TLR9/TLR7-triggered downregulation of BDCA2 expression on human plasmacytoid dendritic cells from healthy individuals and lupus patients

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Abstract Plasmacytoid dendritic cells (pDCs) can produce a large amount of interferon-alpha (IFN-α) upon exposure to TLR9 or TLR7 agonists. Human pDCs have been shown to play an important role in the pathogenesis of systemic lupus erythematosus (SLE) through increased production of IFN-α. So, how to negatively regulate activation of pDCs and how to evaluate the activation of pDC in SLE patients attract much attention. BDCA2 is selectively expressed on human pDCs, acting as a hallmark of human pDCs. In this study, we showed that BDCA2 expression on pDCs decreased along maturation of pDCs, and TLR7 or TLR9 agonists could further significantly downregulate pDCs to express BDCA2, suggesting that the activated pDCs exhibit decreased expression of BDCA2. Functionally, BDCA2 ligation significantly inhibited upregulation of CD40, CD86 and CCR7 expression, IFN-α, IFN-γ and IL-6 production by pDCs stimulated with CpG ODN. Moreover, BDCA2 ligation suppressed CpG ODN-activated pDCs to mediate Th1 response, including T cell proliferation, IFN-γ production, and CD4+CCR5+Th1 development, confirming that BDCA2 is a negative regulator of TLR9-dependent activation of human pDCs. BDCA2 expression on pDCs from SLE patients decreased significantly but IFN-α production of these patients increased markedly as compared to that from healthy donors. Therefore, these results suggest that downregulation of BDCA2 expression on pDCs may reflect the activation of pDCs accumulated in SLE patients, and may be one marker for indication of the disease activity of SLE patients.

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KEYWORDS
Plasmacytoid dendritic cells; TLR9; TLR7; BDCA2; Systemic lupus erythematosus

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Introduction

Toll-like receptor 9 (TLR9) and TLR7 are pattern recognition molecules in innate immunity that not only recognize pathogen-derived components, such as bacterial DNA containing unmethylated CpG motif (CpG ODN) and some DNA or RNA virus, but also recognize some pathogenic autoantibodies, such as immune complexes containing nuclear acids in systemic lupus erythematosus (SLE) patients [1,2]. Upon recognition of ligands, TLR9 or TLR7 can mediate signals to activate innate immune cells and initiate anti-infectious immunity, thus eliminating the invading pathogens. However, numerous studies revealed the roles of TLR9 or TLR7 ligands in the pathogenesis of autoimmune diseases such as SLE through activation of both autoreactive B cells and plasmacytoid dendritic cells (pDCs) [3–5]. So, the mechanistic study for the pathogenic roles of TLR9 or TLR7 in the autoimmune diseases attracted much attention.

Although virtually all types of cells can produce IFN-α/β in response to TLR agonists, up to date, pDCs are generally accepted to be the most potent "natural IFN-producing cells" which produce up to 1000-fold more IFN-α/β than other cell types [6,7]. pDCs can activate many kinds of immune cells including T cells, B cells and NK cells through production of IFN-alpha (IFN-α) [8–10]. Now, it is accepted that over-produced IFN-α by pDCs is an important mechanism for the pathogenesis of SLE by activating autoreactive T cells, maintaining the survival of autoreactive B cells, and inducing monocytes into functional DCs [3,11,12]. Also, pDCs were observed to be infiltrated into the kidney, which may play a pathogenic role in SLE patients with nephritis [12]. In addition, over-activation of pDCs has been shown to be also involved in the pathogenesis of other autoimmune diseases such as psoriasis, Sjögren's syndrome, and multiple sclerosis [13–15]. However, the molecular mechanisms for the negative regulation of pDCs are not fully understood. Therefore, how to control TLR7/TLR9-dependent activation of pDCs will provide promising approach for intervention of autoimmune diseases and contribute to better understand the mechanisms for regulation of autoimmunity.

As a type II c-type lectin, BDCA2 is specifically expressed by human Lin-CD44<sup>dim</sup>CD123<sup>bright</sup>CD45RA<sup>-</sup>pre-pDCs, thus proposing as a hallmark of human pDCs [16,17]. Cross-linking of BDCA2 or other surface Ags, including BDCA4, CD4, and CD123 has been shown to be able to inhibit type I IFN production in TLR-dependent activated pDCs via triggering tyrosine phosphorylation of Syk, increasing intracellular [Ca<sup>2+</sup>] and subsequently leading to the reduced levels of transcripts for type I IFN genes and their responsive genes [18,19]. pDCs have been shown to be over-activated in SLE patients, however, the underlying mechanisms remain to be fully understood. In this study, we show that BDCA2 expression is downregulated on TLR7 or TLR9 agonists-activated pDCs in a type I IFN-independent manner, and that BDCA2 ligation can suppress TLR9-triggered activation of pDCs and subsequently pDC-mediated Th1 response. Importantly, the BDCA2 expression on peripheral pDCs decreases significantly in SLE patients. Moreover, the decreased BDCA2 on pDCs in SLE patients could be partially restored when the disease was alleviated. Therefore, our results demonstrate that TLR9-triggered reduction of BDCA2 on pDCs may be a clinic marker indicating accumulation of activated pDCs in SLE patients and the progression of SLE.

Materials and methods

Reagents

RPMI-1640 medium (PAA Laboratories, Linz, Austria) supplemented with 10% FCS (PAA Laboratories), 2mM L-glutamine, 1% sodium pyruvate, 2×10<sup>−5</sup>M m2 mercaptoethanol (Sigma-Aldrich, St Louis, MO) were used throughout experiments. Recombinant human IL-3, GM-CSF, and IL-4 were purchased from Peprotech (Rocky Hill, NJ). Fluorescence-conjugated anti-human CD40, CD86, HLA-DR Abs and isotype Ig were obtained from B.D. PharMingen (San Diego, CA). Anti-human BDCA2 was obtained from Miltenyi Biotec (Bergisch Gladbach, Germany). Agonist anti-CD40 mAb (5C11) was a gift from Professor Xueguang Zhang (Department of Immunology, Suzhou University, PR China). D-type CpG ODN was a gift from Dr. Dennis M. Klinman (Food and Drug Administration, USA), including same concentrations of D19 and D29. The sequence of D19 is 5′-GGTGCATCGATGCAGGGGGG-3′, and D29 is 5′-GGTGCAGCGGTGACAGGG-3′ [20]. Purified vesicular stomatitis virus (VSV) was kindly provided by Professor Wei Pan (Department of Microbiology, SMMU). Human IFN-α ELISA kit was obtained from PBL Biomedical Laboratories (Piscataway, NJ). ELISA kits of human IFN-α and IL-6 were from R&D Systems (Minneapolis, MN).

Isolation and preparation of immature, mature and activated human pDCs

Buffy coats of healthy volunteers were obtained from Changhai Hospital Transfusion Center (Shanghai, China). PBMCs were prepared from buffy coats by centrifugation through a Ficoll density gradient (Sigma-Aldrich). pDCs precursors (pre-pDCs) or monocytes were isolated from PBMCs using BDCA4 or CD14 magnetic microbeads respectively (Miltenyi Biotec). The purified pre-pDCs were stained with PE-labeled anti-BDCA2 mAb, and more than 95% cells routinely expressed BDCA2. The purified pre-pDCs were cultured in the presence of IL-3 (10ng/ml) for 96h to obtain immature pDCs on day 4. The resulting immature pDCs were stimulated with agonistic anti-CD40 mAb (3μg/ml) for another 48h to obtain mature pDCs on day 6, or the immature pDCs were stimulated with CpG ODN (6μg/ml) alone or CpG ODN plus anti-CD40 mAb (3μg/ml) for another 48h to obtain the activated pDCs on day 6 as described previously [21]. Immature pDCs were stimulated with IC (50μg/ml), or infected with VSV (128pfu/ml) for 4h, and then were cultured in medium supplemented with IL-3 for another 48h to obtain the activated pDCs. Monocytes were cultured in the presence of GM-CSF (500U/ml) plus IL-4 (10ng/ml) for 96h to obtain immature monocyte-derived dendritic cells (MoDC) on day 4. The resulting immature MoDCs were incubated with TNF-α (10ng/ml) for 48h to obtain mature MoDCs on day 6. The mature MoDCs were further stimulated with LPS (1μg/ml) for another 48h to obtain activated MoDCs on day 8 [22]. In some experiments, pre-pDCs at 1×10<sup>5</sup>/ml were
Flow cytometry

Expression of CD86, CD40, HLA-DR, CCR7, CXCXR4, CD62L and BDCA2 on pDCs was analyzed by FACS (FACScalibur and Cellquest software, Becton Dickinson, Mountain View, CA) as previously described [23]. Dead cells were excluded by gating out propidium iodide-positive cells. Geometric mean fluorescence intensity (Geo-MFI) values were used as a semiquantitative presentation of membrane molecule expression.

In vitro priming of human CD4+ T cells by pDCs

CD4+ T cells were isolated using CD4 magnetic microbeads (Miltenyi Biotech), and the purity was routinely greater than 95% as assessed by FACS. The proliferation and activation of CD4+ T cells stimulated by pDCs was detected as previously described [24]. Briefly, pDCs, as stimulator cells, were incubated with CD4+ T cells, as responder cells, for 7 days at indicated ratios. After incubation, the supernatants were collected for measurement of IFN-α, IFN-β and IL-6.

Assay of cytokines

The levels of IFN-α, IFN-β, IL-6, IL-8, IL-12p40, IL-12p70, IL-4, IL-10 and IFN-γ in the supernatants were determined by liquid scintillation spectroscopy (Amersham Pharmacia).

Assay for BDCA2 expression and IFN-α production by pDCs from SLE patients

PBMCs and sera of patients meeting ACR criteria for SLE were obtained from Department of Rheumatology, Changhai Hospital (Shanghai, China). All patients gave written informed consent before collection of blood samples. Disease activity was assessed using SLEDAI score based on the encounter forms, and divided into 4 scales, where 1–5 scores were as 1, 6–10 scores as 2, 11–20 scores as 3, and > 20 scores as 4. PBMCs and sera from 8 healthy individuals were also obtained for use as negative controls. BDCA2 expression on pDCs was detected by FACS. Relative Geo-MFI index was used to compare the expression of BDCA2 on pDCs from SLE patients and healthy volunteers, and calculated as: Geo-MFI of BDCA2+ positive cells/Geo-MFI of isotype staining cells. IFN-α levels in sera were assayed by ELISA.

Isolation of immune complex from sera of SLE patients

ICs were prepared from the sera of SLE patients with active disease on the basis of clinical classification criteria. The sera were passed through a 0.45μm filter and applied to a protein G-agarose column (Invitrogen). ICs were eluted from protein-G column using 100mM glycine buffer (pH 3.4) and then adjusted to pH 7.4 using Tris·HCl (pH 9.0). ICs were concentrated using an Amicon centrifugal filter device with a 10KD limit (NMWL) cutoff (Millipore). Purified ICs were verified by C1q and anti-DNA ELISA assays (Alpha Diagnostics International).

Statistical analysis

Statistical analysis between groups was performed by Student’s t-test. P<0.05 was considered statistically significant.

Results

TLR7, 9 ligand downregulates BDCA2 expression on human pDCs

It was reported that expression of BDCA2 on pDCs is rapidly lost after in vitro culture of pDCs [17]. Interestingly, we found that BDCA2 was highly expressed on freshly isolated pre-pDCs, and the BDCA2 expression was obviously downregulated but still detectable after in vitro culture of human pDCs for 96h (Fig. 1A). In accordance with previous reports that BDCA2 was not expressed on monocytes and MoDCs whether or not matured and activated. However, the expression of BDCA2 was significantly downregulated on the activated pDCs induced by CpG ODN as compared to that on immature pDCs and mature pDCs (Fig. 1B). The data indicated that expression of BDCA2, the specific marker of human pDCs, was downregulated during development of pDCs, and the most significantly decrease of BDCA2 expression was observed on TLR9-activated human pDCs. To investigate whether other activators of pDCs have similar effect on BDCA2 expression, we stimulated immature pDCs with TLR7 agonist, VSV, or ICs from SLE patients respectively, and found that BDCA2 was also downregulated (Fig. 1C). The activated pDCs can secrete large amounts of type I IFN. To investigate whether the downregulation of BDCA2 was a secondary effect of activated pDC-derived type I IFN, we pretreated immature pDCs with neutralizing anti-IFN-α and anti-IFN-α/β receptor before stimulation with CpG ODN. As shown in Fig. 1C, blocking type I IFN pathway had no effect on the downregulation of BDCA2 on the activated pDCs. Therefore, the data demonstrated that BDCA2 expression is downregulated when pDCs are activated by TLR7, 9 agonists, and such downregulation of BDCA2 is not a secondary effect of activated pDC-derived type I IFN.

BDCA2 ligation inhibits TLR9-activated pDCs to express CD40, CD86 and CCR7 and to produce IFN-α, IFN-β and IL-6

To investigate the effects of BDCA2 ligation on the functions of pDCs, we first assessed CpG ODN-induced expression of co-
stimulatory molecules such as CD86 and CD40, and MHC II molecule HLA-DR on pDCs. As shown in Fig. 2A, ligation of pDCs with agonistic anti-BDCA2 mAb AC144 [17] could significantly inhibit CpG ODN-induced upregulation of CD86 and CD40 expression on pDCs. However, the expression of HLA-DR remained almost unchanged. We also detected the effect of BDCA2 ligation on the expression of chemokine receptors, including CXCR4 and CCR7, and CD62L, a homing receptor, on TLR9-activated pDCs. We found that CXCR4 was constitutively expressed on immature pDCs, and was down-regulated on CpG ODN-activated pDCs. However, BDCA2 ligation had no effect on the downregulation of CXCR4 expressed on the activated pDCs. In contrast to CXCR4, CCR7 was inducible expressed on CpG ODN-activated pDCs, however, BDCA2 ligation could significantly inhibit the expression of CCR7 on the activated pDCs. As for CD62L, the activated pDCs expressed higher levels of CD62L than immature pDCs, but BDCA2 ligation had no effect on its expression.
Figure 2  BDCA2 ligation inhibits TLR9-triggered upregulation of CD40, CD86 and CCR7 expression and IFN-α, IFN-β and IL-6 production by human pDCs. pDCs were pre-incubated with anti-BDCA2 mAb AC144 (1 μg/ml) or isotype mIgG1 (1 μg/ml) for 30 min, and then stimulated with CpG ODN (6 μg/ml) for 48 h. (A) pDCs were harvested and analyzed for the expression of HLA-DR, CD86, CD40, CD62L, CCR7 and CXCR4 by FACS. Data were shown as percentage of positive cells. (B) The supernatants of human pDCs were harvested and assessed for the production of IFN-α, IFN-β, IL-6, IL-8, IL-12p70, IL-12p40, IL-4 and IL-10 by ELISA. Data were shown as mean ± SEM of three independent experiments. **P<0.01.
Next, we investigated the effect of BDCA2 ligation on the production of cytokines from CpG ODN-activated pDCs. We observed that ligation of BDCA2 significantly inhibited the CpG ODN-stimulated pDCs to secrete IFN-α, IFN-β, and IL-6 (Fig. 2B). BDCA2 ligation had no effect on the production of other cytokines, including IL-12p40, IL-12p70, IL-4, and IL-10 from CpG ODN-activated pDCs. Therefore, ligation of BDCA2 inhibits TLR9 agonist-triggered activation of pDCs.

**BDCA2 ligation polarizes Th1 response into Th2 response by TLR9-triggered pDCs**

CpG ODN-activated pDCs have been shown to activate CD4+ T cells to proliferate and secrete IFN-γ. The inhibition of CD86 and CD40 expression on pDCs or type I IFNs from activated pDCs by BDCA2 ligation prompted us to further investigate the effects of BDCA2 ligation on pDC-stimulated proliferation and activation of CD4+ T cells in Allo-MLR. As shown in Fig. 3A, BDCA2 ligation significantly inhibited the proliferation of CD4+ T cells stimulated by CpG ODN-activated pDCs. Moreover, ligation of BDCA2 could suppress the CpG ODN-activated pDCs to produce Th1 cytokine, IFN-γ, from CD4+ T cells (Fig. 3B). It has been reported that CCR5 is preferentially expressed on Th1 cells and that CCR4 is preferentially expressed on Th2 cells [25,26]. So, we further investigated the expression of CCR4 and CCR5 on CD4+ T cells stimulated by CpG ODN-activated pDCs.

**Figure 3** BDCA2 ligation inhibits Th1 response primed by TLR9-activated pDC. pDCs were pre-incubated with anti-BDCA2 mAb AC144 (1 μg/ml) or isotype mIgG1 (1 μg/ml) for 30 min, and then stimulated with CpG ODN (6 μg/ml) for 48 h. Then, pDCs were cocultured with allogenic CD4+ T cells at indicated ratios for 7 days. (A) The proliferation of CD4+ T cells was measured using [3H]-TdR incorporation. Data was shown as mean ± SD of triplicate determinants. One representative result was shown from three independent experiments. (B) The level of IFN-γ in the supernatants was measured using ELISA. Data were shown as mean ± SEM of three independent experiments. **P<0.01. (C) Levels of IFN-α in the sera of SLE patients with different SLEDAI and healthy donors. Geo-MFI index was used to compare the levels of BDCA2 expression on pDCs, and calculated as: Geo-MFI of BDCA2+ positive cells/Geo-MFI of isotype staining cells. ● represents the relative Geo-MFI of BDCA2 expression on pDCs from each individuals; — represents the mean value of Geo-MFI index of BDCA2 expression on pDCs from each indicated group. **P<0.01. (D) BDCA2 expression on pDCs from two SLE patients at their various stages of disease activity. PBMCs from SLE patients at indicated disease activity were measured for BDCA2 expression on pDCs using FACS. Data was shown as relative BDCA2 level indicated in the dot plot, which was calculated as Geo-MFI of BDCA2+ cells divided by Geo-MFI of isotype Ig stain.

**Figure 4** Decreased BDCA2 expression on SLE pDCs with the increase of IFN-α levels in SLE serum. PBMC samples from SLE patients and healthy donors were collected and analyzed for the expression of BDCA2 by FACS. (A) Percentage of BDCA2+ cells in PBMCs of SLE patients and healthy donors. *P<0.05 (B) Relative levels of BDCA2 expression on pDCs from SLE patients with different SLEDAI and healthy donors. Geo-MFI index was used to compare the levels of BDCA2 expression on pDCs, and calculated as: Geo-MFI of BDCA2+ positive cells/Geo-MFI of isotype staining cells. ● represents the relative Geo-MFI of BDCA2 expression on pDCs from each individuals; — represents the mean value of Geo-MFI index of BDCA2 expression on pDCs from each indicated group. **P<0.01. (C). Levels of IFN-α in the sera of SLE patients with different SLEDAI and healthy donors. ● represents the level of IFN-α in serum from each individual; — represents the mean value of IFN-α levels in sera from each indicated group. *P<0.05. (D) BDCA2 expression on pDCs from two SLE patients at their various stages of disease activity. PBMCs from SLE patients at indicated disease activity were measured for BDCA2 expression on pDCs using FACS. Data was shown as relative BDCA2 level indicated in the dot plot, which was calculated as Geo-MFI of BDCA2+ cells divided by Geo-MFI of isotype Ig stain.
investigated the expression of CCR4 and CCR5 expression on CD4+ T cells primed by pDC. As shown in Fig. 3C, CpG ODN-activated pDCs could induce more CCR5+ Th1 and less CCR4+ Th2 differentiated from CD4+ T cells, suggesting that CpG ODN-activated pDCs were mainly involved in Th1 response. However, upon ligation of BDCA2 on pDCs, CpG ODN-stimulated pDC-mediated differentiation of CCR5+ Th1 cells was obviously inhibited, whereas more CCR4+ Th2 cells were produced. Altogether, BDCA2 ligation on pDCs can functionally inhibit Th1 response and polarize Th1 response into Th2 response primed by CpG ODN-stimulated pDCs.

Decreased BDCA2 expression on pDCs from SLE patients

It has been shown that SLE patients exhibit the over-activation of pDCs and the elevated IFN-α production. Importantly, the levels of IFN-α in serum are highly correlated with both disease activity and severity of SLE [27,28]. So, as major source of IFN-α, the over-activated pDCs were possibly involved in the pathogenesis of SLE. As described above, BDCA2 was found to be downregulated on pDCs when they were activated, so we wondered whether the expression of BDCA2 on pDCs derived from SLE patients decreased. We collected PBMC samples of SLE patients and healthy donors, and detected the percentage of BDCA2+ pDCs in PBMCs and the relative expression levels of BDCA2 on pDCs. We found that the percentage of BDCA2+ pDCs in PBMCs from SLE patients decreased significantly as compared to that from healthy donors (Fig. 4A), and the levels of BDCA2 expression on pDCs from SLE patients at various stages of the disease were significantly lower than those on pDCs from healthy donors (Fig. 4B). These clinical data suggested that decreased BDCA2 expression level on pDCs and percentage of BDCA2+ pDC (%) in PBMCs were related to the progression of SLE. However, although IFN-α has been accepted to be involved in the pathogenesis of SLE, IFN-α was still undetectable in some SLE patients (Fig. 4C), possibly due to several factors affecting serum IFN-α level, such as metabolic rate. We also obtained blood samples of two patients who were at various stages of SLE activity. As shown in Fig. 4D, BDCA2 levels on pDCs significantly decreased when they had an active SLE, but were partially restored when the disease alleviated. Taken together with the data above showing that BDCA2 is a negative regulator of pDCs and BDCA2 expression is downregulated when pDCs are activated, therefore, levels of BDCA2 on pDCs may be a useful clinician marker indicating the active status of pDCs and the disease activity of SLE patients.

Discussion

DCs express a number of C-type lectin receptors (CLR) that can recognize and bind component of pathogens as well as self-glycoproteins. These CLR include mannose receptor (CD206), Dec-205 (CD205), DC-SIGN (CD209), BDCA2 (CD303) and etc. These surface molecules have been found to be used by pathogens, at least sometimes, or ligands to mediate negative signaling, induce immune tolerance or immune escape. For example, internalization of pathogenic antigens by multiple C-type lectins on DCs may suppress TLR-induced DC activation [29]. Therefore, as a type II C-type lectin specifically expressed on pDCs, BDCA2 has been identified to regulate the function of pDCs. On the other hand, we are interested in the regulation of BDCA2 expression on pDCs, which may give some clues for the investigation of functional regulation of pDCs and mechanistic study of pDCs in the pathogenesis of autoimmune diseases. Unfortunately, natural ligands for BDCA2 are still poorly understood. Previous studies showed that the anti-BDCA2 mAb AC144 is rapidly internalized by pDCs, indicating BDCA2 may play a role in antigen capture [17]. Up to date, known antigens recognized by pDCs include virus and substances containing nuclear acid, such as chromatin released from apoptotic and necrotic cells. Under physiological conditions, pDCs are normally found in peripheral blood and T-area of lymphoid tissues. Thus, it is worthwhile to explore whether BDCA2 is used by some self-antigens to control autoimmunity or to maintain tolerance to self-tissues in hosts, or whether some T cells expressing ligands for BDCA2 exist in body, which are involved in the maintenance of immune tolerance. In this study, we show that the expression of BDCA2 on pDCs is downregulated during the maturation of pDCs. Moreover, TLR7 and TLR9 agonists are able to downregulate the expression of BDCA2 on pDCs more significantly. These data suggest that BDCA2 may exert its primary function at early stages of pDC development and deliver negative signals to antagonize the maturation and activation of pDCs. Once activated by TLR7, 9 ligands, pDCs will lose the negative control by BDCA2, contributing to further activation or over-activation of pDCs. It is also worth investigating whether the lack of BDCA2 ligand is involved in the pathogenesis of some autoimmune disease, such as SLE.

It is well established that DC-derived co-stimulatory signals act together with antigen-specific signal for their efficient induction of proliferation and activation of T cells [30,31]. Conversely, DCs which express high levels of class II molecules but low or moderate co-stimulatory molecules can induce T cell anergy which is involved in the maintenance of T cell tolerance [32,33]. In accordance with these findings, we found that BDCA2 ligation can significantly downregulate CpG ODN-induced expression of co-stimulatory molecules such as CD40 and CD86 on pDCs but doesn’t affect the upregulation of MHC-II molecule expression on CpG ODN-activated pDCs. Thus, the proliferation and activation of allogeneic CD4+ T cells primed by CpG ODN-stimulated pDCs are significantly reduced when pDCs are pretreated with ligation of BDCA2. In addition to cell–cell interaction, BDCA2-mediated inhibition of IFN-α/β production may also possibly contribute to inhibition of Th1 response, because increasing evidence suggest that IFN-α is involved in induction of Th1 response. It has been shown that Th1 cells preferentially express CCR5, and that Th2 cells preferentially express CCR4. In our study, we found a significant reduction in the number of CD4+CCR5+ T cells and a significant increase in the number of CD4+CCR4+ T cells differentiated from T cells stimulated by CpG ODN-stimulated pDCs ligated by anti-BDCA2. So, BDCA2 may be involved in Th2 polarization via affecting pDC functions.

Accumulating evidence demonstrate that pathological process of SLE is related to abnormal elevation of IFN-α which is essential for activation and survival of autoreactive T and B cells [11,34,35]. One predominant feature of SLE is
the break of tolerance to self-antigens, leading to accumulation of immune complexes against nuclear components which are powerful activators of pDCs via TLR7 or TLR9 pathway. Reduction in BDCA2 expression on pDCs in SLE might be a result of pDC activation by immune complex containing nuclear acids through TLR7 or TLR9, and be also an inherited deficiency of a given population which predisposes to suffering from SLE. Although we cannot observe a significant correlation of the decrease in BDCA2 level on pDCs with the progression of the disease because of limited available SLE samples, BDCA2 level is indeed lower in SLE patients at various stages of the disease than that in normal individuals, suggesting that BDCA2 is a sensitive index indicating SLE activity. Moreover, we observed that the decreased BDCA2 levels on pDCs were significantly restored in two patients when their diseases alleviated.

In conclusion, on the basis of our observations that the TLR9-dependent activation of pDCs is negatively regulated by ligation with BDCA2 whose expression is also reciprocally downregulated by TLR7 or TLR9 ligand, we demonstrated the decreased expression of BDCA2 on pDCs from SLE patients may indicate an activated status of pDCs in SLE, and may be a useful clinic marker for indication of the progression of SLE patients.

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