Purinergic Signalling (2024) 20:73–82
https://doi.org/10.1007/s11302-023-09940-3

ORIGINAL ARTICLE

# Human plasmacytoid dendritic cells express the functional purinergic halo (CD39/CD73)

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Received: 12 September 2022 / Accepted: 31 March 2023 / Published online: 14 April 2023
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**Abstract**

Plasmacytoid dendritic cells (pDCs) are a specialized DC subset mainly associated with sensing viral pathogens and high-type I interferon (IFN-I) release in response to toll-like receptor (TLR)-7 and TLR-9 signaling. Currently, pDC contribution to inflammatory responses is extensively described; nevertheless, their regulatory mechanisms require further investigation. CD39 and CD73 are ectoenzymes driving a shift from an ATP-proinflammatory milieu to an anti-inflammatory environment by converting ATP to adenosine. Although the regulatory function of the purinergic halo CD39/CD73 has been reported in some immune cells like regulatory T cells and conventional DCs, its presence in pDCs has not been examined. In this study, we uncover for the first time the expression and functionality of the purinergic halo in human blood pDCs. In healthy donors, CD39 was expressed in the cell surface of 14.0 ± 12.5% pDCs under steady-state conditions, while CD73 showed an intracellular location and was only expressed in 8.0 ± 2.2% of pDCs. Nevertheless, pDCs stimulation with a TLR-7 agonist (R848) induced increased surface expression of both molecules (43.3 ± 23.7% and 18.6 ± 9.3%, respectively), as well as high IFN-α secretion. Furthermore, exogenous ATP addition to R848-activated pDCs significantly increased adenosine generation. This effect was attributable to the superior CD73 expression and activity because blocking CD73 reduced adenosine production and improved pDC allostimulatory capabilities on CD4 + T cells. The functional expression of the purinergic halo in human pDCs described in this work opens new areas to investigate its participation in the regulatory pDC mechanisms in health and disease.

**Keywords** PDCs · Purinergic halo · CD39 · CD73 · Immunomodulation

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## Introduction

pDCs are a specialized dendritic cell (DC) subset that plays a crucial role in the antiviral immune response as the primary type I interferon (IFN-I) producers. This cellular population constitutes a sparse amount of peripheral blood mononuclear cells (PBMCs), ranging between 0.1 and 0.5%. pDCs are defined by surface expression of phenotypic markers such as BDCA-2 (CD303), BDCA-4 (CD304/neuropilin-1), CD123 (interleukin (IL)-3 receptor α-subunit), CD4, CD45R, CD68, and immunoglobulin-like transcript (ILT) 3 and 7 [1]. Identifying these markers gets special attention since the current peripheral blood DC classification recently included a new DC group named Axel-Siglec-6 (AS) DCs. AS-DCs and pDCs share surface phenotypic markers (BDCA-2 +, BDCA4 +, and CD123 +) but show different functionality. pDCs are distinguished by the

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absence of some markers highly expressed in AS-DC, such as CD33, Axl, or Siglec-6 [2].pDCs were initially described as a homogeneous cell population expressing high levels of endosomal TLR-7 and TLR-9 that sense single-stranded RNA (ssRNA) and CpG D.N.A. motifs, respectively. Binding their ligands trigger signaling pathways such as MyD88/IRF7, culminating in IFN-I production and secretion [3, 4]. Nevertheless, human pDC diversification into three stable subpopulations when activated with specific stimuli was recently described. Such differentiated pDC subsets include cells with the ability to produce high amounts of IFN-I, cells specialized in antigen presentation and T-cell priming, and a third group showing both functional capabilities [5]. Hence, due to their plasticity and specialized immunogenic characteristics, pDC functional disbalances have been associated with developing some autoimmune diseases, such as systemic lupus erythematosus (SLE) and psoriasis [6–8]. Therefore, besides their crucial antiviral role, the tolerogenic properties of pDC have become relevant. In this regard, some tolerogenic properties have been described in pDCs, mainly mediated by transforming growth factor-β (TGF-β), IL-6, inducible T cell co-stimulator ligand (ICOSL), OX40L, indolamine 2,3-dioxygenase (IDO), TRAIL, and granzyme B. Such tolerogenic pDC mechanisms have been primarily studied in tumor microenvironments [9–11].

The purinergic halo is an essential regulatory mechanism widely described in lymphoid and myeloid immune cells (regulatory T and B cells, conventional DCs, neutrophils, monocytes, and macrophages, among others) and endothelial cells. This broadly distributed regulatory system is constituted by two ectoenzymes, CD39 and CD73, which generate adenosine from extracellular nucleotides [12]. This strategic immunomodulatory system regulates the extent and magnitude of purinergic signals delivered from and toward immune cells. CD39 (NTPDase-1) is an integral membrane protein of 510 amino acids with seven potential N-linked glycosylation sites and two transmembrane domains essential for catabolic activity and substrate specificity. CD73 (ecto-5′-nucleotidase) is a glycosyl phosphatidylinositol (GPI)-anchored enzyme of 576 amino acids with an N-terminal domain-containing $Zn^{2+}$-binding site, a C-terminal domain that includes the catalytic site, and a short alpha-helix interposed between N- and C-terminal domains. In addition, CD73 also exists in the soluble form [13, 14].

Under steady-state conditions, ATP is located inside the cells, but proinflammatory stimuli, cell stress, hypoxia, or cell death lead to its release. Extracellular ATP is a damage-associated molecular pattern (DAMP) that enhances the inflammatory process. The high extracellular ATP concentration may be regulated through CD39 activity. CD39 phosphohydrolase ATP/ADP into AMP, and in a sequential reaction, CD73 hydrolyzes AMP to adenosine [13, 15]. Extracellular adenosine binds adenosine receptors (A1, A2a, A2b, or A3) [16]. A2a receptor activation results in an increase of cyclic AMP (cAMP) levels and PKA activation, which may, in turn, trigger signaling pathways involved in T-cell anergy induction, generation, and activation of regulatory T cells (Tregs), and differentiation of antigen-presenting cells into tolerogenic cells [17, 18]. Importantly, Tregs express low extracellular CD73, but it is found in cytoplasmic granules at high levels [19]. Moreover, CD73 expression is induced with both proinflammatory (IFNs, TNF-α, IL-1, and $PGE_2$) and anti-inflammatory (TGF-β) stimuli [20, 21]. In a recent investigation, Ray et al. demonstrated the expression of CD73 in pDCs from multiple myeloma patients, demonstrating that pDCs interaction with tumor cells increased CD73 expression in a tumor microenvironment [22]. However, given the relevant function of the purinergic halo in immunomodulation and the limited knowledge of tolerogenic factors in pDCs, this study aimed to analyze the coexpression and functionality of CD39 and CD73 in human blood pDCs in the context of healthy physiology.

## Materials and methods

### Healthy individuals and samples

Peripheral blood samples (120 mL) were obtained under informed consent from thirteen healthy donors. Volunteers were enrolled at the University Clinical Laboratory of the Biochemical Sciences Faculty from the Universidad Autónoma "Benito Juárez" de Oaxaca (UABJO) under the authorization of the Hospital Regional de Alta Especialidad de Oaxaca (HRAEO).

### pDC isolation

PBMCs were obtained by the Ficoll density gradient (Lymphoprep™ STEMCELL Technologies). pDCs were isolated by a double magnetic cell sorting performed with the BDCA-4/Neuropilin-1 MicroBead Kit (Miltenyi Biotec). pDCs were resuspended in culture medium RPMI 1640 with glutagro (Corning) (supplemented with 10% heat-inactivated autologous plasma, 2 mM L-glutamine, 1 mM sodium pyruvate, 0.1 mM nonessential amino acids, 100 U/mL penicillin, 100 mg/ml streptomycin, and 50 nM 2β-mercaptoethanol). Cell numbers and viability after purification were quantified by trypan blue exclusion (0.4%, Sigma).

### pDC stimulation

In total, $2 \times 10^4$ freshly isolated pDCs were cultured in 96-well plates for 24 h at 37 °C in culture RPMI 1640 medium supplemented with IL-3 (10 ng/mL, Miltenyi Biotec) alone or IL-3 plus TLR-7 agonist resiquimod (R848, 2.5 µg/mL, Invivogen) at different time points (6–24 h). Then, supernatants were collected and stored at – 70 °C

for further analysis, and cells were collected for phenotypic analyses. Experiments were run in triplicate.

### Flow cytometry

Freshly purified and cultured pDCs were stained with 7-AAD viability dye (BD Pharmingen) for cell death evidence. The following antibodies were used for phenotypic analyses: anti-BDCA-2/FITC (clone AC144, RRID:AB_2726017), anti-CD4/VioBlue (clone REA623, RRID:AB_2726691), anti-CD33/APC (clone REA775, RRID:AB_2657559), anti-CD39/APC (clone REA739, RRID:AB_2657892), and anti-CD40/PE (clone HB14, RRID:AB_10830710), all from Miltenyi Biotec; anti-CD123/Alexa Fluor 700 (clone 6H6, RRID:AB_2750161) and anti-CD73/Pacific Blue (clone AD2, RRID:AB_2561748), both from BioLegend. For intracellular CD73 detection, cells were fixed in paraformaldehyde at 4% for 30 min and permeabilized with Triton X-100 (0.1%) for 1 h at room temperature. Cells were then washed and stained with the corresponding antibody. Cell acquisition was performed in MACSQuant Analyzer 10 cytometer (Miltenyi Biotec) at the National Laboratory of Cytometry (LABNACIT-UNAM-UABJO-UACH), and data analysis was done with Tree Star FlowJo X software (BD Biosciences, R.R.I.D.:SCR_008520).

### Fluorescence microscopy

Freshly isolated pDCs were fixed with 4% paraformaldehyde in D-PBS 1X, either stained with labeled antibody for surface detection of CD73 or permeabilized with 0.1% Triton X100 for 10 min and subsequently washed with D-PBS 1X and blocked with 10% decomplemented plasma for intracellular CD73 detection. CD73 staining was performed with primary anti-CD73 antibody (clone 7G2, 1:50 dilution; Abcam. R.R.I.D.:AB_879692) and secondary anti-mouse antibody conjugated with Alexa Fluor 488 (1:400; Invitrogen. R.R.I.D: AB_2534069). The wet coverslip was placed with Fluoroshield with DAPI (Sigma) mounting medium on a glass slide to be examined in Zeiss Axio Observer Z1 motorized inverted fluorescence microscope at × 40 objective. Pictures were analyzed with Fiji ImageJ Software (R.R.I.D: SCR_002285).

### IFN-α quantification

IFN-α produced by pDCs was measured in culture supernatants by ELISA. Supernatants were thawed at 4 °C, and the IFN-α assay (Invitrogen ThermoFisher, detection limit 3.2 pg/mL) was done according to the manufacturer's protocol. All samples were run in duplicate.

### Adenosine assays

In total, $5 \times 10^4$ pDCs were kept in culture in 96-well plates in the absence or presence of R848 for 18 h. Then cells were washed with supplemented RPMI 1640 medium without mercaptoethanol and cultured for 1 h in this medium supplemented with ATP (100 μM, Sigma). Supernatants were collected for adenosine detection assays (Cell Biolabs, Inc.), performed according to the manufacturer's protocol. Reading was carried out by fluorometry (Synergy™ H.T.X.). To evaluate the involvement of CD73 in adenosine production, cells were treated with a neutralizing anti-CD73 antibody 10 μg/mL (clone 7G2, Abcam, R.R.I.D.: AB_879692) for 1 h before ATP stimulation. All samples were run in triplicate.

### Primary CD4 + T cell proliferation

PBMCs were obtained by Ficoll-Hypaque gradient from total blood samples. CD4 + T lymphocytes were isolated by negative selection using the MACS CD4 + T-cell isolation kit (Miltenyi Biotec), and lymphocytes were labeled with CFSE (Sigma, 0.5 μM) to assess proliferation by the CFSE dilution method by flow cytometry. Then, T cells were co-cultured in a medium supplemented with 10% FBS with allogenic unstimulated, R848 stimulated, and anti-CD73 neutralizing antibody (anti-CD73) pDCs at 5:1 (T cell:pDC) ratio for 5 days. Following co-culture time, lymphocytes were harvested and stained with anti-CD25 (clone 2A3, R.R.I.D.: AB-2783790) from BD to determine the T cell's activation state.

### Statistical analysis

Statistical analysis was carried out using a Shapiro–Wilk normality test and Wilcoxon matched-pairs signed rank test to nonparametric data. Prism 7 software (GraphPad Software Inc., RRID: SCR_002798) was used to analyze and plot the data.

## Results

### Human blood pDCs express CD39 and CD73

Human blood pDCs were purified by a double-positive magnetic cell sorting with anti-BDCA-4 antibodies. Their phenotype was analyzed in the 7-AAD negative gate (living cells) based on BDCA-2, CD123, CD4, and CD33 expression (Fig. 1a). Pure pDCs were characterized as BDCA-$4^+$, BDCA-$2^+$, $CD123^+$, $CD4^+$, and $CD33^-$ cells. BDCA-$4^+$, BDCA-$2^+$, $CD123^+$, $CD4^-$, and $CD33^+$ cells were considered AS-DCs (11.4 ± 1.9%), and this region was discarded for further phenotypic analysis.

**Fig. 1** CD39 and CD73 expression in freshly purified human blood pDCs. **a** pDCs were selected in the 7-AAD negative gate (living cells) and based on the expression of BDCA-2, CD123, CD4, and CD33. **b** Representative dot plots showing CD39 and CD73 surface expression. The graph shows CD39 + and CD73 + pDC frequencies ($n = 13$). **c** Representative histogram comparing extra *vs.* intracellular CD73 expression. The graphs show the frequency of pDCs displaying extracellular and intracellular staining of CD73 (left) and the corresponding MFI (right). Data are shown as the mean ± SD of $n = 13$ in B and $n = 5$ in C. Wilcoxon matched-pairs signed rank test was used for statistical analysis (extracellular *vs.* intracellular): $^{*}p < 0.05$. **d** Representative fluorescence microscopy images of isotype control, extracellular, and intracellular CD73. The nucleus (blue) and CD73 molecule (green) are observed. **e** Graphic shows ± SD of the mean gray value of signal per area for Alexa Fluor 488 of five images (four extremes and center) per condition, $n = 3$. Mann–Whitney test for compare ranks was used for statistical analysis: $^{***}p < 0.001$ and $^{****}p < 0.0001$

The extracellular CD39 identification in the defined pDC region revealed a 14.0 ± 12.5% frequency of CD39$^{+}$ cells in the analyzed individuals. Conversely, CD73 determination showed very low or null expression on the cell surface (1.5 ± 1.8%) (Fig. 1b). Intracellular analysis of CD73 showed that 8.0 ± 2.2% of pDCs expressed this molecule (Fig. 1c). Moreover, the median fluorescence intensity (MFI) of CD73 tended to be increased in the intracellular compartments (Fig. 1c, right panel). CD73 expression was additionally evidenced by fluorescence microscopy to support the results obtained by flow cytometry. These results showed a more significant number of granules (green) in intracellularly stained pDCs compared to the extracellular condition (Fig. 1d), where this pattern is not observed. In addition, the quantification of the data obtained from the captured images (Fig. 1e) of the isotype control (0.35 ± 0.36), extracellular CD73 (0.48 ± 0.53), and intracellular CD73 (1.57 ± 0.905) corroborates the intracellular localization of CD73 in human blood pDCs.

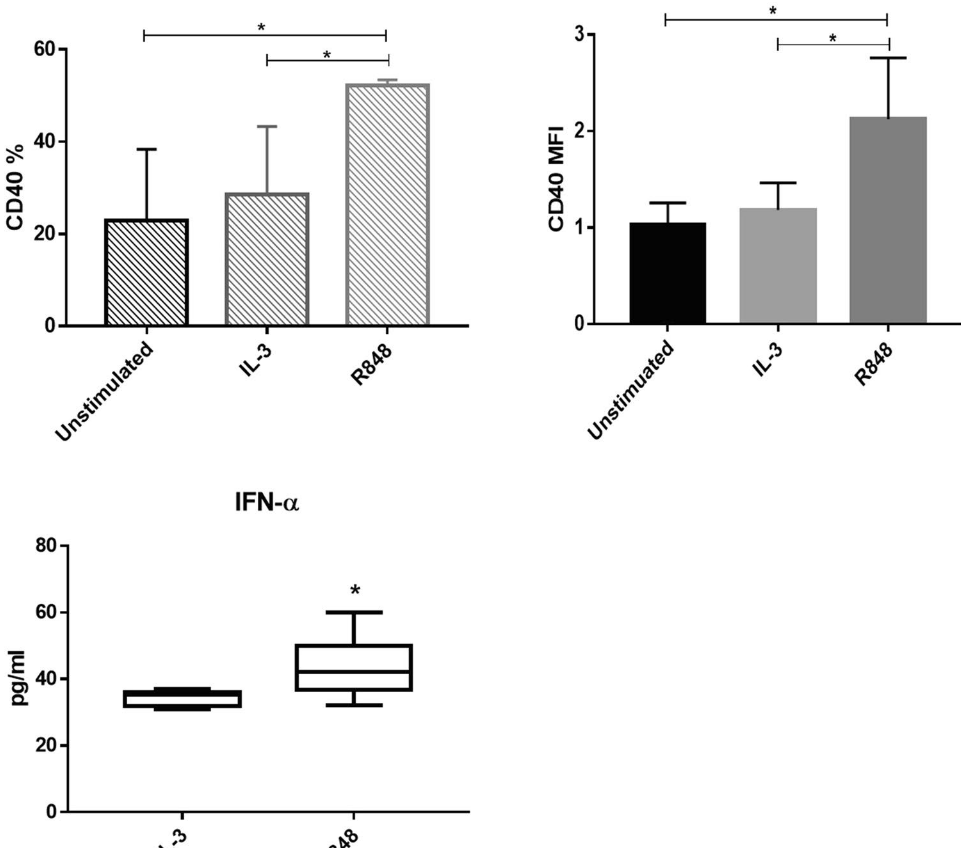


**Fig. 2** TLR7 triggering in human blood pDCs. Unstimulated (freshly isolated), IL-3-stimulated (IL-3), and IL-3 plus R848-stimulated (R848) pDCs were analyzed. Percentage (left) of CD40 + pDCs and CD40 MFI (right). B) IFN-α secretion in the supernatants of cultured pDCs, determined by ELISA ($n = 6$). Data are shown as the mean ± SD. Wilcoxon matched-pairs signed rank test was used for statistical analysis: *$p < 0.05$

## TLR7 triggering in human blood pDCs increases the extracellular expression of the purinergic halo

Once the constitutive expression of the purinergic halo in human blood pDCs was described, these cells were stimulated with R848, an imidazoquinoline that activates pDCs via the TLR-7/MyD88-dependent signaling pathway to induce IFN-α secretion. Aiming to favor in vitro pDC surviving, cells were stimulated with R848, and pDC activation was analyzed through CD40 expression. Hence, to exclude any IL-3-mediated effect on pDC activation, CD40 expression was also examined under unstimulated, IL-3-stimulated, and R848 plus IL-3 (refer to R848) stimulation conditions. Unstimulated and IL-3-stimulated pDCs showed no significant differences in CD40 levels; however, R848-stimulated pDC showed a significantly higher frequency (Fig. 2a, left panel) and CD40 + expression (Fig. 2a, right panel). Likewise, R848 plus IL-3 stimulated pDCs induced significantly higher IFN-α secretion than those cultured with IL-3 alone (Fig. 2b). When compared to the IL-3-stimulated condition, results demonstrated effective R848-mediated pDC activation. Hence, the expression and functionality of the purinergic halo were analyzed under activation conditions.

R848-stimulated pDCs expressed significantly higher levels of CD39 and CD73 than unstimulated pDCs, and interestingly, activated pDCs also showed an increase in the frequency of $CD73^+$ cells (Fig. 3a). Moreover, the percentage of pDCs coexpressing CD39 and CD73 was also significantly superior in R848-stimulated pDCs (Fig. 3b). These results evidenced that pDC activation induced by TLR-7 triggering causes an upregulation of the purinergic halo.

## Activation of human pDCs via TLR-7 increased adenosine generation catalyzed by CD73

Since adenosine generation catalyzed by the purinergic halo depends on CD73 enzymatic activity, we first determined the kinetics of CD73 expression in pDCs after R848 plus IL-3 stimulation. The results showed that the most elevated surface CD73 presence occurred at 18 h upon R848 plus IL-3 exposure (R848-activated pDCs) (Fig. 4a). Then, adenosine generation was evaluated in unstimulated pDCs and R848-activated pDCs after 18 h of culture. When cells were exposed for 1 h to exogenous ATP, R848-activated pDCs generated significantly higher levels of adenosine with respect to unstimulated cells, and this production was abolished in the presence of a neutralizing antibody

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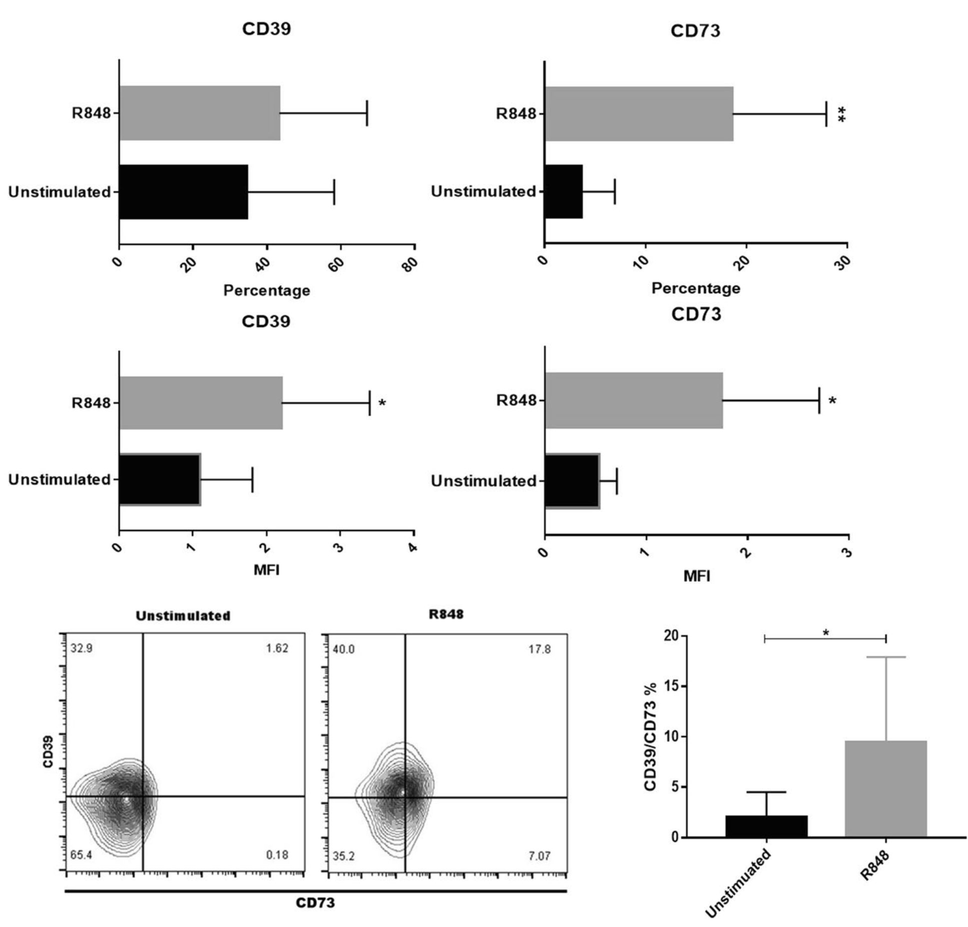


**Fig. 3** R848 activated-pDCs increase extracellular CD39 and CD73 coexpression. **A** CD39 and CD73 expression were evaluated in freshly isolated pDCs (unstimulated) and pDCs stimulated with R848 plus IL-3 (R848). Graphs show the percentage of CD39 + and CD73 + pDCs (upper panels) and their respective MFI (lower panels). **B** Representative dot plots showing CD39 and CD73 coexpression in BASAL and R848-stimulated pDCs. The graph shows the frequency of CD39 + CD73 + pDCs. Data are presented as the mean ± SD of $n = 5$. Wilcoxon matched-pairs signed rank test was used for statistical analysis (unstimulated vs. stimulated cells). *$p < 0.05$; **$p < 0.001$

anti-CD73 (Fig. 4b). These data demonstrate the purinergic halo functionality to generate adenosine in human pDCs.

### Blocking CD73 on activated pDCs improves CD4 + T cells proliferation

It has been reported that the adenosine produced by CD73 affects T cell proliferation. Since CD73 expressed in activated pDCs generates adenosine, a mixed leukocyte reaction assay (co-culture) was performed to evaluate the CD73 involvement in the allostimulatory capacity of pDCs on the proliferation of CD4 + T cells. The results showed that pDC cultures just in the presence of the survival stimulus (IL-3) induced a T cell proliferation around 14.24 ± 2.07%; such T cell proliferation increased when stimulated with R848 plus IL3-activated pDCs (17.14 ± 5.82). Interestingly, this allostimulatory capability was improved, revealed by a statistically significantly higher T cell proliferation (21.4 ± 6.24) (Fig. 5a and b) and greater CD25 expression (Fig. 5c) when CD73 activity was blocked (anti-CD73) in R848 plus IL-3-activated pDCs. Altogether, these data suggest a likely involvement of CD73-produced adenosine on pDCs functional ability to stimulate T cells.

## Discussion

Currently, pDC involvement in the antiviral immune response is widely described, and the knowledge about their functional flexibility and specialized immunogenic characteristics is constantly increasing. Nevertheless, pDC functional disbalances have also been reported in immune tolerance loss and autoimmunity development, mainly related to cell overactivation and IFN-α hypersecretion [23]. However, pDC tolerogenic mechanisms have not been sufficiently described to explore their probable association with these pathologies. Hence, this work directly sought the presence and functionality of the purinergic halo (CD39/CD73) as a likely immunomodulatory mechanism in human blood pDCs.

The purinergic halo is a known regulatory mechanism in Tregs. The importance of these molecules in adenosine generation has been broadly demonstrated [24]. Previously, our research group showed that human tolerogenic monocyte-derived DCs cultured with the immunosuppressive cytokines IL-10/TGF-β displayed higher levels of CD39 expression than their conventional monocyte-derived DC counterparts [25]. Since adenosine production requires both CD39 and CD73 presence, we here evaluated the expression of these molecules and their enzymatic activity in human

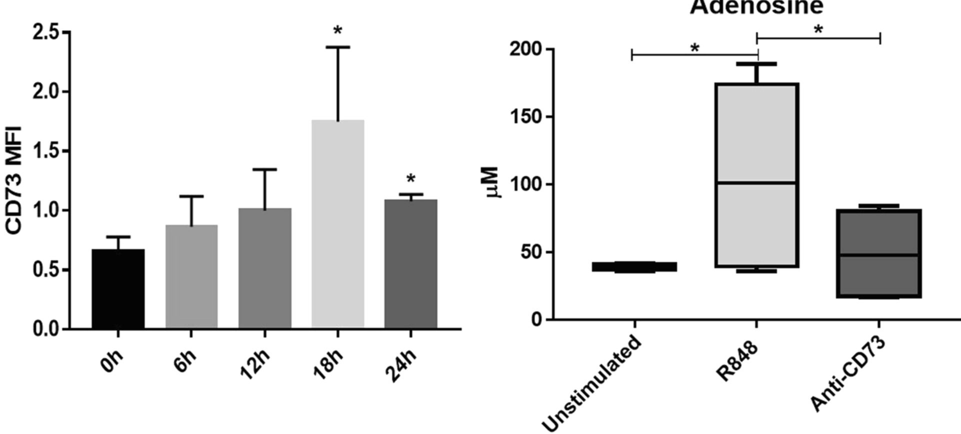


**Fig. 4** CD73-catalyzed adenosine generation in activated human blood pDCs. **A** Kinetics of CD73 expression in R848-activated pDCs. **B** Extracellular adenosine production after exogenous ATP addition. Freshly isolated pDCs (unstimulated) and pDCs activated for 18 h with R848 plus IL-3 in the absence (R848) or presence (anti-CD73) of a blocking antibody against CD73 were incubated for 1 h with ATP. After ATP addition, adenosine levels were measured in the culture supernatants. Data are expressed as adenosine production (µM) with respect to unstimulated pDCs. Graphs show the mean ± SD of two experiments performed in triplicate. Wilcoxon matched-pairs signed rank test was used for statistical analysis: *$p < 0.05$

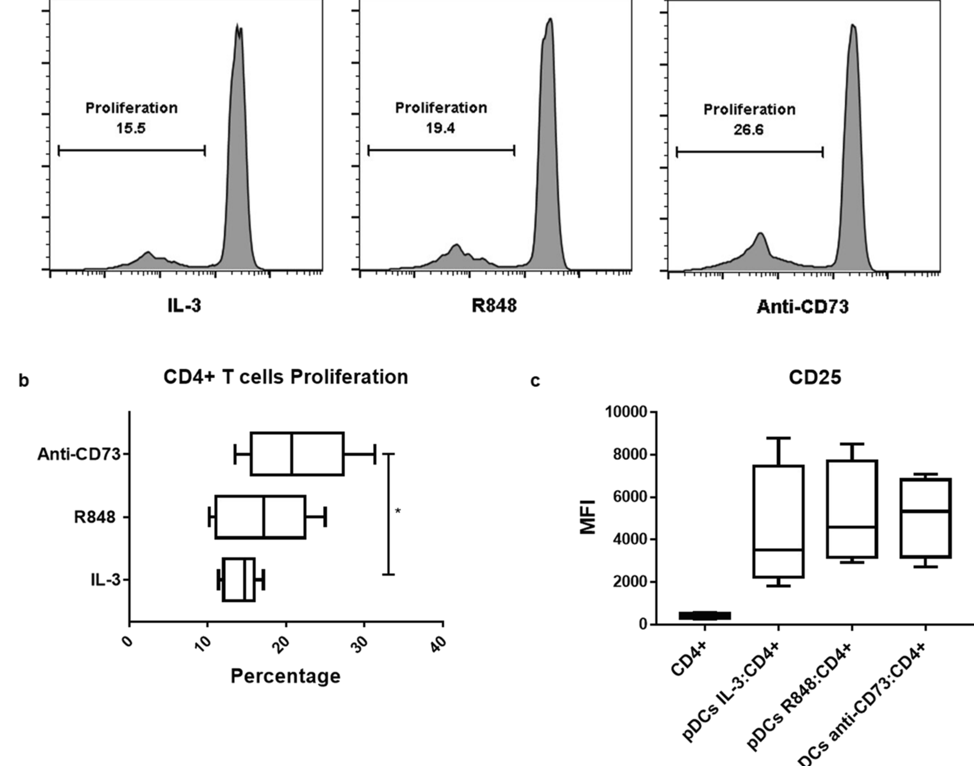


**Fig. 5** CD73 contribution on pDCs APC abilities to allostimulate CD4 + T cells. **a** Histograms of CFSE dilution of CD4 + T cells allostimulated with pDCs previously treated with IL-3 (IL-3), IL-3 plus R848 (R848), or IL-3 plus R848 with Anti-CD73 (anti-CD73). **b** Graphs show the median of percentages ± SD of CD4 + T cell proliferation. **c** CD25 expression on CD4 + T cells cocultured with treated pDCs (IL-3, R848, and anti-CD73). Graphs show the median ± SD of four experiments performed by duplicate. Wilcoxon matched-pairs signed rank test was used for statistical analysis: *$p < 0.05$

blood pDCs to subsequently assess their behavior in pDC-associated pathologies.

For pDC identification, cells were defined as BDCA-$2^+$, BDCA-$4^+$, $CD123^+$, $CD4^+$, and $CD33^-$ cells [26]. The $CD33^+$ cell subset co-purified with pDCs was considered AS-DCs and discarded in further phenotypic analysis. Given their functional differences (especially in IFN-α production), the limited $CD33^+$ cell numbers during activation analysis might not be significant in this study. This work analyzed CD39 and CD73 expression in human blood pDCs. The results revealed an 18.11% frequency of CD39 + pDCs with very low or null extracellular CD73 expression. Similarly, Mandapathil et al.

observed a low CD73 surface but a high intracellular expression in human Treg cells [27]. Likewise, Schuler et al. found that the intracellular localization of CD73 is in cytoplasmic granules in different T-cell subsets [19]. Following up on this background, we performed the intracellular CD73 search in pDCs and observed an 8.09% cell frequency showing this molecule. Additionally, we performed CD73 detection on pDCs by fluorescence microscopy, and data confirmed CD73 expression in pDCs. For the first time, this study demonstrated the CD39/CD73 coexpression on human blood pDC. Low extracellular CD73 levels in some cells have been related to detachment from the cell membrane by GPI anchor cleavage carried out by phosphatidylinositol-phospholipase C (PI-PLC) [28]. However, soluble CD73 retains its catalytic activity [29], and some factors such as hypoxia, ATP, IFN-α, TNF-α, IL-6, and TGF-β may upregulate CD73 expression and enzymatic activity [30]. The interaction between pDCs and multiple myeloma cells has shown increased and regulated CD73 expression in pDC, which might be related to the tolerogenic microenvironment in this milieu [22]. However, the present study revealed that under normal physiological conditions, pDCs from healthy donors also express CD73 and CD39, both key molecules for adenosine production, highlighting the relevance of evaluating whether pDC activation through their receptors (TLR7/9) might influence the expression of these ectonucleotidases.

TLR-7 triggering in human blood pDCs induced an efficient cell activation, as evidenced by the significant increase of CD40 expression and IFN-α secretion (Fig. 2). Regarding that, the study aimed to display IFN-α secretion as a marker for activation and not as an optimized secretion as an antiviral or inflammatory response. The results were analyzed and taken as valid because, despite the low IFN-α levels, a significant difference was obtained between R848-stimulated and unstimulated cells. IL-3, as an in vitro pDC survival factor, did not show substantial changes in the activation with respect to unstimulated pDCs. Therefore, the increase in extracellular CD39 and CD73 coexpression indicates a significant rise of the purinergic halo in pDCs surface induced by TLR-7 activation (Fig. 3).

Given that adenosine generation catalyzed by the purinergic halo depends on CD73 enzymatic activity, adenosine production was quantified at the time of maximum CD73 expression in pDCs after activation established by this study (18 h). The analyses revealed that activated pDCs generated significantly higher adenosine levels than unstimulated cells. It is essential to highlight that, in addition to the purinergic halo, some cell types (i.e., neutrophils) are endowed with adenosine production capability catalyzed by the enzymatic activity of alkaline phosphatase (ALP) [31]. Although ALP activity has not been reported in pDCs, the elevated adenosine production generated by activated pDCs was reverted to the levels of unstimulated pDCs when CD73 enzymatic activity was blocked. Therefore, these results demonstrate that the purinergic halo mediates adenosine generation in human blood pDCs.

The immunomodulatory effect of CD39/CD73 depends on the cell's ability to produce extracellular adenosine. The physiological adenosine concentrations range from the nanomolar level [32]. The adenosine quantification method used in this study has a detection limit of 1.56 μM. Therefore, our results showed that R848-activated pDCs pulsed with exogenous ATP produced sufficient extracellular adenosine to be detected in vitro, suggesting their possible modulatory capabilities.pDCs play a crucial role in the antiviral immune response through their ability to secrete high levels of IFN-I. In addition, pDCs are endowed with antigen-presenting cell (APC) capabilities. Hence, CD73 activity for adenosine production was blocked during an assay of pDC allostimulation on CD4 + T cells to assess the CD73 contribution to pDCs APC abilities. Figure 5 shows that pDCs stimulated an efficient allogeneic lymphocyte proliferation and activation [33] in all the evaluated conditions, and although no significant differences were found between IL-3 cultured and R848-stimulated pDCs, an up tendency to higher proliferation was observed. Such a trend was statistically significant in the proliferation and superior CD25 expression when CD73 functionality was blocked (anti-CD73). It is worth noting that pDC APC efficiency in T cell priming is limited compared to conventional DC [34]. Nevertheless, the evidence that CD73 blocking improves pDC stimulatory capabilities in this study reveals that adenosine production via CD73 activity might modulate pDC function.

Previous studies have shown that adenosine may affect T cell priming; early activation, cytokine production, metabolic activity, proliferation, and effector differentiation on T cells may be abrogated when they are stimulated with adenosine; its absence leads to optimal T cell priming [35]. Due to CD73 working as a limiting step in adenosine production, the results of this study suggest that regulating CD73 activity might influence the pDC effectiveness to prime T cells, focusing attention on the adenosine role.

In summary, the report of the presence and functionality of the purinergic halo (CD39/CD73) in human blood pDCs revealed in this work opens a new research field for studying its involvement in the immunopathogenesis of pDC-imbalance-associated pathologies such as SLE and psoriasis.

**Acknowledgements** The authors thank the National Laboratory of Cytometry (LABNACIT-UNAM-UABJO-UACH), and the doctoral fellowships of Consejo Nacional de Ciencia y Tecnologia [660793 SLSA], [827788 RRWJ], and [AAA]. Dr. Jose Luis Maravillas Montero for his support in the methodological development of this work.

**Author contribution** Conceptualization: T.A.H. and S.T.C.; experimental and methodologic investigation: S.L.S.A, R.R.W.J., and A.A.A.; methodologic support: R.T.M.A., V.R.R., and A.R.S.R.; writing original draft preparation, S.L.S.A. and R.R.W.J.; writing—review and editing: T.A.H. and S.T.C.; supervision: T.A.H. and S.T.C.; funding

acquisition: T.A.H. All authors have read and agreed to the published version of the manuscript.

**Funding** This work was supported by the Consejo Nacional de Ciencia y Tecnologia (CONACyT): grants #285480 and SEP-CONACYT #A1-S-9430, and the Department of Clinical Immunology Research of the Biochemical Sciences Faculty, Universidad Autónoma "Benito Juárez" de Oaxaca.

**Data availability** Not applicable.

## Compliance with ethical standards

**Competing interests** The authors declare no competing interests.

**Ethical approval** All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional research committee and with the 1964 Helsinki Declaration and its later amendments or comparable ethical standards. The study was approved by the Bioethics Committee of the Hospital Regional de Alta Especialidad de Oaxaca (HRAEO-CIC-CEI 013/16).

**Consent to participate** Informed consent was obtained from all individual participants included in the study.

**Competing interest** The authors declare no competing interests.

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