Quantitative analysis of CD79b, CD5 and CD19 in mature B-cell lymphoproliferative disorders

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ABSTRACT

Background and Objective. Distinction between B-cell chronic leukemias can be difficult due to overlap in cell morphology and immunologic features. We investigated, by quantitative flow cytometry, the expression of CD79b, CD5 and CD19 in cells from a variety of B-cell disorders to see whether this analysis adds further information useful to the diagnosis and characterization of these diseases.

Design and Methods. Peripheral blood cells from 6 normal individuals were used as reference controls. The diseases of the 63 patients investigated comprised: 29 chronic lymphocytic leukemia (CLL), six of them with atypical morphology, 6 B-cell prolymphocytic leukemia (PLL), 12 splenic lymphoma with villous lymphocytes (SLVL) and 16 mantle-cell (Mc) lymphoma in leukemic phase. The study was carried out by triple immunostaining with directly conjugated monoclonal antibodies (MoAb) against CD79b, CD5 and CD19 and quantitative estimation of the antigens per cell assessed with standard microbeads (Quantum Simply Cellular).

Results. Compared to normal B-cells, the number of CD19 molecules was significantly lower in cells from all of the B-cell disorders except PLL. The intensity of CD5 in leukemic B-cells was significantly higher in CLL cells, including atypical cases, and Mc lymphoma than in normal B-cells, whilst PLL and SLVL had values similar to those of normal B-lymphocytes. CD79b was expressed at lower levels in all types of leukemic cells compared to normal B-lymphocytes but differences were statistically significant in CLL, Mc lymphoma and SLVL. The number of CD79b molecules per cell was significantly lower in typical CLL than in the remaining B-cell diseases whilst the comparison of CD5 and CD19 intensity between CLL and non-CLL samples failed to show any statistically significant difference.

Interpretation and Conclusions. Distinct antigen density patterns for the various conditions emerged from this analysis: Typical CLL was characterized by moderate CD5 and weak or negative CD79b expression. Atypical CLL had an intermediate pattern of CD79b antigen expression ranging from weak to moderate with bright CD5. Unlike CD5 and CD79b, CD19 did not discriminate the various B-cell disorders but only between normal and leukemic cells.
for distinguishing CLL from non-CLL cases. The discriminatory power of CD79b in other B-cell conditions has not been documented.

In this study we investigated, by quantitative flow cytometry, the expression of CD79b, CD5 and CD19 on cells from a variety of B-cell disorders evolving with a leukemic picture to see whether differences in the intensity of expression of these antigens may disclose patterns which help in the differential diagnosis of the various entities. A PE conjugated CD79b antibody was used as this fluorochrome has a higher sensitivity to detect weakly expressed antigens as is the case of CD79b in CLL.

Design and Methods

Samples

The study was performed at The Royal Marsden Hospital on cryopreserved peripheral blood mononuclear cells from 63 patients with a chronic B-cell lymphoproliferative disorder. These comprised: CLL (29 cases) including 6 cases with atypical morphology (atypical CLL), PLL (6 cases), SLVL (12 cases) and Mc lymphoma in leukemic phase (16 cases). Six samples of cryopreserved peripheral blood mononuclear cells from healthy donors were used to establish reference normal values for the antigen expression of CD79b, CD5, and CD19.

The diagnosis of the various lymphoid diseases was based on cell morphology and immunophenotyping, and when relevant, histology and cytogenetics or fluorescence in situ hybridization (FISH) analysis. Typical CLL had a predominant lymphoid population with circulating cells having nuclear clumped chromatin and scanty cytoplasm and less than 10% larger cells whilst atypical CLL had more than 10% prolymphocytes or immunoblasts (CLL/PLL) or over 15% cleaved or lymphoplasmacytic cells. PLL was defined with the presence of more than 55% of cells with a prominent central nucleolus. Circulating cells in Mc lymphoma were medium-sized with dispersed speckled chromatin and scanty pale cytoplasm, the majority with an indented nucleus; cells from all the latter cases had the t(11;14) demonstrated either by conventional cytogenetics and/or FISH. Lymphocytes in SLVL had condensed chromatin and short and thin cytoplasmic vili. Spleen histology confirmed the diagnosis of SLVL in 8 of the 12 cases in which this was available.

Immunophenotyping showed that all CLL cases had a typical CLL phenotype according to the previous reported scoring system (CD5, CD23, FM C7, CD22 and surface immunoglobulin) with scores ranging from 3 to 5 while the remaining diseases had phenotypes atypical of CLL with low scores (0-2).

Immunostaining

Mononuclear cells were obtained from peripheral blood samples by density gradient centrifugation with Lymphoprep (Nycomed, Oslo, Norway). After separation by density gradient centrifugation with Lymphoprep (Nycomed, Oslo, Norway). After separation and washing the cells were cryopreserved in 10% dimethyl sulfoxide and 4% albumin in phosphate buffered saline (PBS) (final concentrations) and stored in liquid nitrogen. Prior to their use, the cells were thawed at 37°C in a water bath and washed in RPMI 1640 with 10% fetal calf serum. After washing, the cells were resuspended in PBS/azide to a final concentration of 10^6 cells per mL. Cell viability, assessed by trypan blue exclusion, was always over 80%. Studies comparing fresh and cryopreserved cells were not carried out, but we previously reported that there are no differences in the antigen density of CD5 and CD19 between cryopreserved and fresh cells. Because studies in all cases here were performed on cryopreserved cells, results were comparable. The analysis was performed using a triage staining combination with directly labeled MoAb: CD5-FITC (DK23) (Dako), CD79b-PE (CB3-1) (Immunotech S.A., Marseille, France) and CD19-TC (SJ25-C1) (Caltag Laboratories, San Francisco, CA, USA). Mouse immunoglobulins of IgG1 subclass FITC, PE and TC labeled mouse immunoglobulins of IgG1 subclass FITC, PE and TC labeled mouse immunoglobulins of IgG1 subclass FITC, PE and TC labeled microbeads Kit (Sigma, St Louis, Mo., USA) provides a method for the evaluation of the number of molecules of antibody per cell by flow cytometry. Briefly, Quantum Simply Cellular Microbeads Kit (Sigma, St Louis, Mo., USA) provides a method for the evaluation of the number of molecules of antibody per cell by flow cytometry. This kit has a mixture of four types of microbeads coated with different amounts of goat anti-mouse immunoglobulin with a precalibrated antibody binding capacity (ABC). The microbeads react with directly labeled mouse MoAb and serve as a set of standards to calibrate the fluorescence scale of the flow cytometer for each antibody, thus converting the MIF into the number of molecules of antigen expressed per cell. Microbeads were incubated separately with each MoAb and the acquisition was also carried out separately for each reagent using the same procedure as for the clinical samples. For each sample, the ABC value of the isotypic control was subtracted from the ABC value of the positive cells. The microbeads, 50 µL per tube, were incubated with each MoAb separately, washed and further processed as the clinical samples and finally acquired on the flow cytometer under the same conditions.

Flow cytometry analysis

Data acquisition was performed on a FACSscan flow cytometer (Becton Dickinson) using the LYSYS II

Assessment of antibody binding capacity by Quantum Simply Cellular Method

Quantitative analysis was performed as described elsewhere. Briefly, Quantum Simply Cellular Microbeads Kit (Sigma, St Louis, Mo., USA) provides a method for the evaluation of the number of molecules of antibody per cell by flow cytometry. This kit has a mixture of four types of microbeads coated with different amounts of goat anti-mouse immunoglobulin with a precalibrated antibody binding capacity (ABC). The microbeads react with directly labeled mouse MoAb and serve as a set of standards to calibrate the fluorescence scale of the flow cytometer for each antibody, thus converting the MIF into the number of molecules of antigen expressed per cell. Microbeads were incubated separately with each MoAb and the acquisition was also carried out separately for each reagent using the same procedure as for the clinical samples. For each sample, the ABC value of the isotypic control was subtracted from the ABC value of the positive cells. The microbeads, 50 µL per tube, were incubated with each MoAb separately, washed and further processed as the clinical samples and finally acquired on the flow cytometer under the same conditions.
software (Becton Dickinson). For data analysis, Paint-A-Gate software (Becton Dickinson) was used. Peripheral blood mononuclear cells labeled with CD4/CD8/CD3 were used to set the calibration of the instrument and fluorescence compensation. For each tube 10⁴ cells or microbeads were acquired.

Statistical analysis

Results were expressed as median, standard deviation (s.d.) and compared with the Mann-Whitney U test. A two-sided p value ≤ 0.05 was considered statistically significant. Median values were chosen due to the dispersion of values in some diseases, such as B-PLL and atypical CLL.

Results

Normal samples

There was little variability in the expression of the three B-cell associated antigens analyzed in normal samples (Figures 1-3). As shown in Table 1, the median number of CD19 molecules per cell (ABC values) expressed in normal peripheral blood B lymphocytes, was 13±1×10³/cell (range 11-16×10³); 11±2×10³/cell (range 8-13×10³) for CD5 and 43±8×10³/cell (range 29-55×10³) for CD79b.

Leukemic samples (Table 1).

CD19 antigen. CD19 was consistently expressed in cells from all B-cell leukemias. Compared to normal controls, the intensity of antigen expression was significantly lower in all the conditions except in PLL. Over 80% of leukemic samples had less than 10×10³ CD19 molecules per cell and thus, below the lowest value of normal B cells (Figure 1). Cells from very few cases had values within the normal range. There were no statistically significant differences regarding the expression of this marker between CLL and non-CLL cases or among the other groups of B-cell malignancies, although the highest values were found in PLL.

CD5 antigen. CD5 was positive in typical CLL and Mc lymphoma cases, 83% of atypical CLL, 83% of PLL and in 50% of SLVL cases. The intensity of CD5 expression in leukemic B-cells was significantly higher in CLL, including atypical cases, and Mc lymphoma than in normal B-cells (Figure 2). Analyzing the CD5 positive samples, the comparison of CD5 intensity of expression between CLL and non-CLL cases or among other non-CLL B-cell disorders failed to show any statistically significant difference.
However, when we compared the group of PLL plus SLVL versus CLL the differences were significant with lower CD5 expression in PLL and SLVL. CD79b antigen. CD79b was negative in 6 of the 23 CLL cases and consistently positive in the remaining B-cell disorders. Cells from all leukemia and lymphoma patients had lower CD79b expression than normal B-cells, although results were only significantly lower for typical CLL, Mc lymphoma and SLVL. Among the positive cases, CD79b was weakly expressed ($ABC<2\times10^3$) in cells from 88% of CLL samples, 50% of atypical CLL and 17% of SLVL. The number of CD79b molecules per cell was higher than $2\times10^3$ in all Mc lymphoma and PLL cases. Statistical analysis revealed that CD79b expression was significantly lower in typical CLL compared to other B-cell disorders whilst there were no differences among the B-cell leukemias other than CLL albeit there being a trend for PLL cases to have higher CD79b expression. There was also significantly higher CD79b expression in atypical CLL than typical CLL, albeit lower than in the other diseases (Figure 3).

According to our findings, the combination of CD5 and CD79b emerged as a useful marker to distinguish CLL from non-CLL cases and, to some extent, among some of the other B-cell disorders. Figure 4 tries to illustrate the pattern of antigen expression. Typical CLL was characterized by moderate expression of CD5 and weak or negative expression of CD79b. Mc lymphoma showed a homogenous pattern, characterized by a similar intensity of expression of CD5 as that found in CLL but significantly stronger expression of CD79b (Figure 5) whilst SLVL and PLL had weaker CD5 expression and stronger CD79b than that in CLL.

Discussion

We investigated the value of quantitative flow cytometry, analyzing the intensity of expression of three antigens: CD19, CD5 and CD79b to see whether this analysis can be of diagnostic value for differentiating CLL from non-CLL cases and also whether it can distinguish among the B-cell disorders other than CLL.

We have shown that although CD19 discriminates well between normal and leukemic B-cells, it does not help to distinguish among the various conditions. In contrast, the antigen density of CD5 combined with that of CD79b is not only useful for differentiating normal from leukemic B-cells but also helpful in distinguishing among some of the various B-cell disorders. Thus, although Mc lymphoma and CLL had a similar CD5 antigen density, the number of CD79b molecules in Mc lymphoma was significantly higher than in CLL. Of interest was the intermediate pattern of CD79b antigen expression in atypical...
CD79b, CD5 and CD19 expression in B-cell disorders

CLL, ranging from weak to moderate and lying between that in CLL and other B-cell disorders and indeed being significantly higher than in typical CLL. The transmembrane forms of all immunoglobulin (Ig) classes are associated with two glycoproteins, mb1 and B29 that are essential for signal transduction following antigen binding to the Ig molecule.17-19 CD79b (CB3-1) recognizes an external epitope of the B29 component of the surface immunoglobulin receptor complex (B cell receptor).11,20 The B29 protein is expressed early in the cytoplasm in B-cell precursors albeit later than mb-1 (CD79a), CD19 or cytoplasmic CD22, whereas surface expression occurs concomitantly with surface immunoglobulin expression in all B-cells.21-22 A recent study has documented that CLL cells have decreased surface expression of B29 (CD79b) and in some cases, undetectable B29 mRNA.23 Further, most of those cases with detectable m-RNA had point mutations or deletions in the cDNA located in the B29 transmembrane and cytoplasmic domains, probably underlying the loss of signal transduction of CLL cells.23,24 The low number of CD79b molecules per cell exhibited by CLL cells correlates well with low surface immunoglobulin and CD22 expression characteristic of this disease.1,25 Although previous studies have documented that the majority of CLL cases do not stain with CD79b (SN8),1,13 the use of a MoAb directed against a different epitope (CB3-1) and a brighter (PE) and directly labeled antibody allows the detection of small numbers of CD79b molecules in CLL lymphocytes. Indeed, this may correlate with the documented molecular findings of CD79b in CLL.

From the diagnostic point of view, the quantitative estimation of CD79b together with that of CD5 seems to be discriminative between CLL and non-CLL cases whilst the antigen density of CD19 is a good discriminative marker between leukemic and normal B-cells. When considering the antigen density of CD5 and CD79b in the remaining B cell disorders, M c lymphoma exhibited the most homogeneous pattern, clearly different from that in both typical and atypical cases of CLL. There was also a trend for a different pattern of CD5 and CD79b antigen expression in the other two B-cell disorders, PLL and SLVL, with lower numbers of CD5 than in CLL and M c lymphoma and moderate to bright CD79b. Problems of differential diagnosis usually arise between M c lymphoma and atypical CLL, PLL and SLVL and quantitative flow cytometry might be an additional tool for distinguishing between them. Analysis of greater numbers of non-CLL cases e.g. PLL, SLVL and atypical CLL is needed to substantiate these findings.

In addition to the diagnostic value, the differences in antigen expression of CD19, CD5 and CD79b between normal and leukemic samples has potential for monitoring treatment and can be exploited to detect minimal residual disease.

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EC, RM and EM were involved on the immunophenotyping and interpretation of the results. Morphology review was performed by EM. PC carried out the FISH analysis for the 11;14 translocation.

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References


