Venlafaxine inhibits the development and differentiation of dendritic cells through the regulation of p-glycoprotein

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A B S T R A C T

Dendritic cells (DC) are professional antigen-presenting cells that have the ability to detect infectious materials; antigens to T lymphocytes, and serve as a bridge between innate and adaptive immunity [1, 2]. DC have the ability to detect tumor cells, and sample and present antigens to T lymphocytes, while at the same time preventing immune responses against self-tissues [3, 4]. DC are derived from host bone marrow, developing first into immature DC and upon activation, progress to mature DC. Mature DC present antigen in conjunction with co-stimulatory molecules such as B7-families and major histocompatibility complex (MHC) class molecules, and produce a range of cytokines, leading to the initiation of a specific immune response [5]. A critical property of DC is their ability to respond to environmental stimuli, which determine their final maturation or differentiation status [3, 6]. For example, responding to tumor antigens, pathogens binding to Toll-like receptors [7], heat shock proteins, innate cytokines [8], and inflammation cytokines, can lead to DC maturation and subsequent T cell immune responses [9]. Moreover, DC have been recognized as powerful APC, playing a key role in antitumor host responses [10, 11], and inducing primary T-cell responses in vitro and in vivo, which has generated widespread interest in DC-based immunotherapy against several types of cancer [12, 13].

P-glycoprotein (P-gp) is a 170-kDa trans-membrane protein member of a highly conserved superfamily of ATP-binding cassette transport proteins, and is encoded by the mdr-1 gene [14]. In addition to its ability overexpression of P-gp contributes to chemotherapeutic drug resistance [18], and thus remains a critical barrier to successful chemotherapy in cancer patients treated with drugs such as verapamil (VRP), cyclosporine A (CsA) [19], and venlafaxine (VLX) [20]. VRP and CsA, a typical inhibitor of P-gp activity, particularly are used in numerous studies for P-gp function [21, 22]. Recently, P-gp has been reported to be expressed in

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monocyte-derived and interstitial DC during migration toward lymph nodes via afferent lymphatic vessels. In the human skin explants system, it was described that antibodies or drugs that antagonize P-gp, such as VRP, block this migration [16].

Little is known about the role of P-gp in DC maturation, differentiation, and during T cell interaction and the subsequent immune response. The functions of P-gp in immune responses are still unclear. Therefore, in this study, we sought to examine the effects of P-gp down-regulation on the development, maturation, and differentiation of DC and how this affects T cell proliferation and polarization. Taken together, these data identify a novel physiological function for P-gp in DC.

2. Materials and methods

2.1. Animals

Male 8–12-week-old C57BL/6 (H-2Kb and I-Ab) and BALB/c (H-2Kd and I-Ak) mice were purchased from the Korean Institute of Chemistry Technology (Daejeon, Korea). They were housed in a specific pathogen-free environment within our animal facility for at least 1 week before use. All mouse work was approved by the IACUC and was performed in our IACUC approved facility.

2.2. Reagents and Abs

Recombinant mouse (rm)GM-CSF and rmIL-4 were purchased from R&D Systems (Minneapolis, MN, USA). Dextran-FITC (molecular mass, 40,000), and LPS (from *Escherichia coli* 055:B5) were obtained from Sigma-Aldrich. An endotoxin filter (END-X) and an endotoxin removal resin (END-X B15) were acquired from Associates of Cape Cod. Cytokine ELISA kits for murine IL-12 p70, IL-4, IL-6, IL-1α, and IFN-γ were purchased from BD Pharmingen (Rockville, MD, USA). FITC- or PE-conjugated mAbs used to detect the expression of CD11c (HL3), CD80 (16-10A1), CD86 (GL1), IAα β-chain (AF-120.1), H2Kb (AF6-88.5), and CD4 (L3T4), or the intracellular expression of IL-12 p40/p70 (C15.6), and IL-10 (JESS-16E3) by flow cytometry was used to determine the level of Ag expression on CD11c+ DC.

2.3. Generation and culture of DC

DC were generated from murine bone marrow (BM) cells. Briefly, BM was flushed from the tibiae and femurs of C57BL/6 and depleted of red cells with ammonium chloride. The cells were plated in six-well culture plates (10⁶ cells/ml; 3 ml/well) in OptiMEM (Invitrogen Life Technologies, Carlsbad, CA, USA) supplemented with 10% heat-inactivated FBS, 2 mM l-glutamine, 100 U/ml penicillin, 100 µg/ml streptomycin, 5×10⁻⁵ M 2-ME, 10 mM HEPES (pH 7.4), 20 ng/ml rmGM-CSF and rmIL-4 at 37 °C, 5% CO₂. On day 3 of the culture, floating cells were gently removed, and fresh medium was added. On day 6, 80% or more of the nonadherent cells expressed CD11c. In certain experiments, to obtain highly purified populations for subsequent analyses, the DC were labeled with bead-conjugated anti-CD11c mAb (Milenyi Biotec, Gladbach, Germany) followed by positive selection through paramagnetic columns (LS columns; Miltenyi Biotec) according to the manufacturer’s instructions. The purity of the selected cell fraction was >95%.

2.4. Stimulation of DC by P-gp modulators

P-gp modulator (VRP, CsA, and VLX) was dissolved in culture media and was added to cultures of isolated DC in six-well plates (10⁶ cells/ml; 2 ml/well). Media alone was used as negative control. For the analysis of apoptosis, DC were stimulated with LPS or left without any stimuli, and analyzed over time by staining of phosphatidylserine translocation with FITC-annexin V in combination with propidium iodine kit (BD Pharmingen) according to the manufacturer’s instructions.

2.5. Flow cytometric analysis

On day 6, BM-DC were harvested, washed with phosphate buffered saline (PBS) and resuspended in fluorescence activated cell sorter (FACS) washing buffer (2% fetal bovine serum and 0.1% sodium azide in PBS). The cells were first blocked with 10% (v/v) normal goat serum for 15 min at 4 °C and stained with phycoerythrin (PE)-conjugated anti-I-Ab (FACS) washing buffer (2% fetal bovine serum and 0.1% sodium azide in PBS). The cells were first blocked with 10% (v/v) normal goat serum for 15 min at 4 °C and stained with phycoerythrin (PE)-conjugated anti-I-Ab (FACS) washing buffer (2% fetal bovine serum and 0.1% sodium azide in PBS). Table 1

<table>
<thead>
<tr>
<th>Surface Ag</th>
<th>Medium</th>
<th>siRNA</th>
<th>LPS</th>
<th>siRNA + LPS</th>
</tr>
</thead>
<tbody>
<tr>
<td>CD80</td>
<td>74 ± 2 (256 ± 21)</td>
<td>71 ± 1 (241 ± 15)*</td>
<td>86 ± 3 (950 ± 36) #</td>
<td>74 ± 2 (70 ± 28) **</td>
</tr>
<tr>
<td>CD86</td>
<td>75 ± 14 (425 ± 22)</td>
<td>78 ± 2 (421 ± 47)</td>
<td>80 ± 1 (1205 ± 47) #</td>
<td>76 ± 3 (723 ± 86) **</td>
</tr>
<tr>
<td>MHC I</td>
<td>74 ± 2 (87 ± 5)</td>
<td>73 ± 1 (80 ± 1)</td>
<td>81 ± 3 (254 ± 23) #</td>
<td>75 ± 1 (196 ± 23)**</td>
</tr>
<tr>
<td>MHC II</td>
<td>80 ± 1 (230 ± 12)</td>
<td>79 ± 3 (211 ± 23)*</td>
<td>87 ± 2 (470 ± 12) #</td>
<td>85 ± 1 (341 ± 21) **</td>
</tr>
</tbody>
</table>

* ** The statistical significance between samples with and without siRNA of P-gp is indicated (###<0.001 vs unsimulated DC (median); *P<0.01 vs LPS-stimulated DC).

Table 2

<table>
<thead>
<tr>
<th>pg/ml/10⁶ cells</th>
<th>Medium</th>
<th>siRNA</th>
<th>LPS</th>
<th>siRNA + LPS</th>
</tr>
</thead>
<tbody>
<tr>
<td>IL-1α</td>
<td>21 ± 2</td>
<td>23 ± 1</td>
<td>382 ± 23##</td>
<td>240 ± 12**</td>
</tr>
<tr>
<td>IL-12</td>
<td>12 ± 0.5</td>
<td>15 ± 2</td>
<td>630 ± 47##</td>
<td>342 ± 42**</td>
</tr>
</tbody>
</table>

* ** The statistical significance between samples with and without siRNA of P-gp is indicated (###<0.001 vs unsimulated DC (median); *P<0.01 vs LPS-stimulated DC).

Medium siRNA LPS siRNA + LPS

21 ± 2 23 ± 1 382 ± 23## 240 ± 12**

12 ± 0.5 15 ± 2 630 ± 47## 342 ± 42**

### Table 1

siRNA of P-gp inhibits the expression of costimulatory molecules (CD80, CD86 and CD40), MHC class I, and II on LPS-stimulated CD11c+ DC.

<table>
<thead>
<tr>
<th>% of positive cell (MFI)</th>
<th>CD80</th>
<th>CD86</th>
<th>MHC I</th>
<th>MHC II</th>
</tr>
</thead>
<tbody>
<tr>
<td>Medium</td>
<td>74 ± 2 (256 ± 21)</td>
<td>75 ± 14 (425 ± 22)</td>
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</tr>
<tr>
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<td>71 ± 1 (241 ± 15)*</td>
<td>78 ± 2 (421 ± 47)</td>
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<td>79 ± 3 (211 ± 23)*</td>
</tr>
<tr>
<td>LPS</td>
<td>86 ± 3 (950 ± 36) #</td>
<td>80 ± 1 (1205 ± 47) #</td>
<td>81 ± 3 (254 ± 23) #</td>
<td>87 ± 2 (470 ± 12) #</td>
</tr>
<tr>
<td>siRNA + LPS</td>
<td>74 ± 2 (70 ± 28) **</td>
<td>76 ± 3 (723 ± 86) **</td>
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MHC class II), anti-CD80, and anti-CD86 with fluorescein isothiocyanate (FITC)-conjugated anti-CD11c (Pharmingen, San Diego, CA) for 30 min at 4 °C. The stained cells were analyzed using a FACSCalibur flow cytometer (BD Biosciences) (Table 1).

2.6. Cytokines assay

DC were first blocked with 10% (v/v) normal goat serum for 15 min at 4 °C and then stained with FITC-conjugated anti-CD11c (Pharmingen, San Diego, CA) for 30 min at 4 °C. The stained cells were analyzed using a FACSCalibur flow cytometer (BD Biosciences) (Table 1).

2.7. Mixed lymphocyte reaction

Responder T cells, which participate in allogeneic T-cell reactions, were isolated passing through mononuclear cells from spleen of BALB/c mice in a MACS column (Miltenyi Biotec). Staining with fluorescein isothiocyanate (FITC)-conjugated anti-CD4 and anti-CD8 antibodies (BD Pharmingen) revealed that they consisted mainly of CD4+ and CD8+ cells (>95%). The lymphocyte population was then washed twice in PBS and labeled with CFSE, as previously described [23]. The cells were resuspended in 5 μM CFSE in phosphate-buffered saline (PBS). After being shaken for 8 min at room temperature, the cells were washed once in pure fetal bovine serum (FBS) and twice in PBS with 10% FBS. DC (1 × 10^6) or DC exposed to VLX (25 and 50 μM/ml) or LPS (200 ng/ml) for 24 h were cocultured with 1 × 10^5 allogeneic CFSE-labeled T lymphocytes in 96-well plates (Nunc). A negative control (CD4+ and CD8+ lymphocytes alone) and a positive control (CD4+ and CD8+ lymphocytes in 5 μg of Concanavalin A) were included for each experiment. After 4 days, the cells were harvested and washed in PBS. CFSE dilution optically gated lymphocytes were assessed by flow cytometry.

![Fig. 1. Expression of mdr-1 and mdr-3 during DC development. (A) mdr-1 and mdr-3 gene expression levels during DC development for 2, 4, and 6 days. The data are representative of three experiments that gave similar results. (B) Quantitation of mdr mRNA levels by real-time RT-PCR. Samples were taken for RNA preparation, and quantitative real-time RT-PCR analyses were performed. A significant difference in expression between each group was detected. The histogram is from one out of three representative experiments. (C) For P-gp protein analysis, cell lysates were prepared and blotted with anti-P-gp and anti-β-actin Abs. (D) Confocal microscopy analysis of P-gp expression during DC development. The DC were stained for CD11c+ (green), P-gp (red), and DAPI (blue). The data represent the means (±SD) of four separate experiments. (**P < 0.01 vs control groups).](image)
2.8. Quantitative real-time PCR

mdr-1 and mdr-3 PCR primers used were as follows: forward 5′-ACATTCTGCTGACTTGCG-3′ and 5′-TATAGTTTTGGCTGGGCGTGG-3′, reverse 5′-AAAACACCGTCCTGAAGGCT-3′ and 5′-ATTTCTCCAGCACGCGGAC-3′, respectively. Quantitative amounts of each gene were standardized against the GAPDH housekeeping gene. Real-time PCR was performed using a BioRad MiniOpticon System (BioRad Laboratories Ltd, Missouri, USA.) with SYBR green fluorophore. Reactions were performed in a total volume of 20 μl-including 10 μl 2× SYBR Green PCR master Mix (Applied Biosystems, Carlsbad, CA, USA), 1 μl of each primer at 10 μM concentration and 1 μl of the previously reverse-transcribed cDNA template. The protocols used were as follows: denaturation (95 °C for 10 min), amplification was repeated 40 times (95 °C for 30 s, 55 °C for 30 s, 72 °C for 30 s and acquisition temperature for 15 s). For each sample, ddCt (crossing point) values were calculated as the Ct of the target gene minus Dt of the GAPDH gene. Gene expression was derived according to the equation 2−ddCT, and changes in gene expression were expressed as relative to basal.

2.9. siRNA studies

DC were transfected with 100 nM siRNA specific to P-gp (Santa Cruz, CA, USA) and a negative control siRNA according to the manufacturer’s protocol (Santa Cruz). After 24 h incubation, the DC were rinsed with PBS and used for further analysis as described above (Table 2).

2.10. Cytoplasmic extracts and Western blot

The cells (DC) were exposed to VRP, CsA, and VLX for 24 h of incubation at 37 °C, cells were washed twice with cold PBS and lased with modified RIPA buffer (1.0% NP-40, 1.0% sodium deoxycholate, 150 mM NaCl, 10 mM Tris–HCl [pH 7.5], 5.0 mM sodium pyrophosphate, 1.0 mM NaVO4, 5.0 mM NaF, 10 mM/ml leupeptin, and 0.1 mM phenylmethylsulfonyl fluoride) for 15 min at 4 °C. The protein content of cell lysates was determined using the Micro BCA assay kit (Pierce, Rockford, IL). Equivalent amounts of proteins were separated by 10% SDS-PAGE and analyzed by Western blotting using a P-glycoprotein (Abcam, Cambridge, MA, USA) and anti-β-actin (Santa Cruz) mAb for 3 h, as described by the manufacturer of the antibodies. Following the wash for three times with TBST, membranes were incubated with secondary HRP-conjugated anti-mouse IgG for 1 h. After washing, the blots were developed using the ECL system (GE healthcare life sciences, NJ, USA), by following manufacturer’s instructions.

2.11. Statistics

All results were expressed as the means±SD of the indicated number of experiments. Statistical significance was estimated using a Student’s t-test for unpaired observations, and the differences were compared with regard to statistical significance by one-way ANOVA, followed by Bonferroni’s post hoc test. The categorical data from the
fertility test were subjected to statistical analysis via Chi-square test. A P of <0.01 was considered significant.

3. Results

3.1. Up-regulation of mdr-1, mdr-3 and P-gp during DC development

Monocytes, T cells, NK-cells, and skin dendritic cells are known to express the mdr-1 and mdr-3 gene product, P-gp [18, 24]. In the initial series of experiments, we attempted to determine whether mdr gene expression influences DC development. Bone marrow-derived DC were cultured for 2, 4, and 6 days in OptiMEM supplemented with 20 ng/ml of GM-CSF and 20 ng/ml of IL-4. The expression levels of mdr-1 and mdr-3 were assessed using RT-PCR and quantitative real-time PCR, normalizing the amounts of mRNA for targeted genes to the mRNA levels of the GAPDH gene. Our quantitative real-time PCR results clearly indicate that expression of mdr-1 and mdr-3 was highly up-regulated during DC development (Fig. 1A and B). P-gp protein expression was evaluated during the monocyte to immature DC development by confocal microscopy and Western blot analysis. By western blot analysis, we found a pronounced up-regulation of the 170 kDa P-gp protein during differentiation from monocytes to immature DC (Fig. 1C). Cultured DC were co-stained for P-gp and the DC marker, CD11c+. DC expressing P-gp (red) was also positive for DC marker CD11c+ (green), suggesting that these cells are in fact increasing P-gp expression during DC development (Fig. 1D).

3.2. Increased mdr-1 gene expression during DC maturation, but not mdr-3

We found that mdr-1 and mdr-3 genes were essential for DC development, and next examined the individual role of these genes in DC maturation. To determine if expression of mdr-1 and mdr-3 genes is modulated during DC maturation we examined the expression of these genes in LPS-induced DC maturation. As shown in Fig. 2, the expression of mdr-1, but not mdr-3, is up-regulated by DC maturation in a dose- and time-dependent manner. These data indicate that mdr-1 gene expression is required for DC development as well as DC maturation. This was further confirmed on the protein level by western blot analysis (Fig. 2B), as the level of P-gp expression increased during DC maturation. Next, the efflux activity of the transmembrane P-gp was studied using rhodamine dye as a substrate. As shown in Fig. 2C, DC maturation also corresponded with an increase P-gp efflux activity, indicating that P-gp activity was increased during DC maturation.
Fig. 4. VLX treatment suppresses the expression of co-stimulatory molecules and impairs cytokine production in LPS-induced DC maturation. DC were harvested and analyzed using two-color flow cytometry. The cells were gated on CD11c+. VLX was added to the DC for 24 h at concentrations of 25 and 50 μM, and stimulation was induced with 200 ng/ml LPS. Surface molecule expression was then analyzed (A). The histogram shown is from one out of four representative experiments. The numbers indicate mean fluorescence intensity (MFI) (B) DC were stimulated with VRP (20 μM, such as P-gp blocker), or VLX (50 μM, such as P-gp suppressor) for 24 h, with or without LPS. This figure represents the analysis of IL-12p40/p70 and IL-10 expression in CD11c+DC by intracellular cytokine staining after 24 h of LPS stimulation. The numbers indicate the percentages of CD11c+ cells expressing IL-12p40/p70 or IL-10. (C) Analysis of IL-1α, IL-6, IL-10, and IL-12p70 production was measured by ELISA with magnetic bead-purified DC (1 × 10^6 cells) 24 h after LPS stimulation (200 ng/ml). The data represent the means (±SD) of four separate experiments. (**P<0.01 vs control groups).
3.3. VLX suppresses P-gp levels during DC maturation, but not mdr-1 gene expression

To verify the function of P-gp in DC, a Western blot analysis was used to detect changes in P-gp expression following treatment with specific inhibitors, including VRP, CsA, and VLX. Generally, these specific inhibitors of P-gp have been reported to block drug efflux and function of ATP-binding cassette protein subfamily B (ABCB) transport, but not down-regulate the protein expression [25–27]. In preliminary experiments, DC were treated with VRP, CsA, and VLX, and we observed no marked differences in the percentage of dead cells, as evidenced by CD11c+ cell and annexin-V/propidium iodide (PI) staining (data not shown). As shown in Fig. 3A and B, we found that VRP and CsA did not affect expression of P-gp or mdr-1 expression, but VLX treatment significantly decreased the expression level of P-gp. Moreover, VLX treatment suppressed P-gp expression in LPS-induced DC maturation (Fig. 3C).

3.4. P-gp suppressor inhibits DC maturation and activation through IL-12 production

The observed changes in expression of P-gp during DC maturation suggest that VLX exposure may lead to a profound regulation of the phenotypic and functional maturation of DC. We then investigated the effects of VLX on DC maturation. As shown in Fig. 4A, VLX treatment significantly attenuated surface expression of CD80, CD86, MHC classes I and II on the CD11c+ cells in a dose-dependent manner in comparison to the control group. Exposure to VLX in the presence of LPS resulted in impaired expression of MHC classes I and II. Interestingly, a significant down-regulation of the costimulatory molecules, CD80 and CD86, was also observed under these conditions. It is known that inducing DC activation will result in the release of cytokines, including IL-1α, IL-6, IL-10, and IL-12, and this can be modulate DC-mediated T cell immune responses [28, 29]. IL-12 production was identified previously as a specific marker of DC activity, a critical marker for DC maturation, and can be used as a select Th1-dominant adjuvant [30]. We next assess the ability of DC to generate pro-inflammatory cytokines. To do this, we analyzed the production of cytokines following treatment with each P-gp blocker (such as VRP) and suppressor (such as VLX) from both immature and mature DC. As shown in Fig. 4B and C, DC stimulated with VLX expressed small amounts of IL-2, IL-10, and IL-12, in comparison to unstimulated DC. Interestingly, the P-gp blocker VRP had no effect on cytokine levels during DC maturation. These data indirectly indicate that P-gp is necessary for DC maturation and activation.

3.5. P-gp of DC is required for DC-mediated T cells polarization and proliferation

Changes on surface molecule expression and cytokine production from P-gp modulated-DC show that the down-regulation of P-gp
leads to a profound inhibition of the phenotypic and functional changes associated with the maturation of DC. However, these results did not permit us to exclude the possibility that P-gp induces a general inhibition of the physiological functions of DC. Therefore, we sought to determine if VLX-induced decrease of P-gp on DC is associated with the reduced proliferation, differentiation, and cytokine production in T cells. More specifically, we attempted to determine whether the stimulation of DC with a P-gp suppressor alters the ability of the DC cells to activate T cells in a mixed lymphocyte reaction (MLR). As shown in Fig. 5A and B, the LPS-treated DC exhibited a far more profound proliferation than did the controls, whereas treatment with a P-gp suppressor appeared to impair to proliferation responses in the allogeneic T cells elicited by the LPS-stimulated DC. Significant inhibition of T cell proliferation occurred at concentrations of VLX as low as 25 μM in MLR stimulated assays. The concentrations of IL-2, IL-4, and IFN-γ measured in 72-h co-culture supernatants, when cell counts are approximately equal, were also reduced, suggesting a possible cytokine-dependent mechanism and inhibit DC maturation following treatment with P-gp suppressor (VLX).

3.6. P-gp suppressor blocks DC development

While the effects of P-gp suppressor treatment during DC development have not been previously investigated, our data suggest that P-gp is a very important to DC differentiation, maturation, and T cell immunity. To investigate possible roles of P-gp in DC development, a P-gp suppressor, at nontoxic doses, was added to interstitial DC cultures. The addition of the P-gp suppressor VLX markedly inhibited DC development as seen by CD11c-FITC values (Fig. 6). In addition, when analyzing marker surface expression, VLX treatment had suppressive effect on CD86 expression. The DC marker proteins such as CD11c and CD86 were already preferentially localized to the dendritic arbor on day 2, and their polarization increased to immature levels by day 6. The percentage of cells expressing these proteins associated with the dendritic membrane increased significantly during DC development. Moreover, the percentage of cells expressing these proteins associated with the dendritic membrane increased significantly during DC activation.

4. Discussion

This is, to the best of our knowledge, the first report of the effects of P-glycoprotein (P-gp) expression levels on the phenotype, and the differentiation and development capabilities of murine bone marrow-derived DC. The aim of the work described here was to investigate whether regulation of P-gp, a protein previously shown to be involved in the migration of mature DC, altered the development of DC and DC-mediated T cell immunity [16], the functional and development capabilities have not been assessed. The physiological role of P-gp is not completely understood; however, it has been related to multi-drug resistance in cancer cells [31], T cells immunity [32], and DC migration [16]. Previous reports show that P-gp is a trans-membrane protein, a molecule of the ABC transporter superfamily, and is well-known that it mediates efflux of chemotherapeutic agents from the intracellular environment, thereby contributing to drug resistance [33, 34].

In this study, we suggest that P-gp expression alters physiological functions of DC. Down-regulation of P-gp inhibits the maturation and activation of DC, as demonstrated by low expression of costimulatory molecules on DC surfaces and the reduced cytokine levels, including IL-1α, IL-10, and IL-12 (Fig. 4). Moreover, the production of IFN-γ, IL-4, and IL-2 cytokines was significantly decreased in P-gp suppressed-DC in

![Fig. 6. The down-regulation of P-gp inhibits DC development and differentiation. As a measure of development and differentiation, we quantified the percentage of cells staining for each marker protein that was associated with the DC arbor. The DC marker proteins CD11c+ (A) and CD86 were already preferentially localized to the dendritic arbor on day 2, and their polarization increased to immature levels by day 6 (B). The percentage of cells expressing these proteins associated with the dendritic membrane increased significantly during DC development. DC were cultured with GM-CSF, IL-4, with or without VLX then collected and immunophenotyped on the days indicated. Cells were labeled with the designed monoclonal antibodies and analyzed by flow cytometry. The quadrants shown were set based on isotype control staining. The data are representative of three experiments that gave similar results.](image-url)
a co-culture system with T cells (Fig. 5). Functionally, the down-regulation of P-gp in DC inhibited primary T cell stimulatory activity in an allogeneic MLR, and drives Th1/Th2 polarization in both CD4⁺ and CD8⁺ T cells.

DC are the most potent APC that amplify and initiate immune responses. In addition, DC play a crucial role in peripheral tolerance mechanisms, induce different types of immunity and tolerance, recognizing tumors, while capable of preventing immune responses against environmental antigens and self tissues [3, 33, 35-38]. Moreover, DC are among the benign cells affected by anti-cancer drugs, inducing an immune deficient state [39]. Therefore, we investigated the function role of P-pg and the influence of anti-cancer drugs on DC function.

Here, we show that the down-regulation of P-gp inhibits expression of CD80, CD86, MHC I, and MHC II on DC. Moreover, P-gp suppressor modulators and the development of therapeutic adjuvants for the treatment of DC-related acute and chronic diseases.

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References


