Functional heterogeneity among bone marrow-derived dendritic cells conditioned by Th1- and Th2-biasing cytokines for the generation of allogeneic cytotoxic T lymphocytes

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Abstract

Three distinct bone marrow (BM)-derived dendritic cells (BMDC) were expanded from BALB/c BM cells by culture with (i) granulocyte macrophage colony stimulating factor (GM-CSF) plus IL-3, (ii) GM-CSF, IL-3 plus Th1-biasing cytokines (IL-12 and IFN-γ) or (iii) GM-CSF, IL-3 plus Th2-biasing cytokines (IL-4). All of these cells expressed the DC-specific marker CD11c, and were designated as BMDC0, BMDC1 and BMDC2 cells respectively. BMDC1 cells exhibited superior T cell-stimulating activity in allogeneic mixed lymphocyte culture (MLC), while BMDC2 showed inferior stimulating activity. Specifically, BMDC1, as compared with BMDC2, induced a higher frequency of IFN-γ-producing CD8+ T cells in MLC. Moreover, BMDC1, but not BMDC2, were strong inducers of H-2d-specific cytotoxic T lymphocytes (CTL) in MLC. BMDC0 always showed intermediate stimulatory activity; however, when BMDC0 were cultured with IFN-γ, they differentiated into BMDC1-like stimulator cells concomitant with the up-regulation of both MHC antigens and co-stimulatory molecules. In contrast, BMDC2 were refractory to differentiation into superior stimulator cells by treatment with IFN-γ, although this treatment enhanced MHC expression. These findings indicate that Th1- and Th2-biasing cytokines, in addition to their effect on Th cell differentiation, may play a critical role in the functional skewing of DC. These findings have important implications for the development of DC-based immunotherapies.

Introduction

Antigen-presenting cells (APC) play a central role in the triggering of both innate and acquired immunity (1). Among APC, dendritic cells (DC) are the most capable inducers of primary and secondary immune responses (2,3). DC take up foreign antigens through pattern recognition receptors, combine peptide fragments of these antigens with MHC class I and class II molecules, and stimulate naive CD8+ T and CD4+ cells (4,5). In addition, DC can communicate with T cells directly through cell adhesion molecules or indirectly via cytokines such as IL-12, IL-18 and IL-10 (6–9). These interactions influence the differentiation of naive CD4+ T cells into Th1 or Th2 cells that mediate cellular and humoral immunity respectively (10–12).

Recent work has demonstrated functional heterogeneity among DC subsets. In the mouse system, CD11c+CD8α+ lymphoid DC produce high levels of IL-12 (13). Moreover, CD8α+ DC were demonstrated to produce IFN-γ in response to IL-12 (14). Thus, lymphoid DC subsets, which are termed
DC1, appear to be involved in the activation of T_{h1} immunity. In contrast, CD11c^{+}CD8αα^{-}CD11b^{+} myeloid DC, termed DC2, produce low levels of IL-12 compared with DC1 and play a critical role in the development of T_{h2} immunity (13). Since an imbalance of T_{h1}/T_{h2} immunity causes the onset of various immune diseases (15,16), it is of great importance to investigate how the functional maturation of DC is regulated.

In a previous report (17), we proposed that three distinct CD11c^{+} DC subsets, which were expanded from bone marrow (BM) cells under neutral conditions [granulocyte macrophage colony stimulating factor (GM-CSF) plus IL-3], T_{h1}-biasing conditions (GM-CSF, IL-3, IL-12 plus IFN-γ) or T_{h2}-biasing conditions (GM-CSF, IL-3 plus IL-4) exhibited functional differences in the development of T_{h1} or T_{h2} cells from naive T_{h} cells isolated from TCR transgenic mice. In the present paper, we designated these DC subsets as BMDC0, BMDC1 or BMDC2 and investigated their role on the generation of allogeneic cytotoxic T lymphocytes (CTL) in mixed lymphocyte culture (MLC). Among three BMDC subsets, CD11c^{+}CD11b^{+} BMDC1 cells expanded under T_{h1}-biasing conditions expressed the highest levels of MHC class I, MHC class II and co-stimulatory molecules such as B7-1 and CD40, and exhibited the most potent stimulatory activity for the generation of IFN-γ-producing CD8^{+} CTL. In contrast, BMDC2 induced under T_{h2}-biasing conditions poorly supported the induction of allreactive CTL. These data further indicated that T_{h1}- or T_{h2}-biasing cytokines produced during the early immune events may drive the functional maturation of DC and subsequently regulate T_{h1}-dominant cellular immunity or T_{h2}-dominant humoral immunity.

**Methods**

**Animals**

Female BALB/c and C57BL/6 mice were obtained from Charles River Japan (Yokohama, Japan) and used at 5–6 weeks of age.

**Induction of DC from BM cells**

BM cells obtained from BALB/c mouse femora were cultured under three distinct culture conditions in 12-well plates (Costar, New York City) for 4 days. DC0 were induced by culture of BM cells (5×10^{6} cells/well) in the presence of GM-CSF (20 ng/ml) and IL-3 (20 ng/ml). DC1 were induced by culture of BM cells in the presence of IFN-γ (20 ng/ml) and IL-12 (20 U/ml) in addition to GM-CSF plus IL-3. DC2 were induced by culture of BM cells in the presence of IL-4 (20 ng/ml) in addition to GM-CSF plus IL-3. At 2–3 days after the initiation of culture, non-adherent lymphoid cells contaminating in the BM cells were removed from the culture and replaced with fresh medium containing cytokine cocktails. DC cells harvested from 4 day culture of BM cells were used as BMDC subsets. Recombinant IL-12 was kindly donated by Genetics Institute (Cambridge, MA), IL-3, IL-4 and IFN-γ were purchased from Pharmingen (San Diego, CA) and recombinant GM-CSF was purchased from PeproTech EC (London, UK). To examine the conversion of BMDC0 or BMDC2 cells into BMDC1-like stimulator cells, BMDC0 or BMDC2 cells were cultured under neutral conditions (for BMDC0) or T_{h2}-biasing conditions (for BMDC2) for 4 days, then the cells harvested from each culture were pretreated with IFN-γ for 2 days and used in MLC.

**Generation of CTL in mixed lymphocyte culture (MLC)**

C57BL/6 mouse spleen cells (5×10^{6} cells) were co-cultured with either BMDC0, BMDC1 or BMDC2 (2×10^{6} cells), which were inactivated by pretreatment with mitomycin C (60 μg/ml; Kyowa Hakko Kogyo, Tokyo, Japan). Cells were co-cultured for 4 days in round-bottomed 5-ml tube (Falcon; Becton Dickinson, San Jose, CA). After culture, cells were harvested and used for phenotypic analysis, intracellular cytokine expression and cytotoxicity.

**Flow cytometry**

The phenotypic characterization of DC and CTL was carried out using FACSCalibur (Becton Dickinson) and CellQuest software. The mAb used in our experiments [phycoerythrin (PE)-conjugated anti-CD11c mAb, FITC-conjugated anti-CD11b mAb, FITC-conjugated anti-H-2K^{b} mAb, FITC-conjugated anti-I-A^{d} mAb, FITC-conjugated anti-CD1d, FITC-conjugated anti-CD40 mAb, FITC-conjugated anti-CD80 mAb and FITC-conjugated anti-CD11a mAb] were purchased from PharMingen. Detailed procedures for staining and sorting were described previously (18). Fluorescence data were collected with logarithmic amplification. For each sample, data from 10,000 volume-gated viable cells were collected. In some experiments, CD8^{+} T cells isolated from MLC by sorting with FACS vantage (Becton Dickinson) were used for cytotoxicity assay.

**Intracellular cytokine expression**

For the detection of cytoplasmic cytokine expression, cells stimulated with immobilized anti-CD3 mAb for 6 h in the presence of Brefeldin A were first stained with PerCP-conjugated anti-CD8 mAb, fixed with 4% paraformaldehyde, treated with permeabilizing solution (50 mM NaCl, 5 mM EDTA, 0.02% NaN_{3} and 0.5% Triton X-100, pH 7.5), and then the fixed cells were stained with PE-conjugated anti-IL-4 mAb and FITC-conjugated anti-IFN-γ mAb for 45 min on ice. The percentage of cells expressing cytoplasmic IL-4 or IFN-γ was determined by flow cytometry (FACSCalibur) (19). PerCP-conjugated anti-CD8 mAb, PE-conjugated anti-IL-4 mAb and FITC-conjugated anti-IFN-γ mAb were purchased from PharMingen.

**Cytotoxicity assay**

The cytotoxicity mediated by CTL generated in MLC was measured by 4 h ^{51}Cr-release assay as described previously (20). H-2^{d}-specific cytotoxicity was determined using BALB/c-derived P815 mastocytoma cells (H-2^{d}) as target cells. As control, C57BL/6-derived MBL-2 T lymphoma cells (H-2^{b}) were used. The percent cytotoxicity was calculated as described previously (20).

**Results**

**Expansion of three distinct DC subsets from BM cells by culture with different cytokine cocktails**

BALB/c BM cells were cultured under three different conditions for 4 days as follows: (i) in the presence of GM-CSF...
levels of the CD11b marker than BMDC0 and BMDC2. How- stimulator cells for the generation of IFN-

Ever, only a small percentage (11%) of BMDC1 dully CTL. Functional heterogeneity of DC subsets

plus IL-3 (neutral conditions), (ii) in the presence of IL-12 and IFN-γ in addition to GM-CSF plus IL-3 (T_h1-biasing conditions), and (iii) in the presence of IL-4 in addition to GM-CSF plus IL-3 (T_h2-biasing conditions). We designated these BM-derived DC subsets induced by either neutral, T_h1- or T_h2-biasing cytokines as BMDC0, BMDC1 and BMDC2 respectively. As shown in Fig. 1, all BMDC subsets expressed the CD11c antigen, a marker for DC, at the same levels. However, the expression profile of functional cell-surface molecules among the three BMDC subsets is very different. As illustrated in Fig. 1, BMDC1 expressed the highest levels of MHC class I, MHC class II, CD1d, CD40, B7-1 and LFA-1, compared with BMDC0 and BMDC2. BMDC1 also expressed higher levels of the CD11b marker than BMDC0 and BMDC2. However, only a small percentage (11%) of BMDC1 dully expressed F4/80 macrophage marker as well as BMDC0 (6.9%) and BMDC2 (7.1%) (data not shown). Moreover, it was demonstrated that all BMDC subsets expressed high levels of Thy-1 but not CD8 antigens (data not shown). Therefore, BMDC1 appeared to belong to myeloid DC subset but not lymphoid DC subset. BMDC1 also showed unique morphological characteristics, including a small size, a star-like shape and sharp dendrites (Fig. 2). BMDC2 were the largest cells and had long dendrites. BMDC0 had an intermediate size between BMDC1 and BMDC2.

Functional differences among BMDC0, BMDC1 and BMDC2 subsets for the generation of CTL in allogeneic MLC

To demonstrate functional differences among BMDC0, BMDC1 and BMDC2, we compared their stimulating effect on the generation of CTL in allogeneic MLC. MLC was carried out by co-culturing C57BL/6 mouse spleen cells (5 × 10^6 cells) with 2 × 10^5 mitomycin C-treated BALB/c-derived BMDC0, BMDC1 or BMDC2 for 4 days. Consistent with our observation that BMDC1 expressed high levels of MHC and co-stimulatory molecules, critically important for the stimulation of allogeneic T cells, the most potent cell proliferation was induced by stimulation with BMDC1 (data not shown). Cells from MLC stimulated with BMDC1 contained a higher percentage of CD8^+ T cells (23.4%) compared with MLC stimulated with BMDC0 (13.7%) or BMDC2 (11.1%) (Fig. 3A). These data strongly suggested that BMDC1 are the most efficient stimulator cells for inducing expansion of allogeneic CD8^+ T cells. The potent stimulatory effect of BMDC1 on the generation of functional CD8^+ T cells was also demonstrated by investigating intracellular cytokine expression in CD8^+ T cells. The highest percentage (31.6%) of IFN-γ-producing CD8^+ T (TC1) cells was induced in MLC using BMDC1 stimulator cells (Fig. 3B). In sharp contrast, BMDC2 were poor stimulators for the induction of TC1 cells (10.8%). BMDC0 cells always revealed an intermediate stimulatory activity for the induction of TC1 cells (16.8%). Consistent with the efficient function of BMDC1 for the induction of IFN-γ-producing TC1 cells, BMDC1 were also the most effective in stimulating the generation of allogeneic CTL in MLC (Fig. 4). MLC stimulated with BMDC1 stimulator cells showed the highest cytotoxicity against H-2d-positive P815 mastocytoma cells (Fig. 4A), but not against H-2d-positive MBL-2 T lymphoma cells (Fig. 4B). Intermediate levels of CTL activity were induced by stimulation with BMDC0, whereas negligible cytotoxicity was induced by stimulation with BMDC2 (Fig. 4A). To exclude the possibility that the contaminated CD11c^+ BM cells might show potent stimulatory activity on CTL generation, we also examined the different stimulatory activity between BMDC subsets using FACS-sorted CD11c^+ BMDC1 and BMDC2. As shown in Fig. 5(A), CD11c^+ BMDC1 also showed stronger stimulatory activity for the generation of IFN-γ-producing CTL compared with CD11c^+ BMDC2 subsets. Therefore, the functional heterogeneity among BMDC subsets for CTL induction was not derived from contaminated CD11c^+ BM cells. As shown in Fig. 5(B), CD8^+ CTL were responsible for the cytotoxicity induced in MLC by stimulation with BMDC1. From these results, we concluded that BMDC1 cells expanded from BM cells by culture under T_h1-biasing conditions are the most potent stimulator cells for the generation of IFN-γ-producing CD8^+ CTL.

BMDC0, but not BMDC2, can be converted into BMDC1-like stimulator cells by treatment with IFN-γ

We next examined whether it was possible to convert BMDC0 and BMDC2 into BMDC1-like stimulator cells by treatment
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Fig. 2. Morphological characteristics of BMDC subsets: (A) BMDC0, (B) BMDC1 and (C) BMDC2.

Fig. 3. Induction of IFN-\(\gamma\)-producing CD8\(^+\) T cells in MLC by stimulation with BMDC subsets. (A) C57BL/6 mouse spleen cells were stimulated with BALB/c BMDC subsets. Cells from MLC stimulated with BMDC0 (a), BMDC1 (b) or BMDC2 (c) for 4 days were harvested and examined for their CD4/CD8 expression pattern using FACSCalibur. (B) IFN-\(\gamma\)-producing ability in CD8\(^+\) T cells harvested from MLC stimulated with BMDC0 (d), BMDC1 (e) or BMDC2 (f). Intracellular staining of IL-4 and IFN-\(\gamma\) was carried out as described in Methods. Numbers represent the percentage of cells in each quadrant.

with IFN-\(\gamma\) before MLC. BM cells were cultured under neutral conditions (for BMDC0) or Th2-biasing conditions (for BMDC2) for 4 days, then the cells were harvested from each culture and treated with IFN-\(\gamma\) for 2 days before use in MLC. As shown in Fig. 6, treatment with IFN-\(\gamma\) greatly enhanced expression of MHC class I and class II molecules on both BMDC0 and BMDC2, reaching levels as high as those of BMDC1. Interestingly, expression of co-stimulatory molecules such as CD40 and B7-1 was also enhanced by treatment of BMDC0 with IFN-\(\gamma\), while no significant up-regulation of these molecules was observed when BMDC2 cells were treated with IFN-\(\gamma\). Such conversion of BMDC0 into BMDC1-like cells was also induced with lipopolysaccharide stimulation, but not by culture with IL-12 or IL-4 (data not shown). Moreover, removal of IFN-\(\gamma\) from BMDC1 for 2 days caused a marked reduction of MHC molecules and co-stimulatory molecules (data not shown). Therefore, IFN-\(\gamma\) appeared to be the critical factor for the development of BMDC1-like APC.

Consistent with our finding (Fig. 6) that IFN-\(\gamma\)-treatment increased expression of MHC and co-stimulatory molecules on BMDC0, but not BMDC2, IFN-\(\gamma\)-treated BMDC0, but not BMDC2, were efficient stimulators in allogeneic MLC (Figs 7 and 8). Analysis of intracellular cytokine expression (Fig. 7) further demonstrated that the stimulatory activity of BMDC0 for the induction of IFN-\(\gamma\)-producing CD8\(^+\) T (TC1) cells was markedly enhanced by treatment of BMDC0 with IFN-\(\gamma\) for 2 days (Fig. 7A and C). In sharp contrast, no significant increase in the generation of TC1 was observed when BMDC2 were treated with IFN-\(\gamma\) (Fig. 7B and D). The inability of BMDC2 cells to differentiate into BMDC1-like stimulator cells was further confirmed by demonstrating that both untreated and IFN-\(\gamma\)-treated BMDC2 were poor stimulators for the generation of allogeneic CTL in MLC (Fig. 8). In sharp contrast, BMDC0 treated with IFN-\(\gamma\) were very potent stimulators for the genera-
Fig. 5. CD8⁺ CTL are major effector cells induced by MLC stimulated with CD11c⁺ BMDC1. (A) C57BL/6 mouse spleen cells (H-2b) were cultured with FACS-sorted CD11c⁺ BMDC1 or BMDC2 derived from BALB/c (H-2d) BM cells. After 4 day culture, cells were harvested and their cytotoxicity and intracellular IFN-γ expression was examined. The solid bars represent cytotoxicity against P815 mastocytoma cells (H-2d) and the hatched bars represent the percentage of CD8⁺ T cells expressing intracellular IFN-γ. (B) C57BL/6 mouse spleen cells were stimulated with BMDC1 derived from BALB/c BM in MLC for 4 days. After culture, cells were fractionated into CD4⁺, CD8⁺ or CD4⁻CD8⁻ cell populations and their cytotoxicity against P815 mastocytoma cells was measured. The bars represent mean ± SE of triplicate samples.

Discussion

The body is protected from pathogenic antigens by two distinct immune systems, innate immunity and acquired immunity (1,2,20,21). Recent work has suggested that professional APC such as DC and macrophages play a critical role in bridging both immune systems through direct cell–cell contact or the production of cytokines (6–9). For example, NKT cells, one of the major effector cell populations in innate immunity, can be activated by DC through CD1d-restricted presentation of glycolipid antigens together with adhesion molecule-mediated cell–cell interactions and cytokines (8,22). Moreover, DC exhibit potent antigen-processing activity and can present MHC class II-bound antigenic peptides to main-stream CD4⁺ T cells (6,23). When DC interact with NKT or Th cells, DC are conditioned by endogenously produced cytokines such as IL-12, IL-18, IFN-γ, IL-2, IL-10 and IL-4, and these conditioned DC can then activate CD8⁺ T cells to differentiate into functionally matured CTL that produce IFN-γ (6,7,24). Thus, DC play a pivotal role in the initiation and progression of immune responses.

It has recently been suggested that functional heterogeneity may exist among DC subsets (13,14,17,25). CD11c⁺CD8α⁺ lymphoid DC were proposed to be key APC for the development of Th1 immunity (13,14). In contrast, CD11c⁺CD11b⁺CD8α⁻ myeloid DC play a critical role in the development of Th2 immunity (13). However, different results were obtained in analyzing the functions of mouse and human DC subsets (26); it is therefore likely that other DC subsets with different functions may exist. It is now recognized that the Th1/Th2 balance is important for immunoregulation and that its imbalance causes various immune diseases (11,12,15,16). It is therefore critically important to investigate factors that influence the functional maturation of DC and, in turn, affect the differentiation of naive CD4⁺ T cells into Th1 or Th2 cells.

When we initiated the present experiments, our working hypothesis was as follows. Since the polarization of immune responses into Th1- or Th2-dominant immunity is determined by Th-derived cytokines and cytokines produced by innate immune cells may affect the differentiation of functionally distinct DC, and (ii) that DC-derived cytokines may, in part, determine Th1 and Th2 differentiation (3,20). In keeping with our hypothesis, we previously demonstrated that DC differentiated from BM cells in the presence of Th1-inducing cytokines such as IL-12 and IFN-γ facilitate the differentiation of Th1 cells from naive Th cells, while DC induced in the presence of Th2-inducing cytokine IL-4 accelerate the differentiation of Th2 cells (17). In the present paper, we further demonstrated that Th1- and Th2-biasing cytokines may be important for the functional skewing of BM-derived DC subsets with functional heterogeneity for the generation of allogeneic CTL in MLC.

As shown in Figs 3 and 4, BMDC1 expanded from BM cells using Th1-biasing cytokines exhibited the most potent stimulator activity for the induction of IFN-γ-producing CD8⁺ T cells (TC1) and antigen-specific CTL in allogeneic MLC. The higher stimulating activity of BMDC1 compared with BMDC0 or BMDC2 appeared to be related to their increased expression levels of MHC class I, MHC class II and costimulatory molecules such as CD40, B7-1 and LFA-1 (Fig. 1). In allogeneic MLC, it has been demonstrated that MHC class I is critically important for the generation of allogeneic CD8⁺ CTL (27,28). Consistent with this finding, BMDC0 expressing low levels of MHC class I were poor stimulators in MLC (Figs...
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Fig. 7. Enhanced induction of IFN-γ-producing CD8+ T cells in MLC induced by stimulation with IFN-γ-treated BMDC0 but not BMDC2. C57BL/6 mouse spleen cells were co-cultured with BALB/c-derived untreated BMDC0 (A), untreated BMDC2 (B), IFN-γ-treated BMDC0 (C) or IFN-γ-treated BMDC2 (D) for 4 days. After 4 day culture, cells from MLC were harvested and the percentage of IL-4 or IFN-γ-producing CD8+ T cells was examined by intracellular cytokine staining as described in Methods.

Fig. 8. Conversion of BMDC0, but not BMDC2, into BMDC1-like stimulator cells by treatment with IFN-γ. C57BL/6 mouse spleen cells (H-2b) were stimulated with either untreated BMDC0, IFN-γ-treated BMDC0, untreated BMDC2, IFN-γ-pretreated BMDC2 or BMDC1, which were derived from BALB/c BM cells (H-2d). After 4 day MLC, cells were harvested and their cytotoxicity against P815 (H-2d) was measured by 4 h 51Cr-release assay. The bars represent mean ± SE of triplicate samples.

3 and 4). However, when BMDC0 were treated with IFN-γ, they converted into BMDC1-like stimulator cells, concomitant with enhanced expression of both MHC and co-stimulatory molecules (Figs 6–8). Although IL-4 has been reported to stimulate macrophage expression of MHC class II (29), BMDC0 cultured with IL-4 in the presence of GM-CSF plus IL-3 did not convert into BMDC1-like APC (data not shown). Therefore, the differentiation of BMDC1 cells from BM cells appeared to be accelerated by IFN-γ but not by IL-4. However, the fact that lipopolysaccharide also allowed the conversion of BMDC0 into BMDC1-like APC (data not shown) suggested that another factor indifferent to T1 and T2 cytokines might be involved in BMDC1 differentiation from BMDC0. In contrast to BMDC0, BMDC2 could not be differentiated into BMDC1-like stimulator cells by treatment with IFN-γ, even though this treatment strongly enhanced MHC surface expression (Fig. 6). Different characteristics of IFN-γ-treated BMDC0 and BMDC2 include the expression pattern of co-stimulatory molecules such as CD40, B7-1 and LFA-1, which play an important role in CTL generation (30–32). Indeed, we have evidence that LFA-1/ICAM-1 interaction between T cells and DC is critical for CTL generation (data not shown), consistent with previous reports (32). Therefore, the high levels of co-stimulatory molecules, in addition to MHC class I, appeared to be required for CTL generation in MLC. Moreover, we previously found that IL-12-producing ability differs significantly among the
three BMDC subsets (17). Specifically, BMDC0 and BMDC1 produced high levels of IL-12, while BMDC2 produced very low levels of IL-12. This is an important difference, because IL-12 acts as a key cytokine for the development of Th1 and CTL through the induction of IFN-γ (20). Here, we again confirmed that both untreated and IFN-γ-treated BMDC2 produce very low levels of IL-12, while both BMDC0 and BMDC1 can produce significant levels of IL-12 irrespective of IFN-γ treatment (data not shown). Therefore, MHC and co-stimulatory molecule expression and the ability to produce IL-12 appeared to be critically important factors for the induction of Th1- and Tc1-dominant cellular immunity by BMDC subsets.

Consistent with our previous result (17), BMDC1 cells also facilitated the generation of IFN-γ-producing Th1 cells compared with BMDC2 (data not shown). However, we could not demonstrate that BMDC2 stimulated the generation of IL-4-producing Th2 or Tc2 during MLC. This might be because of the difficulty of the induction of IL-4-producing Th2 or Tc2 cells from bulk wild-type mouse spleen T cells in MLC. In allogeneic MLC with spleen cells and BMDC1, MHC class I-reactive CD8+ T cells appeared to be major responder cells rather than CD4+ Th1 cells and conditioned BMDC1 cells could bypass the function of Th1 cells in the generation of CTL. Indeed, we have already demonstrated that Th1-cytokine conditioned BMDC1 allowed the differentiation of CTL from purified CD8+ T cells in the absence of CD4+ T cells (data not shown). Therefore, Th1 cytokine-conditioned BMDC1 will become good tool for immunotherapy.

Recently, DC-based immunotherapy has been applied to malignant and infectious diseases (33,34). In most protocols for immunotherapy, DC expanded with GM-CSF plus IL-4 have been used (35–37). However, our previous (17) and present results strongly suggest that myeloid-type DC expanded in the presence of Th2-biasing cytokine IL-4 may not be optimal for application to DC-based immunotherapy. Although we demonstrated that BMDC1 and IFN-γ-treated BMDC0 are potent stimulators for the development of allogeneic CTL or peptide-specific Th1 cells, it will be necessary to investigate whether these BMDC subsets can process intact protein antigens and support the generation of Th1 and CTL specific to the digested antigenic peptide. Recent studies in mice suggested that lymphoid DC play a critical role in the induction of Th1-dominant immunity, while myeloid DC are important for the induction of Th2-dominant immunity (13). However, we demonstrated that CD11c+CD11b+CD8α myeloid BMDC1 support the development of Th1- and Tc1-dominant cellular immunity. Further studies will be required to select the most suitable DC subsets for DC-based immunotherapy.

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Abbreviations

**APC** antigen-presenting cell

**BM** bone marrow

**BMDC** bone marrow-derived dendritic cells

**CTL** cytotoxic T lymphocyte

**DC** dendritic cells

**GM-CSF** granulocyte-macrophage colony-stimulating factor

**MLC** mixed lymphocyte culture

**References**


