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Full Length Research Paper

The immunomodulatory effects of *Cordyceps sinensis* on dendritic cells derived from chronic myelogenous leukemia (CML)

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Cordyceps sinensis **(CS), a tonic herb in Chinese traditional medicine, is an excellent antitumor agent. However, the underlying mechanisms of its antitumor effects remain unclear. In the present study, we evaluated the role of *C. sinensis* in the differentiation and function of bone marrow-derived dendritic cells (DCs) from patients with chronic myeloid leukemia (CML) and attempted to elucidate its antitumor mechanisms. We isolated mononuclear cells (MNCs) from CML patients. After incubation with granulocyte macrophage colony-stimulating factor (GM-CSF), interlukin-4, and tumor necrosis factor-α (TNF-α), the MNCs developed the morphological characteristics of DCs *in vitro* (CML-DCs). The cells were separated according to phenotype by flow cytometric analyses with DC markers. The activity of IL-12 and the effects of stimulation increased in CML DCs prestimulated by polysaccharide fraction of *C. sinensis* (PSCS) compared to untreated CML-MNCs. Furthermore, the CML-DCs incubated by PSCS resulted in the rapid generation of the costimulatory molecules, CD86 and HLA-DR, and the enhancement of IL-12 expression and stimulatory capacity in allogeneic mixed lymphocyte reaction (MLR). In summary, these results suggested that PSCS can increase T cell immunoresponse and represent a valuable traditional agent for the rapid generation of active DCs. It may also be utilized for vaccine against CML.**

Key words: Dendritic cells, *Cordyceps sinensis*, chronic myeloid leukemia, differentiation.

INTRODUCTION

Cordyceps sinensis, one of the most precious traditional medicines in China, possesses the antitumor activity, antioxidant effects and the capability to modulate the immune system. The chemical constituents of *C. sinensis* include cordycepin (3'-de-oxyadenosine) and its derivatives, including ergosterol, polysaccharides, a glycoprotein and peptides containing alpha-aminoisobutyric acid (Ng and Wang, 2005), amongst others. They have antitumor, antimetastatic, immuno-modulatory, antioxidant, anti-inflammatory, insecticidal, antimicrobial, hypolipidemic, hypoglycemic, anti-ageing, neuroprotective and renoprotective effects (Jeong et al., 2010). Some investigators have indicated that polysaccharide contributes to the anti-tumor and immuno-modulatory activity (Leung et al., 2006; Chen et al., 2006).

However, the mechanisms of the antitumor effect of the polysaccharide fraction of *C. sinensis* (PSCS) remain unclear.

Chronic myelogenous leukemia (CML) is a malignant myeloproliferative disease characterized by the typical

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Philadelphia (Ph) chromosome cytogenetic abnormality. For a long time, it was thought that direct inhibitory effects on tumor cell growth/functions should comprise the major treatment modalities in patients with CML. Recently, it has been recognized that immune-based treatments may play a more central role in the overall antitumor response. For example, the use of dendritic cells (DCs) derived from leukemia has been considered as an attractive approach for antileukemia therapy (Brady et al., 2011). Interactions between DCs and leukemia cells represent an attractive model for the study of DC physiology. Moreover, DCs can be a valuable therapeutic tool for the adjuvant treatment of patients with CML.

However, DC subsets *in vivo* may also be affected by leukemogenesis and may contribute to the escape of leukemic cells from immune control (Kufner et al., 2005). The aim of the present study was to examine the effect of *C. sinensis* on the immunoresponse of CML-DCs and to elucidate its antitumor mechanisms.

MATERIALS AND METHODS

Culture of dendritic cells and Isolation of PSCS

Bone marrow samples were obtained from 17 CML patients at primary diagnosis, including seven females and ten males, aged from 22 to 63 years. All samples were obtained from previously untreated, Ph chromosome positive patients with chronic phase CML. Heparinized BM samples were collected to prepare mononuclear cells (MNCs) by centrifugation in Percoll (Pharmacia Fine Chemicals, AG, Uppsala, Sweden). MNCs were washed twice in RPMI1640 (Gibco, Grand Island, NY) containing 10% heat-inactivated fetal calf serum (FCS; Sigma Chemical Inc., St. Louis, MO). Thereafter, it was cultured in 75 ml tissue culture flasks (Nunclon, Life Technologies, Burlington, Canada) in a volume of 4 mL at a density of 1.5×10^6 cells per milliliter for patients with CML. Cells were incubated in RPMI1640 containing 20% FCS, 50 mM 2-mercaptoethaonoal (Sigma Chemical Inc.), 2 mM glutamine (Gibco), 200 ng/ml granulocyte-macrophage colony-stimulating factor (GM-CSF; Schering Canada Inc, Quebec, Canada), 10 ng/ml tumor necrosis factor-alpha (TNF-α, Amgen Canada Inc, Mississauga, Ontario, Canada) and 200 ng/ml interleukin-4 (IL-4; Schering Canada Inc, Quebec, Canada). DC cultures were maintained at 37°C in humidified air supplemented with 5% CO_2 (CML-DCs).

In the experiments, some DC cultures were further incubated with *C. sinensis* extract (30% Polysaccharides, purchased from Changsha Sunfull Bio-Tech Co., Ltd. NO. 2770804) 200 mg/ml at the time of culture initiation (CML-CS-DCs) or with medium alone (control group). After being cultured for 10 days, cells were harvested for morphological studies (Wright's stain, inverted and electron microscopy), flow cytometric analyses, the mixed lymphocyte reaction assay and enzyme-linked immunosorbent assay (ELISA) measurements.

Phenotypic analysis by flow cytometry

Monoclonal antibodies (mAb) against the following determinants were used in this study: CD1a, CD83, CD80, CD86 and HLA-DR (Pharmingen, San Diego. CA). The antibodies were conjugated with either fluorescein isothiocyanate (FITC) or phycoerythrin (PE). Dual-color immunofluorescence staining and analysis was performed by standard methods. Briefly, cells cultured for 10 days were washed in PBS supplemented with 0.5% bovine serum albumin (BSA) and incubated with the respective mAb for 30 min at 4°C followed by washing in PBS. Stained cells were analyzed by FAC Scan with Lysis II software (Becton Dickinson, San Jose, CA). Cells were electronically gated according to light scatter properties in order to exclude cellular debris and contaminating lymphocytes.

Mixed lymphocyte reaction (MLR)

The freshly prepared allogeneic T lymphocytes with sheep red blood cells (SRBC) (Woodlynlab, Guelph, Canada)-separated derived from normal donors, these cells using as targets in MLR at a constant number of 1×10^5 cells. Dendritic cells were treated with 25 µg/ml mitomycin C (Sigma) at 37°C for 30 min and subsequently washed three times in media (RPMI1640 + 10% FCS) before plating. Decreasing numbers of DCs were mixed with responder PBMCs, added at concentrations of DC: T/1:100, DC:T/1:330, DC:T/1:1000, DC:T/1:3333. They were initiated in 96-plates with U-shaped microwells (Corning Inc. Corning, NY) with RPMI1640 supplemented with 10% FCS. DCs cultured in the absence of responder cells served as the control for background proliferation. Lymphocyte proliferation was measured after 5 days of culture with the addition of 3H-thymidine (1 uCi/well), which was added for 18 h after 54 h of culture. The resulting solution was neutralized using 0.5 N HCL (0.15 ml) and radioactivity was assessed by liquid scintillation counting.

IL-12 detected by ELISA

Cell-free supernatants were collected from untreated or cytokine-treated cultured cells; aliquots of 100 µl were removed (these harvested cells were prestimulated by PMA 20 ng/ml for 48 h) and IL-12 contents were determined by an ELISA kit (R and D systems Minnesota, USA), according to the instructions of the manufacturer.

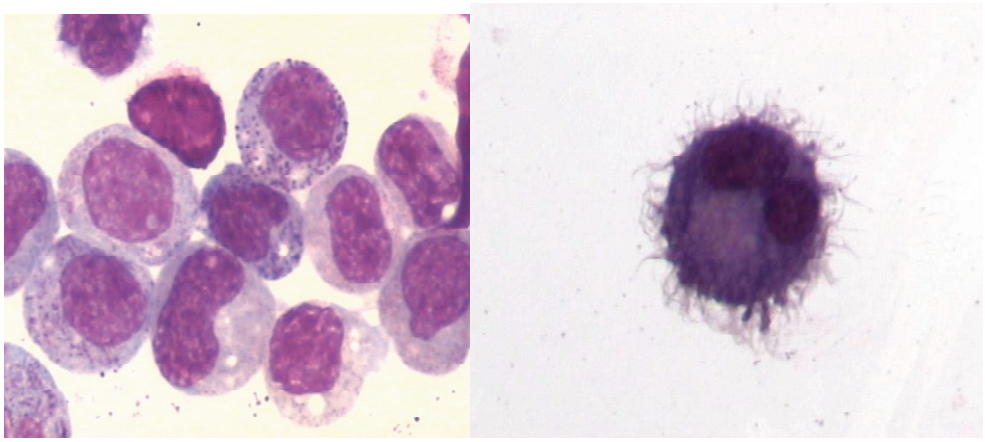
Statistical analysis

For statistical analysis, the ANOVA test paired with Fisher's comparison was used, and a p-value of <0.05 was considered to be significant.

RESULTS

Generation of dendritic cells *in vitro*

Freshly isolated BM-MNCs of CML patients appeared as dispersed, spherical cells with a smooth surface. After 3 to 7 days of culture in the presence of GM-CSF, TNF-α and IL-4 with and without PSCS, these cells appeared large and were aggregated in nonadherent grape-like clusters with short projections emerging from the surface, or as dispersed, nonadherent cells with large-cell bodies and long dendritic projections. Distinct clusters of cells with the dendritic morphology could be seen after 10 days of culture. In particular, cells in the periphery of each cluster displayed a dendritic appearance with delicate membrane projections (Figures 1 to 3). In addition, the medium contained free-floating individual cells. Nonspecific esterase (NSE) was weakly positive in



(A) (B)

Figure 1. Morphology of freshly isolated (A) and cultured CML cells (B). BM-MNCs from patients with CML were isolated and cultured as described in the Materials and Methods (Wright's stain, ×1000).

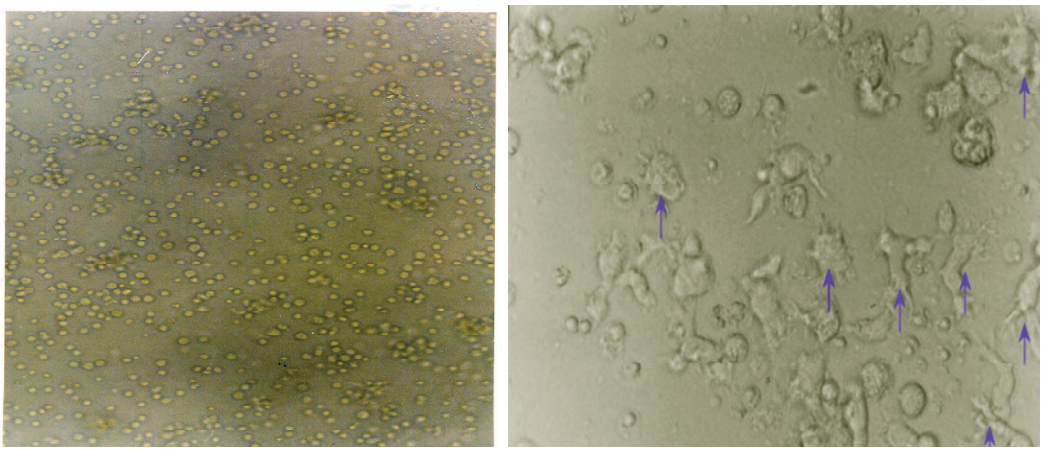


Figure 2. Morphological studies of fresh chronic myeloid leukemia (CML) MNCs (A) and those cultured for 10 days in the presence of GM-CSF, IL-4, PSCS, and TNF-α. A clear population with a typical dendritic-like appearance was observed by inverted light microscopy (A, ×100; B, ×400).

DCs (Figure 4). There were no significant differences in the morphology of DCs generated from CML patients with or without PSCS treatment. In contrast, there was no dendritic-like change in the control group cells.

Phenotypic analysis of cells by flow cytometry

Flow cytometry was performed on cells from each CML patient cultured for 10 days in GM-CSF, IL-4 and TNF-α, respectively, with or without PSCS in each case. Table 1 shows an upregulation of the DC lineage markers, CD1a, CD83 and HLA-DR, and the costimulatory molecules, CD80 and CD86, in CML-DCs compared to the control groups (Figure 5). Notably, the addition of PSCS during the culture of CML-DCs resulted in the appearance of a greater number of differentiated/activated DCs.

Immunostimulatory function of DCs

The effect of equal numbers of BM-derived DCs was assessed by FACS analysis. The results show that a significantly increased allostimulatory capacity was observed after CML-DCs were cultured in the presence of GM-CSF, IL-4, and TNF-α. Furthermore, it was demonstrated that CML-CS-DCs were more effective in stimulating allogeneic T lymphocytes compared to untreated CML-DCs ($p<0.05$) and so the addition of PSCS significantly enhanced the stimulatory effect of

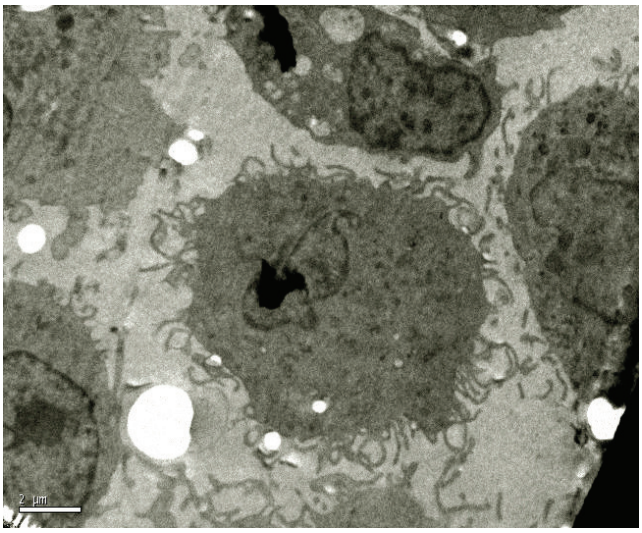


Figure 3. Morphological studies of chronic myeloid leukemia (CML) MNCs after culture for 10 days in the presence of GM-CSF, IL-4, PSCS, and TNF-α. A clear population was shown under electron microscopy (×20,000).

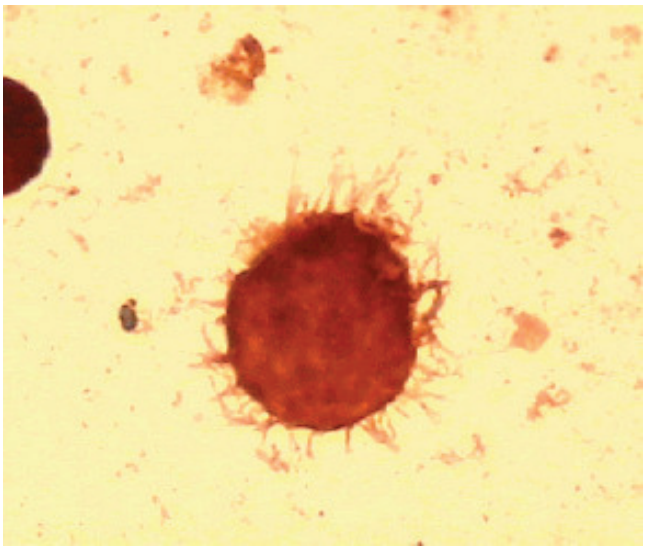


Figure 4. Dendritic cells derived from CML-MNCs are weakly positive for non-specific esterase (NSE; Wright's stain, ×1000).

CML-DCs.

The function of IL-12 secreted by dendritic cells

IL-12 is one of the important parameters characterizing the activation stage of DCs. Thus, it was of interest to evaluate whether CML-DCs or CML-CS-DCs had upregulated IL-12 secretion or not. The level of IL-12 increased by significantly higher amount in CML-DCs (134.37 ± 27.7 pg/ml) that had been prestimulated by PMA than cells in the control group (43.45 ± 11.4 pg/ml, $P<0.05$). Furthermore, we also found that the pretreatment of CML-DCs with PSCS resulted in a significantly enhanced generation of activated DCs that expressed IL-12 (208.22 ± 42.1 pg/ml, $P<0.05$).

DISCUSSION

Dendritic cells (DCs) are a system of potent antigen-presenting cells (APCs), which specialize in the initiation of primary immune responses. DCs are considered to be important elements in the induction of specific antitumor cytotoxic effectors. At present, because of their potential therapeutic implications, the critical role of DCs in cancer patients is under intensive investigation. The interactions between DCs and leukemia cells represent an attractive model for the study of DC physiology (Kufner et al., 2005). Moreover, DCs can be a valuable therapeutic tool for the adjuvant treatment of leukemic patients. Choudhury et al. (1997) generated DCs from the peripheral blood cells of patients with CML. Leukemia cells incubated concurrently with GM-CSF, IL-4, and TNF-α, developed the morphological and phenotypic characteristics of DCs (Choudhury et al., 1997). Leukemic-DCs were potent stimulators of lymphocyte proliferation in specific *in vitro* assays for the functions of DCs. Auto- or allologous T lymphocytes stimulated with leukemia DCs generated in vitro displayed vigorous cytotoxic activity against leukemia cells (Kolb et al., 2004). DC-based immunotherapy may be considered as a new approach for the long-lasting antitumor treatment of patients with leukemia (Tanosaki and Tobinai, 2010).

However, previous studies have revealed that DCs derived from patients with leukemia exhibited a decreased stimulatory activity toward allogeneic T lymphocytes compared to DCs obtained from normal donors (Wang et al., 1999). This may contribute to the escape of leukemia cells from immune control (Wang et al., 1999). Therefore, a safety agent is required to increase the innate immune defense and decrease immune tolerance in DCs in leukemia.

C. sinensis (CS), a herbal medicine, has been used as a popular remedy in China for over 200 years. It is a parasitic fungus that lives on the larvae of lepidoptera. Previous studies revealed that *C. sinensis* possessed important activities that were capable of modulating immune responses and inhibiting tumor growth (Jeong et al., 2011). Zhang et al. (2008) demonstrated that the extracts of *Cordyceps militaris* fruiting bodies inhibited the proliferation of L-60 leukemia cells by inducing cellular apoptosis (Byeon et al., 2011). Park et al. (2005) reported that *C. militaris* inhibited the growth of U937 leukemia cells through the induction of apoptosis. These results revealed that the Cordyceps extracts may have therapeutic potential against human leukemias. The

Table 1. The expression levels of surface molecules on DCs derived from patients with CML, with or without the addition of cytokines (mean ± SD).

Parameter	CD80 (%)	CD86 (%)	CD83 (%)	CD1a (%)	HLA-DR (%)
Control group	18.02±10.87	24.25±15.37	18.42±12.54	11.44±8.27	13.47±10.27
CML-DCs	55.52±20.27*	60.28±17.27*	57.58±27.24*	49.24±22.21*	60.14±16.26*
CML-CS-DCs	63.23±17.57*	81.23±13.29*$^{\nabla}$	59.51±15.62*	48.23±15.23*	88.05±16.87*$^{\nabla}$

Differences between the levels of surface molecules were analyzed using ANOVA. * $p<0.05$: CML-DCs and CML-CS-DCs compared with the control group. $^{\nabla}$ $p<0.05$: significant differences exist between CML-CS-DCs and CML-DCs. CML MNCs were cultured in the absence (control group) or presence (CML-DCs) of GM-CSF, IL-4, TNF-α and the addition of PSCS (CML-CS-DCs) for 10 days.

polysaccharide fraction of *C. sinensis* (PSCS) is the major effective component of the antitumor activity of *C. sinensis*. Zhang's studies showed that the growth of the H22 tumor cell line was significantly inhibited by PSCS (Zhang et al., 2008). Moreover, PSCS significantly enhanced the activity of superoxide dismutase (SOD) in the liver, brain and serum, as well as the activity of glutathione peroxidase (GSH-Px) in the liver and brain in tumor-bearing mice (Ko et al., 2010). PSCS also significantly reduced the level of malondialdehyde (MDA) in the livers and brains of tumor-bearing mice (Chen et al., 2006).

In addition to the direct effects on tumor cells, PSCS may exert several effects on the host immune system that may play a more central role in the overall antitumor response. In the present study, we elucidated the mechanism of PSCS on the host immune system for the generation of a long-lasting antitumor response in CML patients. In a previous study, we characterized the DCs that were generated from untreated patients with CML after 10 days treatment with GM-CSF, TNF-α and IL-4[16]. DCs from patients with CML are able to differentiate, mature and constitutively express MHC class II molecules, the CD80 (B7.1) and CD86 (B7.2) CD1a and CD83. Of note, FACS analyses revealed that a large percentage of the CML-CS-DCs exhibited the typical characteristics of highly activated $CD86^+$ and $HLA\text{-}DR^+$ DCs, which were observed to be generated after CML-DCs were incubated with PSCS.

Previous reports showed that DCs from patients with CML were found to have a lower stimulatory activity than that of DCs derived from normal donors when examined by MLR (Tong et al., 2008). The present results demonstrated that the addition of PSCS to CML-DCs significantly enhanced their stimulatory capacity in an allogeneic MLR, which may have resulted from an increase in the expression of stimulatory molecules by DCs. The antigen capturing and presenting abilities of DCs are developmentally regulated in a process known as maturation. During maturation, the surface expression of HLA-DR molecules by DCs increase several fold and their antigen presentation to T-cells also increases (Kim et al., 2010). Several studies have indicated that costimulatory molecules play a different role in the adjuvant activity of the antigen presenting process. The analysis by Martin et al. (2002) of the expression of B7-1 and B7-2 on the surface of B cells revealed that *Escherichia coli* (LT) enhanced the expression of CD86, but not CD80, while B7-1 appeared to be the predominant B7 molecule involved in the ability of LT (E112K) to augment antibody responses to a co-administered antigen (Cheung et al., 2009; Akaki et al., 2009). Recently, the expression of CD86 has been demonstrated on CD1a+ epidermal DCs, which indicates that CD86 might play a role in the pathogenesis of atopic dermatitis (Akaki et al., 2009). Bonnefont-Rebeix also found that CD86 enables the better activation of antigen presenting (Bonnefont-Rebeix et al., 2006). These findings were consistent with our MLR results. However, Tsang et al. (2001) reported that CD80 can be used to infect human DCs rapidly and efficiently infect and could enhance the efficacy of human DCs in the activation of specific human T-cell populations. This difference may be accounted for by the variation in the source of DCs, and the duration of culture, conditioning media and cell sorting criteria.

IL-12 is generally associated with the capacity of DCs to promote the Th-1-type immune response (Tsai et al., 2009). Notably, we found that CML-DCs can secrete IL-12, which significantly increased after they were cultured with GM-CSF, TNF-α and IL-4.

Furthermore, our results showed that the treatment of CML-MNCs with PSCS resulted in the rapid generation of activated DCs (CML-PSCS-DCs) that secreted IL-12 and correlated with an enhanced T cell response (Raymond and Wilkie, 2004). Several studies have described the effects of IFN-α on DCs from patients with CML; the addition of IFN-α to DCs generated from CML patients significantly enhanced their stimulatory capacity in allogeneic MLR to near normal levels (Wang et al., 1999; Gabriele et al., 2004).

In the present study, CML-CS-DCs resembled DCs generated under similar conditions using IFN-α from patients with CML. This resemblance was not only in terms of their phenotype, but also in their capability to express cytokines, which are generally associated with the competence of DCs to promote a Th-1 type of immune response.

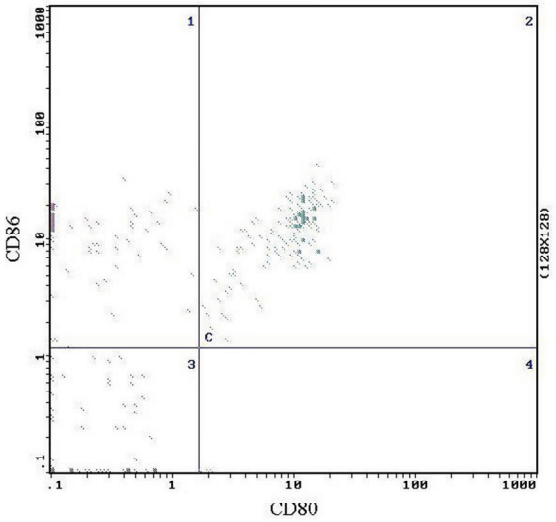
The use of DCs-based immunotherapy in CML patients is aimed at achieving the immuno-mediated control of the

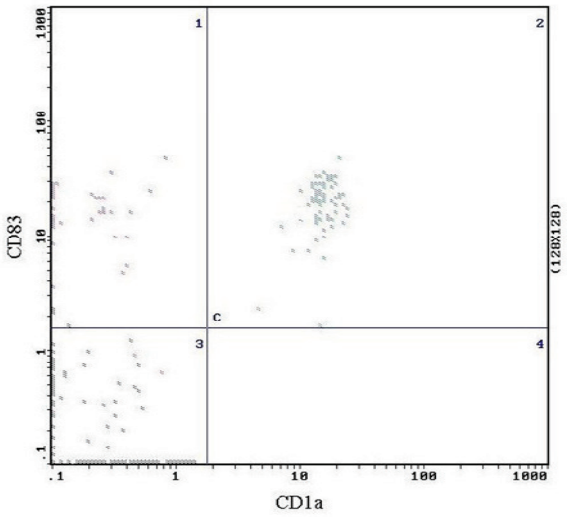
CD80/86

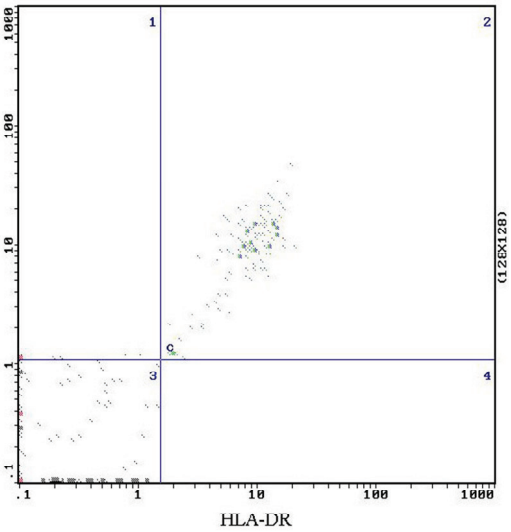
CD1a/CD83

HDA-DR

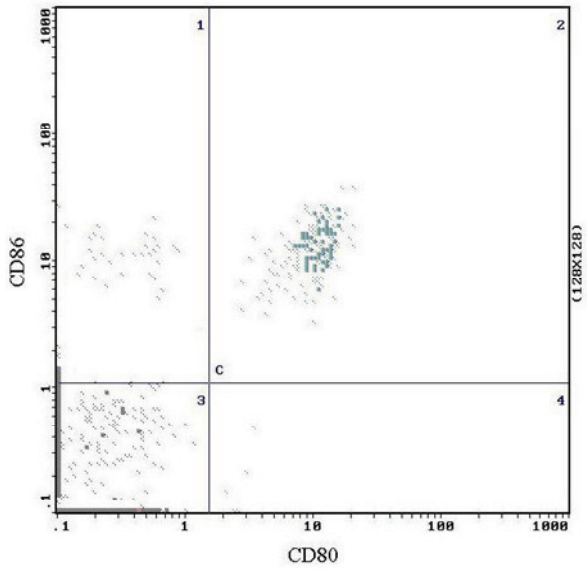
Control group

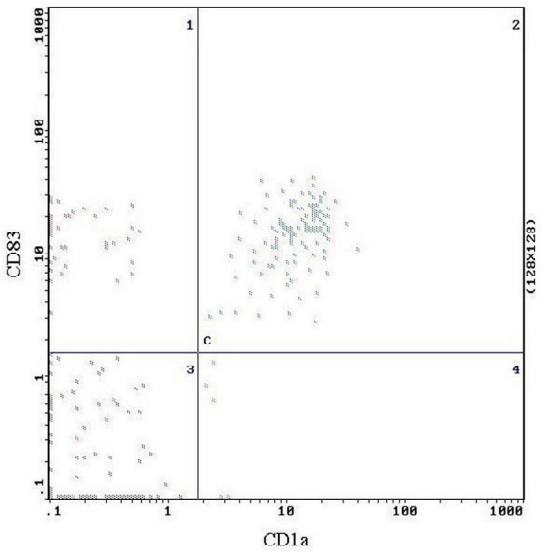


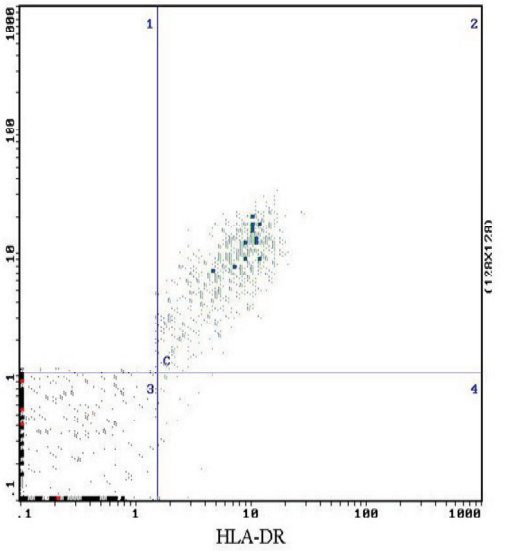




CML-DCs







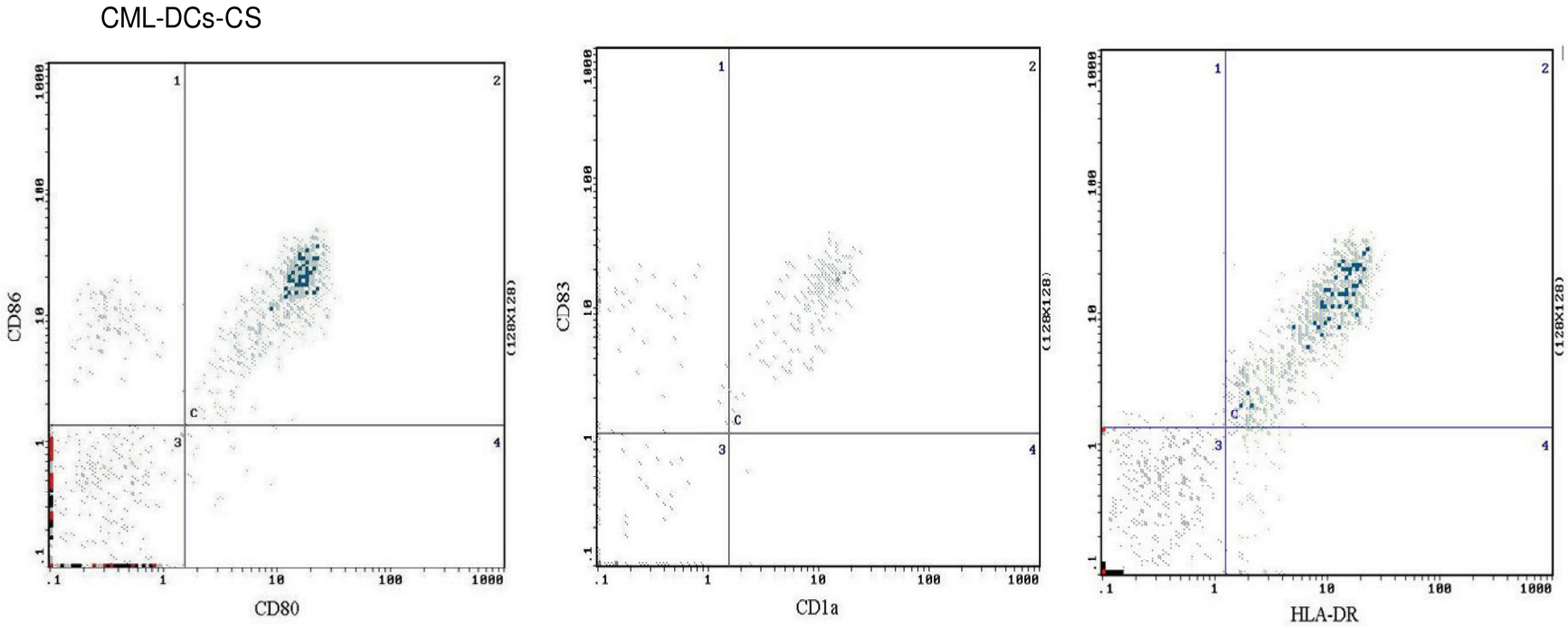


Figure 5. Phenotypic analysis of CML-MNCs by flow cytometry. The molecular expression is demonstrated after cell culture for 10 days in the absence of a cytokine cocktail.

disease, especially when tumor burden is markedly reduced following the use of current gold standard therapeutic strategies (Tanosaki and Tobinai, 2010). This has been a long sought after goal in the clinical setting of therapy-induced minimal residual disease, which may result in an antitumor response without considerable side effects. Our observations demonstrate that highly active DCs, which express Th-1 promoting factors and are capable of efficiently inducing the expansion of allo-T cells, can be generated after cell culture in the presence of *C. sinensis*.

In summary, *C. sinensis* represents a valuable traditional medicinal agent for the rapid generation of activated DCs. It may be utilized for vaccine of patients with CML in the future. We hope that these experiments will open new perspectives for the DC-based therapy of human leukemia.

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REFERENCES

Akaki J, Matsui Y, Kojima H, Nakajima S, Kamei K, Tamesada M (2009). Structural analysis of monocyte activation constituents in cultured mycelia of Cordyceps sinensis. Fitoterapia, 80(3): 182-187.

Bonnefont-Rebeix C, De CCM, Bernaud J, Chabanne L, Marchal TR (2006). DCD86 molecule is a specific marker for canine monocyte-derived dendritic cells. Vet. Immunol. Immunopathol., 109(1-2): 167-176.

Brady MT, Lee J, Ferrone S, Wang ES, Wetzler M (2011). Interferon-γ secretion by t(9;22) acute lymphoblastic leukemia-derived dendritic cells. Leuk. Res., 35(2): 275-277.

Byeon SE, Lee J, Yoo BC, Sung GH, Kim TW, Park HJ, Cho JY (2011). p38-targeted inhibition of interleukin-12 expression by ethanol extract from Cordyceps bassiana in lipopolysaccharide-activated macrophages.

Immuno-pharmacol. Immunotoxicol., 33(1): 90-96.
Chen J, Zhang W, Lu T, Li J, Zheng Y, Kong L (2006). Morphological and genetic characterization of a cultivated Cordyceps sinensis fungus and its polysaccharide component possessing antioxidant property in H22 tumor-bearing mice. Life Sci., 78(23): 2742-2748.
Cheung JK, Li J, Cheung AW, Zhu Y, Zheng KY, Bi CW, Duan R, Choi RC, Lau DT, Dong TT, Lau BW, Tsim KW(2009). Cordysinocan, a polysaccharide isolated from cultured Cordyceps, activates immune responses in cultured T-lymphocytes and macrophages: signaling cascade and induction of cytokines. J. Ethnopharmacol., 124(1): 61-68.
Choudhury A, Gajewski JL, Liang JC (1997). Use of leukemic dendritic cells for the generation of antileukemic cellular cytotoxicity against Philadelphia chromosome-positive chronic myelogenous leukemia. Blood, 89(4): 1133-1142.
Gabriele L, Borghi P, Rozera C, Sestili P, Andreotti M, Guarini A Montefusco E, Foa R, Belardelli F (2004). IFN-alpha promotes the rapid differentiation of monocytes from patients with chronic myeloid leukemia into activated dendritic cells tuned to undergo full maturation after LPS treatment Blood, 103(3): 980-987.
Jeong JW, Jin CY, Kim GY, Lee JD, Park C, Kim GD, Kim WJ, Jung WK, Seo SK, Choi IW, Choi YH (2010). Anti-inflammatory effects of cordycepin via suppression of inflammatory mediators in BV2 microglial cells. Int. Immunopharmacol., 10(12): 1580-1586.
Jeong JW, Jin CY, Park C, Hong SH, Kim GY, Jeong YK, Lee JD, Yoo YH, Choi YH (2011). Induction of apoptosis by cordycepin via reactive oxygen species generation in human leukemia cells. Toxicol. *In vitro.* 25(4): 817-824.
Kim HS, Kim JY, Kang JS, Kim HM, Kim YO, Hong IP, Lee MK, Hong JT, Kim Y, Han SB (2010). Cordlan polysaccharide isolated from mushroom *Cordyceps militaris* induces dendritic cell maturation through toll-like receptor 4 signalings. Food Chem. Toxicol., 48(7): 1926-1933.
Ko WS, Hsu SL, Chyau CC, Chen KC, Peng RY (2010). Compound Cordyceps TCM-700C exhibits potent hepatoprotective capability in animal model. Fitoterapia, 81(1): 1-7.
Kolb HJ, Rank A, Chen X, Woiciechowsky A, Roskrow M, Schmid C, Tischer J, Ledderose G (2004). *In-vivo* generation of leukaemia-derived dendritic cells. Best Practise Res. Clin. Haematol., 17(3): 439-451.
Kufner S, Zitzelsberger H, Kroell T, Pelka-Fleischer R, Salem A, Valle F, Schweiger C, Nuessler V, Schmid C, Kolb HJ, Schmetzer HM (2005). Leukemia-derived dendritic cells can be generated from blood or bone marrow cells from patients with acute myeloid leukaemia: a methodological approach under serum-free culture conditions. Scand. J. Immunol., 62(1): 86-98.
Leung PH, Zhang QX, Wu JY (2006). Mycelium cultivation, chemical composition and antitumour activity of a Tolypocladium sp. fungus isolated from wild *Cordyceps sinensis*. J. Appl. Microbiol., 101(2): 275-278.
Martin M, Sharpe A, Clements JD, Michalek SM (2002). Role of B7 costimulatory molecules in the adjuvant activity of the heat-labile enterotoxin of *Escherichia coli*. J. Immunol., 169(4): 1744-1752.
Ng TB, Wang HX (2005). Pharmacological actions of Cordyceps, a prized folk medicine. J. Pharm. Pharmacol., 57(12): 1509-1519.
Park C, Hong SH, Lee JY, Kim GY, Choi BT, Lee YT, Park DI, Park YM, Jeong YK, Choi YH (2005). Growth inhibition of U937 leukemia cells by aqueous extract of *Cordyceps militaris* through induction of apoptosis. Oncol. Rep., 13(6): 1211-1216.
Raymond CR, Wilkie BN (2004). Th-1/Th-2 type cytokine profiles of pig T-cells cultured with antigen-treated monocyte-derived dendritic cells. Vaccine, 22(8): 1016-1023.
Tanosaki R, Tobinai K (2010). Adult T-cell leukemia-lymphoma: current treatment strategies and novel immunological approaches. Expert Rev. Hematol., 3(6): 743-753.
Tong XM, Yao HP, Qian WB, Zhu LF, Fu ZH, Huang ZL, Jin J (2008).The biological characteristics of dendritic cells derived *in vitro* from myelogeneous leukemia cells and healthy donor cells. Int. J. Lab. Hematol., 30(5): 372-381.
Tsai BY, Lin YL, Chiang BL (2009). Application of interleukin-12 expressing dendritic cells for the treatment of animal model of leukemia.Exp. Biol. Med. (Maywood), 234(8): 952-960.
Tsang KY, Zhu M, Even J, Gulley J, Arlen P, Schlom J (2001).The infection of human dendritic cells with recombinant avipox vectors expressing a costimulatory molecule transgene (CD80) to enhance the activation of antigen-specific cytolytic T lymphocytes. Cancer Res., 61(20): 7568-7576.
Wang C, Al-Omar HM, Radvanyi L, Banerjee A, Bouman D, Squire J, Messner HA (1999). Clonal heterogeneity of dendritic cells derived from patients with chronic myeloid leukemia and enhancement of their t-cells stimulatory activity by IFN-α. Exp. Hematol., 27(7): 1176-1184.
Zhang W, Li J, Qiu S, Chen J, Zheng Y (2008). Effects of the exopolysaccharide fraction (EPSF) from a cultivated Cordyceps sinensis on immunocytes of H22 tumor bearing mice. Fitoterapia, 79(3): 168-173.