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

Jachimowski, L.C.M.

Citation for published version (APA):
THE PLASMACYTOID DENDRITIC CELL SPECIFIC C-TYPE LECTIN BDCA-2 BINDS A NATURAL LIGAND ON HUMAN THYMOCYTES

Loes C.M. Jachimowski¹, Kim I.M. Brandwijk¹, Roos-Marijn Berbers¹, Christel H. Uittenbogaart², Bianca Blom¹

¹Department of Cell Biology and Histology, Academic Medical Center, University of Amsterdam, Amsterdam, The Netherlands
²Departments of Pediatrics and Microbiology, Immunology & Molecular Genetics, David E. Geffen School of Medicine, University of California Los Angeles, Los Angeles, California, USA

Manuscript in preparation
ABSTRACT

Plasmacytoid dendritic cells (pDCs), which are known for their ability to produce large amounts of type I interferons (IFN) upon TLR7 and TLR9 activation, are found in blood and lymphoid tissues, including thymus. The amplitude of the TLR-induced IFN-α response can be regulated by cell surface receptors, such as blood-derived cellular antigen 2 (BDCA-2), which dampens TLR-induced signaling. Thymic pDCs mainly reside at the cortico-medullary (CM) junction and in the medulla where they constitutively produce IFN, but only low amounts. We hypothesized that in the thymus BDCA-2 on pDCs may be engaged by an as yet unidentified ligand to temper IFN production in order to establish an anti-inflammatory state required for optimal T cell development. Surprisingly, our combined results using a cluster-assay and flow cytometric analysis with recombinant BDCA-2 protein demonstrate that pDCs bind to a subset of thymocytes resembling T cells prior to positive selection, since these cells largely lack expression of CD27 and CD69, but express CD4, CD8, CD3 and CD1a. However, we found that pDCs can also bind to thymocytes that express an early medullary phenotype (CD27+CD1a-). Taken together, these results suggest that interaction between pDCs localized at the corticomedullary junction and thymocytes expressing a BDCA-2 ligand may control IFN production in the thymus. Further identification of the BDCA-2 ligand is not only of interest to increase our understanding on the role of pDCs in the thymus, but also to design therapeutic strategies to treat diseases in which excessive IFN concentrations cause pathology, such as lupus or psoriasis.
INTRODUCTION

Plasmacytoid dendritic cells (pDCs) are able to sense single-stranded RNA or double stranded DNA derived from viral or bacterial origin by Toll-like receptor 7 (TLR7) and TLR9, respectively, which will induce the production of massive amounts of type I Interferons (IFN).\textsuperscript{1,2} Type I IFNs are immuno-modulatory cytokines that alert many cells of the immune system\textsuperscript{3-8} thereby providing fast and early defense against pathogens and initiation of innate and adaptive immune response. pDCs produce 100-1000 fold more IFN-α in response to inactivated herpes simplex virus compared to other immune cells.\textsuperscript{19} In addition, pDCs are activated upon sensing endogenous nucleic acids like self-DNA and self-RNA that can be released during necrosis and apoptotic cell death.\textsuperscript{30-32} Adversely, the ability of pDCs to respond to self-RNA and self-DNA contributes to the pathogenesis of autoimmune diseases such as systemic lupus erythematosus (SLE), psoriasis and Sjögren’s syndrome (SS).\textsuperscript{33-38} In patients with SLE, pDCs are constantly activated by complexes of self-DNA and auto-antibodies.\textsuperscript{39,40}

Due to the ongoing production of type I IFN, activation and maturation of myeloid DCs (mDCs) causes stimulation of auto-reactive T cells leading to increased pathology.\textsuperscript{20}

In addition to blood and secondary lymphoid tissues, pDCs are also present in the thymus, where they reside in the medulla and at the cortico-medullary (CM) junction.\textsuperscript{21,22} The medulla is the area dedicated for the process of negative selection of T cells (i.e. deletion of potentially autoreactive T cells) and subsequent differentiation into functionally mature CD4\textsuperscript{+} and CD8\textsuperscript{+} T cells.\textsuperscript{23} In contrast, rearrangements of T cell receptor (TCR) genes and the process of positive selection (i.e. recognition of self-MHC/peptide at intermediate affinity) take part in the thymic cortex.\textsuperscript{24} The role for pDCs in the thymus is largely unresolved, although they may be involved in the development of Tregs in the thymus.\textsuperscript{25,26} Previously, we showed that human pDCs in the thymus, but not in other lymphoid organs, constitutively produced type I IFN.\textsuperscript{27} The T cells that harbor a type I IFN signature are mainly mature T cells present in the thymic medulla.\textsuperscript{27} In the mouse, type I IFN suppressed murine T and B cell development in vivo.\textsuperscript{28} In line with this, IFN-α also suppressed the development of human hematopoietic progenitor cells into T cells in vitro by inhibition of early and late steps in T cell development (29 and manuscript in preparation). Induction of constitutive expression of type I IFN in thymic pDCs could well be mediated by self-DNA/RNA or mitochondrial DNA\textsuperscript{30} in complex with the antimicrobial peptide LL-37, which is constitutively expressed mainly in the thymic medulla.\textsuperscript{11,12,27} As LL-37/nucleic acid complexes only induced low levels of type I IFN, but not TNF-α in thymic pDCs,\textsuperscript{27} it is likely to assume that activation of thymic pDCs is tightly controlled to avoid a pro-inflammatory state in the human thymus. Hence, while type I IFNs play an antiviral and immune-regulatory role in the periphery, at low concentrations they may have a regulatory role in the thymus to control thymopoiesis.

It remains elusive how thymic pDCs are restricted to produce only low amounts of type I IFN and no TNF-α, as to possibly maintain an anti-inflammatory state in the thymus. It is known that pDCs express several receptors that can regulate the amplitude of the TLR induced type I IFN response, including blood dendritic cell antigen-2 (BDCA-2) and immunoglobulin-like transcript 7 (ILT-7).\textsuperscript{30,32} BDCA-2 (CD303, CLEC4C) is a type II transmembrane glycoprotein and a member of the C-type lectin (CLR) superfamily, which is selectively expressed on human pDCs.\textsuperscript{33} CLR are
pattern recognition receptors (PRRs) that are able to recognize glycan structures on host cells and pathogens. BDCA-2 was shown to bind asialo-galactosyl-oligosaccharides, although the natural ligand for BDCA-2 has yet to be identified. Crosslinking of BDCA-2 by antibodies, however, has been shown to interfere with TLR9-induced activation of pDCs, thereby inhibiting secretion of IFN type I, TNF-α and IL-6. Another known function of BDCA-2 is the inhibition of soluble TNF-related apoptosis inducing ligand (TRAIL) secretion. Furthermore, BDCA-2 crosslinking led to receptor internalization, antigen uptake, Ca\(^{2+}\)-influx and signaling via a FcεRlγ-dependent pathway. BDCA-2 antibody-derived-peptides were presented via major histocompatibility complex (MHC) class II to CD4\(^+\) T cells, showing that BDCA-2 may deliver its potential ligand into an antigen-processing compartment in pDC. In addition, BDCA-2 can be bound by glycoproteins of viruses, including gp120 of human immunodeficiency virus (HIV)-1, E2 protein of Hepatitis C virus (HCV) and Hepatitis B surface antigen (HBSAg) of Hepatitis B virus (HBV). Thus, certain viruses may abuse BDCA-2 to dampen type I IFN production and mitigate the antiviral response.

To increase our understanding of the role of pDCs in the human thymus and how these cells may be restrained to produce excessive amounts of inflammatory cytokines we focused on BDCA-2. We hypothesized that a natural ligand of BDCA-2 may be expressed in the human thymus to provoke BDCA-2 signaling resulting in reduced inflammatory cytokine production. Results obtained in cluster assays revealed that pDCs may interact with thymocytes. Moreover, we observed that recombinant BDCA-2 protein bound to different subsets of thymocytes. One subset phenotypically resembled immature T cells prior to positive selection residing in the cortex based on the observation that the majority of thymocytes binding BDCA-2 lacked expression of CD69 and CD27, but expressed CD4, CD8 and CD1a. Another subset was enriched for T cells that have been positively selected and reside in the medulla, as they expressed CD27, CD69, CD4 and CD8. Expression of CD1a on these BDCA-2 binding medullary T cells is in line with the notion that they are not functionally mature T cells. Our results suggest that interaction of pDCs with thymocytes expressing a ligand for BDCA-2 may potentially occur at the CM junction or in the medulla. These interactions may result in impaired type I IFN production by pDCs possibly to allow differentiation of T cells in the medulla. Collectively, these data not only further our knowledge on the role of pDCs in the thymus, but in addition is a first step towards identification of a natural BDCA-2 ligand, which may have therapeutic value to treat autoimmune diseases.

**MATERIAL & METHODS**

**Human primary cells and cell lines**

Human fetal thymus tissues were obtained from elective abortions (Bloemenhoven clinic, Heemstede). Gestational age was determined by ultrasonic measurements and ranged from 14 to 20 weeks. Postnatal thymus tissue (PNT) was obtained from surgical specimens removed from children up to three years of age undergoing open heart surgery (Leids Universitair Medisch Centrum, Leiden, The Netherlands). PNT tissue was mechanically disrupted and pressed through a stainless steel mesh. To obtain leukocytes, the single cell suspension of thymocytes were put on a Ficoll-Hypaque density gradient (Lymphoprep; Nycomed Pharma,
Oslo, Norway). Peripheral blood of healthy volunteers was used upon donor consent (Sanquin Bloodbank, Amsterdam, The Netherlands). Monocytes and peripheral blood lymphocytes (PBLs) were isolated from PBMCs by a Percoll (Amersham Biosciences) gradient step. 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 Gen2.2 was cultured as described before. 42

Reagents
The following reagents were used: 100µg/ml Mannan (Sigma Aldrich), 10mM Ethylenediaminetetraacetic acid (EDTA) (Invitrogen), 10 mM EGTA (Sigma), TSA buffer (TSA buffer contains TSM buffer (Tris buffer (20mM Tris-HCL, pH 7, 150 mM NaCl, 1mM CaCl₂, 2mM MgCl₂) + 1% bovine serum albumin).

Flow cytometry
Recombinant BDCA-2~Fc chimera (R&D systems) was added to the thymocyte suspension in TSA buffer. The Fc tail of recombinant BDCA-2~Fc chimera was detected by addition of anti-human IgG Goat F (ab) PE (Southern Biotech) or mouse-anti-human IgG1 alexa647 (Life technologies). Cell suspensions were stained with the following monoclonal antibodies: CD1a, CD4, CD8, CD27, CD69 (Biolegend) or isotype controls (BD Biosciences) coupled to either fluorescein isothiocyanate (FITC), phycoerythrin (PE), PE-cyanine (PE-Cy7) allophycocyanin (APC) or APC-Cy7. For viability staining we used 7-amino-actinomycin (7AAD) solution (ebioscience) or DAPI. Samples were analyzed on a LSRII (BD) and analyzed using FlowJo software version 7 (Treestar).

Recombinant BDCA-2 binding assay
Streptavidin-coupled beads (TransFluoSpheres, Carboxylate-modified microspheres 488/645, Invitrogen) were coated with recombinant recBDCA-2~Fc chimera (R&D systems) via Goat-anti-HuFc-bio. Beads with and without recBDCA-2~Fc were incubated for 45 minutes with 50,000 monocytes, peripheral blood lymphocytes (PBL) or thymocytes. Beads were pre-incubated with and without EDTA or mannan for 30 minutes at room temperature in the dark prior to addition of the cells. Cells were added and incubated for 45 minutes at 37 degrees. All steps in the protocol were performed in TSA buffer, to provide calcium in the environment. Samples were measured using the LSRII (BD) measuring beads in PERCP channel.

Cluster assay
CFSE (Invitrogen) labeled Gen2.2 cells and CellTrace Violet dye (Invitrogen) labeled fetal or postnatal thymocytes were mixed in a 1:1 ratio. Cells were co-incubated for 90 minutes in TSA buffer in the presence or absence of EDTA before FACS analysis. The percentage of clustering cells (double positive events) was depicted. The percentage of clusters (n) was calculated relative to the number of single positive Gen2.2 (o) and single positive thymocytes (p) as follows: (2*n)/(2*n+o+p)*100%.
Statistical analysis
Data were subjected to two-tailed paired Student’s t-test analysis using Graphpad Prism 5 for Windows (Graphpad software, San Diego, USA). Data are considered significant when at least $P<0.05$.

RESULTS
Recombinant BDCA-2 binds to subsets of thymocytes and PBLs, but not to monocytes
In order to study possible natural binding partners for BDCA-2 in the thymus we made use of soluble recombinant BDCA-2 (recBDCA-2) coupled to Streptavidin-beads. This is a well-established binding assay in which the percentage of cells binding fluorescent beads indicates the presence of cellular CLR-specific ligands. Thymocytes, but also peripheral blood lymphocytes (PBLs) and monocytes were incubated with soluble recBDCA-2-beads and binding was measured by flow cytometry (Figure 1). RecBDCA-2-beads specifically bound a subset of thymocytes (19.7%) when compared to control beads (2.4%) (Figure 1A-B). Binding of recBDCA-2 to monocytes was also observed, but this was considered unspecific, since control beads also bound to monocytes (Figure 1A-B). PBLs also specifically bound recBDCA-2-beads (percentage ± SD) (8.0% ± 2.6) as compared to control beads (3.2% ± 0.6) (Figure 1A-B), but the percentage of cells that bound was lower compared to thymocytes. RecBDCA-2 binding to thymocytes could not be blocked by addition of the carbohydrate mannan (Figure 1A-B), suggesting that the ligand for BDCA-2 does not contain a mannan moiety. Addition of EDTA, which chelates $Mg^{2+}$ and $Ca^{2+}$ ions and is necessary for optimal CLR binding, did reduce binding of recBDCA-2-beads both to thymocytes and to PBLs (Figure 1A-B). In a flow cytometric recBDCA-2 binding assay, addition of EGTA which only chelates $Ca^{2+}$ ions, also showed a clear decrease in recBDCA-2 binding to thymocytes, although addition of EDTA almost completely reduced binding of recBDCA-2 to thymocytes. These results suggest that BDCA-2 has a natural ligand in the human thymus, and may also be present on a subset of PBLs.

Clustering of pDCs and thymocytes is mediated by a C-type lectin
As we were mostly interested in the role of pDCs in the thymus we further focused on expression of a BDCA-2 ligand on thymocytes. Therefore, we investigated the interaction between pDCs and thymocytes using a cluster assay. To accomplish this we made first use of the pDC model cell line Gen2.2 that expresses BDCA-2 (data not shown and 42). Thymocytes were isolated from human PNT or FT and co-cultured with Gen2.2 cells in a 1:1 ratio. To distinguish both cell types by flow cytometry, Gen2.2 cells and thymocytes were labeled with a different cell membrane dye and then co-incubated in the presence of TSA buffer with or without EDTA. A time course experiment revealed that optimal clustering was achieved after 90 min of co-incubation (data not shown). The level of clustering was determined by flow cytometry after 90 min and is shown as the percentage of double positive events (Figure 2A). We observed that Gen2.2 cells clustered with thymocytes (Figure 2A). No difference was observed between PNT or FT (data...
Figure 1. Soluble recBDCA-2 binds to a subset of human thymocytes. (A) Thymocytes, monocytes and PBLs were incubated for 45 minutes with recBDCA-2-coated beads or control beads in the absence (TSA buffer only) or presence of 100 µg/ml mannan or 10 mM EDTA. Binding of beads was analyzed by flow cytometry. Numbers indicate percentages of bead binding cells. Shown is one representative experiment out of two. (B) Percentages of bead-binding thymocytes, monocytes and PBLs as analyzed in panel A. Shown are mean values ± SD of monocytes (2 donors) and PBLs (2 donors) and the values of thymocytes (1 donor). (C) Flow cytometric analysis of fetal thymocytes after staining with an isotype control (grey histogram) or soluble recBDCA-2 and the secondary antibody against FcγRII in the absence (black line) or presence of 10 mM EDTA (dashed line) or 10 mM EGTA (dotted line).
not shown). The percentage of double positive events was reduced in the presence of EDTA, suggesting that Gen2.2-thymocyte clustering was Ca\(^{2+}\)-dependent. In Figure 2B the results are shown for multiple experiments indicating that reduced clustering between Gen2.2 cells and thymocytes in the presence of EDTA is statistically significant. We conclude that Gen2.2 cells and thymocytes have the potential to form clusters likely mediated by a CLR.

**Involvement of BDCA-2 in cluster formation between pDCs and thymocytes**

The next question that we aimed to address is whether clustering between Gen2.2 cells and thymocytes is mediated by interaction of BDCA-2 and its ligand. Therefore, we tested a set of different anti-BDCA-2 antibodies that potentially have a neutralizing capacity. First, we wanted to confirm that these antibodies specifically stained the pDC cell line Gen2.2 using flow cytometric analysis (Figure 3A). We observed that all antibodies bound to Gen2.2 cells only, but not to an irrelevant cell line lacking BDCA-2 expression (data not shown). Then, we performed cluster-assays using Gen2.2 cells and thymocytes in the presence of these antibodies to address whether cluster formation could be inhibited. Unfortunately, none of the anti-BDCA-2 antibodies were capable of preventing clustering of pDCs and thymocytes (Figure 3B). While

![Figure 2: Clustering of pDCs and thymocytes is Ca\(^{2+}\)/Mg\(^{2+}\) dependent.](image)

(A) The pDC cell line Gen2.2 was labeled with CFSE and thymocytes were labeled with CellTrace violet. Clustering of pDC and thymocytes was determined by flow cytometric analysis after culturing in the presence or absence of EDTA for 90 minutes. Numbers represent percentages of cells in each quadrant. Shown is a representative experiment out of 7 experiments. (B) Clustering was performed as described in panel A. The percentages of CFSE-CellTrace violet pDC/thymocyte clusters in the control condition were set at 100%. Relative to this the percentages of clusters in the presence of EDTA were calculated. Shown are 7 independent experiments (** *P* < 0.01).
this may suggest that clustering is not mediated by BDCA-2 and its ligand, it may also indicate that the anti-BDCA-2 antibodies lack neutralizing capacity.

**BDCA-2 ligand is expressed on immature thymocytes**

The observation that BDCA-2 might have a natural ligand in the human thymus prompted us to identify what thymocyte subset binds to recBDCA-2. To study this, thymocytes from PNT and FT were incubated with recBDCA-2 in combination with selected monoclonal antibodies to electronically gate on thymocytes in different developmental stages. Using flow cytometry we detected that recBDCA-2 bound on average to 23% (range 13-36%) of the total thymocyte population (Figure 4A-B). The population of thymocytes that bound recBDCA-2 was enriched for the expression of CD1a, CD4 and CD8 (CD1a+ DP) (Figure 4C). The majority of these CD1a+DP thymocytes lacked expression of CD69 and CD27, although some enrichment was observed in the CD1a’CD27’ and
Figure 4: Human immature double-positive thymocytes express a ligand for BDCA-2. (A) Fetal thymocytes were incubated with soluble recBDCA-2 in TSA buffer and stained with a secondary antibody against Fc-IgG1. Gated are the recBDCA-2- thymocytes (-) and the recBDCA-2+ thymocytes (+). (B) Flow cytometric analysis of fetal thymocytes as in panel A showing five different donors. (C) Flow cytometric analysis of thymocytes incubated with soluble recBDCA-2 and co-stained for surface expression of CD27, CD1a, CD69, CD4 and CD8. Dotplots show the phenotypes of thymocytes that are gated as recBDCA-2- and recBDCA-2+ cells depicted in panel A. Numbers in each quadrant indicate percentages of cells.
SIX

BDCA-2 ligand expressed on thymocytes

CD1a+CD69+ subsets when compared to the recBDCA-2 nonbinding population (Figure 3C). Also, these cells expressed CD8β with low intensity TCRαβ expression (data not shown). Functionally mature single positive (SP) CD4 or SP CD8 T cells that lack CD1a were underrepresented in the cells that bind recBDCA-2. Taken together, this indicates that the ligand of BDCA-2 is preferentially expressed on a subset of DP thymocytes that has not been positively selected yet, but that also a subset of positively selected cells is enriched in the BDCA-2 binding population.

DISCUSSION

Here, we show that recBDCA-2 binds to subsets of human thymocytes and may be involved in clustering of thymic pDCs and thymocytes. We found that the elusive ligand for BDCA-2 may preferentially be expressed on a subset of immature CD1a+DP thymocytes that are at a stage prior to positive selection based on the observation that the BDCA-2 binding thymocytes largely lack CD27 and CD69. However, BDCA-2 also bound to a subset of CD27+CD69+ positively selected CD1a+DP T cells, but not to CD4 or CD8 SP T cells that have lost CD1a expression. Collectively, our data may add to our understanding on the nature of pDC-thymocyte interactions and may contribute to unravel the role of pDCs in the thymus.

Previously, it was reported that recombinant BDCA-2 bound to monocytes and that this binding could be blocked by addition of an anti-BDCA-2 antibody. In our search for a natural BDCA-2 ligand in the thymus we used monocytes as a positive control. Unexpectedly, however, we observed that monocytes bound BDCA-2 non-specifically as control beads also bound monocytes and the addition of EDTA did not block the binding of BDCA-2 beads to monocytes. Riboldi et al. used recombinant BDCA-2 tetramers conjugated to PE for flow cytometric analysis and showed that monocytes bound BDCA-2. In our studies we used BDCA-2-Fc, which was also used by Riboldi et al. in selected experiments, but we were unable to confirm their findings. It is unclear whether in their hands the BDCA-2-Fc binding to monocytes could be inhibited by addition of the anti-BDCA-2 antibody. In contrast to BDCA-2 binding to monocytes, we did observe that EDTA blocked BDCA-2 binding to thymocytes providing circumstantial evidence that this is a Ca²⁺-dependent process as expected from C-type lectins. When interpreting the results of the cluster assays it should be noted, however, that EDTA is not specific for BDCA-2 and that another C-type lectin, i.e. dendritic cell immunoreceptor (DCIR), is expressed on pDCs as well. As DCIR was identified as mannose/fucose-binding lectin, addition of mannan to the cluster assay using pDCs and thymocytes should reveal whether DCIR is involved.

BDCA-2-Fc bound to a subset of thymocytes that after phenotyping by flow cytometry were identified as CD1a+DP cells. Considering the fact that these thymocytes largely lack expression of CD27 and CD69, which are hallmarks of positively selected T cells in the medulla of the human thymus, it is likely that the thymocytes expressing the BDCA-2 ligand have not been positively selected and thus reside in the cortex. This would suggest that interaction of CD1a+DP thymocytes with pDCs may occur at the CM junction where pDCs reside. Different scenarios can be envisioned. First, BDCA-2 ligand expressing thymocytes are halted at the CM border by pDCs as these thymocytes may have possibly failed to undergo positive selection in the cortex.
and are not granted access into the medulla. Reducing the levels of type I IFN produced by pDCs may somehow be involved in this. Second, thymocytes expressing the BDCA-2 ligand are positively selected in the cortex, but have not yet upregulated CD27 and CD69 and are on their way to the medulla. If this is the case it is reasonable to assume that these T cells should be granted free passage to the medulla for further differentiation and upregulation of CD27 and CD69. As type I IFN inhibits T cell differentiation (29; manuscript in preparation) the site of entry into the medulla should be devoid of type I IFN, which can be achieved by ligation of BDCA-2 on pDCs. Another subset of thymocytes expressing the BDCA-2 ligand as well were CD1a+DP T cells that did express CD27 and CD69 and therefore are likely to reside in the medulla. In the mouse it was previously shown that peripheral pDCs home to the thymus in a CCR9-dependent manner where they contribute to negative selection of peripheral antigen-specific thymocytes.51 Potentially, pDCs that produce low levels of IFN-α27 as a result of BDCA-2 engagement by ligand expressed on thymocytes play a role during negative selection of T cells.

The identity of the of the natural ligand for BDCA-2 on the CD1a’DP thymocytes remains elusive. BDCA-2 is able to bind to asialo-galactosyl-oligosaccharides34, which are posttranslational modifications removing α-glycosidically linked sialic acid residues from carbohydrate groups of glycoproteins. Removal is done by glycosidases called neuraminidases or sialidases. In humans, at least four mammalian sialidase homologs have been described, including NEU1, NEU2, NEU3, NEU4.52 Expression profiling of NEU genes at different stages during T cell development in the thymus has not been reported, but this is of interest to further pinpoint the subsets of thymocytes expressing the BDCA-2 ligand and to gain inside in the nature of this ligand. It is known that differential sialylation of glycoproteins plays a role during T cell maturation. For example the ability of the CD8 molecule to interact with class I MHC is developmentally regulated as decreased sialylation of class I MHC increased CD8 binding.53 Similarly, it can be envisioned that the strength of pDC and T cell interaction is managed at the level of BDCA-2 by altering the glycosylation state of its ligand. This may hypothetically affect the process of T cell selection. Furthermore, identification of the BDCA-2 ligand may have prospective value to treat autoimmune diseases such as SLE or psoriasis. In SLE, pDCs accumulate in lesions, where they are a major source of elevated IFN-α/β levels in the serum54, induce the differentiation of auto-antibody producing plasma cells7;55 and activate auto-reactive T cells.20 In psoriasis, pDCs are activated via self-DNA that is released in the extracellular milieu and when complexed with antimicrobial peptide LL-37 can induce a strong type I IFN response.12 Given that BDCA-2 ligation by antibodies regulates the levels of TLR-induced IFN-α production29, we speculate that therapeutic advantage can be gained by treatment of SLE or psoriasis with recombinant BDCA-2 ligand to temper IFN-α production. Collectively, our results pave the way towards identification of a BDCA-2 ligand, which will not only increase our understanding on the role of pDCs in T cell development, but in addition open avenues to treat autoimmune diseases.
REFERENCE LIST


26 Hanabuchi S, Ito T, Park WR et al. Thymic stromal lymphopoietin-activated plasmacytoid dendritic cells induce the generation of FOXP3+ regulatory T...

27 Colantonio AD, Epeldegui M, Jesiak M et al. IFN-
alpha Is Constitutively Expressed in the Human
Thymus, but Not in Peripheral Lymphoid Organs.

28 Lin Q, Dong C, Cooper MD. Impairment of T and
B cell development by treatment with a type I

29 Schmidlin H, ontje W, root F et al. Stimulated
plasmacytoid dendritic cells impair human T-cell

30 Ries M, Schuster P, Thomann S et al. Identification
of novel oligonucleotides from mitochondrial DNA
that spontaneously induce plasmacytoid dendritic

31 Dzionek A, Sohma Y, Nagafune J et al. BDCA-2, a
novel plasmacytoid dendritic cell-specific type
II C-type lectin, mediates antigen capture and is a
potent inhibitor of interferon alpha/beta

32 Cao W, Bover L, Cho M et al. Regulation of TLR7/9
responses in plasmacytoid dendritic cells by
BST2 and ILT7 receptor interaction. J.Exp.Med.
2009;206:1603-1614.

33 Dzionek A, Fuchs A, Schmidt P et al. BDCA-2, BDCA-3,
and BDCA-4: three markers for distinct subsets of dendritic cells in human peripheral

34 Riboldi E, Daniele R, Parola C et al. Human C-type
lectin domain family 4, member C (CLEC4C/
BDCA-2/CD303) is a receptor for asialo-galactosyl-

35 Cao W, Zhang L, Rosen DB et al. BDCA2/Fc epsilon
Ri gamma complex signals through a novel BCR-
like pathway in human plasmacytoid dendritic

36 Riboldi E, Daniele R, Cassatella MA, Sozzani S,
Bosisio D. Engagement of BDCA-2 blocks TRAIL-
mediated cytotoxic activity of plasmacytoid

37 JaehnPS, Zaeanker KS, Schmitz J, Dzionek A. Functional
dichotomy of plasmacytoid dendritic cells: antigen-
specific activation of T cells versus production of type

38 Florentin J, Aouar B, Dental C et al. HCV
glycoprotein E2 is a novel BDCA-2 ligand and acts
as an inhibitor of IFN production by plasmacytoid

39 Martinelli E, Cicala C, Van RD et al. HIV-1 gp120
inhibits TLR9-mediated activation and IFN-α
secretion in plasmacytoid dendritic cells. Proc.

40 Xu Y, Hu Y, Shi B et al. HBSAg inhibits TLR9-
mediated activation and IFN-alpha production

Downregulation of CD1 marks acquisition of
functional maturation of human thymocytes and
defines a control point in late stages of human T

42 Chaperot L, Blum A, Manches O et al. Virus or TLR
agonists induce TRAIL-mediated cytotoxic activity
2006;176:248-255.

43 Geijtenbeek TB, van KY, van Vliet SJ et al. High
frequency of adhesion defects in B-lineage acute

44 Lepenies B, Lee J, Sonkaria S. Targeting C-type
lectin receptors with multivalent carbohydrate

45 van KY, Rabinoich GA, Protein-glycan interactions
in the control of innate and adaptive immune

46 Meyer-Wentrup F, Benitez-Ribas D, Tacken PJ et al.
Targeting DCIR on human plasmacytoid dendritic
cells results in antigen presentation and inhibits

47 Bloem K, Vuist IM, van der Plas AJ et al. Ligand binding and signaling of dendritic cell
immunoreceptor (DCIR) is modulated by the
glycosylation of the carbohydrate recognition

48 Bloem K, Vuist IM, van den Berk M et al. DCIR
interacts with ligands from both endogenous and

49 Vanhecke D, De Clercq G, Plum J, Vandekerkhove
B. Characterization of distinct stages during the
differentiation of human CD69+CD3+ thymocytes

50 Vanhecke D, Verhasselt B, De SM et al. Human
thymocytes become lineage committed at an
early postselection CD69+ stage, before the
1997;159:5973-5983.

51 Hadeiba H, Lahl K, Edalati A et al. Plasmacytoid
dendritic cells transport peripheral antigens
to the thymus to promote central tolerance.

52 Miyagi T, Yagamichi K. Mammalian sialidases:
physiological and pathological roles in cellular

53 Daniels MA, Devine L, Miller JD et al. CD8
binding to MHC class I molecules is influenced
