

Figure 3. *Effects of Toho-1 on antigen presentation-associated molecules on PMDC05 cells. PMDC05 cells stimulated with or without LPS and IFN-γ in the presence or absence of Toho-1 at a concentration of 1, 10 or 20 μM were analyzed for expression of CD80, CD86 and HLA-DR by flow cytometry. Values indicated are mean fluorescence intensities (MFI). Representative data of three experiments are shown.*

Effects of Toho-1 on antigen presenting ability of PMDC05 cells. PMDC05 cells, which were cultured with LPS with IFN-γ and Toho-1 simultaneously for 6 h, were used as stimulator cells in MLC assay. Antigen-presenting ability of PMDC05 cells was evaluated by determining ^{3}H-thymidine incorporation in responder cells. Toho-1 enhanced antigen-presenting ability of PMDC05 cells in comparison with solvent control (DMSO) (Figure 6).

Effects of Toho-1 on antigen-specific CTL-inducing ability of PMDC05 cells. Mutant-type WT1-specific CTL induction from PB $CD8^{+}$ T-cells was compared among untreated PMDC05 cells, Toho-1 exposed PMDC05 cells and Necrostatin-1 exposed PMDC05 cells as stimulator cells. Exposure of Toho-1 to PMDC05 cells increased the generation of WT1-specific CTLs in co-culture for two weeks, but Necrostatin-1 did not show such an effect (Figure 7).

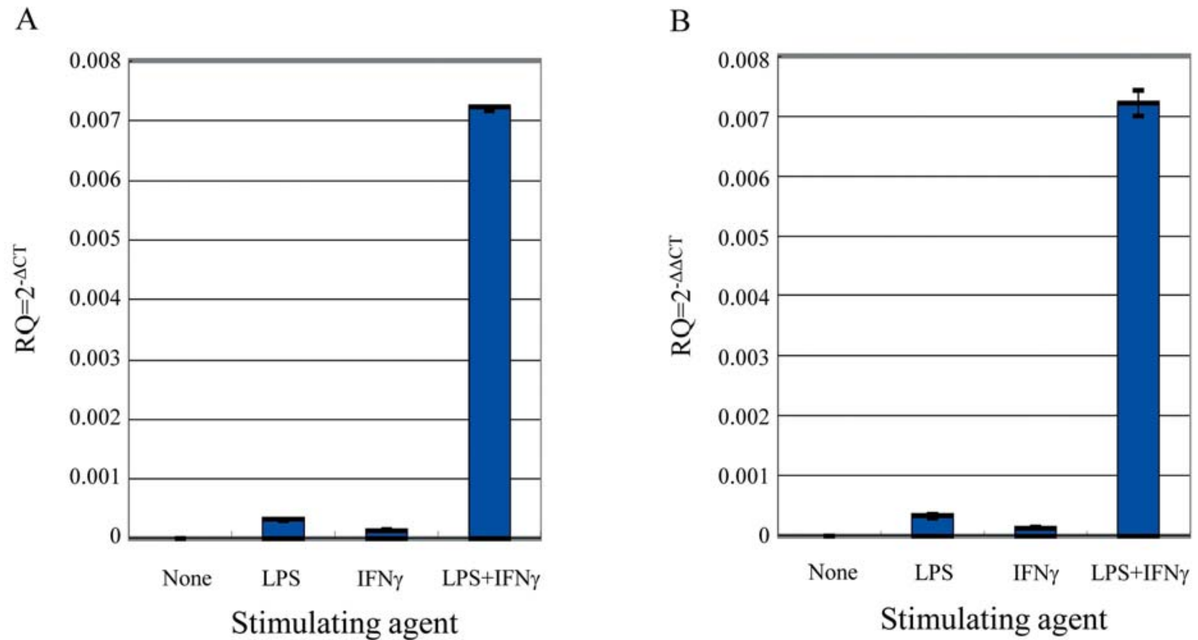

Figure 4. *Enhancement of IDO mRNA expression in PMDC05 cells by stimulation with LPS and IFN-γ. IDO mRNA was quantified by RT/RQ-PCR. The amounts of each transcript are shown by the comparative CT method. A: The relative quantification of sample mRNA against β-actin was determined by the formula of 2−ΔCT (described in Materials and Methods). B: The relative quantification of sample mRNA against calibrator sample (PMDC05 cells without stimulation) RNA was determined by the formula of 2−ΔΔCT (described in Materials and Methods). Another experiment rerurned identical results.*

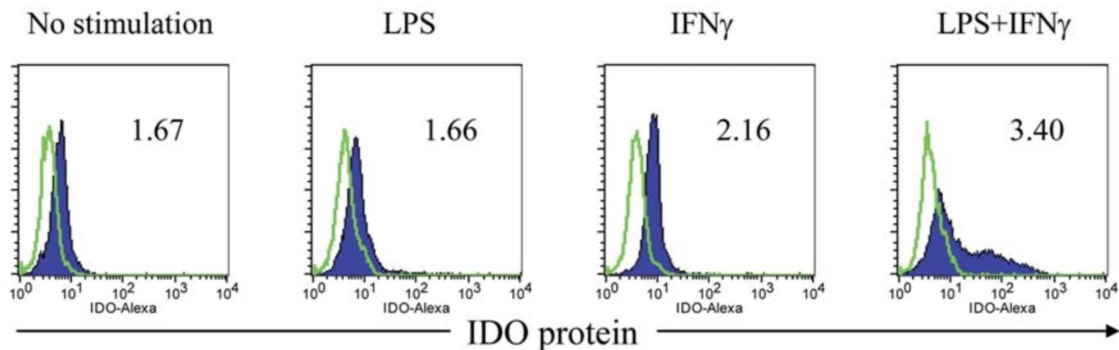

Figure 5. *Enhancement of IDO protein expression in PMDC05 cells by stimulation with LPS and IFN-γ. PMDC05 cells were analyzed for the expression of IDO protein by flow cytometry. Values in the Figure are mean fluorescence intensities (MFI). Representative data from among three experiments are shown.*

Discussion

We previously reported that PMDC05 cells stimulated with LPS-alone were able to enhance antigen-presenting ability and tumor antigen-specific CTL-inducing ability (21). Since the expression of antigen presentation-associated molecules in PMDC05 cells was much increased by stimulation with LPS with IFN-γ compared to LPS alone (Figure 2 and 3), PMDC05 cells stimulated with LPS with IFN-γ were thought to have a more potent antigen-presenting ability. However, when we use PMDC05 cells stimulated with LPS/IFN-γ as antigen-presenting cells, our major concern is IDO, which possesses immunosuppressive function in activated antigen-presenting cells. PMDC05 cells stimulated with LPS with IFN-γ increased their expression of *IDO* mRNA and IDO protein (Figures 4 and 5). Therefore, IDO expressed in PMDC05 cells could function as an immunosuppressive agent and reduce the LPS/IFN-γ-associated enhancement of

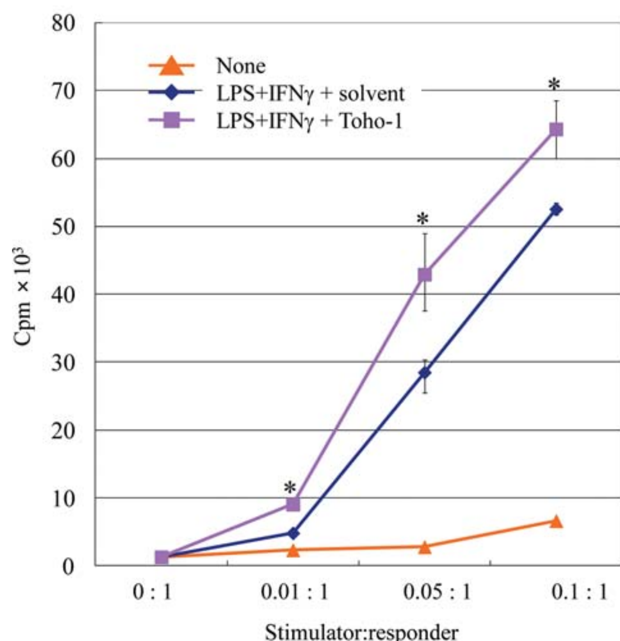

Figure 6. *Enhancement of antigen-presenting ability of PMDC05 cells by Toho-1. PMDC05 cells were cultured in medium including LPS/IFN-γ with or without Toho-1 for 6 h. Antigen-presenting ability was assayed by mixed leukocyte culture using ^{3}H-thymidine incorporation consisting of graded numbers of PMDC05 cells as stimulator cells and 2×10^5/well healthy PB-MNCs as responder cells. PMDC05 cells were cultured without stimulation, with LPS and IFN-γ with solvent (dimethyl sulfoxide), or with LPS and IFN-γ with Toho-1 for 6 h before mixed to the leukocyte culture. The statistical differences between Toho-1 and solvent control were evaluated with the Student's t-test. Significance at *$p<0.05$. The other two experiments gave similar results.*

antigen presentation by PMDC05 cells. Thus, we tried to inhibit the function of IDO expressed in activated PMDC05 cells by using a novel IDO inhibitor, Toho-1.

Regarding the effects of IDO inhibitors on the expression of antigen presentation-associated molecules of DCs, a novel IDO1-selective inhibitor, INCB024360, has been reported to inhibit apoptosis of DCs induced by IDO and increase the number of DCs with high expression of CD86 (24). However, it was reported that another IDO inhibitor, 1-MT, did not affect the expressions of antigen presentation-associated molecules, which had been enhanced in human pDCs activated with ODN 2006 (Toll-like receptor-9 ligand) (9). As to the effects of Toho-1 on the expression of antigen presentation-associated molecules, PMDC05 cells exposed to Toho-1 exhibited a baseline or a slight increase in their expression of CD80, CD86 and HLA-DR at a concentration of 1 μM. However, higher concentrations of Toho-1, such as 10 μM and 20 μM, reduced the expression of these molecules in a dose-dependent manner (Figure 3). In a separate study, we investigated the effects of Toho-1 and Necrostatin-1 on IDO protein expression of cord blood (CB) monocyte-derived DCs activated with LPS and IFN-γ. CB-monocyte-derived DCs were used for their clear expression of IDO when stimulated with LPS and IFN-γ. The expression of IDO protein in CB-monocyte-derived DCs was not influenced by the exposure to these IDO inhibitors (Figure 8). These findings were thought to be in accordance with the mechanism of action of these IDO inhibitors, which is competitive. In the present study, we investigated the effects of Toho-1 on the antigen-presenting ability and antigen-specific CTL-inducing ability of PMDC05 cells. Treatment with Toho-1 enhanced antigen presentation (Figure 6) and CTL induction (Figure 7) of PMDC05 cells when activated with LPS with IFN-γ. DC-based immunotherapy is a promising strategy in the treatment of tumors. Recently, IDO inhibitors have been applied in clinical trials in combination with immunotherapy against tumors. It has been reported that a new tumor vaccination therapy, which was developed in combination with 1-MT and tumor antigen peptide, was efficient for inhibiting IDO activity *in vitro* and displayed favorable antitumor effects *in vivo* (25). In addition, DCs treated with IDO inhibitors have been introduced for generating antigen-specific CTLs in adoptive immunotherapy (25). Together with the findings of our current study, an IDO inhibitor seems to provide a potent approach for enhancing the function of DCs, which could be applied in adoptive transfer immunotherapy.

In conclusion, we investigated the effects of a novel IDO inhibitor, Toho-1, on a pDC cell line and revealed its applicability for enhancing the antigen-presenting ability of PMDC05 cells when activated by stimulators such as LPS with IFN-γ. Our results indicate that Toho-1 is efficient for potentiating antigen presentation of DCs, including PMDC05 cells, and might be applicable for DC-based immunotherapy in tumors and severe viral infections.

Conflicts of Interest

Authors declare that they have no conflicts of interest.

References

1 Mellor AL and Munn DH: IDO expression by dendritic cells: tolerance and tryptophan catabolism. Nat Rev Immunol *4*: 762-774, 2004.

2 Grohmann U, Fallarino F and Puccetti P: Tolerance, DCs and tryptophan: much ado about IDO. Trends Immunol *24*: 242-248, 2003.

3 Stone TW and Darlington LG: Endogenous kynurenines as targets for drug discovery and development. Nat Rev Drug Discov *1*: 609-620, 2002.

4 Munn DH, Shafizadeh E, Attwood JT, Bondarev I, Pashine A and Mellor AL: Inhibition of T cell proliferation by macrophage tryptophan catabolism. J Exp Med *189*: 1363-1372, 1999.

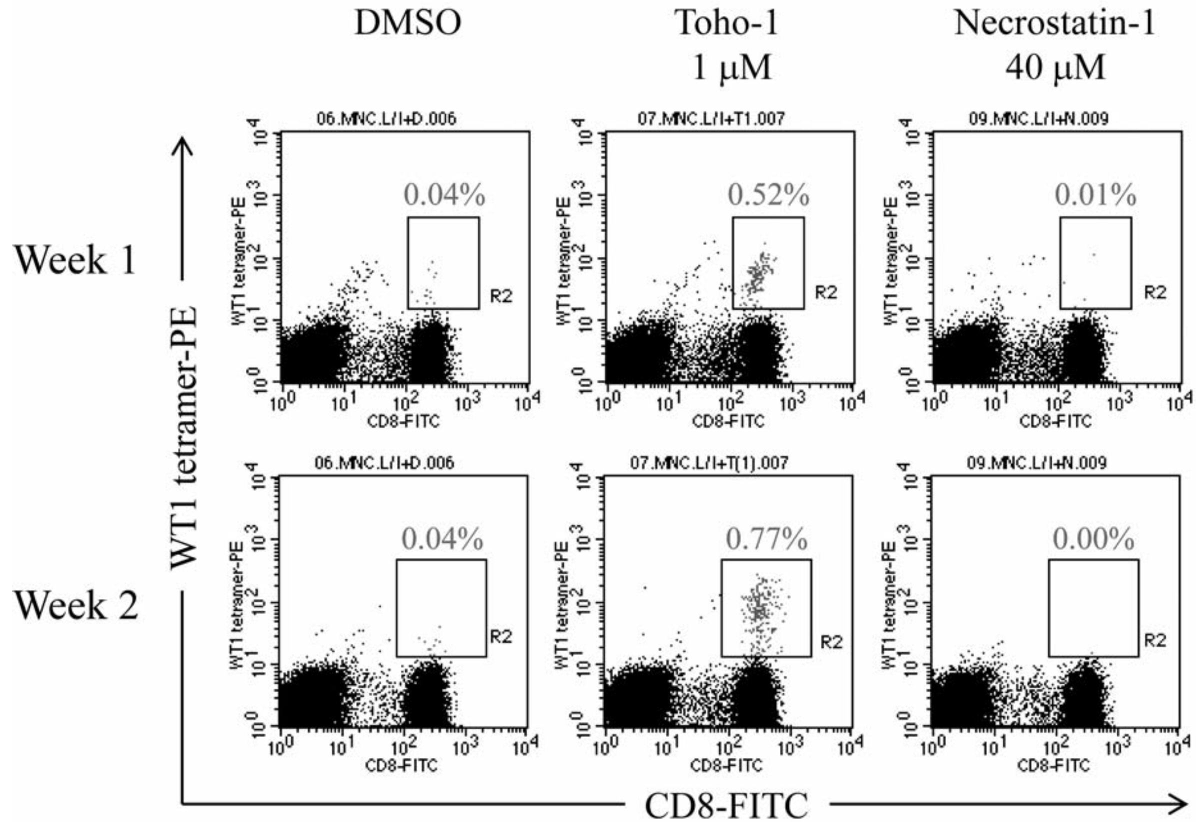

Figure 7. *Effects of Toho-1 on WT1-specific CTL induction from CD8+ T-cells stimulated with LPS and IFN-γ-activated PMDC05 cells. CD8+ T-cells from peripheral blood of a healthy door were cultured with WT1 peptide-pulsed and LPS and IFN-γ-stimulated PMDC05 cells in medium containing Toho-1 (1 μM) or Nectrostatin-1 (40 μM) for two weeks. The cells co-cultured for one or two weeks then double-stained with FITC-labeled CD8 antibody and PE-labeled WT1 tetramer. Values are the percentage of WT1 tetramer+/CD8+ T-cells among the cells in lymphocyte gate of forward scatter/side scatter dot plots. Data from one representative experiment, are shown.*

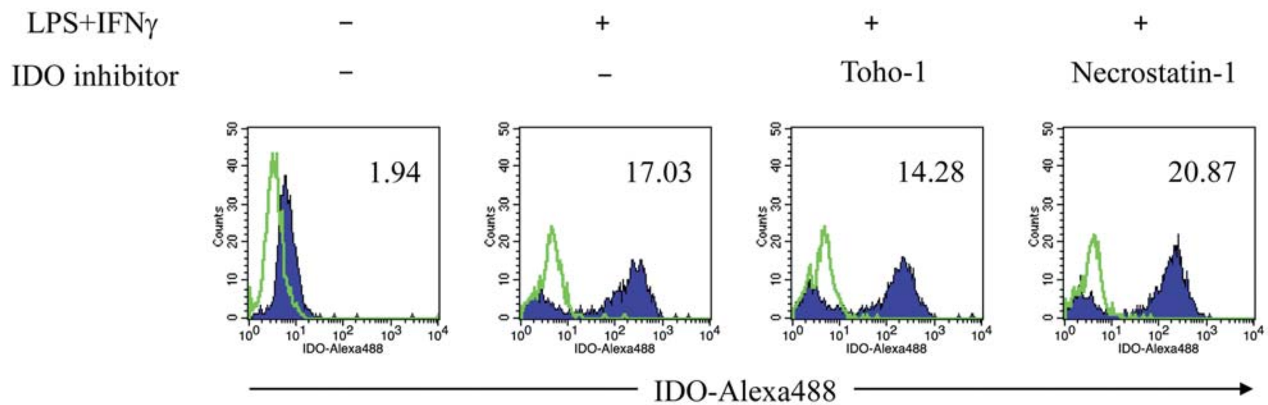

Figure 8. *Lack of effect of Toho-1 and Necrostatin-1 on IDO expression of moDCs. Monocytes were isolated by incubating cord blood MNCs in a plastic culture dish and removing non-adherent cells from the dish. Immature cord blood moDCs were induced from monocytes by culturing plastic adherent cells in RPMI1640 with 10% FBS, 50 ng/ml granulocyte/macrophage-colony stimulating factor (GM-CSF) and 5 ng/ml IL4 for six days. Immature moDCs were matured by adding 0.1 μg/ml LPS and 1,000 U/ml IFN-γ for 24 h. Cord blood-derived moDCs were exposed to Toho-1 (1 μM) or Necrostatin-1 (40 μM) during 24 h of the maturation process. Mature cord blood-derived moDCs were collected from the culture dish by pipetting and occasionally using a Cell Scraper and processed for staining with IDO. Data from one representative experiment, are shown.*

5 Lee GK, Park HJ, Macleod M, Chandler P, Munn DH and Mellor AL: Tryptophan deprivation sensitizes activated T cells to apoptosis prior to cell division. Immunology *107*: 452-460, 2002.

6 Frumento G, Rotondo R, Tonetti M, Damonte G, Benatti U and Ferrara GB: Tryptophan-derived catabolites are responsible for inhibition of T and natural killer cell proliferation induced by indoleamine 2,3-dioxygenase. J Exp Med *196*: 459-468, 2002.

7 Terness P, Bauer TM, Rose L, Dufter C, Watzlik A, Simon H and Opelz G: Inhibition of allogeneic T cell proliferation by indoleamine 2,3-dioxygenase-expressing dendritic cells: mediation of suppression by tryptophan metabolites. J Exp Med *196*: 447-457, 2002.

8 Fallarino F, Grohmann U, Vacca C, Orabona C, Spreca A, Fioretti MC and Puccetti P: T cell apoptosis by kynurenines. Adv Exp Med Biol *527*: 183-190, 2003.

9 Chen W, Liang X, Peterson AJ, Munn DH and Blazar BR: The indoleamine 2,3-dioxygenase pathway is essential for human plasmacytoid dendritic cell-induced adaptive T regulatory cell generation. J Immunol *181*: 5396-5404, 2008.

10 Guillemin GJ, Smythe G, Takikawa O and Brew BJ: Expression of indoleamine 2,3-dioxygenase and production of quinolinic acid by human microglia, astrocytes, and neurons. Glia *49*: 15-23, 2005.

11 Odemuyiwa SO, Ghahary A, Li Y, Puttagunta L, Lee JE, Musat-Marcu S and Moqbel R: Cutting edge: human eosinophils regulate T cell subset selection through indoleamine 2,3-dioxygenase. J Immunol *173*: 5909-5913, 2004.

12 Rezakhanlou AM, Habibi D, Lai A, Jalili RB, Ong CJ and Ghahary A: Highly efficient stable expression of indoleamine 2,3 dioxygenase gene in primary fibroblasts. Biol Proced Online *12*: 9028.

13 Beutelspacher SC, Tan PH, McClure MO, Larkin DF, Lechler RI and George AJ: Expression of indoleamine 2,3-dioxygenase (IDO) by endothelial cells: implications for the control of alloresponses. Am J Transplant *6*: 1320-1330, 2006.

14 Puccetti P and Grohmann U: IDO and regulatory T cells: a role for reverse signalling and non-canonical NF-kappaB activation. Nat Rev Immunol *7*: 817-823, 2007.

15 Huang L, Baban B, Johnson BA, 3rd and Mellor AL: Dendritic cells, indoleamine 2,3 dioxygenase and acquired immune privilege. Int Rev Immunol *29*: 133-155.

16 Muller AJ, DuHadaway JB, Donover PS, Sutanto-Ward E and Prendergast GC: Inhibition of indoleamine 2,3-dioxygenase, an immunoregulatory target of the cancer suppression gene Bin1, potentiates cancer chemotherapy. Nat Med *11*: 312-319, 2005.

17 Tanaka M, Li X, Hikawa H, Suzuki T, Tsutsumi K, Sato M, Takikawa O, Suzuki H and Yokoyama Y: Synthesis and biological evaluation of novel tryptoline derivatives as indoleamine 2,3-dioxygenase (IDO) inhibitors. Bioorganic & medicinal chemistry *21*: 1159-1165, 2013.

18 Narita M, Watanabe N, Yamahira A, Hashimoto S, Tochiki N, Saitoh A, Kaji M, Nakamura T, Furukawa T, Toba K, Fuse I, Aizawa Y and Takahashi M: A leukemic plasmacytoid dendritic cell line, PMDC05, with the ability to secrete IFN-alpha by stimulation *via* Toll-like receptors and present antigens to naive T cells. Leuk Res *33*: 1224-1232, 2009.

19 Narita M, Kuroha T, Watanabe N, Hashimoto S, Tsuchiyama J, Tochiki N, Saitoh A, Satoh N, Furukawa T, Toba K, Fuse I, Aizawa Y and Takahashi M: Plasmacytoid dendritic cell leukemia with potent antigen-presenting ability. Acta Haematol *120*: 91-99, 2008.

20 Watanabe N, Narita M, Yamahira A, Nakamura T, Tochiki N, Saitoh A, Kaji M, Hashimoto S, Furukawa T, Toba K, Fuse I, Aizawa Y and Takahashi M: Transformation of dendritic cells from plasmacytoid to myeloid in a leukemic plasmacytoid dendritic cell line (PMDC05). Leuk Res *34*: 1517-1524, 2010.

21 Yamahira A, Narita M, Nakamura T, Watanabe N, Kaji M, Taniguchi T, Hashimoto S, Furukawa T, Toba K, Aizawa Y, Kuzushima K and Takahashi M: Generation of antigen-specific cytotoxic T lymphocytes using a leukemic plasmacytoid dendritic cell line as antigen presenting cells. Leuk Res *35*: 793-799, 2011.

22 Yamahira A, Narita M, Ishii K, Jayathilake RM, Iwabuchi M, Satoh N, Uchiyama T, Taniguchi T, Hashimoto S, Kasahara N, Faure E, Bogan B, Takizawa J, Sone H and Takahashi M: Enhancement of antigen presenting ability in the leukemic plasmacytoid dendritic cell line (PMDC05) by lentiviral vector-mediated transduction of CD80 gene. Leuk Res *36*: 1541-1546, 2012.

23 Jurgens B, Hainz U, Fuchs D, Felzmann T and Heitger A: Interferon-gamma-triggered indoleamine 2,3-dioxygenase competence in human monocyte-derived dendritic cells induces regulatory activity in allogeneic T cells. Blood *114*: 3235-3243, 2009.

24 Liu X, Shin N, Koblish HK, Yang G, Wang Q, Wang K, Leffet L, Hansbury MJ, Thomas B, Rupar M, Waeltz P, Bowman KJ, Polam P, Sparks RB, Yue EW, Li Y, Wynn R, Fridman JS, Burn TC, Combs AP, Newton RC and Scherle PA: Selective inhibition of IDO1 effectively regulates mediators of antitumor immunity. Blood *115*: 3520-3530, 2010.

25 Yi YM, Zhang G, Zeng J, Huang SC, Li LL, Fang R, Jiang GM, Bu XZ, Cai SH and Du J: A new tumor vaccine: FAPtau-MT elicits effective antitumor response by targeting indolamine2,3-dioxygenase in antigen presenting cells. Cancer biology & therapy *11*: 866-873, 2011.

Received April 7, 2014
Revised June 4, 2014
Accepted June 5, 2014