Early Expression of Glycophorin C during Normal and Leukemic Human Erythroid Differentiation

Jean-Luc Villeval, Caroline Le Van Kim, Ali Bettaieb, Najet Debili, Yves Colin, Bouchra El Maliki, Dominique Blanchard, William Vainchenker, and Jean-Pierre Cartron


ABSTRACT

Glycophorins C and D (GPC and GPD) are two erythrocyte glycoproteins which originate from the same gene but differ in their NH₂-terminal residues. The cell surface expression of these glycoproteins during normal and erythroid differentiation has been investigated with monoclonal and polyclonal antibodies and has been compared to the expression of glycophorin A (GPA), the major sialoglycoprotein of human red cells. Using glycosylation-independent antibodies (monoclonal or polyclonal), GPC or GPA was detected in erythroid and nonerythroid cell lineages. However, a glycosylation-dependent monoclonal antibody (MR4-130) detected an epitope on GPC which appears to be erythroid specific, suggesting that lineage specificity of this glycoprotein is related to some carbohydrate structures. During normal erythroid differentiation, GPC was expressed early at the level of erythroid progenitors (part of erythroid burst-forming unit and erythroid colony-forming unit) as detected with a glycosylation-independent monoclonal antibody (APO 3), whereas GPA is only present during terminal erythroid differentiation. The MR4-130 epitope was not coordinately expressed on the cell surface with the GPC molecule in the erythroid differentiation, since it was detected at the level of the more mature erythroid colony-forming unit slightly later than the GPC polypeptide.

In four erythroleukemic patients, blast cells blocked at discrete stages of the erythroid differentiation were also investigated with antibodies and complementary DNA probes for GPA and GPC. GPA was immunologically detected in three of four cases, and its cell surface expression was correlated with the amount of specific mRNA in the cells, as seen by Northern blot analysis. GPA was immunologically detected on the blast cells of all four patients. However, in two cases including one with positive expression of GPA, the MR4-130 epitope was absent from the GPC molecule. By Northern blot analysis, we found that the GPC/GPD mRNA was present at a high level in all four patient samples. Western blot analysis of GPC and GPD in two of these patients revealed that these mRNAs were mostly translated into the GPD molecule, suggesting that these glycoproteins might be differently processed in certain cases of erythroleukemia.

INTRODUCTION

Human erythrocytes carry at least four main species of sialoglycoproteins known as GPA, GPC, and GPD, which have been extensively investigated and have been used as models of integral membrane proteins (for review, see Ref. 1). These GPs have a high content in sialic acid which contributes to the negative charge of red cells. Moreover, they carry blood group antigens, respectively, and have extensive structural homologies, suggesting that they have derived by duplication of a common ancestral gene. GPA and GPD are minor components of the red cell membrane carrying the blood group Gerbich determinants. Several lines of evidence indicate that these GPs play a critical role in regulating membrane deformability and mechanical stability of red cells (2) by interacting via protein 4.1 with the membrane skeleton (3). Recently, it was suggested that GPC and GPD are encoded by the same gene (4) and that GPD might be a shortened form of GPC (5). Indeed, these two GPs differ in their NH₂-terminal region (4) but are immunologically (6) and structurally (5) indistinguishable in their COOH-terminal domain.

Since the red cell membrane glycoporphins are now well characterized, they represent useful markers to follow the sequential steps of erythroid differentiation, and GPA has been already used for this purpose (7). GPA is synthesized during terminal erythroid differentiation but is detected slightly earlier with PoAbs which recognize peptidic epitopes than with MoAbs which react with carbohydrate-dependent determinants (8). However, even with a PoAb, GPA is not detected on the surface of BFU-E and CFU-E (9). Only preliminary data, however, have been obtained concerning the expression of GPC during normal (10) and leukemic differentiation (11). In the present study, we have compared the expression of GPA and GPC during normal and leukemic erythroid differentiation using specific antibodies, and we have investigated their biosynthesis by Northern blot analysis of their mRNA in four patients suffering from erythroleukemia.

MATERIALS AND METHODS

Samples. Normal bone marrow and blood samples obtained from normal donors after informed consent were collected on heparin. In addition, the blast cells from 4 patients presenting an early erythroid leukemia were analyzed; 3 of these 4 cases have been previously described (11).

Isolation of the Cells. Cells from blood and normal marrow were separated by a Ficoll-metrizoate gradient centrifugation (Lymphoprep; Norgaard, Norway). Adherent and nonadherent cells were separated by a 2-h incubation. T-cells were separated from other nucleated cells by rosette formation with 2-amino-ethylisothiouronium bromide-treated sheep red blood cells (12). Granulocytes were isolated by sedimentation on dextran followed by a Ficoll-metrizoate gradient centrifugation. The remaining red cells in the granulocyte cell pellet were removed by lysis with ammonium chloride (13). Platelets were obtained from platelet-rich plasma.

Antibodies. The antiserum directed against GPA and GPD (L 857) was produced by immunization of rabbits with purified GPD (4). Monoclonal antibodies anti-GPC, MR4-130, and APO 3 were obtained from P. Rouger (14) and W. Dahr (15), respectively. Two previously described antibodies against GPA, a murine MoAb (LICR-LON-R18) and the MN (11) and Ss (16) blood group antigens, respectively.
conjugated to fluorescein 500-fold diluted (Bioart, Meudon, France). Cells were either spread on a slide and examined with a Zeiss microscope (Oberkochen, Federal Republic of Germany) equipped with fluorescence epipolarization or analyzed by flow cytometry. Rabbit antiserum against either GPA or GPC was applied on cytopinned cells after 1-min fixation by methanol; their binding was revealed by a goat anti-rabbit IgG Fab'2 fragment conjugated to rhodamine (Cappel, Cochranville, PA). Normal rabbit serum was used as a control. Fluorescence labeling was performed on the different types of separated hematopoietic cells as well as on hematopoietic colonies grown in plasma clot (21).

Cell Sorting. Flow cytometric analyses and cell sorting were performed on fresh light-density marrow cells stained by MoAbs MR4-130, APO 3, and R 18 using a FACS 440 (Becton Dickinson, Mountain View, CA) or an ATC 3000 (Odam, Wissembourg, France). Marrow cells labeled by a fluorescent conjugated Fab'2 fragment or by an irrelevant IgG1 followed by the fluorescent conjugated Fab'2 fragment were used as a negative control. Cells were sorted under sterile conditions and collected into positive and negative fractions. Unfractionated marrow cells and cells sorted into positive and negative fractions were assayed for hematopoietic progenitors or were cytocentrifuged for May-Grünwald-Giemsa staining.

Clonogenic Assays. Clonogenic assays for BFU-E, CFU-E, CFU-GM, and CFU-MK were performed by either the methylcellulose technique (23) or the plasma clot technique (21). Stimulating factors were a combination of 5% supernatant of the Mo cell line and of 1 IU/ml of human erythropoietin (Terry Fox Laboratory, Vancouver, Canada) for erythroid progenitors, 5% supernatant of the 5637 cell line for CFU-GM, or 2.5% phytohemagglutinin leukocyte-conditioned medium for CFU-MK.

Complementary DNA Probes and Northern Blot Analysis. Blood cells (>90% of blasts) from the 4 erythroleukemic patients were obtained by separation on a Ficoll gradient, and total RNAs were prepared according to published procedures (24, 25). Aliquots of 10 μg were resolved by electrophoresis on 6% formaldehyde/1% (w/v) agarose gels and transferred onto nitrocellulose membranes according to the method of Thomas (26). Hybridizations with 32P-labeled complementary DNA inserts for GPC (27) and GPA (28) were performed as previously described (27).

Western Blot Analysis. The presence of the GPC and GPD molecules on the blasts from Patients 2 and 3 were investigated by immunoblotting technique using the polyclonal and monoclonal (APO 3) antibodies according to Towbin et al. (29).

RESULTS

Specificity of Antibodies against Human Glycophorins C and D. The specificity of three antibodies directed against the erythrocyte glycophorins, GPC and GPD, was examined in detail. The structure recognized by the monoclonal antibody MR4-130 has been originally described as directed against the blood group Gerbich antigens (14) which are carried by GPC and GPA (for review, see Ref. 1). This antibody, however, recognized the NH2-terminal residues of GPC and did not bind to GPD as deduced from inhibition studies with purified glycoprotein fractions (15). Furthermore, the binding to GPC involved sialic acid residues. We found that MR4-130 antibody could be used for binding studies on intact cells, but it did not react properly on either Western blots or by immunoprecipitation. The monoclonal antibody APO 3 reacted with a trypsin-sensitive but neuraminidase-resistant determinant located in the NH2-terminal region of GPC (15). Western blot studies carried out with red cell membrane preparations further indicated that APO 3 reacted with GPC but not with GPD (Fig. 1). It may be deduced from these data that MR4-130 and APO 3 are both directed against GPC without cross-reactivity for GPD. However, the MR4-130 epitope was glycosylation dependent, whereas the APO 3 epitope was not. The rabbit antibody L 857 was obtained by immunization with a purified GPD preparation. The immune serum was absorbed on intact erythrocytes in order to select glycosylation-independent antibodies directed against the intracellular or intramembranous domain of the minor glycophorins. As expected, the absorbed antibody reacted with both GPC and GPD in Western blot analysis (Fig. 1) since both glycoproteins share a common C-terminal region. We subsequently investigated the reactivity of these antibodies directed against GPC and GPD and those against GPA with hematopoietic cells.

Expression of GPC and GPD by Normal Hematopoietic Cells. Among peripheral blood cells, the MoAb or PoAb directed against GPA stained only red cells as previously reported (7, 9, 10). In contrast, a different pattern was observed with antibodies directed against GPC and GPD. MR4-130 stained only red cells by indirect immunofluorescence, whereas APO 3 stained leukocytes as well. APO 3 gave significant fluorescence labeling with all T-cells (rosette-forming cells) and very weak staining, just above background with B-cells (nonadherent, non-rosetting cells) and monocytes (adherent cells). Granulocytes and platelets were negative. The reactivity of the rabbit serum (L 857) was similar to that of APO 3.

When tested on marrow cells, the antibodies against GPA
stained only erythroblasts which were characterized morphologically under the light microscope and by double labeling experiments using the anti-CA 1 or anti-hemoglobin polyclonal antibodies. Identical results were obtained with the MR4-130 MoAb. The specificity of the anti-GPA antibodies and MR4-130 MoAb for the erythroid lineage was further confirmed by the analysis of CFU-GM-, BFU-E-, and CFU-MK-derived colonies in indirect immunofluorescence experiments. All these antibodies stained only erythroid colonies as ascertained by their morphology and by double labeling experiments. Furthermore, no staining was observed when erythropoietin was omitted from the culture medium.

APO 3 also gave a bright fluorescence on erythroblasts. However, other marrow cells (negative for hemoglobin or CA 1) were also stained, but with a very weak intensity of labeling. These cells could not be identified. The L 857 antisera labeled erythroid cells strongly as well as some myeloid precursors which were identified by a staining with 80H5 (anti-CD15: X hapten, 3-fucose-N-acetyl lactosamine).

In order to more precisely investigate the reactivity of APO 3 and MR4-130 on hematopoietic precursors, marrow cells were analyzed after indirect immunofluorescence labeling by flow cytometry. At first, using R 18 as a positive control, two cell populations were clearly identified by their fluorescence intensity. Ninety-six % of the cells were negative and superimposed with the negative controls, while 4% of marrow cells were clearly positive which, after sorting, were identified as erythroblasts. In contrast, a complex curve was observed when using MR4-130 and APO 3 without clear-cut cell populations. Cells were subsequently analyzed for their fluorescence intensity and their forward angle light scattering (Fig. 2). Cells with the maximum autofluorescence (myeloid precursors) have a high forward angle light scattering and can be easily removed by selecting gates as shown in Fig. 2. Accordingly, an irrelevant IgG1 MoAb gave less than 0.4% positive cells in this gate. Morphological analysis of the cells sorted with MR4-130 and APO 3 is reported in Table 1. MR4-130 positive population (3.7%) was constituted by 80% of erythroblasts. In addition, numerous naked nuclei from acidophilic erythroblasts were present but were not enumerated. A minor population (14%) of myeloid precursors was present which could be either truly positive cells or a cell contaminant since, after reanalysis, the sorted population was 90% positive for MR4-130. The APO 3 positive population (6%) included a large fraction of blast cells (Table 1: myeloblasts plus blasts, 46%) besides the erythroid cells. These immature cells showed a low intensity of fluorescence labeling and an intermediate or high forward angle light scattering. Some of these blast cells were myeloblasts; others had the morphological appearance of early erythroid cells.

Therefore, the first part of this study indicated that, in contrast to GPA, GPC or molecules immunologically related to GPC are expressed not only on the erythroid lineage but also on other cell lineages such as T-cells, although at low antigenic density. However, the carbohydrate-dependent epitope identified by MR4-130 was restricted to the erythroid lineage.

GPC Is Expressed Earlier Than GPA during Erythroid Differentiation. In one set of experiments, the reactivity of MR4-130 and anti-GPA antibodies with blood BFU-E-derived colonies from Day 5 to Day 12 of culture was investigated. The erythroid origin of these colonies was deduced from double labeling with the anti-CA 1 antibody. MR4-130 stained erythroid colonies as early as Day 5 of culture. In contrast, R 18 did not recognize the BFU-E-derived colonies until Day 8; labeling was significant from Day 9 of culture and was complete at Day 10. A double staining was also performed between MR4-130 and the rabbit serum against GPA at Day 7 of culture. The majority of BFU-E-derived colonies stained by MR4-130 remained negative with the rabbit anti-GPA, indicating that GPC can be detected on the membrane of erythroid cells earlier than GPA in the erythroid differentiation even when investigations are carried out with a PoAb.

In a second set of experiments, MR4-130 was used in cell-sorting analyses. Clearly positive and negative bone marrow cells were sorted. All the morphologically identifiable erythroblasts were found in the positive fraction containing from 8% to 10% of marrow cells. In addition, all typical proerythroblasts and blast cells classified as erythroid cells by their strong basophilia with a developed ergastoplasm were present in the positive fraction. Clonogenic assays were performed, and the results are summarized in Table 2. Hematopoietic progenitors of all cell lineages (BFU-E, CFU-GM, and CFU-MK) were found in the negative fraction, and a significant percentage (31%) of CFU-E was found in the positive fraction. These CFU-E corresponded to progenitors which gave small erythroid
Expression of GPA, GPC, and GPD in Erythroleukemias. The cell surface expression of GPs on the blasts from the 4 erythroleukemic patients was investigated, and the results are summarized in Table 4. In 3 patients, leukemic cells resembled normal erythroid cells by their immunophenotype. Patient 4 showed a more mature phenotype with the expression of GPA detected by MoAb and PoAb on the majority of the cells. In Patient 2, 60%, 1%, and 35% of the blast cells were stained by MR4-130, R 18, and the PoAb directed against GPA, respectively. In Patient 1, no significant staining was observed with anti-GPA antibodies and MR4-130; the rare positive cells corresponded to erythroblasts. In contrast, APO 3 antibody as well as Cell types
<table>
<thead>
<tr>
<th></th>
<th>Unsorted cells (100%)</th>
<th>MR4-130 positive cells (3.7%)</th>
<th>APO 3 positive cells (6%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Blasts</td>
<td>1</td>
<td>0</td>
<td>27</td>
</tr>
<tr>
<td>Myeloid cells</td>
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<td></td>
<td></td>
</tr>
<tr>
<td>Proerythroblasts</td>
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<td>Acidophilic erythroblasts</td>
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<td>Lymphocytes</td>
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<tr>
<td>Monocytes</td>
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<td></td>
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<tr>
<td>Plasma cell</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>BFU-E</td>
<td>305 ± 64 (26)</td>
<td>17 ± 12 (12)</td>
<td>64 ± 2 (3)</td>
</tr>
<tr>
<td>Day 7 BFU-E</td>
<td>2457 ± 286 (88)</td>
<td>167.5 ± 23.5 (53)</td>
<td>243.5 ± 26 (93)</td>
</tr>
<tr>
<td>Day 14 BFU-E</td>
<td>2816 ± 161 (47)</td>
<td>185 ± 157 (12)</td>
<td>296 ± 10 (7)</td>
</tr>
<tr>
<td>CFU-GM</td>
<td>10 ± 26 (26)</td>
<td>4 ± 1 (2)</td>
<td>0 ± 0 (0)</td>
</tr>
<tr>
<td>Day 7 CFU-GM</td>
<td>2457 ± 314 (74)</td>
<td>185 ± 157 (12)</td>
<td>296 ± 10 (7)</td>
</tr>
<tr>
<td>Day 14 CFU-GM</td>
<td>2816 ± 161 (47)</td>
<td>185 ± 157 (12)</td>
<td>296 ± 10 (7)</td>
</tr>
</tbody>
</table>

* Mean ± SD.

Numbers in parentheses, percentage.

Table 2 Sorting of human bone marrow cells with the MR4-130 MoAb

A cell sorting was performed only on the fluorescence intensity. Bright fluorescent cells were sorted in the positive fraction. Cells were cultured at a concentration of 5.10⁶ cells/ml for MR4-130 positive cells and 1.10⁶ cells/ml for MR4-130 negative cells in the CFU-E, BFU-E, and CFU-GM assays, whereas they were plated at a concentration of 15.10³ cells/ml and 2.10² cells/ml, respectively, for the CFU-MK assay. Results are expressed as the number of progenitors per 1.10⁵ cells.

Table 3 Sorting of human bone marrow cells with MR4-130 and APO 3 MoAbs

A biparametric cell sorting (fluorescence intensity and forward angle light scattering) (see Fig. 2) was performed. Cells were plated at a concentration of 5.10⁶ cells/ml for MR4-130 positive cells, 35.10⁴ cells/ml for APO 3 positive cells, and 1.10² cells/ml for MR4-130 negative cells in the CFU-E, BFU-E, and CFU-GM assays, whereas they were plated at a concentration of 15.10³ cells/ml and 2.10² cells/ml, respectively, for the CFU-MK assay. Results are expressed as the number of progenitors per 1.10⁵ cells.

Table 4 Morphological analysis of unsorted versus sorted MR4-130 and APO 3 positive marrow cells

<table>
<thead>
<tr>
<th>Cell types</th>
<th>Unsorted</th>
<th>MR4-130 positive</th>
<th>APO 3 positive</th>
</tr>
</thead>
<tbody>
<tr>
<td>Blasts</td>
<td>100</td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td>BFU-E</td>
<td>305 ± 64</td>
<td>17 ± 12</td>
<td>64 ± 2</td>
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<td>Day 7 BFU-E</td>
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<td>185 ± 157</td>
<td>296 ± 10</td>
</tr>
<tr>
<td>CFU-GM</td>
<td>10 ± 26</td>
<td>4 ± 1</td>
<td>0 ± 0</td>
</tr>
<tr>
<td>Day 7 CFU-GM</td>
<td>2457 ± 314</td>
<td>185 ± 157</td>
<td>296 ± 10</td>
</tr>
<tr>
<td>Day 14 CFU-GM</td>
<td>2816 ± 161</td>
<td>185 ± 157</td>
<td>296 ± 10</td>
</tr>
</tbody>
</table>

* Mean ± SD.

Numbers in parentheses, percentage.
Fig. 3. Schematic diagram of the expression of GPA and GPC during normal erythroid differentiation. p BFU-E, primitive BFU-E (Day 16); m BFU-E, mature BFU-E (Day 12).

Table 4. Phenotype of the blast cells in four cases of erythroleukemia

<table>
<thead>
<tr>
<th>Patient</th>
<th>GPA MoAb (R 18)</th>
<th>GPA PoAb</th>
<th>GPC MoAb (MR4-130)</th>
<th>GPC PoAb (MR4-130)</th>
<th>CAI* PoAb</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>100</td>
<td>40</td>
</tr>
<tr>
<td>2</td>
<td>1</td>
<td>35</td>
<td>60</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>3</td>
<td>23</td>
<td>63</td>
<td>0</td>
<td>42</td>
<td>49</td>
</tr>
<tr>
<td>4</td>
<td>58</td>
<td>82</td>
<td>60</td>
<td>ND</td>
<td>ND</td>
</tr>
</tbody>
</table>

CAI, carbonic anhydrase 1; ND, not determined.

In the last patient (Patient 3), leukemic cells differed from a normal erythroid cell by its phenotype. Indeed, about half of the blast cells from Patient 3 express GPA, but GPC was undetectable with MR4-130. In contrast, L 857 serum and APO 3 faintly stained the blast cells as observed in Patient 1. Our results suggest an abnormal processing of the GPC/GPD molecules, especially of the GPC glycosylation, in these leukemic cells. In order to further discriminate between the expression of GPA and GPD, immunostaining of the proteins extracted from the blast cells of Patients 2 and 3 was performed using the L 857 antiserum and APO 3 (Fig. 4). In both patients, GPD was clearly detected with the PoAb. The apparent molecular weight of the GPD was slightly higher in the two patients than in control red cells, suggesting an abnormal glycosylation. On Western blots, APO 3 revealed low amounts of GPC on the erythroblasts of Patient 3.

To investigate whether the antibody staining in blast cells was correlated with gene transcription, the level of mRNAs specific of GPA and GPC was determined by Northern blot analysis (Fig. 5). The GPC mRNA was sized at 1.1 kilobases as in other tissues (4) and was detectable at a high level in blasts from all patients, including those which express no significant amount of the MR4-130 epitope (Patients 1 and 3). Using the same Northern blot, the GPA mRNAs were detected in significant amounts only in cells from patients in which GPA had been previously detected by immunological techniques (Patients 3 and 4). A very low level of GPA mRNAs was present in Patients 1 and 2. The size of the GPA mRNAs (1.1, 1.7, and 2.4 kilobases) was similar to that previously reported in fetal and adult erythroblasts and in the K562 cell line (28, 31). The 3.6-kilobase mRNA detected with the GPA probe could represent an unspliced precursor, since it was detected only when

Fig. 4. Detection of GPC and GPD by Western blot in protein extracts from normal red cells and blasts from Patients 2 and 3. Electrophoresis and blotting of membrane proteins as in Fig. 1. A, immunoblotting with L 857 polyclonal antibody 50-fold diluted followed by a detection with 125I-labeled Protein G. Lane 1, normal red blood cells; Lane 2, Patient 2; Lane 3, Patient 3. The high-molecular-weight material corresponds to IgG bound to the cell surface and revealed by the 125I-labeled Protein G. B, immunoblotting with APO 3 MoAb (culture supernatant 500-fold diluted). Binding was revealed by phosphatase alkaline-conjugated goat IgG directed against mouse immunoglobulin. Lane 1, normal red blood cells; Lane 2, Patient 3. A similar but weaker band which could not be photographed was obtained in Patient 2.

Fig. 5. Level of GPC/GPD and GPA mRNAs in the blast cells from Patients 1 to 4. After electrophoresis and transfer to nitrocellulose (see "Materials and Methods"), 10 μg of total RNAs were hybridized first with the GPC complementary DNA probe and then rehybridized with the GPA complementary DNA probe. Stringent washes were done in 1 mM NaH2PO4, pH 7.7, containing 18 mm NaCl, 0.1 mm EDTA, and 0.1% (w/v) sodium dodecyl sulfate at 60°C for 10 min. The number of each lane corresponds to that of each patient in Table 2.
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total RNAs were analyzed by electrophoresis and not when purified polyadenylate-containing RNA was used.

DISCUSSION

In this paper, we have investigated the expression of GPA and GPC/GPD during normal human erythroid differentiation and in erythroleukemias. Several lines of evidence indicate that expressions of GPA and GPC/GPD during hematopoietic differentiation clearly differ. We found that GPA, in contrast to GPA (7, 9, 30), was not an erythroid-specific protein. When using the APO 3 MoAb which recognizes a glycosylation-independent epitope of GPA, a molecule identical or immunologically related to GPA was found on lymphocytes, monocytes, and presumably on myeloid precursors but with a much lower antigenic density than on the erythroid lineage. It is well established that this molecule is GPA, since the expected mRNA was recently detected in all these cell types. However, further analyses are necessary to characterize the GPA molecule of nonerythroid cells. Some glycosylation-dependent epitopes of the GPA molecule may be lineage specific. Indeed, the MR4-130 MoAb which defines a sialic acid-dependent epitope on GPA has an erythroid-restricted reactivity. From the present study, it cannot be firmly concluded whether this phenomenon is related to an erythroid-specific glycosylation of GPA or to a glycosylation determinant which is predominant on the erythroid GPA. The presence of some myeloid precursors in the MR4-130 positive cell fraction in sorting experiments suggests that the second hypothesis is more likely. Cell type-specific O-linked carbohydrate structures are well documented for leukosialin, another sialoglycoprotein of the hematopoietic series (32). It was shown that leukosialin is an ubiquitous molecule among hematopoietic cells which displays cell lineage-specific O-glycosylation. Supporting this hypothesis a MoAb called GA3 was produced recently which identifies an O-glycosylation-dependent leukosialin epitope which, like the MR4-130 epitope on GPA, appears to be erythroid restricted (33).

GPC is expressed early during erythroid differentiation, whereas GPA is only synthesized during terminal erythroid differentiation (7, 9, 30). Indeed, GPA could be detected with APO 3 on a part of BFU-E and on CFU-E. This early synthesis of GPA during erythroid differentiation is in agreement with a recent report which indicates the presence of GPA on normal proerythroblasts (34). In addition, the expression of the MR4-130 epitope was found to be maturation dependent, since it was detected later in the erythroid differentiation than the APO 3 epitope. MR4-130 did not react with BFU-E but bound to a fraction of CFU-E. The MR4-130 positive CFU-E gave rise to small colonies, suggesting that they correspond to the more differentiated types of these progenitors (35). Maturation-dependent glycosylation of other erythroid sialoglycoproteins has been previously reported. Gahmberg et al. (8, 36) have shown, using PoAb and MoAb, that O-glycosylation of GPA gradually increases during erythroid differentiation, its glycosylation being complete at the stage of the polychromatophilic erythroblast. However, these results were not confirmed by a recent report using two MoAbs, including one which is not sugar dependent (37). In contrast, leukosialin has been clearly demonstrated to exhibit a maturation-dependent O-glycosylation (33).

We have also investigated whether GPD, which represented a shortened variant of GPC, could have a different tissue distribution than GPA. In fact, the PoAb directed against both GPC and GPA had a cellular reactivity very close to that of a GPC-specific antibody, suggesting that GPD and GPA have a coordinate expression. However, further studies using continuous cell lines are necessary to determine whether GPA and GPD may have a dissociated expression in certain cell types since no antibody is specific for GPD alone.

Finally, we compared the expression of GPA and GPC/GPD in four cases of erythroleukemia undiagnosed by morphological criteria (11) with blast cells blocked at different stages of the erythroid maturation. Important differences in the expression of these glycoporphins have been found. Based on the immunological detection of GPA, these cases have been classified into three groups corresponding to discrete stages of the normal erythroid differentiation (11). Northern blot analysis indicated a direct relationship between the cell surface expression of GPA and the level of its specific mRNAs. In contrast, while the GPC molecule was detected in all cases with the APO 3 antibody, the cell surface expression of the MR4-130 epitope on blast cells did not perfectly correlate with the classification established previously (11), since in one patient this MoAb was not reactive with the blast cells expressing GPA. In fact, this result can be explained by an incomplete glycosylation of the GPC molecule and probably also by a posttranscriptional control of GPC/GPD expression. Two lines of evidences support this last hypothesis. First, Western blot analysis indicated that the GPC/GPD mRNA is preferentially translated into an incompletely glycosylated GPC molecule. Second, the large amounts of GPC/GPD mRNA present in blast cells from all patients did not correlate with the level of these molecules.

In conclusion, our studies indicate that GPA and GPC/GPD are useful markers to follow the differentiation of the erythroid cell lineage. GPA is expressed earlier than GPA, but evidences are accumulating that GPA has a wider distribution than GPA on hematopoietic cells. Interestingly, however, erythroid specificity of GPC appeared to be acquired by maturation-dependent carbohydrate structures. Investigations on the cell surface expression and transcription of GPA and GPC/GPD molecules from erythroleukemic cells suggest an abnormal processing of the GPC/GPD molecules. Further studies should indicate whether this is due to the leukemic process or to a posttranscriptional control of GPC/GPD production in immature cells.

ACKNOWLEDGMENTS

We are indebted to Z. Mishal and A. Katz for their help in cell-sorting experiments. The authors wish also to thank Dr. P. A. W. Edwards for providing R 18, Dr. W. Dahr for APO 3, Dr. C. G. Gahmberg for the anti-GPA PoAb, Dr. P. Mannoni for 80H5, and Dr. P. Rouger for MR4-130. We are grateful to Drs. J. London and C. Rahuel for providing the complementary DNA probe for GPA and to J. M. Massé for photographic assistance.

REFERENCES

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