original paper

haematologica 2001; 86:1261-1269
http://www.haematologica.it/2001_12/1261.htm

Interleukin-3 receptor α chain (CD123) is widely expresssed in hematologic malignancies

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Background and Objectives. The hematopoietic system is controlled by growth factors (cytokines) which can influence cell survival, proliferation, differentiation and functional activation. The study of cytokine receptor expression by flow cytometry could allow us to differentiate between normal and tumoral cells.

Design and Methods. We analyzed the expression of the interleukin-3 (IL-3) receptor α chain (CD123) in 22 normal samples and in a wide panel of hematologic malignancies using flow cytometry. We found that CD123 was expressed in the myeloid progenitor subpopulation but in contrast, normal lymphoid progenitors lacked CD123. We analyzed the CD123 expression pattern in 64 patients with acute leukemia, 45 with acute myeloid leukemia (AML) and 19 with acute lymphocytic leukemia (ALL) (13 B-cell lineage ALL and 6 T-cell lineage ALL).

Results. All the AML cases except two patients with M7 and all the B-cell lineage ALL patients were CD123 positive. In contrast, all the T-cell lineage ALL cases tested were CD123 negative. We also studied the CD123 expression pattern in 122 patients with a B-cell chronic lymphoproliferative disease (B-CLPD). CD123 was positive in three situations: 1) typical cases of hairy cell leukemia showed a specific, strong CD123 expression, 2) a subgroup of atypical chronic lymphocytic leukemia with a marked CD11c expression was also CD123 positive, and finally 3) transformed B-CLPD showed the phenomenon of transition from CD123 negativity to CD123 positivity simultaneuosly with morphologic changes.

Interpretation and Conclusions. In summary, our data show high expression of IL-3 receptor α chain in hematologic malignancies. Given the high frequency of CD123 reactivity in blast cells in contrast to in normal precursors, this antigen could be applied to the study of minimal residual disease in acute leukemia. CD123 is expressed with a characteristic pattern in cases of hairy cell leukemia.

Key words: acute leukemia, B-cell lymphoproliferative disorders, CD123 expression, IL-3 receptor α chain, hairy cell leukemia, minimal residual disease.

Normal hematopoiesis is a multistep process in which lineage development occurs as a consequence of the ordered effect of a number of growth factors and of the expression of determined transcription factors. Interleukin- 3 (IL-3), like other cytokines, stimulates cell cycle progression, and differentiation, whilst inhibiting apoptosis of hematopoietic cells. The mechanisms by which cytokines transduce their regulatory signals have been evaluated by identifying the involvement of specific protein kinase cascades and their downstream transcription factor targets.[1-3] IL-3 induces tyrosine phosphorylation. This stimulation results in the activation of JAK proteins.[4] The Jak activation is capable of recruiting and activating different STATs. The activated STAT molecules move to the nucleus and induce the transcription of regulated genes.[5] Some of the genes which are induced by STAT stimulate proliferation, whereas others serve to inhibit the Jak/STAT signal transduction pathway. IL-3 can also induce anti-apoptotic pathways which can result in the phosphorylation of pro-apoptotic proteins.[1] Identification and characterization of these pathways have considerably improved our knowledge of leukemogenesis.[1,6-9]

The normal hematopoietic process is characterized by sequential expression of surface markers which allows recognition of various stages of cell differentiation and assessment of commitment to different lineages.[10] The earliest stem cell with the ability of self-renewal seems to be $CD34^-$ $CD45^+$. On activation this cell can acquire $CD34^+$ and oth-

er signal-regulatory proteins such as CD90, AC133 and CD117. Antigen expression is probably a gradually increasing or decreasing process reflecting the fluent maturation progress and at the end of this process differentiated cells display distinct patterns of antigen expression.[11] Acute myeloid leukemias (AMLs) are considered to be clonal disorders involving early hematopoietic progenitor cells. Differences and similarities in phenotype, genotype and biology are described for leukemic cells and normal hematologic progenitors. One potential difference between normal and leukemic cells lies in their response to hematopoietic growth factors.[12] Recently, CT Jordan *et al.* have shown that the IL-3α chain receptor (CD123) is a unique marker for human acute myelogenous leukemia stem cells.[13] The differences in the phenotype between leukemic cells and normal hematologic progenitors can be used in minimal residual disease (MRD) analysis.[14]

We analyzed CD123 expression in peripheral blood and in bone marrow from healthy donors and its expression in different types of neoplastic cells in order to distinguish better the phenotypic properties of normal and tumoral cells and to define new monoclonal antibody combinations which could be used in MRD studies.

Design and Methods

Patients

Bone marrow (BM) n=12 , peripheral blood (PB) n=7 and non-neoplastic lymph nodes (LN) n=3, from healthy donors were used as controls. Sixty-four BM samples from patients with acute leukemia (AL) were analyzed for the expression of the IL-3α chain receptor (IL-3αR). The patients were categorized as follows: 45 with acute myeloid leukemia (AML) and 19 with acute lymphoblastic leukemia (ALL), 13 with B-cell lineage ALL and 6 with T-cell lineage ALL. The diagnosis of AL was based on standard morphologic and immunophenotypic criteria.[15,16] We also studied 122 samples of BM, PB or lymph nodes from patients with B-cell chronic lymphoproliferative disorders (B-CLPD). The diagnosis of the subgroup of B-CLPD was made in accordance with the REAL recommendations:[17] 77 chronic lymphocytic leukemia (CLL), 12 mantle-cell lymphoma, 5 follicular lymphoma, 7 hairy cell leukemia, 2 Burkitt´s lymphoma and 20 other different B-cell CLPD. For every case the immunophenotype score was calculated following the recommendations of Matutes *et al.*[18]

Flow cytometry analysis

Sample preparation. The number of cells was quantified by microscopy and adjusted to 2×10^6 in each tube. The immunophenotypic analysis was performed on lysed whole BM samples with direct conjugated monoclonal antibodies (MoAbs). Antigen expression was analyzed using triple combinations of the following MoAbs conjugated with fluorescein isothiocyanate (FITC), phycoerythrin (PE), peridinine chlorophyll protein (PerCp) or phycoerythrin-cyanine 5 (PE/Cy 5) fluorochrome tandem. The MoAbs used in the study were: CD22 (4KB128 FITC), glycophorin A (JC 159 PE), CD41 (5B 12 PE), IgM (rabbit anti-human, PE), CD79a (HM57 PE) and TDT (HT-6 FITC) from DAKO, Glostrup, Denmark; CD15 (MMA-FITC), CD34 (8G12-FITC, PE), HLA-Dr (L243 PetCp), CD10 (W8E7 FITC), CD 20 (L27 PE), CD2 (S5.2 FITC), CD33 (67.6 PE), CD7 (4H9 FITC), CD45 (2D1 PerCp), CD13 (L138 PE), CD14 (M0P9 FITC), CD3 (SK7 PerCp), CD4 (Leu 3 FITC), CD5 (Leu 1 FITC), CD8 (Leu 2 PE) purchased from Becton Dickinson, San José, CA, USA (BDIS); CD19 (SJ25-C1 PE/Cy 5) and MPO (H-43-5 FITC) from Caltag Laboratories, Burlingame, CA, USA; CD 123 (9F5 PE), CD10 (HI10a, Cy-Chrome) from Pharmingen, San Diego, CA, USA; CD36 (FAG-52 FITC) from Immunotech, Marseille, France.

In B-CLPD samples a different panel of monoclonal antibodies was used to study the complete immunophenotype of each clonal sample: FMC7-FITC (Harlan SeraLab, Sussex), CD103-FITC (Immunoquality, Groningen), CD-10 PE-Cy5, CD123 (9F5 PE), (Pharmingen, San Diego, CA, USA), CD19-RPe/Cy5, CD22-FITC and CD79b-FITC (Dako), CD5-PE, CD23-PE, CD25-PE, CD20-PE, CD10-FITC, CD11c-PE, CD10-FITC, CD11c-PE (Becton Dickinson, San José, CA, USA). The clonality study of B-lymphocytes was undertaken using a triple reagent consisting of a combination of κ-FITC, λ-PE and CD19 PE/Cy5 in a single tube (K/L, Simultest® purchased from Becton Dickinson, San José, CA, USA and CD19-PE/Cy5 from Caltag, San Francisco, USA).

Direct immunofluorescence was performed by incubating 2×10^6 cells with the specific MoAb for 15 minutes in the dark at room temperature. An isotype-matched negative control (BDIS) was used in all cases to assess background fluorescence intensity. Cells were lysed (FACS Lysis solution, BDIS) for 3 to 5 minutes and centrifuged at 250 g for 5 min. The cells were washed twice with phosphate buffered saline (PBS) before being resuspended in PBS and examined.

Table 1. CD123 expression in patients with acute leukemia and B-cell chronic lymphoproliferative disorders.

	Diagnoses							
	AML	*ALL-B*	*ALL-T*	*CLL*	*MCL*	*FL*	*HCL*	*BL*
Samples analyzed	45	13	6	77	12	5	7	2
Samples $CD123^+$	43	13	0	3	1	1	6	1
MFI (mean ± SD)	69±40	119±84	–	41±42	25±148	13±12	120±286	40±12

AML: acute myeloid leukemia; ALL-B: B-cell lineage acute lymphoblastic leukemia; ALL-T: T-cell lineage acute lymphoblastic leukemia; CLL: chronic lymphocytic leukemia; MCL: mantle cell lymphoma; FL: follicular lymphoma; HCL: hairy cell leukemia; BL: Burkitt's lymphoma; MFI: mean fluorescence intensity; SD: standard deviation.

Data acquisition and analysis

Measurements were performed on a FACScan flow cytometer (BDIS). For data acquisition the LYSIS-II (BD) software program (BDIS) was used. At least 10,000 events/tube were measured. The PAINT-A-GATE PRO software program (BDIS) was employed for further data analysis. Thresholds for positivity were based on isotype negative controls. Analytical gates were set on desired viable cells based on forward light scatter and side light scatter. The positivity threshold was 20% for all markers except for cytoplasmic or intranuclear antigens for which a 10% threshold was used.

The normal precursor cells were analyzed using a two-step acquisition procedure. In the first step, acquisition of 10,000 cells was performed and information stored for all these events. In the second step, a minimum of 300,000 cells was measured, information being stored only for the precursor cells, which were acquired employing a preestablished CD34 live gate.

The level of fluorescence in cells which expressed CD123 was measured in arbitrary units as the mean fluorescence intensity (MFI).

Results

Normal expression of the IL-3 αR in bone marrow, peripheral blood and lymph nodes

In control BM from healthy volunteers, CD123 was expressed in 0.27% (0.1%-0.6%) of the total nucleated cells. In the precursor cell compartment, the percentage of CD123 expression was 53%, ranging between 29% and 78% of the $CD34^+$ cells. We studied the expression of this interleukin receptor in myeloid and lymphoid progenitor subpopulations. In the myeloid compartment ($CD34^+CD33^+CD19^-$) CD123 was positive in 63% (38%-100%) of cells with a dim-moderate intensity (MFI= 33 ± 21). In contrast, CD123 was negative in normal lymphoid progenitors ($CD34^+CD33^-CD19^+CD10^+$) (Figure 1). To confirm these findings, myeloid $AC133^+$ cells were isolated by positive immunomagnetic selection (Miltenyi Mini-Macs Bergisch Gladbach, Germany). Ninety-two percent of the $AC133^+$ sorted cells expressed CD123 with a moderate intensity (Figure 2). The $CD34^+AC133^-CD123^-$ cells represent a more immature progenitor than $CD34^+AC133^+CD123^+$ cells. These findings are in agreement with earlier studies.[13,19] It is possible that IL-3 αR expression could vary among different lineages and developmental stages.

We also analyzed CD123 expression in PB. In our samples, cells with the strongest CD123 expression corresponded to dendritic cells, monocytes were positive for CD123 but showed a very faint reactivity and there was a minor population of B-cells expressing CD123. Granulocytes appeared to be CD123 negative.

We tested the CD123 expression in 3 normal lymph nodes. In all the cases the $CD19^+$ cells showed dim CD123 expression (MFI= 16± 12) and T-cells were CD123 negative.

IL-3 αR expression in AML

CD123 was expressed in 42 out of 45 AML samples assayed (93%). Regarding the FAB classification, CD123 expression was detected in all subtypes except in patients with a megakaryoblastic phenotype (n=2) (Figure 3).

The IL-3 αR pattern was similar in all the AML cases. CD123 was expressed in the totality of the blast cell population with a homogeneous pattern. The intensity of the expression was moderate (MFI= 69±40) (Table 1). Only in one patient was the intensity of the CD123 expression high (MFI= 236±182).

CD34 expression was analyzed in the blast cells of AML patients. We found no differences in CD123 expression between $CD34^+$ and $CD34^-$ cases.

Specific molecular lesions in AML were studied in order to detect differences in IL-3 αR. Six patients were PML/RARα positive, two patients had MLL rearrangements, and two patients were CBFβ/MYH11 positive. No differences in the CD123 pattern expression were found in patients with these molecular lesions.

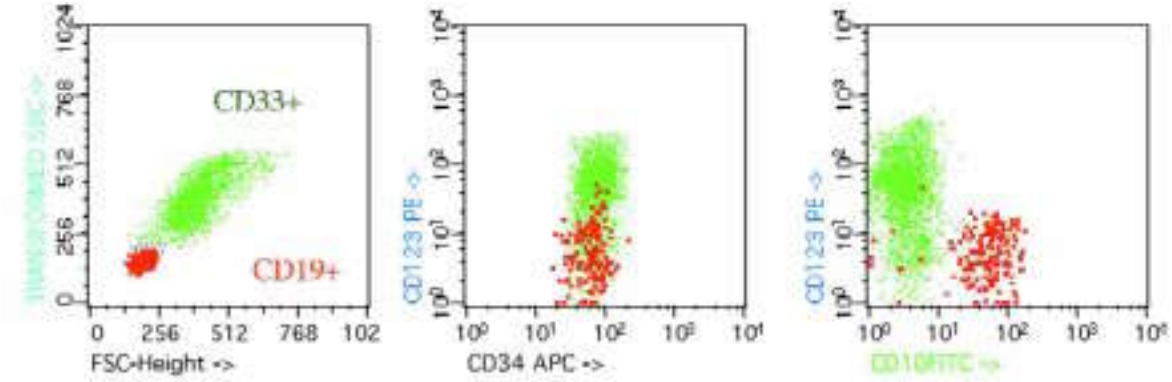

Figure 1. Absence of CD123 expression in normal lymphoid precursors: red dot plots (CD34$^+$ CD33$^-$ CD19$^+$ CD10$^+$) showed lower FSC/SSC pattern than myeloid progenitors (green dot plots). Normal lymphoid progenitors were CD123 negative.

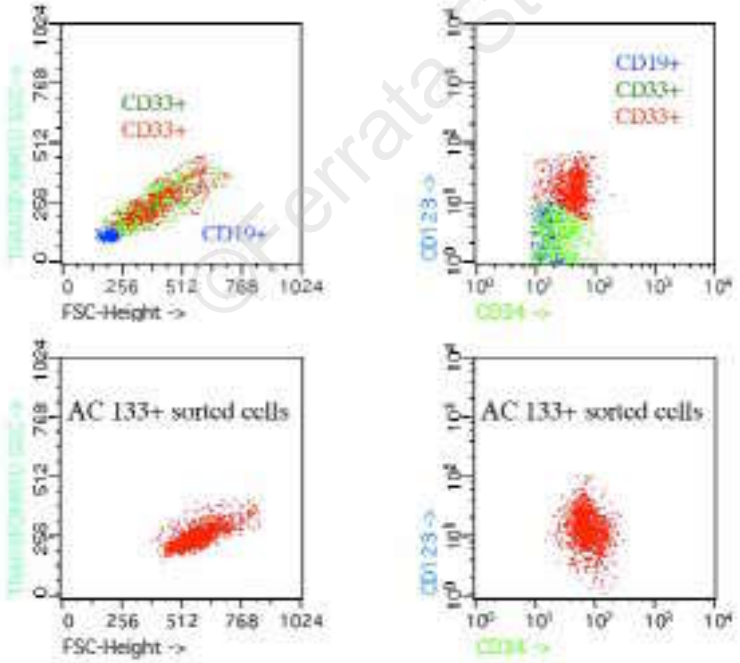

Figure 2. CD123 expression in normal myeloid precursors. Red and green dot plots (CD34$^+$ CD33$^+$CD19) showed higher FSC/SSC pattern than lymphoid progenitors (blue dot plots). A subpopulation of myeloid progenitors were CD123$^+$ with moderate intensity (red dot plots). CD34$^+$ myeloid cells were isolated with AC 133$^+$ minibeads. Most of the sorted cells expressed CD123 (bottom right).

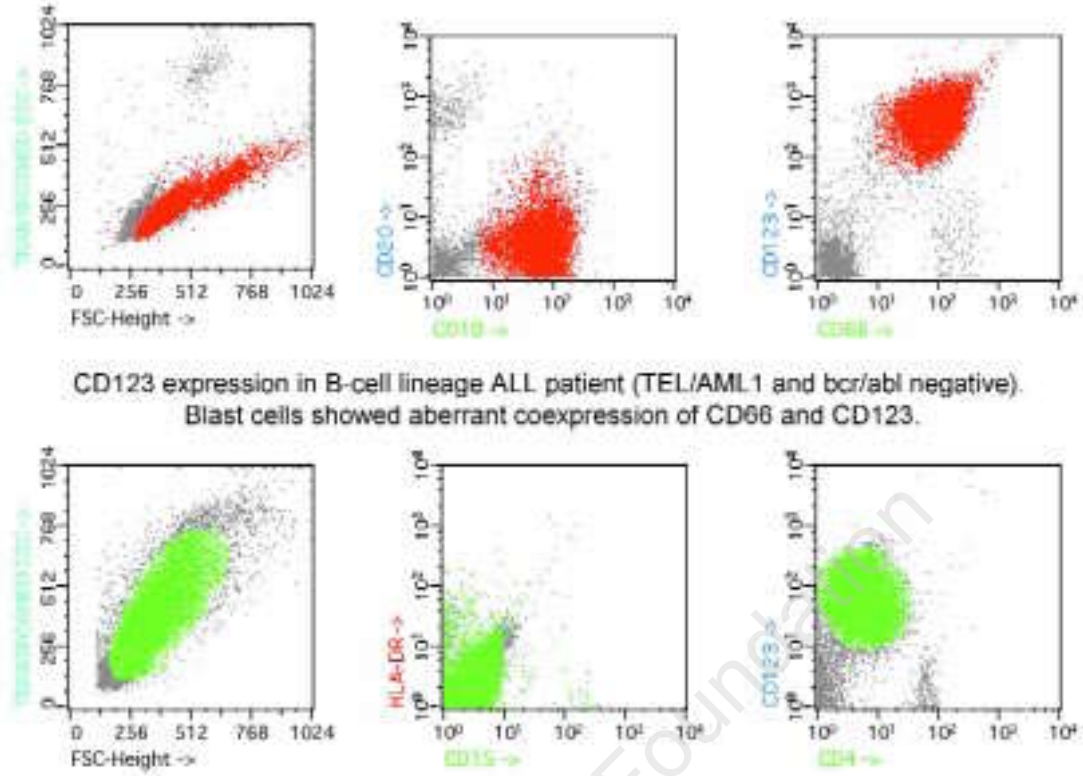

Figure 3. CD123 expression in B-cell lineage ALL patient (TEL / AML1 and bcr / abl negative). Blast cells showed aberrant coexpression of CD66 and CD123. CD123 expression in acute promyelocytic leukemia (PML / RARa positive). Blast cells showed the characteristic phenotype CD34$^-$, CD15$^-$, HLA-DR$^-$ with CD123 expression.

IL-3 αR expression in ALL

In ALL samples, IL-3 αR was restricted to the B-cell lineage. Molecular lesions were studied in order to compare the CD123 expression in different biological subgroups. Three patients were bcr/abl positive and one patient had c-myc rearrangements. No differences in CD123 expression were found in these patients harboring specific molecular lesions. CD123 was expressed in all B-cell ALL samples (n=13) and this high expression was in striking contrast to lack of expression of CD123 on normal lymphoid precursors. The intensity of the expression in ALL was higher than in AML samples (MFI= 119±84) (Table 1). In all the cases, the totally blast population showed a homogeneous CD123 pattern. In contrast, all T-cell ALL cases (n=6) tested were CD123 negative.

IL-3 αR expression in B-cell chronic lymphoproliferative disorders

We analyzed IL-3 αR expression in 122 samples of BM, PB or lymph nodes from patients with B-CLPD. In the CLL group (n=77), 7 patients showed CD123 reactivity MFI= 41±42) (Table 1). All these patients had an atypical morphology, the lymphoid cells were large with abundant cytoplasm and with cytoplasmic projections. Matutes´score for these patients was >4, but all of them showed immunophenotypic differences with respect to typical CLL i.e. strong expression of CD11c and loss of CD25.

Interestingly, 3 out of 4 patients with aggressive B-cell CLPD (two patients with transformed NHL from mantle and follicular lymphoma and one patient with Burkitt's lymphoma) expressed CD123 with a moderate intensity.

The results in the hairy leukemia group were striking. In 6 out of 7 patients studied CD123 was positive with strong intensity, (MFI=120±288) (Table 1). This CD123 pattern was characteristic of hairy cell leukemia (HCL) and we did not detect it in the other types of B-CLPD (Figure 4). Only the patient with a CD25$^-$ variant form of HCL did not show IL-3 αR expression.

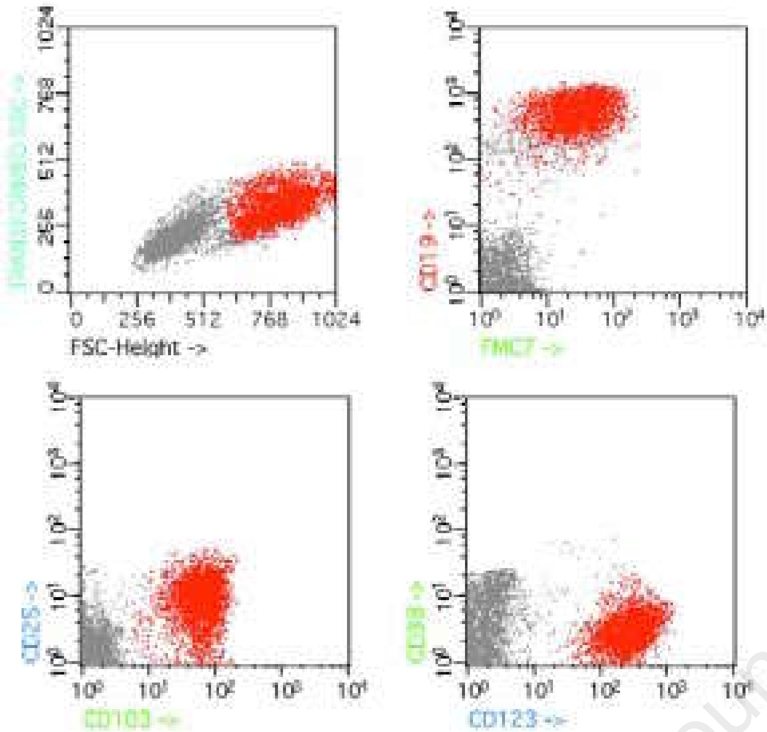

Figure 4. CD 123 expression in hairy cell leukemia. These cells showed a high FSC and the characteristic immunophenotype: B-cell associated antigens, CD5—, CD11c++,CD25+ CD103+, SIg+ and CD123+ with strong intensity.

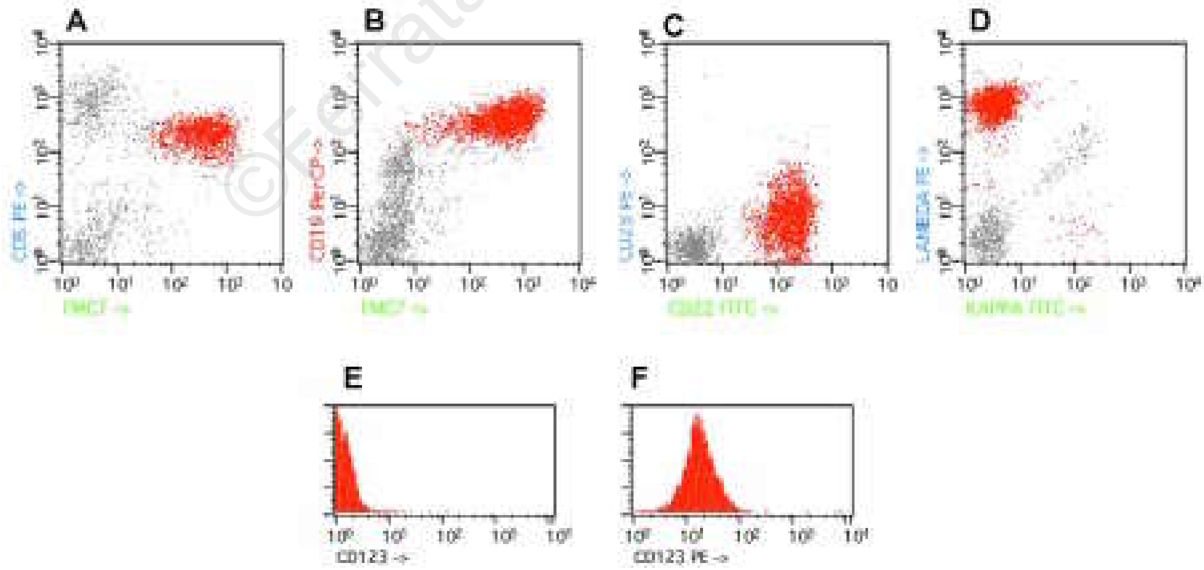

Figure 5. (A, B, C, D): Immunophenotype of a patient with a mantle cell lymphoma CD5+ CD19+FMC7+ CD22+CD23- SIg+. Blastic transformation in the same patient showed the phenomenon of transition from CD123 negativity (E) to CD123 positivity (F).

Discussion

Interleukin-3 is a regulatory glycoprotein known to support the survival, proliferation, and development of progenitor cells from multiple hematopoietic lineages.[1-3] In normal hematopoiesis, IL-3 not only stimulates the proliferation of hematopoietic progenitor cells but also helps to maintain cellular viability via the suppression of apoptosis.[7-9]

IL-3 acts through activation tyrosine-kinases. However, the effects of aberrant regulation of these molecules on signal transduction and the apoptotic pathways in transformed hematopoietic cells are still under discussion.[1] The high proportion of CD123 expression detected in our study in acute leukemia samples of myeloid (95%) and B-lymphoid lineage (100%), are in agreement with previous studies which suggest a possible role of IL-3 in leukemogenesis.[13] IL-3 and other cytokines promote the differentiation of progenitor cells, suggesting that in these leukemic diseases the oncogenic events happen at a stage of precursor cells that possess IL-3 receptors.[7,22] IL-3 may also act as a tumor promoter, inducing the proliferation of leukemic cells and inhibiting apoptosis.[9,23] Earlier experiments have shown that IL-3 plays an important role in the leukemogenesis of lymphoid and myeloid cells, inducing these cells to grow autonomously.[6,9] Some reports have described the relation between IL-3 and transcriptor factors associated with the myeloid leukemia, such as the AML1 and homeobox genes.[28-30] These studies suggest that two events are necessary for leukemogenesis: a block in myeloid differentiation, mediated by the inappropriate expression of the gene, and an increase in progenitor proliferation, mediated by IL-3.[27] Given this fact, the IL-3αR would be an important molecule in the leukemic process. CT Jordan *et al.* analyzed α signal transduction activity in the MAPK, Akt and STAT5 pathways in $CD123^+$ cells but did not detect phosphorylation in response to IL-3 stimulation. However, there are other possible mechanisms by which CD123 may affect the leukemia process. For example, CD123 could mediate the activation of the complex JaK/STAT pathway phospholyrating molecules other than STAT5, MAPK and Akt. Alternatively, CD123 could have no functional role in leukemia biology and the overexpression of CD123 could be an aberrant and non-functional expression. Another point to note is that the functional IL-3 receptor is composed of α and β chains and both are required for signal transduction by the IL-3 cytokine.[1] Jordan *et al.* examined the expression of the CD131 (IL-3 β chain) by flow cytometry on AML specimens and they never detected CD131 expression in the $CD34^+$ compartment.[13] The authors reasoned that physiologically relevant levels of IL-3β chain might fall below the limits of sensitivity of flow cytometry. However, if the cells do not express CD131, the expression of CD123 is perhaps not relevant to IL-3 signaling. Further experiments to explore the biological role of CD123 are being performed in our laboratory.

We investigated IL-3 αR expression in bone marrow and in peripheral blood from normal samples in order to establish normality patterns. In our study, normal lymphoid precursors did not show CD123 reactivity and only a proportion of myeloid progenitors expressed CD123 with a moderate intensity. These findings are in agreement with results from previous authors[13] who found a small CD123 expression in the normal $CD34^+$ population while CD123 was negative in the more primitive $CD34^+/CD38^-$ compartment. In contrast with the small expression of receptor in normal hematopoietic precursors, we found IL-3 αR expression in the majority of myeloid and B-cell leukemic cells. This feature may be useful in MRD studies.[14] Our data show that the combined use of CD123/CD34 with B-cell associated antigens such as CD19/CD10 may be a great help for identifying residual leukemic cells in B-cell leukemia cases. In addition, the $CD123^+/CD34^+/CD33^+$ is a minor phenotype in normal BM. The detection of cells with this phenotype in a very superior number to the standard range could suggest the presence of myeloid leukemic cells. The wide expression of this marker in the majority of AML and B-cell lineage ALL cases analyzed and the low CD123 expression in normal hematopoietic stem cells suggest that IL-3 αR could be a suitable target for immunotherapeutic intervention.[24-26]

Regarding the CD123 reactivity in B-CLPD, our findings suggest that the presence of this marker in these diseases has a different meaning. The most important use of CD123 in B-CLPD would be in differential diagnosis, especially in HCL. In our study, all the typical HCL cases analyzed showed a characteristic pattern with a strong expression of CD123. We did not detect this pattern in any other sample of B-CLPD. To the best of our knowledge, this feature of the immunophenotype of HCL has not been described before. Despite the small number of cases analyzed, CD123 provides a good marker for the specific diagnosis of HCL. CD123 was also detected in a few cases of CLL. In these samples the CD123 expression was dim. All these CD123 positive CLL samples tested showed an atypical morphology. It should be pointed out that CD123 was

expressed in the majority of aggressive or transformed B-CLPD analyzed. We observed the acquisition of CD123 simultaneously with blast transformation in one patient with a mantle-cell lymphoma (Figure 5). In these cases the biological role of CD123 would be identical to that in acute leukemia.

In conclusion, we have shown that CD123 is a good marker of myeloid and B-cell lymphoid leukemic cells and this feature could be used in MRD analysis. CD123 is expressed with a characteristic pattern in HCL cases and this antigen could be important in the differential diagnosis of B-CLPD. Nevertheless, further analysis is necessary to understand better the biological function of the IL-3 αR and the mechanisms by which this receptor transduces possible aberrant regulatory signals in malignant cells. If the biological role of the CD123 is confirmed, this antigen could be used as a target in the selective destruction of malignant cells in the majority of myeloid and B-cell leukemia patients.

Contributions and Acknowledgments

LM was primarily responsible for the article, was responsible for the analysis and interpretation of data and drafted the article. JFN was responsible for its conception and revised it critically for important intellectual content. The remaining authors have taken responsiblity for the following parts: OL, MJC and MB: laboratory experiments; AA: cytogenetic data; SB: collection of clinical data and JS: final approval of the version to be submitted. Order of authorship: authors are listed according to a criterion of decreasing individual contribution to the work, with the exception of the last author who had a major role in revising and preparing the final version of the article.

Funding

Luz Muñoz is recipient of a FIJC-99/ESP-GLAXO grant from the José Carreras International Leukemia Foundation.

Disclosures

Conflict of interest: none.

Redundant publications: no substantial overlapping with previous papers.

Manuscript processing

This manuscript was peer-reviewed by two external referees and by Dr. Marie-Christine Bené, who acted as an Associate Editor. The final decision to accept this paper for publication was taken jointly by Dr. Bené and the Editors. Manuscript received August 28, 2001; accepted October 19, 2001.

Potential implications for clinical practice

Immunophenotyping, together with cytologic, karyotypic and molecular approaches, retains a crucial place in the diagnosis and management of leukemia.[31] Flow cytometry analysis of CD123 expression might be a useful complementary tool in the diagnosis of hairy cell leukemia[32] and in the detection of minimal residual disease in acute leukemia.

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