Assessment of distribution of CD34 epitope classes in fresh and cryopreserved peripheral blood progenitor cells and acute myeloid leukemic blasts

FRANCESCO LANZA, SABRINA MORETTI, BARBARA CASTAGNARI, FABIO MONTANELLI, ANGELA LATORRACA, LUISA FERRARI, ANTONELLA BARDI, MASSIMO DOMINICI, DIANA CAMPIONI, MELISSA DABUSTI, NADIA PIVA, GIANLUCA LODI,* ROBERTO REVERBERI,* GIANLUIGI CASTOLDI

Sezione di Ematologia, Dipartimento di Scienze Biomediche e Terapie Avanzate, Università di Ferrara; *Servizio Immunotrasfusionale, Azienda Ospedaliera, S. Anna, Ferrara, Italy

ABSTRACT

Background and Objectives. So far several reports have described changes in the expression of surface antigens in progenitor cells and blasts following cryopreservation. However, there are no data on the effects of cryopreservation on the expression of the three CD34 epitope classes, and on their relationship with the clonogenic capacity of PBPC collected by leukapheresis.

Design and Methods. In order to analyze the effects of freezing/thawing procedures (-80°C storage for 3 months) and use of dimethylsulfoxide (DMSO) on the immunophenotype profile and colony production of peripheral blood progenitor cells (PBPC) in apheresis products derived from 20 patients with stage 0-III non-Hodgkin’s lymphoma (nHL), a flow cytometry study was undertaken using different CD34 monoclonal antibodies (MoAbs) capable of recognizing the 3 epitope classes of CD34 molecule (class III: HPCA-2/ FITC, HPCA-2/PE, 581/FITC, 581/PE; class II: Q-Bend 10/PE; class I: ICH3/PE, B13C5-PE, Immuno-133-PE). CD34 epitope expression was also analyzed in thawed CD34+ blasts obtained from 14 patients with acute myeloid leukemia (AML), who were analyzed using a larger number (#17) of CD34 epitope class I, II, and III reactive MoAbs.

Results. Under our experimental conditions it was found that class III and class II CD34 epitopes (differentially resistant to enzymatic cleavage with neuraminidase, chymopapain and glycoprotease) are better preserved than class I epitope – sensitive to degradation – after cell exposure to cryoprotectant DMSO and the freezing/thawing procedures. Results further showed a concomitant decrease in class I CD34+ counts and in BFU-E colony production. A significant increase in CD34 antigen expression levels (i.e., antibody binding capacity, ABC) by cryopreserved cells stained with CD34 epitope class III, and class II reactive MoAbs was also documented, while no changes after cryopreservation was noted using class I-reactive MoAbs. The slight increase in the percentage of CD34+ cells detected after frozen storage was correlated to a concomitant decrease in the number of more mature myeloid cells (CD15+, CD13+, CD33+). Compared to pre-cryopreservation values, a slight reduction in class I CD34 epitope expression was also found in thawed CD34+ AML blasts.

Interpretation and Conclusions. As far as the reduction of class I CD34 epitope is concerned, it may be hypothesized that the freezing procedure, use of DMSO, and/or lysis methodology may either damage a CD34 subset, or induce distinct alterations of the CD34 glycoprotein, possibly determining a reduction in their immunoreactivity with some CD34 MoAbs. In conclusion, this study has shown that exposure to the cryoprotectant DMSO and the freezing/thawing procedures modifies the distribution of CD34 epitopes as well as the clonogenic capacity of PBPCs from nHL patients, and CD34+ blasts from AML. These findings need to considered when selecting CD34 MoAbs for enumeration and positive selection of stem/progenitor cells for research and clinical purposes.

©1999, Ferrata Storti Foundation

Key words: CD34, epitope class, cryopreservation, colony forming units

The CD34 molecule is a one-pass transmembrane glycoprotein with a molecular weight of 105-120 kilodalton in either the reduced or unreduced form. The full length CD34 protein is composed of 385 amino acids, and contains nine sites for N-glycosylation and a number of sites for O-glycosylation, that are essential constituents of the three epitopes of the molecule. More recently, a truncated form of CD34 protein has been characterized, which contains only 323 amino acids. While the intracellular cytoplasmic domain is shorter in the CD34 splice variant, the extracellular and the transmembrane regions of the two forms of CD34 are identical. CD34 is also rich in sialic acid, and its biochemical composition suggests a mucin-like structure and resembles in some respects leucosialin (CD43). CD34 antigen is expressed on hemopoietic stem and progenitor cells, small vessel endothelial cells, and a subset of fibroblasts. Seven CD34 MoAbs have
been clustered at the 3rd and 4th Workshop on Leukocyte Differentiation Antigens (Oxford, 1986 and Vienna, 1988), and a further 25 MoAbs were verified as recognizing the CD34 molecule during the 5th and 6th International Workshops on Leukocyte Differentiation Antigens (Boston, 1983; Osaka, 1996), the most direct evidence being reactivity with cells transfected with CD34 cDNA and binding to CD34 protein. Based on current knowledge, the epitope specificity of CD34 MoAbs could be classified into three distinct groups according to the different sensitivities of the epitopes to enzymatic cleavage (which has been performed using neuraminidase, chymopapain and glycoproteinase), reactivity with fibroblasts and high endothelial venules, and cross-blocking experiments. We know, in fact, that glycoproteinase from Pasteurela haemolytica specifically cleaves only proteins containing sialylated O-linked glycans, while neuraminidase cleaves sialic acid residues. On the basis of these data, it can be speculated that class I epitopes are more proximal to the extracellular side of the cell membrane than the class I and II epitopes.

Over the few last years, PBPC have been increasingly used to provide rapid and durable hemopoietic reconstitution following high dose chemotherapy and radiotherapy. However, the quality of PBPC apheresis products has to be monitored in all the processing steps, which include cryopreservation, and subsequent thawing and reinfusion. Traditionally, either the CFU-GM test or flow cytometry measurement of CD34-expressing cells has been used to evaluate the potential of PBPC autografts for restoring hemopoiesis after high-dose therapy. There are accumulating data showing a significant correlation between numbers of transplanted CD34+ cells/kg and hemopoietic reconstitution. So far several studies have investigated the effects of cryopreservation on the expression of surface antigens in progenitor cells and blasts. Most of the data have focused on the analysis of CD7, CD13, CD33 and CD34 molecules during the freezing/thawing procedures in the presence of cryoprotectants such as DSMSO. However, there are no data on the effects of cryopreservation on the distribution of expression of CD34 epitopes or on its relationship with the clonogenic capacity of PBPC collected by leukapheresis.

In order to analyze the effects of short-term cryopreservation (3 months at –80°C using 10% DSMSO and 4% human serum albumin as a cryoprotective agent without rate-controlled freezing) on the immunophenotype profile and colony production of PBPC apheresis products from 20 patients with stage 0-III non-Hodgkin’s lymphoma (nHL), a flow cytometry study was undertaken using different CD34 MoAbs capable of recognizing the 3 epitope classes of CD34 molecule. The effect of cryopreservation on the expression of different CD34 epitopes was also evaluated through the use of a flow cytometer and 17 distinct CD34-reactive MoAbs on CD34+ (HPCA-2+) blasts taken from 14 patients with acute myeloid leukemia (AML).

**Design and Methods**

**Subjects**

Non-Hodgkin’s lymphoma. Fresh and cryopreserved peripheral blood mononuclear cells (PBMC) obtained from 20 consecutive patients with stage 0-III nHL (aged between 16 and 56; mean: 35 years) were investigated within 1 hour from their collection and processing. Diagnosis of lymphoma was based on morphologic, immunologic, cytochemical, cytogenetic, and molecular genetic criteria. No bone marrow contamination of lymphoma cells was documented at the time of the study. All patients achieved a remission state at the time of PBMC collection. The mobilization regimen was cyclophosphamide 7 g/m² plus rhG-CSF (filgrastim, 5-15 µg/kg/day). PBMC were collected by apheresis. Written informed consent for PBPC collection and autologous transplantation was obtained from each patient.

Acute myeloid leukemia. Light density (<1.077 g/ml) peripheral blood cells obtained from 14 patients with CD34+ (HPCA-2/PE) AML were investigated before and after cryopreservation. Diagnosis of leukemia was based on morphologic, cytochemical, cytogenetic, molecular genetic, and immunologic criteria. According to the FAB classification, the distribution of AM L subtypes was as follows: M0: 1 pt; M1: 5 pts; M2: 5 pts; M4 3. AM L patients had a blast percentage higher than 60% in all specimens analyzed (mean 74%). The CD34+ acute leukemia samples included in this study were selected using the HPCA-2/PE MoAb. Using HPCA-2/PE MoAb, the range of CD34 positivity on leukemic cells was 45-82% AM L patients were treated with a DAT (daunorubicin, cytosine arabinoside, thioguanine)-based induction regimen, followed by consolidation chemotherapy.

**Collection of PBPC, processing and cryopreservation**

PBMC were collected by apheresis from nHL patients in remission state during hematologic recovery which followed the mobilization regimen administered (cyclophosphamide 7 g/m² plus rhG-CSF (filgrastim, 5-15 µg/kg/day). PBPC were collected by means of apheresis using CS-300 Plus blood cell separator (OmnixTM, Baxter, Milan, Italy). Each apheresis session consisted in processing 10-15 liters of blood. Acid citrate-dextrose (ACD) was used as anticoagulant. Samples were collected immediately after apheresis and analyzed by flow cytometry with saturating concentrations of CD34 MoAbs and irrelevant isotope-matched MoAbs.

Peripheral blood mononuclear cell samples were mixed with an equal volume of cryoprotective solution consisting of 20% DSMSO and 8% human serum albumin. The final cell concentration was adjusted to <5 x 10⁹/L. The suspensions were frozen in cryo-
preservation bags (Cryocyte freezing container, Baxter) without rate-controlled freezing in an electrical freezer at -80°C (Harareus-Votsch).

Twenty specimens from each donor were stored for 3 months in order to study the effect of cryopreservation on the immunophenotypic profile of stem/progenitor cells.

For rapid thawing, freezing tubes were taken from the freezer and placed in a 37°C water bath, they were then diluted slowly 10 fold with a calcium- and magnesium-deprived PBS buffer. Samples were also taken from freezing tubes, before and after cryopreservation, for trypan blue dye exclusion tests.

Cell counting and leukocyte differential was performed using an automated hematology analyzer (Technicon H3, Bayer, Milan). The white cell differential was also determined microscopically on May-Grünwald-Giemsa stained films.

Flow cytometry analysis of class I, II, and III CD34 epitopes

PBMC apheresis products and light density cells obtained from AMI samples were analyzed before and after cryopreservation with a Faccscan flow cytometer (Becton Dickinson, San José, CA, USA) equipped with a 15 mW argon-ion laser. The instrument was calibrated with FITC (fluorescein isothiocyanate) and PE (R-phycocerythrin) beads provided by Becton Dickinson. Data were analyzed with negative controls using Lys II and Cell Quest research software. As negative control, we used an appropriate and similarly titered isotype control.20 Over 50,000 viable cells (containing at least 100 CD34+ cells) were analyzed for each sample at a flow rate of approximately 300 particles per second.

The enumeration and the quantitative analysis of the expression levels of the various CD34 MoAbs was performed on fresh and cryopreserved PBMC apheresis products using a modified version of the Milan protocol.8 This is a no-lyse and wash technique based on direct CD34 immunofluorescence staining and between 0.9-1.1 x 10^4 MFI.18,24

Based on the analysis of the minimum detection threshold of our flow cytometer, and on variation in ABC values for CD34 M oAbs in the specimens analyzed (evaluated within and between different specimens), we estimated that the lower limit of quantification was between 0.5-0.7 x 10^3 MFI/cell (indirect staining) and between 0.9-1.1 x 10^3 ABC/cell. Below this limit the enumeration of antigenic determinants was considered unreliable and not reproducible.18

Monoclonal antibodies to CD34

A large series of CD34 epitope class I, II, and III reactive MoAbs were used in this study. PBMC apheresis products from nHL were investigated by using 6 different CD34 MoAbs: HPCA-2/FITC (class III, BDIs), HPCA-2/PE (BDIs), 581-FITC (class III, Caltag), 581/PE (Caltag), Q-Bend 10-PE (class II, Immunotech); ICH3-PE (class I, Caltag), Immu-133 (class I, Immunotech). For the analysis of class I, and class II CD34 epitopes, we used only PE conjugates, since recent data have indicated that FITC-labeled class I and class II CD34 MoAbs provide less reliable results than the PE-conjugated forms.25-27

The expression of CD34 on leukemic cells was assessed by using 17 different unconjugated CD34
MoAbs (Class I: Immu-133, Immu-409, 14G3, B13C5; Class II: Q-Bend 10, 43A1, MD34.1, MD34.3, MD34.2, 4A1, 9066, 9069; Class III: CD34-9F2, 8G12, 581, 553, 563). This series of CD34 MoAbs was provided by Prof. M. Greaves (London, UK), and were intended for analysis during the fifth Workshop on Leukocyte Differentiation Antigens held in Boston, November 1993.2,4

Negative controls were used in all experiments. As negative controls, appropriate and similarly sized isotype-matched non-relevant MoAbs (mouse IgG1, IgG2a, IgM; Dakopatts, Silenus, Becton, Caltag) were employed. Light density cells from AML samples were washed twice in PBS before their incubation with CD34 MoAbs. Human AB serum was added prior to antibody incubation to avoid non-specific binding of MoAbs to Fc receptors. All samples were further incubated for 20 minutes with a lysing buffer (Facyslysing solution, Becton Dickinson) to eliminate red blood cells.

Purified CD34 MoAbs (including the isotype controls) were utilized using an indirect immunofluorescence technique. As a second step we employed FITC-conjugated goat F(ab') Ig fragments specific for mouse IgG is included in the mixture. These beads mimic cells with different antigen densities. The minimum detection threshold of microbeads is 5% CO2 in air. After 14 days of culture, colonies of more than 50 cells were scored under an inverted microscope. All cultures were performed in triplicate.

Assessment of cell viability and FAS antigen expression by flow cytometry

The assessment of cell viability was made with a Facscan flow cytometer (Becton-Dickinson). Cells were stained with either propidium iodide solution (50 µg/mL) or 7-ADD nucleic acid dye. Expression of FAS antigen was evaluated on PBMNC using the DX2-FITC MoAb (anti-FAS from Bender, Austria) in combination with PE-conjugated NC using the DX2-FITC MoAb (anti-FAS from Bender, Austria). Expression of FAS antigen was evaluated on PBMNC using the DX2-FITC MoAb (anti-FAS from Bender, Austria) in combination with PE-conjugated NC using the DX2-FITC MoAb (anti-FAS from Bender, Austria).

Statistical analysis

Expression of class I, II, and III CD34 epitopes and counts of CFU-GM, CFU-GEMM, BFU-E and of other biological parameters were compared using parametric and non-parametric statistics (paired Student's t test, Wilcoxon's test, linear regression model, multivariate regression analysis). When pertinent one way analysis of variance (ANOVA) and chi square or Fisher's tests were used to compare means and frequencies.

Results

Determination of CD34+ counts for epitope class I-, II-, and III-reactive MoAbs in fresh and cryopreserved PBPC

Effects of short-term cryopreservation (90 days storage) on the expression of CD34 antigens for class I, II, and III reactive MoAbs are shown in Table 1. Flow cytometry results showed that class II and class III CD34+ counts were higher in cryopreserved PBPC.
Distribution of CD34 epitopes in cryopreserved cells

Prepared with pre-cryopreservation values (Table 2). The number of positive events detected in the negative controls samples was less than 1% in all cases examined. As previously mentioned (see Design and Methods section: flow cytometry analysis of class I, II, and III CD34 epitopes), viable cells were identified using a nucleic acid dye staining method (propidium iodide or 7-AAD) (Figure 1).

The quantitative analysis of CD34 antigens showed that the cryopreservation procedures induced a significant increase in ABC values for class

<table>
<thead>
<tr>
<th>MoAb</th>
<th>Pre-cryopreservation</th>
<th>Post-cryopreservation</th>
</tr>
</thead>
<tbody>
<tr>
<td>HPCA-2 (FITC) class III</td>
<td>2.9±3.2</td>
<td>&lt; 0.01</td>
</tr>
<tr>
<td>HPCA-2 (PE) class III</td>
<td>3.0±3.3</td>
<td>&lt; 0.01</td>
</tr>
<tr>
<td>581 (FITC) class III</td>
<td>2.6±3.1</td>
<td>&lt; 0.05</td>
</tr>
<tr>
<td>581 (PE) class III</td>
<td>2.7±3.3</td>
<td>&lt; 0.05</td>
</tr>
<tr>
<td>QBEND-10 (PE) class II</td>
<td>2.7±3.3</td>
<td>ns</td>
</tr>
<tr>
<td>BI3C5 (PE) class I</td>
<td>2.3±2.7</td>
<td>ns</td>
</tr>
<tr>
<td>ICH3 (PE) class I</td>
<td>2.3±2.7</td>
<td>ns</td>
</tr>
<tr>
<td>IMMU-133 (PE) class I</td>
<td>1.7±1.0</td>
<td>ns</td>
</tr>
</tbody>
</table>

*Percentage of positive cells expressed as mean±standard deviation (SD) ns = not significant (paired test).

Table 1. Distribution of CD34 epitope classes in PBPC before and after cryopreservation (direct immunofluorescence).*

Table 2. Percent variation of CD34 cell positivity for the various CD34 MoAbs after cryopreservation (direct immunofluorescence).

Figure 1. Flow cytometry analysis of fresh (A, B) and cryopreserved (C, D) PBSC obtained from patients with non-Hodgkin’s lymphoma. Viable CD34+ cells were identified and quantified using the cell viability dye 7-AAD in combination with CD34 epitope class III, II, and I-reactive MoAbs. Simply Cellular beads were used for the calculation of ABC units, i.e. number of antigenic determinants expressed by CD34+ progenitors stained with the various CD34 MoAbs.
III and class II CD34 MoAbs (Table 3). However, compared to baseline values (pre-cryopreservation), the antigen density expressed by thawed PBPC labeled with class I CD34 MoAbs was only slightly increased (ICH3 MoAb) or even decreased with the remaining two class I-reactive MoAbs (BI3C5 and Immu-133 MoAbs) (Table 3) (Figure 1).

The clonogenic capacity of PBPC before cryopreservation and after thawing was evaluated by means of standard 14-day culture assays. These data are shown in Table 4. A comparative analysis of the effects of cryopreservation on CD34 epitope expression and numbers of CFU derived from PBPC apheresis products showed a concomitant decrease in class I CD34+ counts and in BFU-E colony production (Table 4).

The slight increase in the percentage of CD34+ cells detected after cryopreservation was correlated to a concomitant decrease in the number of more mature myeloid cells (CD15; CD13; CD33) (data not shown).

Effects of cryopreservation on cell viability were evaluated by flow cytometry using a multiparameter staining approach. The mean cell viability (±SD) decreased from 99.3±2.1% (pre-cryopreservation values) to 84.2±14.2% after three months of cryopreservation (Table 5). Table 5 also shows results derived from the analysis of either FAS (CD95) antigen expression or DNA staining in CD34-expressing cells labeled with CD34 epitope class I, II, and III-reactive MoAbs.

Determination of CD34+ counts for epitope class I-, II-, and III-reactive MoAbs in fresh and cryopreserved AML blasts

Compared to pre-cryopreservation values, a reduction in class I CD34 epitope expression was also found in thawed CD34+ blasts obtained from 14 patients with acute myeloid leukemia (AML), who were analyzed using a larger series (#17) of CD34 epitope class I, II, and III-reactive MoAbs. In contrast, the cytofluorimetric analysis of acute leukemic cells after frozen storage at –80°C for three months showed that class III CD34+ counts increased in comparison to those found on fresh cells.

The quantification of CD34 antigens showed that our flow cytometry analysis on frozen-thawed AML cells was associated with a significant increase in ABC values for several class III and class II CD34 MoAbs. An increase in ABC values was also observed in thawed AML cells stained with three out of 4 class I CD34 MoAbs, as compared with corresponding results from fresh aliquots. Antigen density was decreased in cryopreserved cells labeled with 14G3 class I MoAb, in comparison to those obtained from the fresh samples (Table 6).

Discussion

In this paper we evaluated the effects of short-term cryopreservation (3 month storage) on class I, II and III CD34 epitope expression in a series of leukapheresis products from lymphoma patients and leukemic sam-
samples from CD34⁺ acute myeloid leukemia.

In previous studies, many groups including ours have shown a great variability in results obtained within and between the CD34 MoAb classes in progenitor cells taken from bone marrow, PBPC, umbilical cord blood, and acute leukemia samples. On the basis of these data, many authors have postulated that the classification into three epitope classes could underestimate the extent of antigenic, epitopic diversity on the extracellular domains of CD34 molecule. Moreover, data derived from cross-block

<table>
<thead>
<tr>
<th>MoAb (epitope class)</th>
<th>pre-cryo-preservation (%)</th>
<th>post-cryo-preservation (%)</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>CD34-9F2 (III)</td>
<td>38.9±34.4</td>
<td>35.9±24</td>
<td>ns</td>
</tr>
<tr>
<td>8G12 (III)</td>
<td>54.8±30.7</td>
<td>51.7±22.3</td>
<td>ns</td>
</tr>
<tr>
<td>S81 (III)</td>
<td>58.3±31.6</td>
<td>62.4±22.8</td>
<td>ns</td>
</tr>
<tr>
<td>5S3 (III)</td>
<td>58.2±31.8</td>
<td>63.4±22.4</td>
<td>ns</td>
</tr>
<tr>
<td>S63 (III)</td>
<td>60.0±30.6</td>
<td>64.9±22.6</td>
<td>ns</td>
</tr>
<tr>
<td>QBEND-10 (II)</td>
<td>55.0±29.5</td>
<td>60.6±22.1</td>
<td>ns</td>
</tr>
<tr>
<td>43A1 (II)</td>
<td>27.9±34.7</td>
<td>28.8±29.5</td>
<td>ns</td>
</tr>
<tr>
<td>MD34.3 (II)</td>
<td>56.8±30.3</td>
<td>61.5±25.0</td>
<td>ns</td>
</tr>
<tr>
<td>MD34.1 (II)</td>
<td>52.6±28.9</td>
<td>52.9±24.6</td>
<td>ns</td>
</tr>
<tr>
<td>MD34.2 (II)</td>
<td>56.8±30.3</td>
<td>62.2±24.6</td>
<td>p &lt; 0.01</td>
</tr>
<tr>
<td>4A1 (II)</td>
<td>54.8±30.2</td>
<td>54.8±25.8</td>
<td>ns</td>
</tr>
<tr>
<td>9066 (II)</td>
<td>50.5±30.7</td>
<td>54.5±23.4</td>
<td>ns</td>
</tr>
<tr>
<td>9069 (II)</td>
<td>48.3±30.4</td>
<td>50.2±25.7</td>
<td>ns</td>
</tr>
<tr>
<td>Immu-133 (I)</td>
<td>50.4±30.0</td>
<td>46.4±20.2</td>
<td>p &lt; 0.05</td>
</tr>
<tr>
<td>Immu-409 (I)</td>
<td>14.5±22.1</td>
<td>4.7±13.6</td>
<td>p &lt; 0.05</td>
</tr>
<tr>
<td>14G3 (I)</td>
<td>53.7±30.4</td>
<td>53.4±19.2</td>
<td>p &lt; 0.05</td>
</tr>
<tr>
<td>B13C5 (I)</td>
<td>34.4±22.1</td>
<td>36.7±22.4</td>
<td>p &lt; 0.05</td>
</tr>
</tbody>
</table>

*Data are expressed as mean percentage±SD; **MESF = molecular equivalent of soluble fluorochrome; ns = not significant (paired test).

A reduction in class I and class II CD34 epitope expression was also found in CD34⁺ blasts obtained from 14 patients with acute myeloid leukemia (AML), who were analyzed using a larger series (#17) of CD34 epitope class I, II, and III reactive MoAbs. The slight increase in the percentage of CD34⁺ cells after cryopreservation was correlated to a concomitant decrease in the number of more mature myeloid cells (CD15⁺, CD13⁺, CD33⁺). As far as the increase in the antibody binding capacity (ABC) observed in cryopreserved cells stained with CD34 epitope class III reactive MoAbs is concerned, many explanations have been offered by authors. Recent studies have shown that class I and class II MoAbs cannot be labeled with charged fluorochromes such as FITC, because of the presence of a large number of acid residues near the binding site of the antibody. Furthermore, class I and class II MoAbs have a strong negative charge, and their conjugation to negatively charged fluorochromes such as FITC induces a significant reduction in the binding affinity of the antibody. As a consequence, class I and class II reactive MoAbs should be labeled with fluorochromes such as PE in order to obtain the most reliable results. The fact that FITC is the most negatively charged fluo-
rochrome may explain the finding that higher ABC values were detected in samples analyzed using FITC-conjugated class III MoAbs, than in those using PE-labeled CD34 MoAbs.37

The large variation in antigen density observed in frozen-thawed CD34+ cells could be due to a number of additional factors, such as poor reproducibility and unreliability of Simply Cellular beads in detecting CD34 surface antigen expression in cryopreserved cells, the differences in antibody binding affinities, as well as the lysis procedure used in this study. Recent data have shown that binding of CD34 MoAbs could be affected by lysis and fixation procedures, and that the binding of the class I antibody Immu-133 was most markedly decreased.38-40 In a study by Basso et al. the percentage of CD34 positivity obtained with NH₄Cl or with Q-Lyse lysis solution was significantly higher than that obtained with FACS lysing solution (up to eight times higher in cord blood).39 On the basis of these data, it cannot be excluded that the lysis methodology may interfere with the binding of CD34 MoAbs to the cells examined in our study. As far as the antibody binding affinities are concerned, it should be take into account that the three epitope subsets, or induce distinct alterations of the CD34 glycoprotein, possibly causing a reduction in their expression cells, the differences in antibody binding affinities of some class I-reactive MoAbs (BI3C5, ICH 3).

In conclusion, this study has shown that exposure to the cryoprotectant DMSO and freezing/thawing procedures modify the distribution of CD34 epitopes as well as the clonogenic capacity of PBPCs from NHL patients, and CD34+ blasts from AM L. These findings need to considered when selecting CD34 MoAbs for enumeration and positive selection of stem/progenitor cells for research and clinical purposes.41

Contributions and Acknowledgments

FL and GLC were responsible for the conception of the study. FL wrote the paper. SM and LF contributed to the flow cytometric analysis of leukemic samples. BC, FM, and MD performed the CD34 cytofluorimetric assay of PBSC. AL, NP, GL and RR were involved in the processing and cryopreservation of PBSC. AL contributed to the cytofluorimetric analysis of FAS antigen. MD, DC, and AB carried out the clonogenic assay.

The criteria for the order in which the authors appear are based on the amount of work performed in this study.

We are indebted to Prof. M. F. Greaves (Leukaemia Research Fund, Institute of Cancer Research, London) for providing us with the purified CD34 MoAbs.

Funding

This study was supported in part by a grant from Istituto Superiore Sanità (project: Blood), MURST 60%, and “Progetto Università-Ospedale di Ferrara”, Italy.

Disclosures

Conflict of interest: none.

Redundant publications: no substantial overlapping with previous papers.

Manuscript processing

Manuscript received April 9, 1999; accepted August 6, 1999.

References


