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Determination of normal expression patterns of CD86, CD210a, CD261, CD262, CD264, CD358, and CD361 in peripheral blood and bone marrow cells by flow cytometry

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ABSTRACT

In 2010, new monoclonal antibodies were submitted to the 9th International Workshop on Human Leukocyte Differentiation Antigens, and there are few studies demonstrating normal expression patterns of these markers. Thus, the objective of this study was to determine the normal patterns of cell expression of CD86, CD210a, CD261, CD262, CD264, CD358, and CD361 in peripheral blood (PB) and bone marrow (BM) samples by flow cytometry. In the present study, CD86 was expressed only in monocytes and B lymphocytes in PB and in monocytes and plasma cells in BM. Regarding CD210a expression, in PB samples, monocytes and NK cells showed weak expression, while neutrophils, B and T lymphocytes, and basophils showed weak and partial expression. In BM samples, expression of CD210a was observed in eosinophils, monocytes, and B and T/NK lymphocytes. Weak expression of CD210a was also observed in neutrophilic cells and plasma cells. All B cell maturation stages had weak expression of CD210a except for immature B cells, which did not express this marker. In the present study, no cell type in PB samples showed positivity for CD261 and, in BM samples, there was very weak expression in neutrophilic series, monocytes, and B lymphocytes. Conversely, plasma cells showed positivity for CD261 with a homogeneous expression. For CD262, there was weak expression in monocytes, neutrophils, and B lymphocytes in PB samples and weak expression in monocytes, B lymphocytes, and plasma cells in BM samples. The evaluation of CD264 showed very weak expression in B cells in PB samples and no expression in BM cells. Very weak expression of CD358 was observed in neutrophils, monocytes, and B lymphocytes in PB and BM samples. In addition, in BM samples, plasma cells and T lymphocytes showed weak expression of CD358. In relation to the maturation stages of B cells, there was weak expression in pro-B cel, pre-B cell, and mature B cell. In the present study, it was possible to observe expression of CD361 in all cell types analyzed in PB and BM samples. The analyzed markers presented varied profiles of expression and, in some cases, these profiles were different from those observed in other studies. Further studies are needed to evaluate these molecules, mainly in relation to a possible application in the diagnosis of hematological malignancies or as new therapeutic targets for the treatment of hematological neoplasms or autoimmune diseases.

1. Introduction

Immunophenotyping by flow cytometry evaluates individual cells in suspension for the presence or absence of specific antigens. It can be used to identify deviations from the normal pattern of cell antigen expression [1]. Each population of normal cells has a specific immunophenotype, and abnormal phenotypes can be found in hematological malignancies [2]. Over time, several classification systems have been proposed for hematological neoplasms, grouping these diseases according to their morphological, histological, molecular, and

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Table 1
Description of the MoAbs used in the study.

MoAb	Fluorochrome	Code	Clone	Manufacturer
CD86	PE	1P-531-T100	BU63	Exbio, CZ
CD210a	PE	556013	3F9	BD Biosciences, USA
CD261	PE	1P-403-C100	DR-4-02	Exbio, CZ
CD262	PE	1P-461-C100	DR5-01-1	Exbio, CZ
CD264	PE	1P-519-C100	TRAIL-R4-01	Exbio, CZ
CD358	PE	1P-404-C100	DR-6-04-EC	Exbio, CZ
CD361	PE	1P-643-T100	MEM-216	Exbio, CZ
CD19	Pe-Cy7	557835	SJ25C1	BD Pharmingen, USA
CD38	APC-H7	656646	HB7	BD Biosciences, USA
CD10	APC	340923	HI10a	BD Biosciences, USA
CD3	PercP	PC-514-T100	UCHT1	Exbio, CZ
CD34	PercP Cy5.5	T9-664-T100	581	Exbio, CZ
CD8	FITC	AO7756	B9.11	Beckman Coulter, USA
CD4	PB	PB-359-T100	MEM-241	Exbio, CZ
CD45	PO	PO-684-T100	HI30	Exbio, CZ
CD20	V450	642274	L27	BD Biosciences, USA

Note: MoAb, monoclonal antibody; PB, Pacific Blue; PO, Pacific Orange.

Table 2
Schematic representation of the studied cell populations.

Compartment	Cell Type	Immunophenotype
Bone Marrow	Eosinophils	forward- and side-scatter
	Neutrophilic series	forward- and side-scatter
	Monocytes	forward- and side-scatter, CD38+
	Plasma cells	CD19+, CD38+++
	B lymphocytes	CD19+
	BI lymphocytes	CD19+, CD34+, CD10++, CD20-
	BII lymphocytes	CD19+, CD34-, CD10+, CD20-/+
	BIII lymphocytes	CD19+, CD34-, CD10+ weak, CD20+
	BIV lymphocytes	CD19+, CD34-, CD10-, CD20++
	T/NK lymphocytes	forward- and side-scatter, CD45+
Peripheral Blood	Eosinophils	forward- and side-scatter
	Neutrophils	forward- and side-scatter
	Monocytes	forward- and side-scatter
	Basophils	forward- and side-scatter
	B lymphocytes	CD19+
	T lymphocytes	CD3+
	NK cells	forward- and side-scatter, CD3- and CD19-

phenotypic characteristics [3,[4]. The classification of hematological neoplasms currently used worldwide was proposed by the World Health Organization and utilizes information on these characteristics to categorize hematological malignancies [4]. Flow cytometric immunophenotyping of clinical specimens can provide a rapid screen for these neoplasms and plays a key role in diagnosis and classification [1]. Using flow cytometry, it is possible to distinguish neoplastic cells from other cell populations based on differences in antigen expression. For some antigens, these differences are observed in positivity or negativity of expression, and for others, the intensity of expression is altered (an increased or decreased intensity of staining is achieved with fluorochrome-labeled antibodies) [2].

Studies demonstrate that various abnormalities of antigen expression on red cells, platelets, granulocytes, and monocytes are important for the diagnosis of hematological malignancies, such as myelodysplastic syndromes (MDS) [4–6]. In addition, it is known that normal B cells have phenotypic characteristics similar to B cells found in lymphomas [7]. Since most lymphomas mimic the normal stages of B-cell maturation, one of the classification criteria is based on phenotypic expression, the stage of differentiation, and the location of neoplastic B cells in the lymph node [4]. Thus, it is necessary to know the expression profile of the immunophenotypic markers in normal B cells to evaluate the expression of these markers in lymphoma cells. Immunophenotyping by flow cytometry is a sensitive method able to identify specific aberrations in both immature and mature hematopoietic cells in bone marrow (BM) and peripheral blood (PB) samples [4–6],[4–6]. Immunophenotyping by flow cytometry uses monoclonal antibodies (MoAb) in the diagnosis of neoplasms that were developed against epitopes of molecules present on the surface of leukocytes denominated *Clusters of Differentiation* (CDs). The CD nomenclature was proposed and established at the first International Workshop and Conference on Human Leukocyte Differentiation Antigens (HLDA) in Paris, 1982. To date, more than 370 CDs have been identified and many are widely used in basic research, in the diagnosis of immunodeficiencies and neoplasms, and in the monitoring and treatment of diseases [8]. In 2010, new monoclonal antibodies were submitted to the 9th HLDA Workshop, and few studies have demonstrated the normal expression patterns of these markers in PB and BM samples. As neoplastic cells may present changes in the phenotypic expression of some antigens, these markers were identified as possible new targets for research and, mainly, as new tools for the differential diagnosis of mature B cell neoplasms [9,10]. Of all monoclonal antibodies submitted to the 9th HLDA Workshop, seven were selected and evaluated in this study: CD86, CD210a, CD261, CD262, CD264, CD358, and CD361. They were selected for this study because previous studies have shown that these antibodies are reactive with hematopoietic cell lineages [9,10]. The objective of this study was to determine normal patterns of cell expression for these markers in PB and BM samples by comparing the mean fluorescence intensity (MFI) of stained and unstained cells (negative control).

2. Subjects and methods

2.1. Samples

For the evaluation by flow cytometry, eight samples of BM aspirate (males 36.4 ± 25.1 years and females 23.3 ± 5.3 years) and 10 samples of PB (males 27.8 ± 3.7 years and females 31.4 ± 7.6 years) were analyzed. BM samples were obtained from patients with suspected hematological malignancies not confirmed by immunophenotypic screening. Samples were collected in EDTA at the University Hospital of the Federal University of Santa Catarina and at the Hematology and Hemotherapy Center of Santa Catarina, Brazil, after patients had signed the informed consent form according to the local Ethics Committee (CEPSH-UFSC no. 746.486/2014).

2.2. Monoclonal antibodies

The panel of monoclonal antibodies used for labeling PB samples comprised CD8 FITC, CD3 PercP, CD19 Pe-Cy7, CD38 APC-H7, CD4 Pacific Blue, CD20 V450, and CD45 Pacific Orange and the panel used for labeling BM samples comprised CD34 PercP, CD19 Pe-Cy7, CD10 APC, CD38 APC-H7, CD20 Pacific Blue, and CD45 Pacific Orange (Table 1). All MoAbs investigated in this study (CD86, CD210a, CD261, CD262, CD264, CD358, and CD361) were conjugated with the

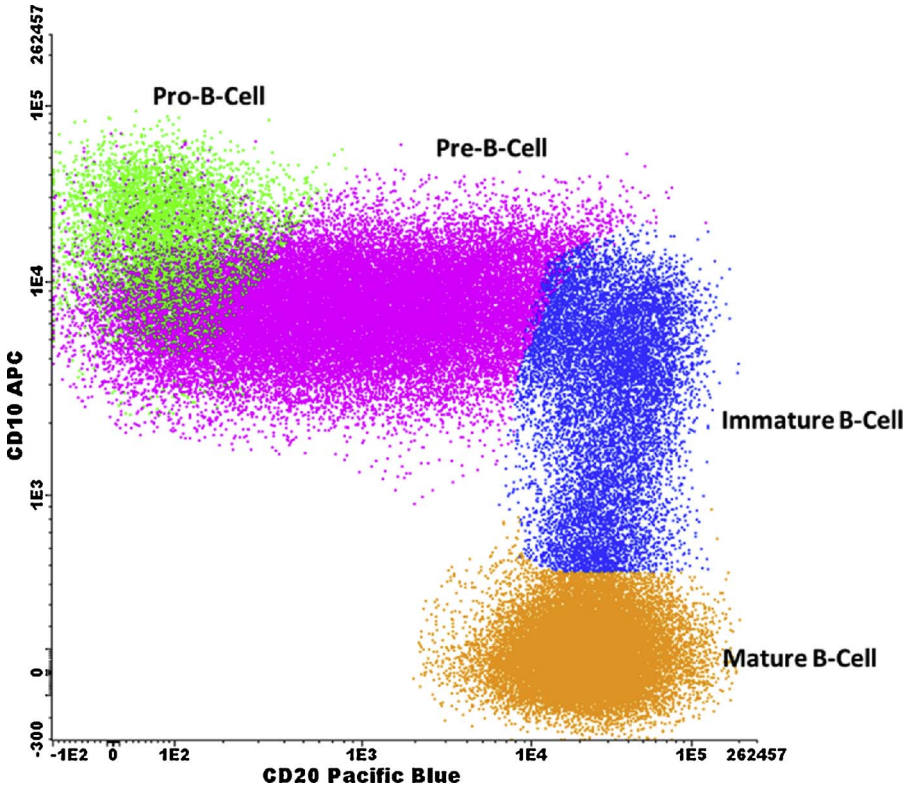

Fig. 1. Representative figure of the analysis of the maturation stages of B lymphocytes according to CD20 and CD10 expression.
Representative dot-plot of CD20 Pacific Blue versus CD10 APC showing B cells (CD19+) maturation stages in which pro-B cells are CD34+, CD10++ and CD20-; pre-B cells are CD34-, CD10++, CD20-/++; immature B cells are CD34-, CD10+ weak and CD20++; and mature B cells are CD34-, CD10- and CD20++.

Table 3
MFI and statistical significance (*p*-value) of cell types evaluated in PB samples.

Cell type	MFI	MFI	p	MFI	p	MFI	p	MFI	p	MFI	p	MFI	p	MFI	p
	NC	CD86		CD210a		CD261		CD262		CD264		CD358		CD361	
Eosinophils	523	602	0.016*	673	< 0.0001*	575	0.060	635	0.008*	588	0.296	661	0.002	6629	< 0.0001*
Neutrophils	121	113	0.517	265	< 0.0001*	134	0.585	207	< 0.0001*	97	0.019*	197	< 0.001*	11096	< 0.0001*
Monocytes	94	1046	< 0.0001*	394	< 0.0001*	121	0.123	242	< 0.0001*	71	0.035*	165	< 0.001*	9341	< 0.0001*
Basophils	57	53	0.353	132	< 0.0001*	84	1.000	75	0.028*	36	0.023*	109	0.315	2809	< 0.0001*
B lymphocytes	37	168	< 0.0001*	140	0.001*	128	0.011*	139	0.001*	95	0.003*	208	< 0.001*	3351	< 0.0001*
T lymphocytes	43	43	0.739	231	< 0.0001*	99	0.023*	72	< 0.0001*	30	0.190	76	< 0.001*	988	< 0.0001*
NK cells	47	45	0.912	237	< 0.0001*	57	0.481	70	0.005*	39	0.105	73	0.063	637	< 0.0001*

NC: negative control (unstained cells)

Table 4
MFI and statistical significance (p-value) of cell types evaluated in BM samples.

Cell type	MFI	MFI	p	MFI	p	MFI	p	MFI	p	MFI	p	MFI	p	MFI	p
	NC	CD86		CD210a		CD261		CD262		CD264		CD358		CD361	
Eosinophils	580	1136	0.038*	2730	< 0.0001*	1366	0.059	795	0.021*	705	0.334	2614	0.007*	11689	0.007*
Neutrophilic series	166	242	0.038*	593	< 0.0001*	315	0.005*	468	0.001*	191	0.161	539	0.001*	12379	< 0.0001*
Monocytes	135	1598	< 0.0001*	385	< 0.0001*	433	0.003*	489	< 0.0001*	135	0.161	504	< 0.0001*	15687	< 0.0001*
Plasma cells	228	597	< 0.0001*	1221	0.0011*	1821	< 0.0001*	447	0.002*	535	0.065	560	< 0.0001*	19732	< 0.0001*
B lymphocytes	87	131	0.208	227	0.003*	194	0.007*	153	0.047*	116	0.105	187	0.007*	2999	< 0.0001*
B-I lymphocytes	54	84	0.096	395	0.023*	176	< 0.0001*	126	0.001*	79	0.268	206	< 0.0001*	2388	< 0.0001*
B-II lymphocytes	86	122	0.310	295	0.038*	247	< 0.0001*	158	0.033*	116	0.384	196	0.008*	2634	< 0.0001*
B-III lymphocytes	97	148	0.111	271	0.007*	207	0.004*	157	0.046*	135	0.313	229	0.001*	4619	< 0.0001*
B-IV lymphocytes	100	177	0.161	222	0.032*	164	0.182	169	0.145	130	0.546	203	0.061	3259	< 0.0001*
T/NK lymphocytes	51	84	0.010*	186	< 0.0001*	84	0.015*	110	0.003*	68	0.105	117	0.005*	1539	< 0.0001*

NC: negative control (unstained cells).

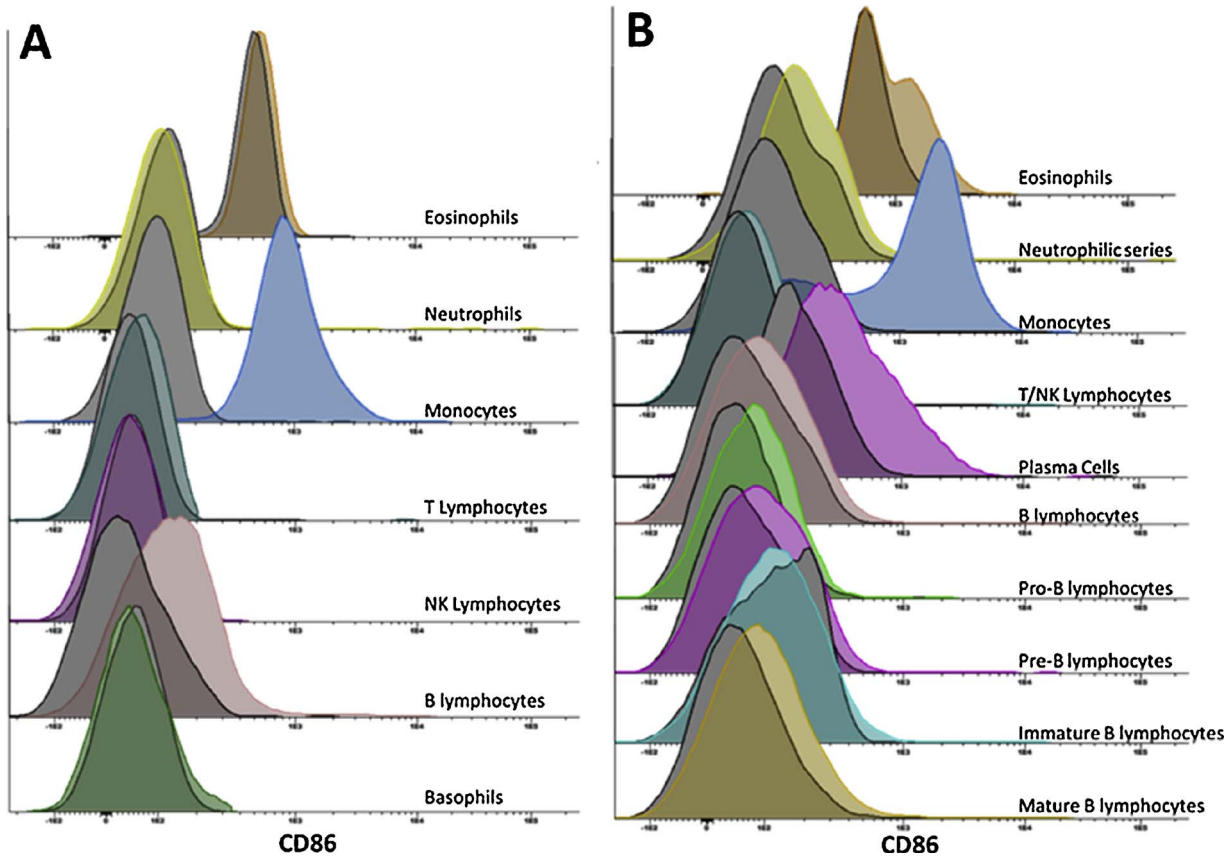

Fig. 2. Histograms demonstrating the expression of CD86 in PB and BM cell types.
Grey histograms represent unstained cells and colored histograms represent cells stained with CD86. (A) PB samples: histograms showing the expression of CD86 in eosinophils, neutrophils, monocytes, T lymphocytes, B lymphocytes, NK cells and basophils. (B) BM samples: histograms showing the expression of CD86 in eosinophils, neutrophilic series, monocytes, T/NK lymphocytes, plasma cells and B lymphocytes included for maturation stages (pro-B, pre-B, immature B cell and mature B cell).

fluorochrome Pe.

2.3. Immunophenotyping by flow cytometry

Briefly, 100 μL of each sample was incubated with the MoAbs for 15 min at room temperature and protected from light. After this period, erythrocytes were lysed with the lysing solution (BD FACS Lysing Solution diluted 1/10) and incubated at room temperature in the dark for another 10 min. Samples were centrifuged for 5 min at 300*g*, and the supernatant was discarded. The pellet was suspended in PBS saline (Laborclin, Brazil) to adjust cell suspension to 1×10^6 cells/500 μL.The acquisition of 50,000 events was performed on a BD FACS Canto II (Becton Dickinson – BD, San Jose, USA) flow cytometer using the software BD FACS Diva version 6.1.2 (BD, San Jose, USA). Data analysis was performed using the software Infinicyt version 1.7.1 (Cytognos, Salamanca, Spain). The threshold was set at 33,000 on the forward-scatter channel (FSC) to avoid, as much as possible, losing small lymphocytes as their sizes can be very close to that of debris (particles smaller than cells), dead cells, and non-lysed red blood cells. For the analysis, files were merged using the Infinicyt software to overlap the parameters of interest and generate histograms and cell MFIs. Granulocytes, eosinophils, and monocytes were identified by their forward- and side-scatter characteristics, whereas other cell types were identified by the expression of specific markers based on the phenotypes described in Table 2. The combination of CD20, CD10, CD34, and CD19 (pan-B) is well established for the analysis of B cell differentiation by separating them into four subpopulations according to the loss of CD10 expression and acquisition of CD20 expression [11] (Fig. 1).

2.4. Quality control

Instrument setup, calibration, and quality control were performed daily during the study period using the commercial standard reagents Cytometer Setup and Tracking Beads (BD Biosciences, USA) and Immunotroll Cells® (Beckman Coulter, USA). In addition, fluorochrome compensation was standardized and checked periodically according to Euroflow Consortium recommendations [12]. Fluorescence-minus-one (FMO) control was also performed [13].

2.5. Statistical analysis

Statistical analysis was carried out using the software SPSS version

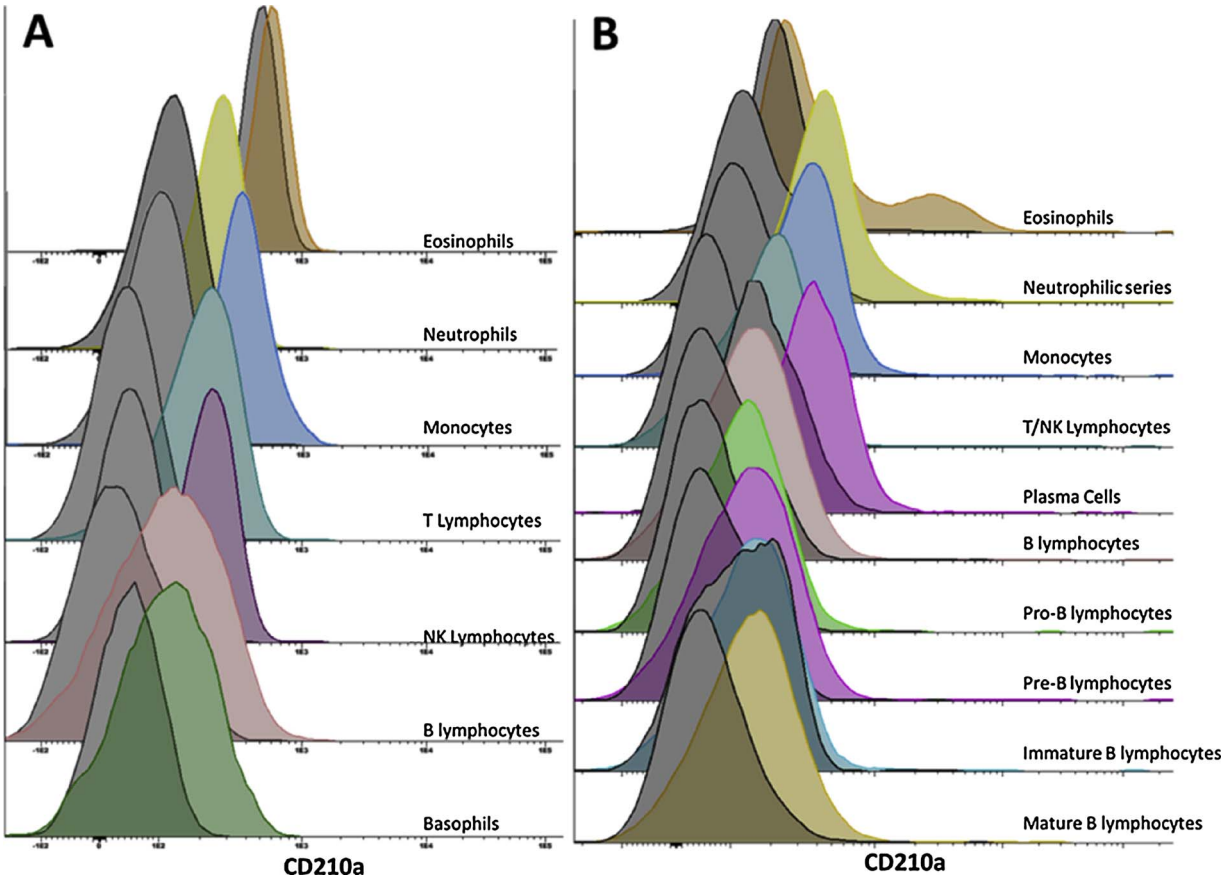

Fig. 3. Histograms demonstrating the expression of CD210a in PB and BM cell types.
Grey histograms represent unstained cells and colored histograms represent cells stained with CD210a. (A) PB samples: histograms showing the expression of CD210a in eosinophils, neutrophils, monocytes, T lymphocytes, B lymphocytes, NK cells and basophils. (B) BM samples: histograms showing the expression of CD210a in eosinophils, neutrophilic series, monocytes, T/NK lymphocytes, plasma cells and B lymphocytes included for maturation stages (pro-B, pre-B, immature B cell and mature B cell).

22.0 (USA). In all cases, differences were considered statistically significant when $p \leq .05$. The MFI of stained and unstained cells were compared using the *t*-test for parametric data and the Mann–Whitney test for non-parametric data.

3. Results and discussion

Tables 3 and 4 show the mean MFI of all samples and the statistical significance of the expression of each marker in the different cell types analyzed in PB and BM samples. These Tables present the statistical analysis comparing MFI of stained and unstained cells of the same type (negative control; NC). Although the expression of some markers (MFI) in different cells showed statistical significance ($p < .05$) when compared with the MFI of their NC, it was not always possible to observe these differences in histograms. For example, difference in the expression of CD86 and CD210a in eosinophils in relation to NC could not be observed in the histograms, but when MFI values were compared, they were statistically different. However, in the majority of cases, the statistical difference was consistent with the results observed in histograms.

3.1. CD86 expression in monocytes and B lymphocytes in PB and in monocytes and plasma cells in BM

CD86 is a costimulatory molecule whose pathway is well characterized and involves CD28/CTLA-4 receptors present on T cells. In humans, CD28 is constitutively expressed on 95% of CD4+ T cells and 50% of CD8+ T cells [14]. Some studies show that CD86 is expressed in low intensity in non-activated dendritic cells [15] and constitutively in monocytes/macrophages [16] and mature germ-center B cells [9]. The expression of CD86 on antigen-presenting cells (APCs) is enhanced by the presence of pathogens and by cytokines that are produced in response to these pathogens, resulting in T-cell activation. The expression of CD86 is primarily limited to APCs; however, CD86 has recently been shown to be expressed on T cells, but the functional significance of its expression is not well understood [17]. In the present study, it was possible to visualize positive expression only in monocytes and B lymphocytes (weak expression) in PB samples (Fig. 2A). These data corroborate the results reported by Vasilevko and colleagues [16] and Llinàs and colleagues [9]. In BM samples, expression of CD86 was observed in monocytes and in plasma cells (Fig. 2B).

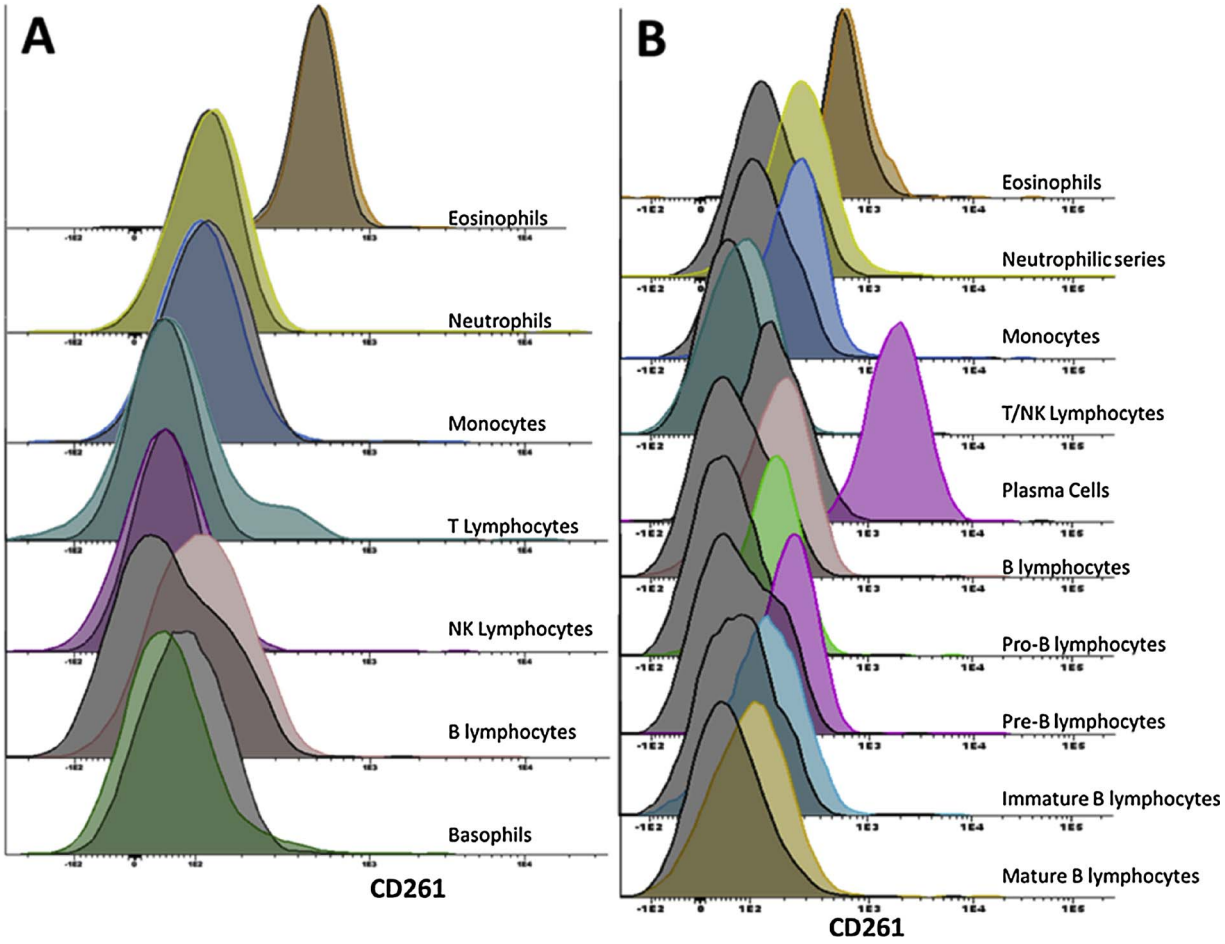

Fig. 4. Histograms demonstrating the expression of CD261 in PB and BM cell types.
Grey histograms represent unstained cells and colored histograms represent cells stained with CD261. (A) PB samples: histograms showing the expression of CD261 in eosinophils, neutrophils, monocytes, T lymphocytes, B lymphocytes, NK cells and basophils. (B) BM samples: histograms showing the expression of CD261 in eosinophils, neutrophilic series, monocytes, T/NK lymphocytes, plasma cells and B lymphocytes included for maturation stages (pro-B, pre-B, immature B cell and mature B cell).

3.2. CD210a expression in most hematopoietic cells

Interleukin 10 (IL10) has been shown to stimulate B-cell survival, proliferation, and differentiation [18]. However, the main functions of IL10 in immunity are immunosuppression, inhibition of pro-inflammatory cytokines synthesis, and down-regulation of MHC and costimulatory molecules [19,20]. These functions are mediated by the interleukin 10 receptor (IL10R), which consists of two subunits, IL10Rα and IL10Rβ [21]. IL10Rα is also known as CD210a, whose extracellular region contains two fibronectin type III domains [10]. Most hematopoietic cells constitutively express low levels of CD210a [22], specifically monocytes, monocytoid dendritic cells, and T and B lymphocytes (pro-B, pre-B, immature, mature, germinal center, and memory cells) [10]. In another study conducted by Llinàs and colleagues [9], it was observed that germinal center B cells express CD210a, but this marker was absent in other B cell populations [9]. In PB samples (Fig. 3A), monocytes and NK cells showed weak expression, and neutrophils, T lymphocytes, B lymphocytes, and basophils, in addition to weak expression, showed partial positivity. In BM samples (Fig. 3-B), partial expression was observed for eosinophils. Monocytes and B and T/NK lymphocytes presented weak expression, corroborating data from Matesanz-Isabel and colleagues [10]. Weak expression in neutrophilic series and plasma cells was also observed, contrary to the results of Matesanz-Isabel and colleagues [10], in which expression in these cell types was absent. When evaluating B cell maturation, weak expression of CD210a was observed for all stages, except for immature B cells, which had no expression of this marker. This result differs from the reported by Matesanz-Isabel and colleagues [10], who found weak positivity of CD210a in all B maturation stages.

3.3. Expression of TRAIL receptor family in leukocytes

Apoptosis can be controlled by two pathways, the extrinsic (receptor-mediated) pathway and the intrinsic (mitochondria-mediated) pathway. Numerous ligands of the tumor necrosis factor (TNF) superfamily are involved in apoptosis. A member of this family is TRAIL/Apo2L/TNFSF10. Failure to undergo apoptosis has been implicated in tumor development and resistance to cancer therapy. Tumor necrosis factor-related apoptosis-inducing ligand (TRAIL) is characterized as a powerful activator of programmed cell death in tumor cells with no

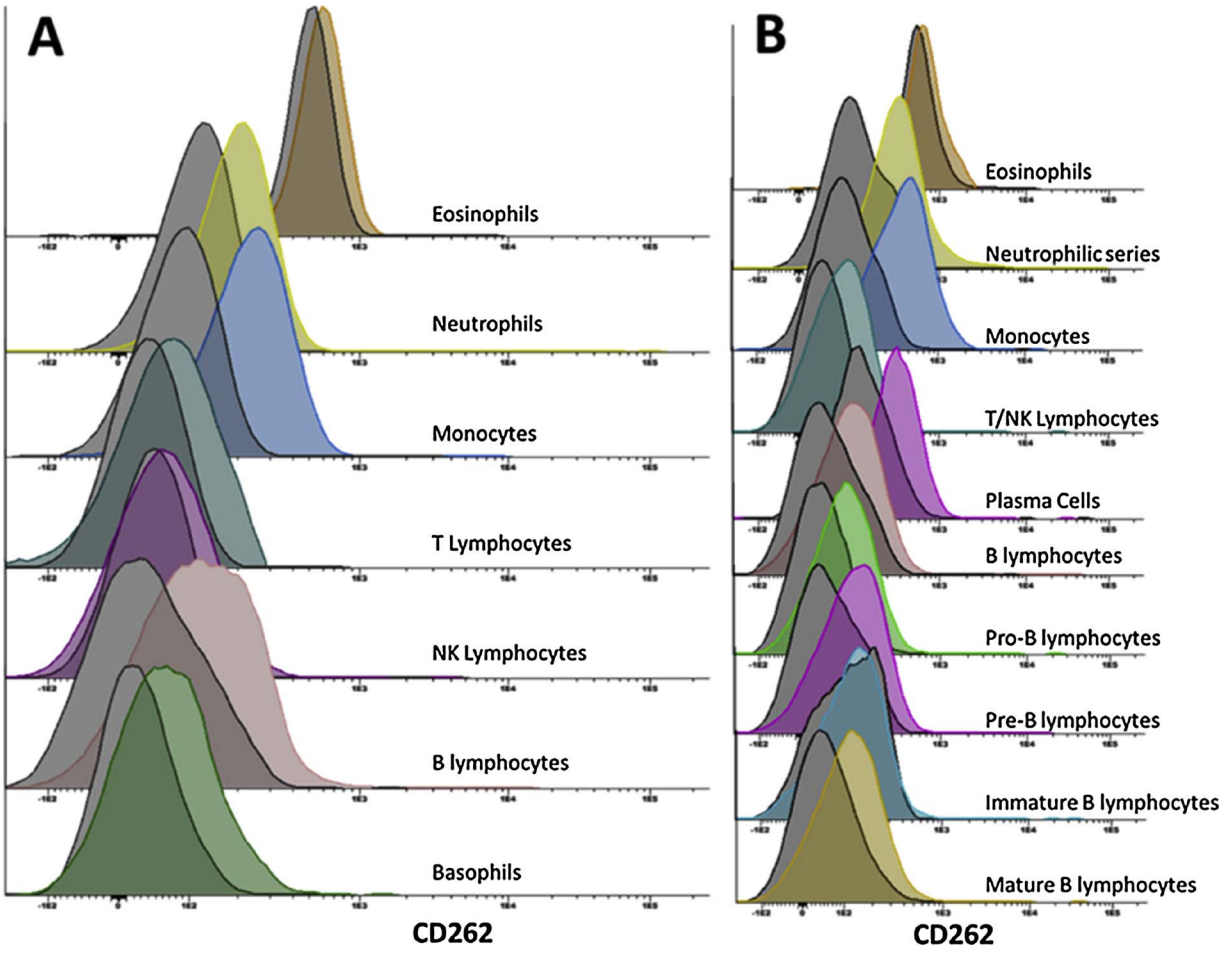

Fig. 5. Histograms demonstrating the expression of CD262 in PB and BM cell types.
Grey histograms represent unstained cells and colored histograms represent cells stained with CD262. (A) PB samples: histograms showing the expression of CD262 in eosinophils, neutrophils, monocytes, T lymphocytes, B lymphocytes, NK cells and basophils. (B) BM samples: histograms showing the expression of CD262 in eosinophils, neutrophilic series, monocytes, T/NK lymphocytes, plasma cells and B lymphocytes included for maturation stages (pro-B, pre-B, immature B cell and mature B cell).

toxicity against normal tissues. TRAIL binds to five receptors: four type I transmembrane proteins, TRAIL-R1 (CD261, DR4), TRAIL-R2 (CD262, DR5), TRAIL-R3, and TRAIL-R4, and one soluble receptor (OPG) [23]. The TRAIL receptor family is related to several cellular events, such as inflammation, septic shock, and apoptosis. CD261 and CD262 are type 1 transmembrane proteins related to apoptosis induction [24], as they contain death domains (DD) through which TRAIL transmits an apoptotic signal. TRAIL triggers apoptosis via interaction with TRAIL death receptors present in target cells. These intracellular DD of TRAIL-R1 and TRAIL-R2 have been found to be essential for induction of apoptosis following receptor ligation [23]. In the present study, no cell type evaluated in PB showed positivity for CD261 (Fig. 4A). In BM samples, there was very weak expression of CD261 in neutrophilic series, monocytes, and B lymphocytes (Fig. 4B). However, plasma cells showed positivity with a homogeneous expression, differently from that observed in a study by Llinàs and colleagues [9], in which these cells were considered negative. Thus, the expression of this marker could be useful in the diagnosis of plasma cell neoplasms. Regarding B cell maturation stages, very weak expression of CD261 was observed in all B cell stages. CD262 (TRAIL-R2) is also a type 1 transmembrane protein related to the induction of apoptosis [24]. Regarding CD262, there was weak expression in monocytes, neutrophils, and B lymphocytes (Fig. 5A). Llinàs and colleagues observed that expression of CD262 was only slightly positive in memory B cells [9]. In BM samples, weak expression in monocytes, B lymphocytes, plasma cells, and in pro-B, pre-B, and mature B cells was also observed (Fig. 5B). Another apoptosis-related protein, CD264, was analyzed. CD264 (TRAIL-R4) is a transmembrane protein whose function is related to the inhibition of apoptosis [24]. For lacking a functional DD, it acts as an antagonist receptor competing with the death receptors TRAIL-R1 and TRAIL-R2 for binding to TRAIL. Therefore, this receptor is involved in the negative regulation of apoptosis by sequestering TRAIL and stimulating pro survival signals [23]. The evaluation of CD264 showed that there was no expression in PB cells of healthy subjects, except in B cells, which presented very weak expression (Fig. 6A). Llinàs and colleagues [9] observed expression of CD264 with moderate intensity in unswitched memory B cells

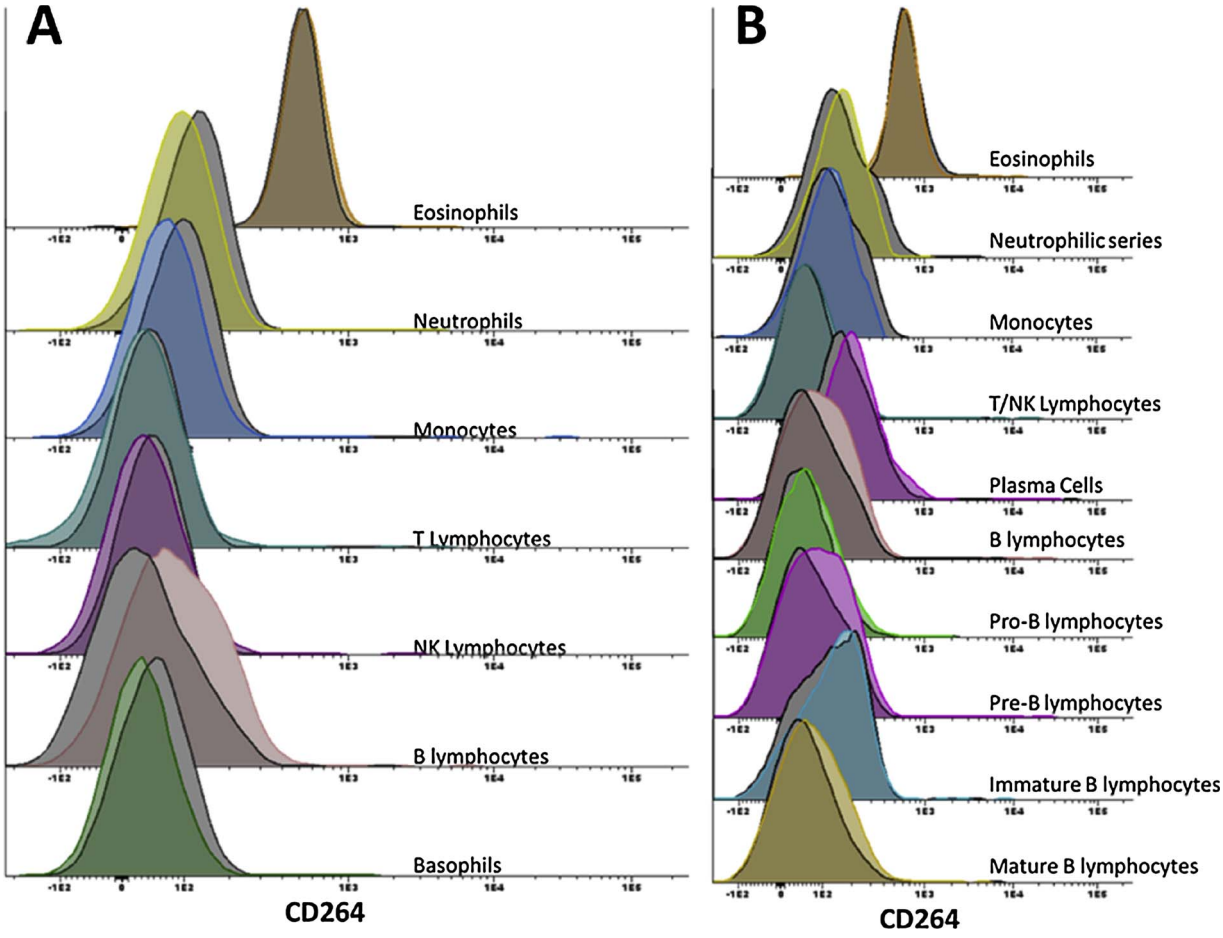

Fig. 6. Histograms demonstrating the expression of CD264 in PB and BM cell types.
Grey histograms represent unstained cells and colored histograms represent cells stained with CD264. (A) PB samples: histograms showing the expression of CD264 in eosinophils, neutrophils, monocytes, T lymphocytes, B lymphocytes, NK cells and basophils. (B) BM samples: histograms showing the expression of CD264 in eosinophils, neutrophilic series, monocytes, T/NK lymphocytes, plasma cells and B lymphocytes included for maturation stages (pro-B, pre-B, immature B cell and mature B cell).

and pre-germinal B cells [9]. CD264 was not expressed in BM cells (Fig. 6B).

3.4. CD358 is widely expressed in hematopoietic cells

CD358 is a type 1 transmembrane receptor, member 21 of the tumor necrosis factor receptor superfamily (TNFSF21) [9],10]. Over-expression of CD358 enables its cytoplasmic death domain to induce apoptosis and triggers the NF-κB and c-Jun N-terminal kinase (JNK) pathways [2]. It can also promote apoptosis mediated by BAX and by the release of cytochrome c from the mitochondria into the cytoplasm [25]. According to Matesanz-Isabel and colleagues [10] and Llinàs and colleagues [9], this receptor is widely expressed in hematopoietic and non-hematopoietic cells, including tumor cells. In PB, it is present mainly in monocytes and, to a lesser extent, in B and T lymphocytes [9],10]. In the present study, very weak expression of CD358 was observed in neutrophils, monocytes, and B lymphocytes in PB and BM samples (Fig. 7A and B). In addition, in BM samples, plasma cells and T lymphocytes showed weak expression. In relation to B cell maturation stages, there was weak expression in pro-B, pre-B, and mature B cells. In contrast, in the study by Matesanz-Isabel and colleagues [10] and Llinàs and colleagues [9], CD358 expression was observed in pro-B, pre-B, and immature cells in BM samples, suggesting that this molecule might be involved in B-cell development during the initial stages.

3.5. CD361 shows expression in all leukocyte cells analyzed

CD361 (EVI2b: ectopic viral integration site 2B) is a type 1 transmembrane protein [10] found in non-immune cells and with broad leukocyte expression but poorly characterized whose function remains unknown [26]. Matesanz-Isabel and colleagues [10] observed that CD361 is expressed in mature B cells, T lymphocytes, monocytes, granulocytes, NK cells, and plasmacytic dendritic cells. In the present study, it was possible to observe expression in all analyzed cell types in PB (eosinophils, neutrophils, B and T lymphocytes, NK cells, and basophils) (Fig. 8A). Expression of CD361 was also observed in all

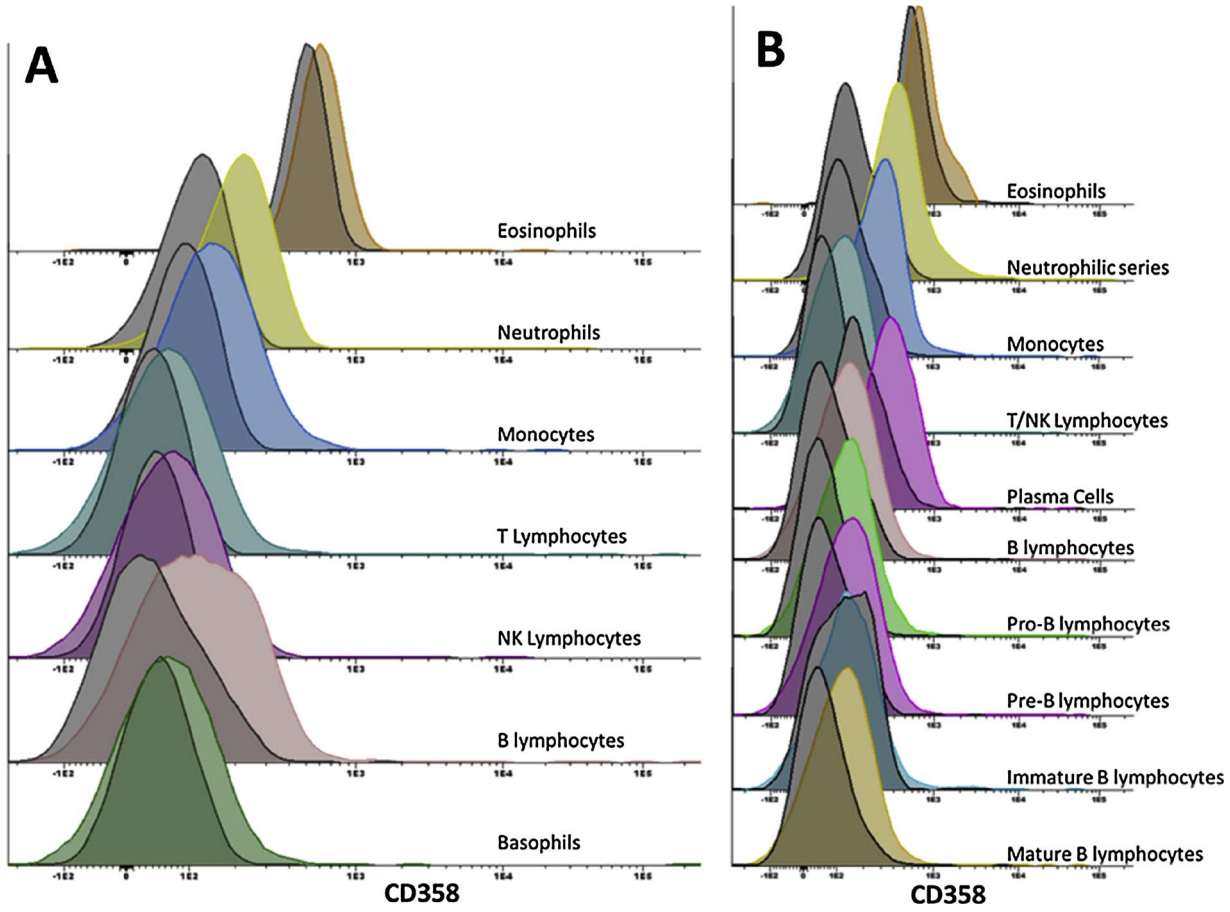

Fig. 7. Histograms demonstrating the expression of CD358 in PB and BM cell types.
Grey histograms represent unstained cells and colored histograms represent cells stained with CD358. (A) PB samples: histograms showing the expression of CD358 in eosinophils, neutrophils, monocytes, T lymphocytes, B lymphocytes, NK cells and basophils. (B) BM samples: histograms showing the expression of CD358 in eosinophils, neutrophilic series, monocytes, T/NK lymphocytes, plasma cells and B lymphocytes included for maturation stages (pro-B, pre-B, immature B cell and mature B cell).

analyzed cell types in BM samples, including all B cell maturation stages (Fig. 8B). These data corroborate those found by Matesanz-Isabel and colleagues [10], in which expression of CD361 was found in all B cells (pre-B, immature, mature, germinal center, memory, and plasma cells), T lymphocytes, monocytes, granulocytes, NK cells, and plasmacytic dendritic cells. CD45 is considered a common leukocyte antigen (pan-leukocyte) because it is expressed only in cells of the hematopoietic system. All hematopoietic cells, with the exception of platelets and mature erythrocytes, are CD45 positive [27]. CD361 also showed expression in all leukocyte cells present in PB and BM samples. Comparison of CD45 and CD361 expressions can be observed in Fig. 9.

The analyzed markers are not expressed in a single lineage but in several leukocyte cell lines evaluated in PB and BM samples. The results of the present study differ from data presented in other studies for some markers, mainly CD210a and CD261. Further research is needed to evaluate these molecules in relation to their applicability in the diagnosis of hematological malignancies, including the differential diagnosis of subtypes with phenotypic overlap, such as some mature B cell neoplasms. Furthermore, since these molecules are expressed on the cell surface, some may prove to be new therapeutic targets for the treatment of hematological neoplasms or autoimmune diseases, as has been proven for other CD molecules.

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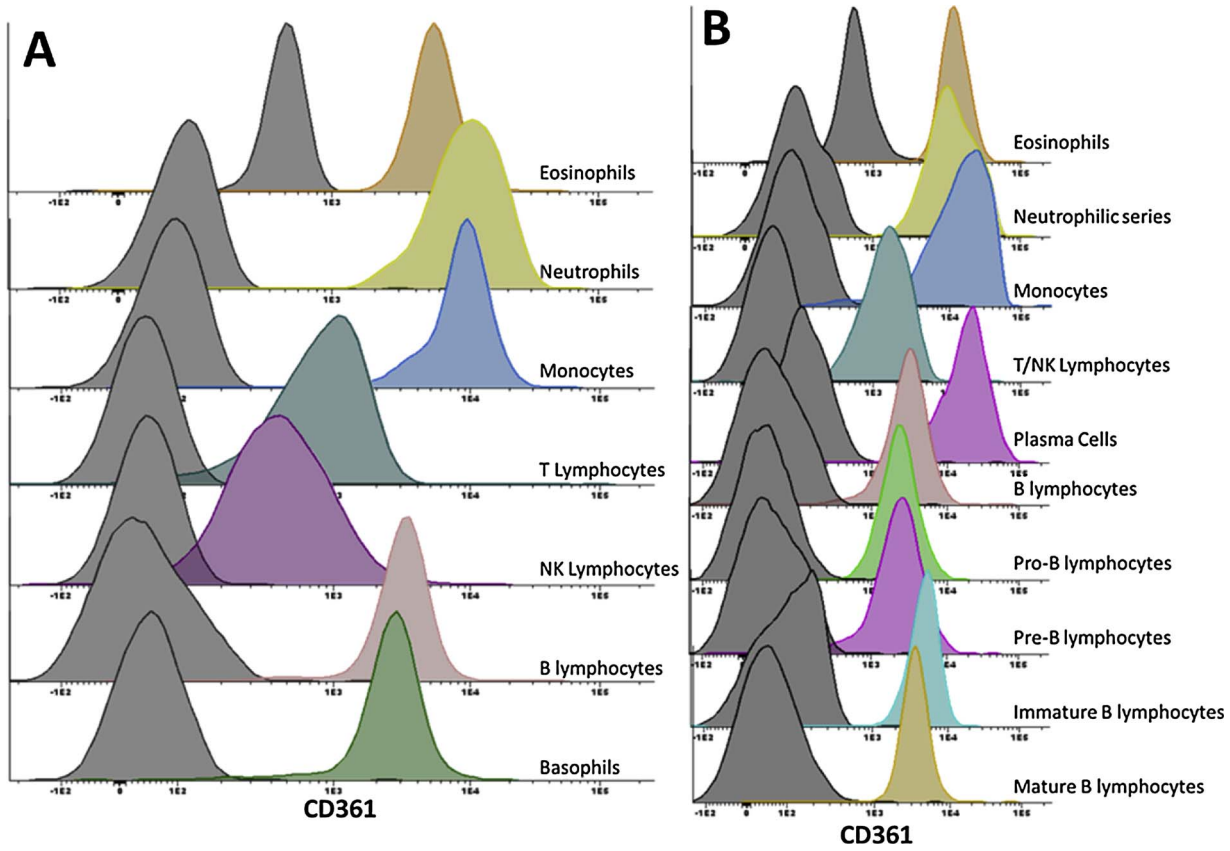

Fig. 8. Histograms demonstrating the expression of CD361 in PB and BM cell types.
Grey histograms represent unstained cells and colored histograms represent cells stained with CD361. (A) PB samples: histograms showing the expression of CD361 in eosinophils, neutrophils, monocytes, T lymphocytes, B lymphocytes, NK cells and basophils. (B) BM samples: histograms showing the expression of CD361 in eosinophils, neutrophilic series, monocytes, T/NK lymphocytes, plasma cells and B lymphocytes included for maturation stages (pro-B, pre-B, immature B cell and mature B cell).

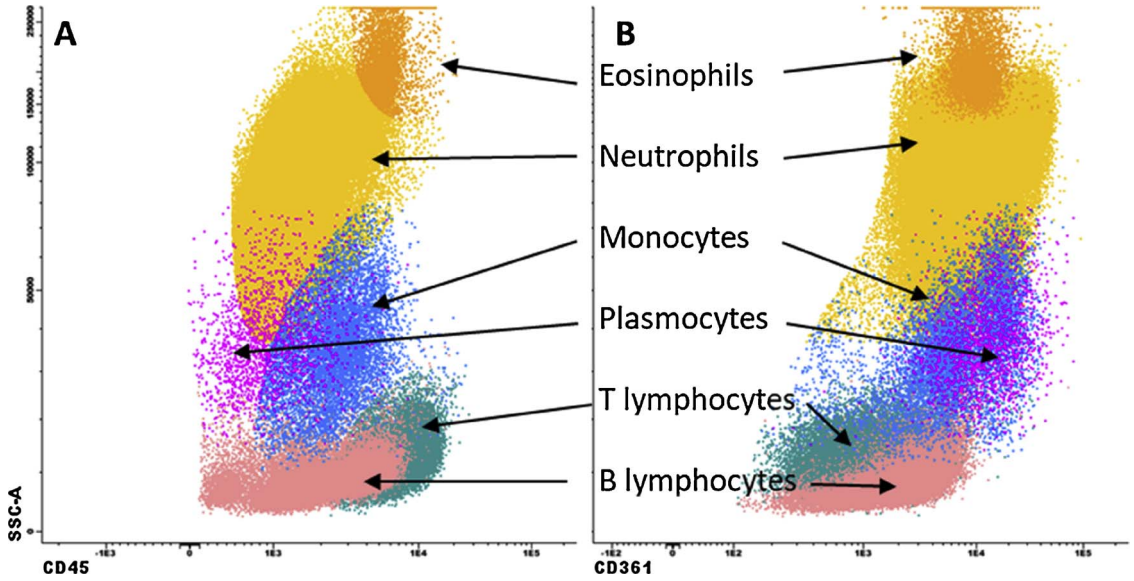

Fig. 9. Comparison between expression profile of CD45 and CD361 in leukocytes of BM samples.
(A) Dot-plot showing the expression of CD45 in the leukocyte cells in the BM samples;
(B) Dot-plot showing the expression of CD361 in the leukocyte cells in the BM samples.

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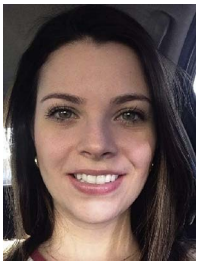

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