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Identification of CD123$^+$ myeloid dendritic cells as an early-stage immature subset with strong tumoristatic potential

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Abstract

CD123 has been identified as a specific surface marker for plasmacytoid dendritic cells (PDCs). However, CD123 has recently been shown to be expressed on freshly isolated or in vitro generated myeloid dendritic cells (MDCs). In this article, we investigated whether the expression of CD123 on monocyte-derived MDCs was related to their function, especially to tumor-inhibiting potential. MDCs were induced from cord blood CD14$^+$ monocytes with granulocyte-macrophage colony-stimulating factor (GM-CSF) and interleukin-4 (IL-4) for 7 days, and then CD123$^+$ cells were isolated by positive immunomagnetic cell selection. We observed that CD123$^+$ cells lost monocyte CD14 expression, acquired immature myeloid dendritic cell phenotype and morphology. They exerted more significant endocytosis and less antigen-presenting function than CD123$^-$MDCs which are often referred to as typical MDCs. Meanwhile, CD123$^+$ MDCs exhibited more significant tumor-inhibiting activity toward hematological tumor cell lines of U937 and Jurkat even at a low effector:target ratio. CD123$^+$ MDCs expressed higher level of cytoplasmic TNF-α-related apoptosis-inducing ligand (TRAIL), but no detectable surface TRAIL and very little soluble TRAIL. Pretreatment with recombinant human TRAIL receptor 2:Fc fusion protein significantly reduced the tumor-inhibiting effect of CD123$^+$ MDCs, but not of CD123$^-$ MDCs. Overall, our data demonstrated that CD123$^+$ MDCs were an early-stage immature DC subset, with a significant tumor-inhibiting activity partially via involvement of enhanced cytoplasmic TRAIL.

Keywords: CD123; Myeloid dendritic cells; Tumor-inhibiting activity; TRAIL

Abbreviations: DCs, dendritic cells; PDC, plasmacytoid dendritic cell; MDC, myeloid dendritic cell; GM-CSF, granulocyte-macrophage colony-stimulating factor; IL-4, interleukin-4; TNF, tumor necrosis factor; TRAIL, TNF-α-related apoptosis-inducing ligand; Ab, antibody; mAb, monoclonal antibody; FCS, fetal calf serum; FACS, fluorescence activated cell sorter; MFI, mean fluorescence intensity; MLR, mixed lymphocyte reaction; E:T ratio, effector target ratio; TRAIL-R2:Fc, recombinant human TRAIL receptor 2:Fc fusion protein; SEM, standard error of the mean.

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1. Introduction

Human dendritic cells (DCs) contain several phenotypically and functionally distinct subpopulations. Two subsets of blood DCs have been described according to the differential expression of CD11c and CD123 (interleukin (IL)-3 receptor α chain) [1]. The typical myeloid DCs (MDCs), express CD11c, often lack expression of CD123 and have been proposed to be the key initiators of Th1 responses [2]. On the other hand, the $CD11c^-$ DCs, often referred to as plasmacytoid DCs (PDCs), express high levels of CD123 antigen, and are APC candidates regulating Th2 T cells. MDCs can be generated from monocytes after culture with combinations of granulocyte-macrophage colony-stimulating factor (GM-CSF) and interleukin-4 (IL-4) [3], whereas the survival of pDCs in vitro is interleukin (IL)-3-dependent and GM-CSF-independent [4]. However, some recent reports suggest that PDC-specific marker CD123 was also expressed on MDCs. In freshly isolated DCs, less than 1% were double positive ($CD11c^+$ $CD123^+$), and this double positive cells increase significantly after overnight incubation [5]. In classic myeloid culture system basically consisting of GM-CSF and IL-4, monocytes or $CD34^+$ cells can also differentiate into DC population functioning as typical MDCs but phenotypically expressing both CD11c and CD123 [6,7]. $CD123^+$MDCs might be a distinctive subset both in terms of function and differentiation stage.

As highly specialized APCs, DCs are responsible for induction of primary immune responses. Immature DCs are localized in the peripheral tissues, express low levels of costimulatory molecules and MHC molecules, and have high endocytic activity. Upon exposure to various microbial and inflammatory products, DCs mature and migrate into lymphoid tissues to interact with T and B cells [8–11]. In cancer settings, immature DCs first capture and digest dead cancer cells, then process their proteins into peptide epitopes, while mature DCs finally present these epitopes to specific T cells [12–14]. Besides their role as APCs, DCs have been recently demonstrated to exert cytotoxicity or cytostasis on some tumor cells [15,16]. In murine tumor models, injection of DCs can inhibit growth of tumors to some degree [11,17]. In rats, DCs possess cytotoxic activity toward NK-sensitive YAC-1 cells [18]. In humans, activated DCs appear to suppress growth of a variety of tumor cell lines in vitro [14]. Furthermore, Fanger et al. [15] have shown that a peripheral blood subset of $CD11c^+$ DCs, but not $CD123^+$ pDCs, stimulated by IFN-γ or IFN-α can induce cellular apoptosis in several tumor cells via involvement of tumor necrosis factor (TNF) family apoptosis-inducing pathway. These observations have led to the belief that, the role of DCs includes effecter function as well as antigen processing and presentation. However, there have been no reports concerning the characteristics of $CD123^+$ MDCs that bear myeloid and plasmacytoid DC surface marker.

In this article, we obtained novel information that $CD123^+$ MDCs derived from cord blood monocytes represented a population of early-stage immature MDCs, with more significant tumor-inhibiting activity than typical MDCs.

2. Materials and methods

2.1. Reagents

Human recombinant IL-4 was kindly provided by Ono Pharmaceutical Co. Ltd. (Osaka, Japan). Recombinant human GM-CSF was purchased from RHC Corporation (Suite, CA). The reagents mentioned above were dissolved in serum-free RPMI1640 (Life Technologies, Gaithersburg, MD) at 10× working concentration, aliquoted and stored at −80 °C. Before use, cytokines were diluted with fresh complete medium to working concentrations. FITC- or PE-labeled mouse anti-human CD14, CD1a, CD83, CD86, HLA-DR, CD11c, CD123 and their isotype control Abs, as well as non-conjugated mouse anti-human TRAIL mAb and FITC-labeled secondary Ab, were all obtained from BD PharMingen (San Diego, CA). Indirect immunomagnetic isolation kits for $CD14^+$ monocytes, $CD4^+$ T cells and $CD123^+$ cells were purchased from Miltenyl Biotec (Bergisch Gladbach, Germany).

2.2. Cell lines

The following human cell lines were originally purchased from Japan Type Culture Collection (JTCC, Tokyo): Jurkat (acute T-cell leukemia), U937 (histocytic lymphoma), HL60 (promyelocytic leukemia). The cell lines were grown under standard cell culture conditions.

2.3. Generation and isolation of monocyte-derived $CD123^+$ MDCs

Normal human cord blood was obtained with informed consent. The mononuclear cells were obtained by density gradient centrifugation on Histopaque 1077

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(sigma). Monocytes were isolated by positive immunomagnetic cell selection with anti-CD14-microbeads. To generate MDCs, monocytes were cultured at a density of 1×10^6/ml in the presence of GM-CSF (800 IU/ml) and IL-4 (1000 IU/ml) in RPMI 1640 medium supplemented with 10% fetal calf serum in 5% CO_2 at 37 °C. On day 7, CD123-positive cells were isolated by indirect immunomagnetic isolation kits, according to the manufacturer's instruction. Briefly, the cell suspension was labeled with PE-conjugated anti-CD123 monoclonal antibodies and subsequently with anti-PE antibody conjugated with magnetic microbeads. Labeled cells were positively selected with a magnet as $CD123^+$ MDCs, while isolated negative cells were named as $CD123^-$ MDCs. The positive cell preparations were >95% pure as confirmed by flow cytometric analysis. For some experiments, they were resuspended at 4×10^5/ml in the medium containing FCS, GM-CSF and IL-4 as described above for 48 h, then their supernatant was harvested for subsequent experiments.

2.4. Flow cytometric analysis

All analyses were performed by FACS Calibur flow cytometer using CellQuest software (Becton Dickinson, Mountain View, CA). The change of phenotype along DC differentiation was assessed by two-color immunofluorescence staining with CD11c-FITC in combination with CD123-PE as described [19]. Phenotypes of DCs selected on day 7 were determined following their direct single-color staining with FITC-conjugated mAb specific for CD86, CD83, CD14, CD1a and PE-conjugated mAb for HLA-DR. The cell surface or intracellular protein expression of TRAIL was assessed as described [20]. Briefly, aliquots of DCs were washed with cold PBS, resuspended in 1× FACS fixation solution (Immunotech) at 5×10^5 cells/0.1 ml at room temperature for 15 min. After one wash, cells were resuspended in FACS permeabilizing solution (Immunotech) or PBS at 5×10^5 cells/0.1 ml for the subsequent stain of intracellular or cell surface protein, respectively. Cells were then incubated with non-conjugated anti-TRAIL (2 μg/ml) mAb and FITC-labeled second mAb at room temperature for 30 min. Following the staining procedures, cells were fixed with 1% paraformaldehyde and analyzed. Relevant isotype negative control antibodies were used (gated arbitrarily as 1% positive) to allow determination of the percentage of positive event.

2.5. Morphologic analysis

Monocyte-derived $CD123^+$ and $CD123^-$ cells on day 7 of the culture were isolated by indirect immunomagnetic isolation. Morphologic analysis was performed by scanning electron microscopy and by the forward and side scatter profiles analyzed by flowcytometry as described [15,20].

2.6. Antigen-uptake experiment (phagocytosis assay)

To measure the endocytic activity of MDCs, a total of 2×10^5 $CD123^+$ and $CD123^-$ MDCs were incubated with 1 mg/ml of FITC-dextran (40,000 MW, sigma) at 37 °C and 0 °C (as a control) for 1 h. Cells were then washed four times with ice-cold PBS supplemented with 2% fetal calf serum (FCS) and analyzed by FACSCalibur. The level of antigen-uptake by DCs was calculated by the difference in mean fluorescence intensity (MFI) between the test (37 °C) and control (0 °C) for each sample.

2.7. Induction of lymphocyte proliferation

Cord blood $CD4^+$ T cells purified with anti-CD4 microbeads (5×10^4 cells/well) were mixed with irradiated $CD123^+$ or $CD123^-$ MDC at a stimulator:responder ratio of 1:5 in triplicate in 96-well U-bottomed plates for 5 days. Eighteen hours before harvesting the cells, ^{3}H-thymidine (1 μCi/well, Amersham Pharmacia Biotech) was added to each well. ^{3}H-thymidine uptake was counted in a liquid β-scintillation counter (MicroBeta Trilux Scntillation Counter; Wallac, Turku, Finland). Responses were reported as means cpm ± SEM for triplicate wells.

2.8. Tumor growth-inhibition assay

DC-mediated inhibition of tumor growth was measured by a 24 h ^{3}H-TdR uptake assay. Isolated $CD123^+$ or $CD123^-$ MDCs used as effector cells were cocultured with tumor target cells (1×10^3/100 μl/well) in 96-well plates at a graded effector:target (E:T) ratio of 5:1–40:1. Jurkat, U937 and HL60 cells were used as target cells. In some experiments, the effector cells (4×10^4/well) and their cell-free supernatant (100 μl/well) were pretreated with a TRAIL-R2:Fc (Alexis Biochemicals) (20 μg/ml) or isotype control human IgG1 (20 ng/ml) for 1 h before addition of tumor cells at an E:T of 10:1. Plates were incubated for 24 h and for an additional 24 h in the presence of 1 μCi/well of ^{3}H-TdR. ^{3}H-TdR incorporation was measured by means of a liquid scintillation counter. DCs did not incorporate a significant amount of ^{3}H-TdR (less than 1500 cpm), while tumor cells usually produced 20,000–100,000 cpm depending on the tumor line. TRAIL-R2:Fc and IgG1 exhibited no effect on target cells. The data were presented as the percentage of inhibition calculated from the following formula: % inhibition = (1-test cpm/control cpm) × 100%, where test cpm was ^{3}H-TdR incorporation by tumor cells cultured with DCs, and control cpm was ^{3}H-TdR incorporation by tumor cells cultured alone.

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2.9. Release of soluble TRAIL from DCs

DC-free supernatant collected as described above was measured for soluble TRAIL by ELISA using a commercial kit (R&D Systems) according to the manufacturer's recommendations.

2.10. Statistical analyses

Paired statistical analysis was performed by using the Student's 2-tailed *t-test.*

3. Results

3.1. CD123 expression progressively declined during MDC differentiation

Cord blood dendritic cells were generated from monocytes in the presence of GM-CSF and IL-4 which consisted of classical MDC-inducing system. We followed the change of CD123 and CD11c expression during DCs differentiation by two-color immunofluorescent staining at individual time points of the procedure. At day 0–1, $CD14^+$ monocytes differentiating towards DCs were almost all $CD123^+CD11c^+$. Subsequently, the fluorescence intensity of CD123 expression rapidly decreased, while the $CD123^+$cells still comprised a large population (44–61%) in the culture on day 7 (Fig. 1A). From days 7–10, the pattern of CD123 expression was essentially stable. Until day 14, $CD123^+$ cells constantly decreased to about 40%. On the other hand, $CD11c^+$ cell frequency remained stable (>90%) throughout the culture period, although they down-regulated the MFI of CD11c expression (Fig. 1B and C).

3.2. $CD123^+$ MDCs exhibited an immature dendritic cell phenotype

$CD123^+$ cells were immunomagnetically selected at the culture period of 7 days. We examined the $CD123^+$ populations for their expression of markers reported to be expressed on DCs. The $CD123^+$ populations lost CD14 expression (data not shown), and exhibited high levels of CD86 costimulatory molecules, moderate level of HLA-DR and human DC-specific marker CD1a, indicating that they were induced into DCs. However, neither $CD123^+$ nor $CD123^-$ population expressed detectable levels of maturation-specific marker CD83, showing that

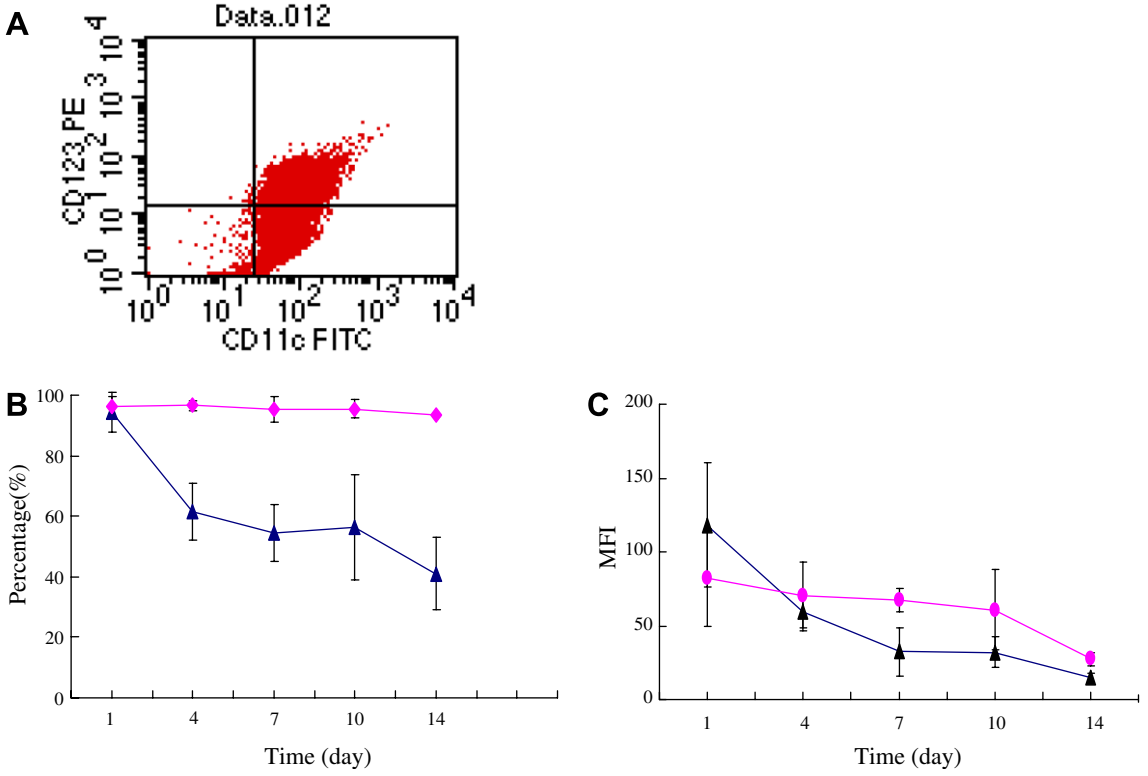

Fig. 1. CD123 and CD11c expression during the period of DC differentiation. Monocytes were induced into DCs with GM-CSF and IL-4. CD11c and CD123 expression was assessed by flow cytometry during the course of ex vivo culture for 14 days. The coexpression of CD123 and CD11c on cells on day 7 in a representative of three independent experiments is shown (A). The positive rate (B) and MFI (C) of CD123 (▲) and CD11c (♦) expression are expressed as means ± SEM in three independent experiments.

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they were immature DCs, and the lower expression of CD1a and HLA-DR on $CD123^+$ MDCs suggest that $CD123^+$ MDCs were more immature than $CD123^-$ counterparts within the fraction (Fig. 2).

3.3. $CD123^+$ MDC represented a subset of small-sized immature DCs

As $CD123^+$ MDCs exhibited different phenotype from $CD123^-$ MDCs which represented typical myeloid DCs, we investigated whether the morphologic feature of $CD123^+$ MDCs was distinct. As shown in Fig. 3A, $CD123^+$ MDCs were smaller than $CD123^-$ counterparts when they were observed under 3000 magnification with scanning electron microscopy, while the short and thick projections on $CD123^+$ MDCs were similar to those on the negative fraction. Consistent with this, $CD123^+$ MDCs were smaller in size and lower in granularity as indicated in the forward and side scatter profiles of flow-cytometric analysis (Fig. 3B).

3.4. $CD123^+$ MDCs exhibit higher antigen-uptake and lower T-cell stimulating activity

We then assessed whether $CD123^+$ and $CD123^-$ cells exhibited different ability to internalize FITC-dextran that would mimic pathogens. As seen in Fig. 4A, almost all of DCs from both subsets endocytosed dextran. During our experiments, a notable variation in antigen-uptake activity of DCs was observed among tested donors. However, the amount of median fluorescence intensity internalized by $CD123^+$ subset was more than corresponding $CD123^-$ fraction from each donor (Fig. 4B). Furthermore, Fig. 5 showed that $CD123^+$ MDCs significantly increase T-lymphocyte proliferation as compared with T cells cultured for 5 days in the absence of DCs. However, the T-cell stimulating activity of $CD123^+$ MDCs was nearly 2-fold less effective when compared with that of $CD123^-$ MDCs. These results suggest that $CD123^+$ MDCs represent a more immature DCs subset.

3.5. Growth-inhibiting effect of $CD123^+$ MDCs on human hematological tumor cells

Many tumor cells of hemopoietic-origin are sensitive to TRAIL-mediated apoptosis. To determine whether $CD123^+$ MDCs can affect the tumor cell growth through TRAIL, TRAIL-sensitive HL60, Jurkat and U937 were used as target cells in our study. We performed 48-h growth inhibition assays by co-culturing DCs with target tumor cell lines. As shown in Fig. 6A and B, at a high E:T ratio of 40:1, the growth-inhibiting effect exhibited no statistically significant difference between $CD123^+$ and $CD123^-$ MDCs. At E:T ratio of 20:1 or 10:1, $CD123^+$ MDCs exhibited more significant inhibitory activity against U937 and Jurkat than $CD123^-$ population. Evident cellular debris was observed under the light microscope when tumor cells were cocultured with $CD123^+$ MDCs (data not shown). However, no different growth-inhibiting effect between $CD123^+$ MDCs and $CD123^-$ MDCs against HL60 was observed at all E:T ratios examined (Fig. 6C).

3.6. Involvement of cytoplasmic TRAIL in the enhanced tumor-inhibiting activity of $CD123^+$ MDCs

Human immature DCs and $CD11c^+$ DCs could directly mediate apoptosis of tumor cells via TRAIL under certain conditions [16,20]. To investigate whether TRAIL was responsible for the enhanced growth inhibition effect of $CD123^+$ MDCs, we first measured cell surface and cytoplasmic expression of TRAIL on $CD123^+$

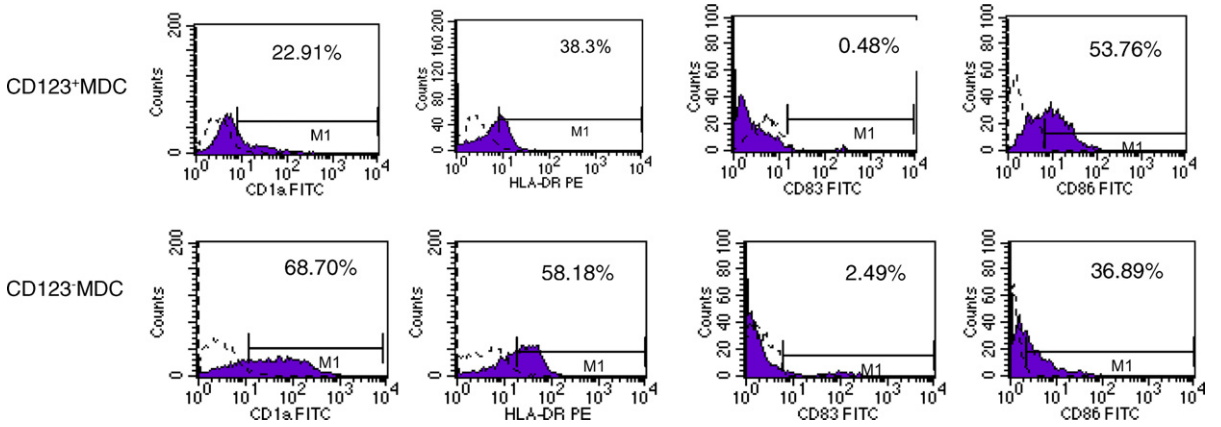

Fig. 2. Immunophenotypic characteristics of $CD123^+$ and $CD123^-$ MDCs. Monocyte-derived $CD123^+$ and $CD123^-$ MDCs were isolated by immunomagnetic beads. Then, they were analyzed for CD1a, HLA-DR, CD83, and CD86 expression by flow cytometry. Profiles obtained with isotypic control mAbs are shown in open histograms, whereas those obtained with the specific mAbs are shown in filled histogram. These data are representative of three independent experiments.

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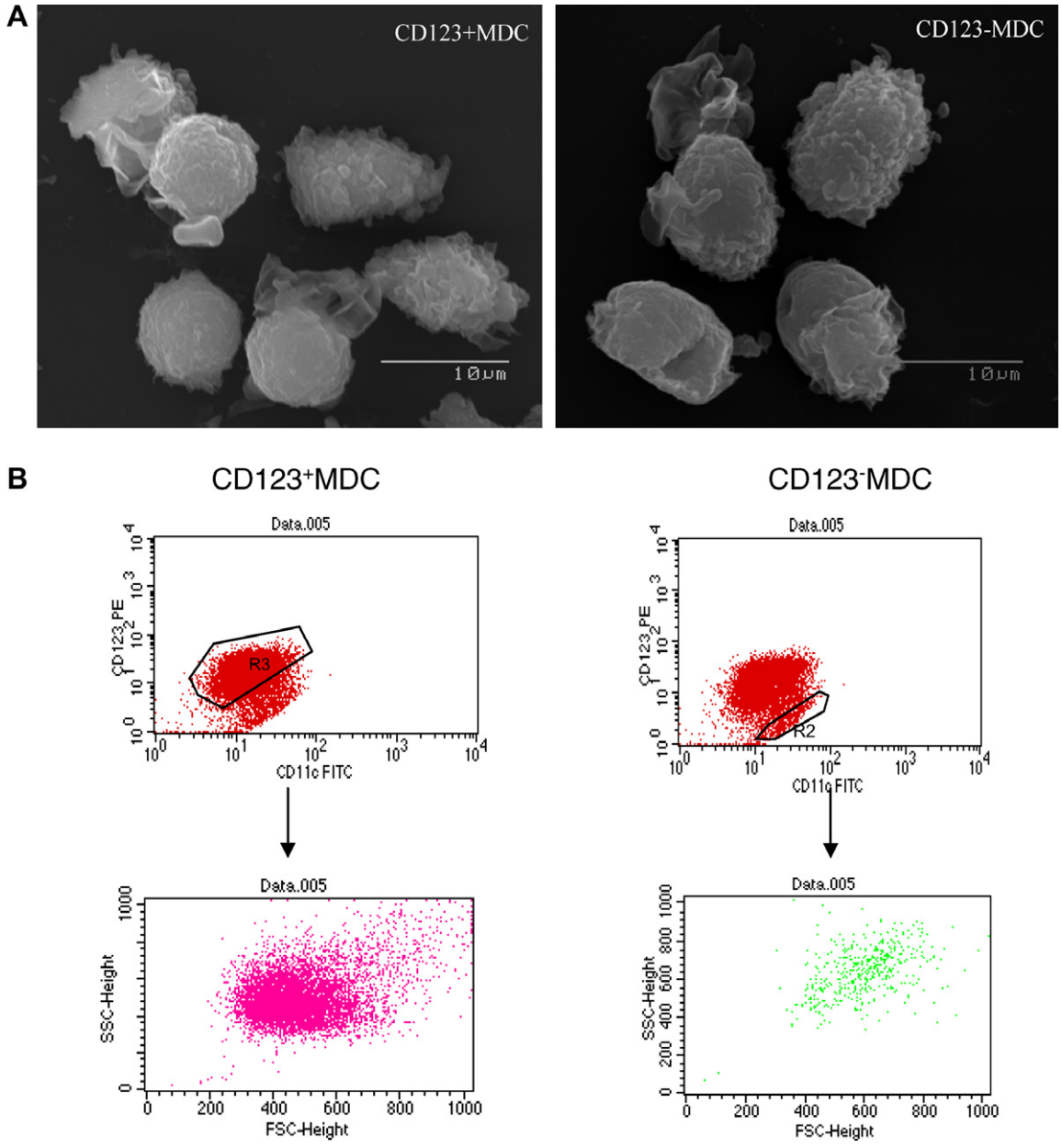

Fig. 3. Morphology of CD123$^+$MDCs in comparison with CD123$^-$MDCs. CD123$^+$ and CD123$^-$ MDCs were isolated from monocytes cultured for 7 days in the presence of GM-CSF and IL-4. (A). The pictures illustrate the morphology of MDCs with scanning electron microscopy (3000×). (B). Forward light scatter (FSC) and side light scatter (SSC) are compared between CD123$^+$ MDCs and CD123$^-$ MDCs. DCs were labeled with anti-CD123 PE and anti-CD11c FITC on day 7 of the culture. CD123$^+$ MDCs (R3) and CD123$^-$ MDCs (R2) were shown on the corresponding forward/side scatter parameters.

MDCs isolated at the culture period of 7 days. As revealed in Fig. 7, cytoplasmic TRAIL was more strongly expressed on CD123$^+$ MDCs compared with CD123$^-$ MDCs, while TRAIL was never detected on their surface. Furthermore, we pretreated the CD123$^+$ MDCs and CD123$^-$ MDCs with TRAILR2:Fc to block TRAIL. As shown in Fig. 8, the anti-tumor potential of CD123$^+$ MDCs against Jurkat, U937 and HL60 was reduced significantly by TRAIL-R2:Fc, whereas the effect of CD123$^-$ MDCs was slightly blocked. Isotype human IgG1 exerted no effect either on CD123$^+$MDCs or CD123$^-$ MDCs (data not shown). These results provide

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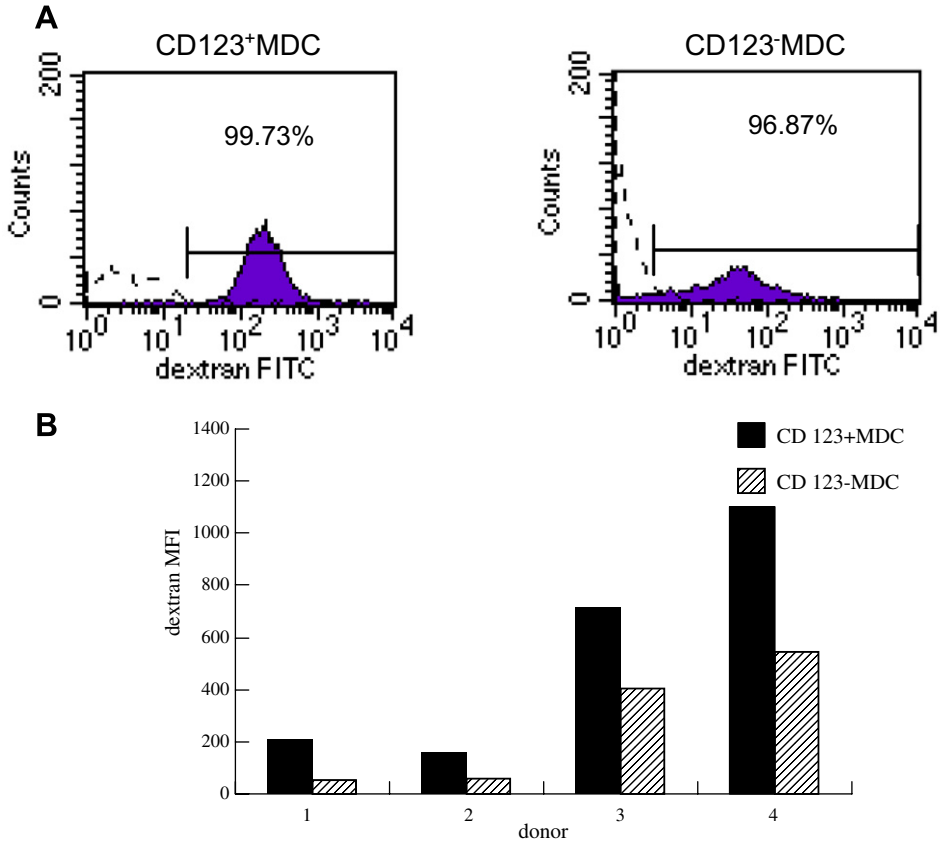

Fig. 4. CD123$^+$MDCs are more efficient than CD123$^-$ MDCs in FITC-dextran uptake. CD123$^+$ MDCs and CD123$^-$MDCs were incubated with FITC-dextran at 0 °C (as control; open histogram) or at 37 °C (filled histogram) for 1 h. After washing with cold buffer, the capacity of cells to take up FITC-dextran was determined by flow cytometry. Data as % positive cells from one representative donor (A) and MFI of four donors (B) are shown.

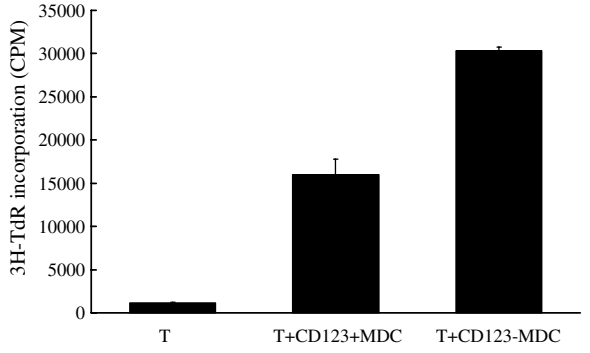

Fig. 5. CD123$^+$MDCs exhibited less efficiency in induction of MLR. Allogeneic cord blood T cells (5×10^4) were incubated alone or with irradiated CD123$^+$ or CD123$^-$ MDCs at a responder:stimulator ratio of 5:1. Proliferation of T cells was determined by thymidine incorporation after culture for 5 days. Results are shown as the mean cpm ± SEM in one of three separated experiments.

evidence that cytoplasmic TRAIL may partially contribute to the enhanced tumor-inhibiting activity of CD123$^+$ MDCs.

3.7. Role of soluble TRAIL in CD123$^+$ MDC-mediated growth inhibition

To ascertain whether MDCs mediate growth inhibition by releasing soluble TRAIL, we measured TRAIL in the supernatant of MDCs by ELISA. As shown in Fig. 9, very low soluble TRAIL was detected (6.79 pg/ml) in the supernatant of CD123$^+$ MDCs, as in that of CD123$^-$ MDCs. Pretreatment of DC-free supernatant with TRAIL-R2:Fc exhibited no inhibiting affect on tumor cells (data not shown).

4. Discussion

In this article, we investigated the characteristics of CD123$^+$ MDCs derived from cord blood monocytes. Our results show that CD123$^+$ MDCs represent earlier-stage immature MDC subset. They significantly inhibited proliferation of human hematological tumor lines partially through enhanced cytoplasmic TRAIL, indicating that in addition to

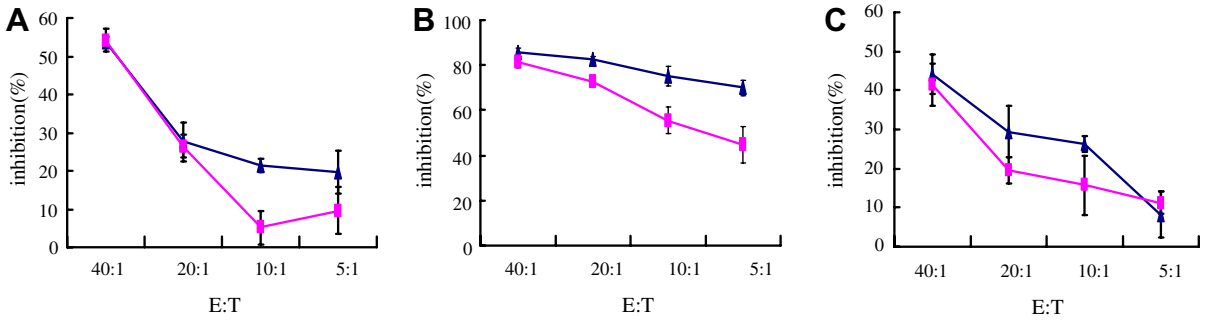

Fig. 6. DC-mediated growth inhibition against (A) U937, (B) Jurkat and (C) HL60. Human tumor cell lines (1×10^3/well) were cocultured with isolated $CD123^+$ MDCs (▲) or $CD123^-$ MDCs (■) at indicated effector:target (E:T) ratio for 24 h. The cultures were pulsed with ^{3}H-TdR for another 24 h. The results are presented as the percentage of growth inhibition against tumor cells in the mean ± SEM of triplicate wells. The presented results are from a representative experiment of three (A) and four (B and C) similar experiments performed.

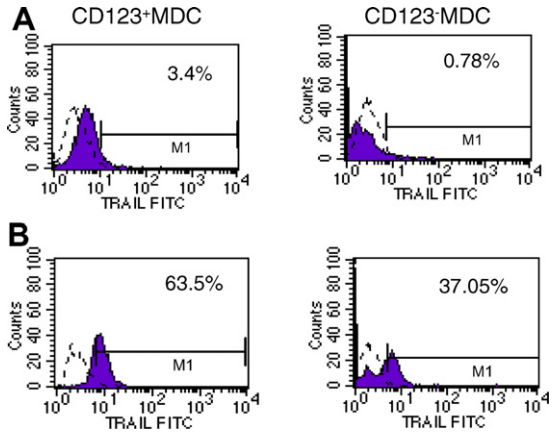

Fig. 7. Cell surface (A) and cytoplasmic (B) expression of TRAIL in $CD123^+$ MDCs and $CD123^-$ MDCs. Monocytes were induced into DCs with GM-CSF and IL-4 for 7 days, and then $CD123^+$MDCs were isolated by indirect immunomagnetic isolation kits. Either untreated (cell surface) or FACS permeabilizing solution treated (cytoplasmic) MDCs were consecutively co-incubated with the antibodies specific for TRAIL. The labeled cells were analyzed in a FACScan flowcytometer. The data are from a representative experiment of three performed, showing overlays of single-color histograms of isotype control (open histograms) and specific antibodies (filled histograms) fluorescence.

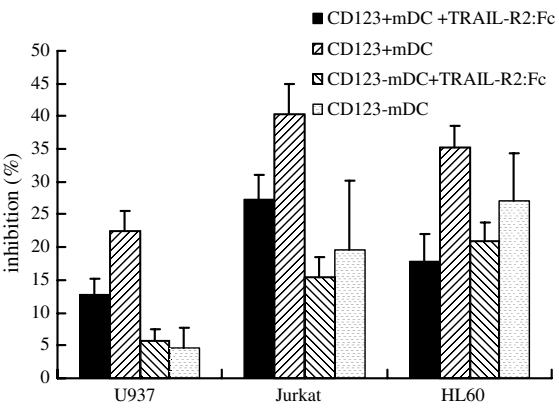

Fig. 8. Enhanced anti-tumor effect of $CD123^+$ MDCs is partially mediated by TRAIL. $CD123^+$ MDCs (4×10^5/ml) were pre-treated with TRAIL-R2:Fc (20 μg/ml) for 1 h. Then they were cocultured with the indicated tumor cells at an E:T of 40:1 for 24 h, and for an additional 24 h, with ^{3}H-thymidine before being harvested. The results are presented as the mean percentage of inhibition of tumor cell proliferation ± SEM of triplicate wells. Similar results were obtained in three independent experiments.

their predominant role for uptake, processing and presenting tumor antigen to T cells, the immature MDCs in early-stage differentiation could also serve as a component of innate immunity in controlling tumor growth.

The in vitro culture system consisting of GM-CSF and IL-4 has been reported to provide a classic model for inducing MDCs [21]. Physiologically, cord blood monocytes might have a greater chance to be exposed to GM-CSF because higher serum levels of GM-CSF have been detected in cord blood than in adult blood [22]. Therefore, we chose to use cord blood to study the characteristic of $CD123^+$ MDCs. We confirmed here that highly purified $CD14^+$ cord blood monocytes differentiated into cells with MDC features in this culture system, as evidenced by the constant expression of myeloid marker CD11c throughout the culture period and absence of CD304 (BDCA-4) expression which was a selective marker of PDCs induced in vitro (data not shown).

MDC population has been noted to be heterogeneous [23]. We showed here that MDCs derived from cord blood monocytes included $CD123^+$ and

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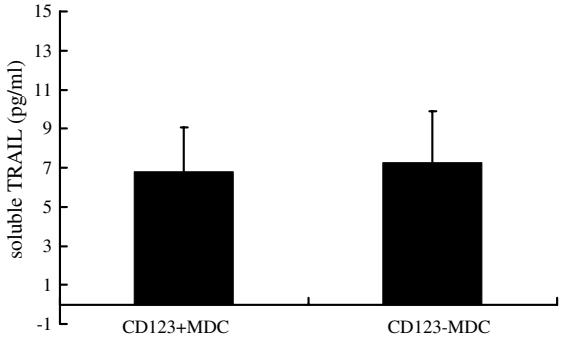

Fig. 9. $CD123^+$ MDCs released little soluble TRAIL. $CD123^+$ MDCs and $CD123^-$ MDCs were isolated and resuspended at 4×10^5/ml after culture for 48 h in the presence of GM-CSF and IL-4. Cell-free supernatant was collected and assessed for the presence of TRAIL using specific ELISA. The results are means ± SEM of five experiments obtained from different donors.

$CD123^-$ subpopulations. It has been reported that CD123 was a selective marker of lymphoid dendritic lineage cells [4,24], and it was maintained during all stages of monocytic and granulocytic differentiation [25]. However, the presence of CD123 marker in the MDC population was not a surprising finding, since the expression of CD123 molecules on the surface of DCs generated from peripheral blood monocytes in the presence of GM-CSF and IL-4 was described previously [5,6], but the functional significance of the $CD123^+$ cells is unknown.

We demonstrated that $CD123^+$ MDCs display morphologic and phenotypic characteristic of immature DCs. In the culture on day 7, $CD123^+$ cells showed morphology of immature DC-like cells with short and thick veils and dendritic processes (Fig. 3A). In accordance with their immature state, these cells do not express the maturation-specific marker CD83, but expressed the DC-associated markers. The expression of CD1a together with the costimulatory molecules CD86 in concert with HLA-DR molecules, suggested that these cells might already possess potent stimulatory capabilities that are characteristic of MDCs. We confirmed that $CD123^+$ MDCs stimulate allogeneic MLR although it was less effective than $CD123^-$ population which expressed higher levels of CD1a and HLA-DR. Curiously enough, those results were independent of the level of the CD86 expression, because $CD123^+$ population expressed significantly higher levels of CD86 than $CD123^-$ population, suggesting that other costimulatory molecules such as OX40L, RANKL, or 4BBL may be involved [26]. We also measured IL-12p70 in the supernatant of $CD123^+$ and $CD123^-$ MDCs and found both subpopulations release very little IL-12p70 (data not shown). In contrast to their lower ability to stimulate allogeneic MLR, $CD123^+$ MDCs exhibited higher efficiency of FITC-dextran uptake which usually represents the function of immature DCs. These features might make $CD123^+$ MDCs more effective in capturing and processing tumor antigen in tumor immunity in vivo.

We are also concerned whether $CD123^+$ MDCs exert direct anti-tumoral effector function, since it has recently been reported that DCs possess the machinery of tumoricidal and tumoristatic activity [15,16]. Our previous report also showed that DCs derived from cord blood monocytes could kill tumor cell lines after stimulating by inflammatory factors [27]. In the present study, $CD123^+$ MDCs profoundly suppressed U937 and Jurkat cell proliferation in coculture system compared with $CD123^-$ MDCs. Similar tendency was observed for HL60 cell line although there was no statistical difference. The differential sensitivity of tumor cells to DCs indicates the complexity of DC-tumor interactions as well as heterogeneity of the individual tumor line. Our results also indicate that the growth-inhibiting effect of $CD123^+$ MDCs is not positively associated with the maturation status of DCs. This conclusion is based on the findings that $CD123^-$ MDCs, which were more mature than $CD123^+$ MDCs, failed to enhance the effect. These results indicate that immature DC-mediated tumor growth inhibition mainly depends on the population of $CD123^+$ MDCs, especially at a low E:T ratio.

The exact mechanisms of $CD123^+$ MDC-mediated tumor growth inhibition could not be determined completely. Chapoval et al. reported that DCs inhibited tumor cell growth through cellular contact with tumor cells [15]. There has been a few reports as for the role of TNF family ligands in tumoristic activity [16,28]. In our study, we observed that increased cytoplasmic TRAIL was partially responsible for the enhanced tumor-inhibiting effect of $CD123^+$ MDCs against Jurkat and U937 cells, based on the evidence that cytoplasmic TRAIL was higher in $CD123^+$ MDCs and TRAIL-R2:Fc could partially reduce this effect. Our conclusion is consistent with the reported observation in which the measles virus induces cytoplasmic but not cellular surface TRAIL expression on DCs, which specifically mediates cytostatic effect [29]. We further determined in our system, whether

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the tumor-inhibiting activity of $CD123^+$ MDCs via cytoplasmic TRAIL was through soluble TRAIL either secreted or cleaved from DCs in the supernatants. However, we found that neither $CD123^+$ MDCs nor $CD123^-$ MDCs produced functional soluble TRAIL, indicating that $CD123^+$ MDC-mediated tumoristic activity was soluble TRAIL-independent.

Our data provide novel information that human immature MDCs not only uptake, process and present tumor antigens, but also directly participate in the growth inhibition of tumors. Considering the in vivo distribution of immature MDCs in normal tissues as well as their increased numbers in cancer tissues of patients [30], our findings strongly indicate that early-stage immature MDCs are indirectly and directly involved in elimination of newly generated cancer cells and in control of tumor progression in vivo.

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