Regulation of the multifaceted functions of human plasmacytoid dendritic cells: a polyphonic policy

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EXPRESSION OF THE C-TYPE LECTIN BDCA-2 ON PLASMACYTOID DENDRITIC CELLS IS DIRECTLY REGULATED BY THE TRANSCRIPTION FACTOR SPI-B

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ABSTRACT

Plasmacytoid dendritic cells (pDCs) are the main producers of type I Interferons (IFN) in response to bacterial or viral nucleic acids, which are sensed by Toll like receptors (TLR)-9 and TLR7, respectively. In addition, pDCs differentiate into mature DCs that acquire the ability to activate T cells. The pDC specific C-type lectin blood-derived cellular antigen 2 (BDCA-2) has a role in reducing the TLR-induced production of type I IFN and stimulating antigen uptake. It has remained elusive how expression of BDCA-2 is regulated. Previously, we reported that the transcription factor Spi-B is crucial for pDC development and survival. Here we show by using overexpression and knockdown approaches that Spi-B also regulates expression of BDCA-2 by directly binding to the promoter region of BDCA-2. Collectively, our data provide further evidence that Spi-B is a key regulator in pDCs that alters their function in favor of antigen presentation at the cost of type I IFN expression.
INTRODUCTION

Dendritic cells (DCs) recognize and internalize different pathogenic products via pathogen recognition receptors (PRRs) thereby steering appropriate immune responses to eradicate intruders. Plasmacytoid dendritic cells (pDCs) form a distinct DC population that are able to detect pathogens via the recognition of single-stranded RNA or double stranded DNA by Toll-like receptor 7 (TLR7) and TLR9, respectively. Ligation of TLR7 and TLR9 in the early endosomal compartment results in rapid and massive secretion of type I interferons (IFN). Type I IFNs are pleiotropic antiviral cytokines that can directly inhibit viral replication, but also initiate innate and adaptive immune responses, including activation of natural killer (NK) cells and conventional DCs (cDCs), induction of monocyte differentiation into cDCs, increasing the ability of DCs to cross-present exogenous antigens to CD8+ T cells and promote their clonal expansion, differentiation of B cells into antibody secreting plasma cells and polarization of T cells. In addition to foreign nucleic acids, pDC are also able to sense self-DNA and self-RNA released during necrotic and apoptotic cell death. When unceased, production of type I IFNs by pDCs can be deleterious for the host as reflected by human immunodeficiency virus (HIV)-induced immune activation or the causality of the IFN signature and the severity of autoimmune diseases such as systemic lupus erythematosus (SLE), psoriasis and Sjögren’s syndrome (SS). In SLE patients, pDCs are constantly activated by immune complexes consisting of self-DNA and auto-antibodies that engage FcγRII. Due to the ongoing production of type I IFN, autoimmune T cells are stimulated via the activation and maturation of mDCs.

To regulate the amplitude of the type I IFN response, pDCs express several surface receptors, including blood dendritic cell antigen-2 (BDCA-2) (also known as CD303, CLEC4C). BDCA-2 is a type II transmembrane glycoprotein that belongs to the C-type lectin superfamily. Its expression is unique on human pDCs, but it remains elusive how BDCA-2 expression is regulated. C-type lectins are PRRs that recognize glycan structures on host cells and pathogens, and can function as uptake receptors. BDCA-2 signals via the FcεRγ-Syk signaling pathway, which interferes with TLR9-induced activation of pDCs, thereby inhibiting IFN type I secretion and TNF-related apoptosis inducing ligand (TRAIL)-mediated cytotoxic activity. Furthermore, peptides coupled to a monoclonal antibody that targeted BDCA-2 were internalized and presented via major histocompatibility complex (MHC) class II to CD4+ T cells. While the natural ligand for BDCA-2 is elusive, BDCA-2 can bind to asialo-galactosyl-oligosaccharides. Moreover, viral glycoproteins, including human immunodeficiency virus (HIV)-gp120, Hepatitis C virus (HCV)-E2 protein and Hepatitis B virus (HBV)-Hepatitis B Surface Antigen (HBsAg), bind BDCA-2 and contribute to the downregulation of type I IFNs.

A key player in pDC biology is the transcription factor Spi-B, which is an ETS family member that is expressed in CD34+ precursor cells, mature B cells and in pDC, but not cDCs. Overexpression of Spi-B in hematopoietic progenitor cells (HPCs) blocks T cell, B cell and NK cell development but promotes pDC development, showing that Spi-B regulates lineage commitment during hematopoiesis. This, together with the result that pDC development was impaired when Spi-B expression was inhibited, reinforced the notion that Spi-B is a critical
transcription factor for pDC development. Downregulation of Spi-B resulted in a significant decrease in pDC numbers due to the induction of apoptosis by direct regulation of the anti-apoptotic gene Bcl2-A1. Given the central role of Spi-B in pDC development and survival we examined whether Spi-B might regulate expression of BDCA-2.

We confirmed and extended the observation that expression of BDCA-2 was downregulated in pDC upon TLR7 and TLR9 ligation. We show that this not only coincided with the downregulation of Spi-B, but we also demonstrate that Spi-B through direct binding to the promoter of BDCA-2 regulates its expression. While these results reveal part of the molecular network that regulates the expression of this C-type lectin, it also provides insight in the mechanism that pDCs use to control type I IFN production. This contributes to our understanding how aberrant IFN production, as observed in autoimmune diseases such as SLE or HIV infection may be controlled.

MATERIAL & METHODS

Human primary cells and cell lines

Peripheral blood of healthy volunteers was used upon donor consent (Sanquin Bloodbank, Amsterdam, The Netherlands). Peripheral blood mononuclear cells were obtained via Ficoll-Hypaque density gradient (Lymphoprep; Nycomed Pharma, Oslo, Norway) followed by immunomagnetic enrichment by depletion of CD3/CD14/CD16/CD19/CD20 expressing cells coupled to anti-FITC beads (Miltenyi Biotec, Bergisch Gladbach, Germany). Postnatal thymus tissue (PNT) was obtained from surgical specimens that were removed from children up to three years of age undergoing open heart surgery (LUMC, Leiden, The Netherlands). PNT tissue was mechanically disrupted and thymocytes were isolated via a Ficoll-Hypaque density gradient (Lymphoprep). BDCA-4+ pDCs from PNT were enriched using immunomagnetic bead selection with the BDCA-4+ cell separation kit (Miltenyi Biotec). Subsequently, the BDCA-4+ enriched fraction was sorted by flow cytometry for expression of CD123+CD45RA+ on a FACSAria (BD biosciences). Purity of all sorted populations was ≥99%. The use of all human tissues was approved by the Medical Ethical committee of the Academic Medical Center and was contingent on obtaining informed consent, in accordance with the Declaration of Helsinki. The pDC cell line CAL-1 was cultured in RPMI-1640 medium (Invitrogen) supplemented with 8% FCS (Hyclone, Thermo Fisher, Waltham, MA, USA), penicillin 50 U/ml, streptomycin (50 µg/ml) (Roche) and maintained at 37°C and 5% CO₂. The following reagents were used for cell stimulations: R848, CpG-A (ODN2216), CpG-B (ODN2006) (all at 10 µg/ml; Invivogen, San Diego, California, USA).

Lentiviral constructs and transductions

Human cDNA sequence for Spi-B coupled to a mutated estrogen receptor (ER) was ligated into the multiple cloning site of the pHEFTIG vector. pHEFTIG-Spi-B-ER-IRE-RES-green fluorescent protein (GFP) or the empty (control) vector pHEFTIG-IRE-RES-GFP were used as viral constructs for overexpression of Spi-B. To knockdown Spi-B in CAL-1 cells, short hairpin (sh)RNA to Spi-B was cloned into a doxycycline (dox) inducible lentiviral backbone (pLV711-tH1-Spi-B-sh-GFP) or the irrelevant (sh targeting Renilla
Spi-B directly regulate S Bdca-2 expression

For transduction experiments, supernatants from transfected (X-tremeGENE 9 DNA transfection reagent, Roche) HEK293T cells were collected, filtered and frozen at -80°C until use. Transduction of CAL-1 cells was done using virus supernatant on retronectin-coated plates (30µg/ml Takara Biomedicals, Kyoto, Japan) for six hours at 37°C and 5% CO₂. To induce nuclear translocation of ER-tagged Spi-B, cells were analyzed after two days of culture for expression of GFP followed by addition of 0.5 µM 4-hydroxytamoxifen or DMSO (4HT; Sigma-Aldrich, St Louis, MO, USA). For shRNA expression, transduced CAL-1 cells were cultured in the presence or absence of dox (1µg/ml) for 2 days.

**Flow cytometry**

For flow cytometry we used anti-human monoclonal antibodies coupled to either fluorescein isothiocyanate (FITC), phycoerythrin (PE), PE-cyanine (PE-Cy7), allophycocyanin (APC) or APC-Cy7 directed against CD3, CD14, CD19, CD40 (Biolegend), CD123 (eBioscience), BDCA-2, BDCA-4 (Miltenyi), CD20, CD16, CD45RA (BD) and matching isotype controls (Dako, BD). For viability staining we used DAPI. Samples were measured using a LSRII or sorted using a FACSAria (BD) and analyzed with FlowJo software (Treestar).

**PCR**

Total RNA was extracted from the cells using Trizol reagent (Invitrogen). RNA concentration and quality were determined via Nanodrop spectrophotometer (Thermo Fisher Scientific). Equal amounts of RNA were reverse transcribed into cDNA using High Fidelity cDNA synthesis kit following manufacturer’s protocol (Roche). cDNA was synthesized using the PCR machine (PTC-200, MJ Research) and analysis was performed on the iCycler (Biorad) for quantitative PCR (qPCR) using SYBR Green supermix (Biorad) and specific primer sets to identify BDCA-2, CD40, Bcl2-A1, CD40 promoter and BDCA-2 promoter #1, 3, 8, 10, 14 and 15 (Supplementary table 1). Samples were analyzed in triplicates and normalized to housekeeping genes: GAPDH, HPRT and β-actin.

**Chromatin immunoprecipitation assay**

Spi-B~ER-GFP+ CAL-1 cells were incubated for four hours with or without 4HT. Chromatin immunoprecipitation (ChIP) was performed according to an adapted version of the Upstate ChIP kit protocol (Upstate Biotechnology) by using 3µg of polyclonal anti-ER antibody (Santa Cruz Biotechnology) or 3µg of normal rabbit Immunoglobulin (Ig)G (Invitrogen). The precipitated chromatin was then purified using the QIAamp DNA mini kit (Qiagen) and analyzed by qPCR on the qCycler (Biorad). Primerset were made for various parts of the promoter region before the ATG site of BDCA-2 (Supplementary table 1).

**Western blot analysis**

Proteins of the whole cell lysates of sorted isolated human pDCs were separated by SDS-PAGE and transferred to a nitrocellulose membrane. Blocking was performed with TBST plus 5% milk. Spi-B protein was detected using a Spi-B antibody (kindly provided by Lee Ann Sinha, State
University of New York, Buffalo, USA). Actin (I-19, Santa Cruz Biotechnology) was used as a control. Visualization of the protein bands was performed via addition of chemiluminescent substrate and horseradish peroxidase (HRP)-conjugated secondary antibody (DAKO).

RESULTS

TLR activation downregulates BDCA-2 expression on human pDCs

It remains incompletely understood how expression of BDCA-2 in pDCs is regulated. To study this, we isolated pDCs from peripheral blood and stimulated the cells with the TLR9 ligands CpG-A or CpG-B, or the TLR7 ligand R848. The next day pDCs were analyzed by flow cytometry after staining with a monoclonal antibody for BDCA-2. We observed that expression of BDCA-2 was downregulated in all the conditions tested as compared to the medium control cultured cells (Figure 1). This confirms and extends the findings reported previously that TLR ligation is involved in the downregulation of BDCA-2.40

The transcription factor Spi-B is downregulated after TLR engagement on pDCs

To gain insight in the molecular mechanism controlling expression of BDCA-2 we focused on the role of the Ets transcription factor Spi-B, which is specifically expressed in pDCs as compared to cDCs34 and is crucial for the development of human pDCs.36 First, we analyzed the kinetics in expression of Spi-B in pDCs after stimulation with various TLR ligands. Western blot analysis was performed on lysates from pDCs that were cultured for 5 hrs or 24 hrs in the presence of CpG-A or CpG-B. We observed that stimulation for 5 hrs did not affect Spi-B protein levels, whereas Spi-B levels were reduced after 24 hrs in all conditions tested as compared to freshly isolated pDCs (Figure 2A). QPCR analysis showed that CpG stimulation, in particular CpG-B stimulation, upregulated Spi-B mRNA levels after 2 hrs and downregulated Spi-B mRNA levels after 4-18 hrs (Figure 2B). We conclude that TLR ligation in time downregulates Spi-B both at the mRNA and protein level.

Figure 1: Activation of pDCs regulates BDCA-2 expression. Sorted peripheral blood pDCs were cultured overnight in medium only or in the presence of CpG-A, CpG-B or R848 (10µg/ml). BDCA-2 expression was analyzed by flow cytometry after overnight TLR ligation (black line) and was compared to medium (filled dark grey histogram) and the isotype control (light grey histogram). RCN, relative cell number.
five Spi-B directly regulate BDCA-2 expression

BdCA-2 is a direct target gene of Spi-B

Given the observation that both BDCA-2 and Spi-B are downregulated with similar kinetics in activated pDCs we hypothesized that Spi-B may be involved in regulating BDCA-2 expression levels. Hence, we studied whether BDCA-2 is a direct target of Spi-B. To this aim we investigated whether Spi-B directly binds the promoter area of BDCA-2 using Chromatin immunoprecipitation (ChIP) assays. First, we generated a 4-hydroxy-tamoxifen (4HT)-regulatable Spi-B-estrogen receptor (Spi-B~ER) fusion retroviral construct that allows 4HT-induced nuclear transport of transcriptionally active Spi-B. Green fluorescent protein (GFP) is used as a marker gene and expressed from the same retroviral promoter separated by an IRES sequence to allow independent tracing of transduced cells. We transduced the model pDC cell line CAL-1 with Spi-B~ER and sorted GFP+ cells. Spi-B~ER transduced CAL-1 cells were cultured for 4 hrs with 4HT or DMSO after which we immunoprecipitated the chromatin with either an anti-ER antibody or a control rabbit IgG antibody. As a positive control we analyzed the CD40 promoter and observed that Spi-B bound to the CD40 promoter consistent with our previous reports (Figure 3A). Seventeen different primer sets for the BDCA-2 promoter region were

Figure 2: Activation of pDCs downregulates Spi-B levels. (A) Sorted human pDCs were compared for the expression levels of Spi-B protein by Western blot analysis. pDCs were stimulated for 5 hrs or 24 hrs with either CpG-A or CpG-B and compared to freshly isolated pDC. (B) Sorted human pDCs were cultured in the presence of CpG-A or CpG-B for 0 hr, 2 hrs, 4 hrs, 6 hrs and 18 hrs. Spi-B mRNA levels after activation were compared to Spi-B mRNA levels of freshly isolated pDC by qPCR. mRNA levels were normalized to GAPDH, β-actin and HPRT expression.
designed for every 500bp up to 9kb upstream of the transcriptional start site of BDCA-2. We analyzed the DNA fragments that were bound to anti-ER or control IgG by qPCR, and detected Spi-B~ER binding to three of the regions investigated in the promoter sequence of BDCA-2, including PR3, PR14 and PR15, but not in the other regions (Figure 3B). This result suggests that BDCA-2 is a direct target gene of the transcription factor Spi-B.

**Spi-B regulates expression of BDCA-2 mRNA in pDCs**

Since we observed that transcription factor Spi-B can directly bind to the BDCA-2 promoter area we were interested to validate the ChIP results by overexpressing Spi-B levels in CAL-1 cells and analysis of BDCA-2 mRNA levels. To test this we sorted Spi-B~ER or EV GFP-transduced CAL-1 cells and cultured them for four hours in the presence of 4HT or DMSO followed by RNA isolation. mRNA expression levels were measured by qPCR. As expected, mRNA levels of Spi-B and the validated Spi-B target genes CD40 and Bcl2-A1 were increased in Spi-B~ER transduced CAL-1 cells as compared to control CAL-1 cells (Figure 4A). Furthermore, Spi-B overexpression in CAL-1 cells resulted in a 4-fold increase in BDCA-2 mRNA levels in the presence of 4HT after four hours (Figure 4A). When Spi-B~ER sorted CAL-1 cells were cultured over a period of three days we still observed increased Spi-B and CD40 mRNA expression levels as compared to control

![A] human CD40 gene

![B] human BDCA-2 gene

**Figure 3: BDCA-2 is a direct target of Spi-B.** (A) Binding of Spi-B to the CD40 promoter region was assessed by ChIP of the Spi-B~ER protein in transduced CAL-1 cells using an anti-ER antibody or irrelevant IgG control antibody incubated with or without 0.5µM 4HT. The DNA that was pulled down was analyzed for CD40 promoter binding as a positive control (B) Binding of Spi-B to the BDCA-2 promoter region was analyzed by BDCA-2 promoter primers designed for every 500 bp of the ~9kb promoter region upstream BDCA-2. Six promoter regions are depicted, three regions show specific binding of Spi-B~ER.
CAL-1 cells (Figure 4B). However, while BDCA-2 mRNA levels initially increased in Spi-B-ER CAL-1 cells in the presence of 4HT after 24 hrs, thereafter the levels decreased although these remained higher than Spi-B-ER transduced CAL-1 cells in the absence of 4HT (Figure 4B). The kinetics for Spi-B mRNA levels were similar to that observed for BDCA-2 mRNA levels,

Figure 4: Ectopic expression of Spi-B in CAL-1 cells induces BDCA-2 mRNA expression. (A) Relative Spi-B, CD40, Bcl2-A1 and BDCA-2 mRNA levels in CAL-1 cells transduced with either Spi-B-ER or EV and cultured for 4 hours in the presence of 4HT or DMSO. Analysis of mRNA levels was done by qPCR. CD40 and Bcl2-A1 mRNAs were analyzed as positive controls. Spi-B, CD40, Bcl2-A1 and BDCA-2 mRNA levels were normalized to GAPDH, β-actin and HPRT. Expression of mRNAs in EV transduced CAL-1 cells in the presence of 4HT was set to 1. Spi-B, CD40, Bcl2-A1 data are shown in one representative experiment out of two as mean values ± SD of relative expression measured in triplicate. BDCA-2 expression is shown as mean value ± SD of relative expression of three experiments measured in triplicate. (**P < .01) (B) Relative Spi-B, BDCA-2 and CD40 mRNA levels were determined by qPCR in CAL-1 cells transduced with either Spi-B-ER (square symbols) or EV (round symbols) and cultured in the presence of 4HT up to 72 hours. Spi-B, BDCA-2 and CD40 mRNA levels were normalized to GAPDH, β-actin and HPRT. Expression of mRNA in EV transduced CAL-1 cells and addition of 4HT at time point 0 hour was set to 1. Shown are mean values measured in triplicate of relative mRNA expression of one representative experiment out of two. (C) Relative BDCA-2 mRNA levels after transduction of CAL-1 cells with either Spi-B shRNA or control Renilla shRNA and cultured in the presence of doxycycline (dox) for 24 hours. BDCA-2 mRNA levels were normalized to GAPDH, β-actin and HPRT. Shown are mean values ± SD of relative expression of mRNA in three experiments measured in triplicate. (**P < .05)
although less pronounced. To further strengthen our observations that Spi-B and BDCA-2 have a similar expression pattern, we downregulated Spi-B using a lentiviral construct in which Spi-B shRNA expression was regulated with addition of doxycycline to the cells. Indeed, CAL-1 cells transduced with Spi-B shRNAs expressed lower levels of BDCA-2 mRNA as compared to control (Renilla) shRNA transduced CAL-1 cells and DMSO controls (Figure 4C). Collectively, these results confirm that Spi-B controls the expression of BDCA-2.

### Spi-B regulated BDCA-2 protein expression in pDCs

To substantiate our findings we addressed whether BDCA-2 protein levels are consistent with the mRNA data and we performed flow cytometric analysis on Spi-B~ER transduced CAL-1 cells. We observed that BDCA-2 protein levels were increased on CAL-1 cells after ectopic expression of Spi-B~ER and culture in 4HT, but not in the absence of 4HT or on control transduced CAL-1 cells (Figure 5). Only a small shift in mean fluorescent intensity (MFI) of BDCA-2 was observed, but this might be due to the fact that CAL-1 cells constitutively express BDCA-2 already at high levels. Upregulation of CD40 was more pronounced in Spi-B~ER CAL-1 cells cultured in 4HT (Figure 5A). Taken together, we show that Spi-B is involved in the regulation of BDCA-2 expression. Moreover, these results suggest that reduced BDCA-2 expression levels on pDC after TLR7/9 activation may directly result from downregulating Spi-B levels.

### DISCUSSION

Here we report that expression of the C-type lectin BDCA-2 is controlled by the Ets-factor Spi-B in human pDCs. While Spi-B overexpression increased BDCA-2 expression, Spi-B knockdown resulted in reduced BDCA-2 expression. This, together with the results of a ChIP assay demonstrating that Spi-B binds the BDCA-2 promoter at several locations, provides evidence that BDCA-2 is a direct target of Spi-B. Collectively, our data reinforce that Spi-B has a key role in pDCs, not only to regulate the development and survival of pDCs, but also to control effector functions of pDCs.

Spi-B is constitutively expressed in pDCs (this study and others) and eventually downregulated when either TLR7 or TLR9 is ligated by agonists. We observed an increase in Spi-B mRNA expression shortly after TLR ligation, which was most pronounced after activation with CpG-B for 2 hrs. Prolonged TLR activation, however, reduced Spi-B mRNA expression which is consistent with the reduced expression of Spi-B protein observed after 24 hrs. How Spi-B expression is regulated and is affected by the TLR signaling pathway has not yet been resolved. In the mouse, there is evidence that the lymphoid-specific transcriptional coactivator POU class 2 associating factor 1 (POU2AF1, also known as OBF-1, Bob.1, OCA-B) controls Spi-B expression, at least in B cells. While this has not been confirmed in human B cells, it also remains elusive whether POU2AF1 is expressed in pDCs. Downregulation of Spi-B expression may also be regulated by microRNAs, which act by repressing gene expression through targeting of the 3’-untranslated region (UTR) of mRNAs resulting in either mRNA degradation or translation inhibition, or a combination of both. Recently, we reported that TLR signaling induced miR-146a. We
investigated whether the transcript of Spi-B may be targeted by miR-146a. However, despite the fact that the 3'-UTR of Spi-B mRNA is predicted to contain miR-146a binding sites, we were unable to observe a consistent reduction in Spi-B protein levels in miR-146a transduced CAL-1 cells (unpublished observations). Alternatively, post-translational modifications of Spi-B protein may alter its stability. It was shown that casein kinase II (CKII) phosphorylates Spi-B on four serine residues, which decreases both the stability and the transcriptional activity of the protein. This, together with the fact that expression of CKII is regulated by NF-κB, which is activated upon TLR signaling, may contribute to decreased Spi-B protein levels. However, the exact regulatory mechanism of Spi-B expression in pDCs is still unclear and needs to be further investigated.

We identified BDCA-2 as a direct target of Spi-B. Hence, it is reasonable to assume that TLR-induced downregulation of Spi-B contributes to decreased BDCA-2 expression levels. Given that BDCA-2 ligation contributes to the downregulation of the TLR-induced IFN-α/β response, we assume that the downregulation of Spi-B abrogates the ability of pDCs to control type I IFN responses after encountering a virus. Consistent with this notion, it is evident that viruses gain by high expression levels of Spi-B and BDCA-2 in pDCs, since this will prevent an optimal

Figure 5: Ectopic expression of Spi-B upregulates BDCA-2 protein. (A) Flow cytometric analysis of BDCA-2 expression on Spi-B-ER and EV transduced CAL-1 cells. Cells were cultured overnight in the presence of 4HT or DMSO and analyzed for the expression of GFP, BDCA-2 and CD40. Depicted are Spi-B-ER transduced cells in the presence of 4HT (black line) and DMSO (dashed line) in EV transduced CAL-1 cells in the presence of 4HT (grey filled histogram) and DMSO (dark grey filled histogram). RCN, relative cell number. Isotype control is depicted in light grey in the histogram. (B) BDCA-2 protein expression was measured on transduced CAL-1 cells and shown as the mean fluorescence intensity ± SD of two experiments.
anti-viral response through type I IFN production. Additional benefit from Spi-B as a host cell factor is gained by certain viruses, such as John Cunningham (JC)-virus, bovine leukemia virus and Epstein-barr virus, as Spi-B can bind to the viral promoter/enhancer thereby inducing the activation of early viral gene expression of these viruses.46-48 Conversely, aberrant and constitutive production of type I IFN by pDCs may result from decreased expression of BDCA-2 resulting in the lack of inhibition of TLR-induced responses. This may be the case in patients suffering from the autoimmune disease SLE, who have high type I IFN serum concentrations due to constitutive activation of pDCs by autoantibody-nucleic acid complexes.15,16,49 It is notable that BDCA-2 levels on pDCs from SLE patients are decreased when compared to healthy individuals.40 Considering our results, we speculate that expression of Spi-B is reduced in SLE-pDCs compared to healthy pDCs as well, but this requires further investigation. If true it will be of interest to examine how Spi-B expression can be increased in SLE-pDCs thereby increasing BDCA-2 expression and hence dampen the ongoing type I IFN production.

In conclusion, we show that after TLR7/9 ligation the levels of both Spi-B and BDCA-2 are downregulated. As Spi-B transactivates the expression of the BDCA-2 gene this adds Spi-B as a novel player in the network to regulate type I IFN production in pDCs.

REFERENCE LIST

five

Spi-B directly regulate SBDca-2 expression


27 Cao W. BDCA2/Fc epsilon RI gamma complex signals through a novel BCR-like pathway in human plasmacytoid dendritic cells. 2007


Supplementary table 1: Primer sequences

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