Use of a Monoclonal Antibody (GA3) to Demonstrate Lineage Restricted O-Glycosylation on Leukosialin During Terminal Erythroid Differentiation

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A murine monoclonal antibody (GA3) obtained by immunizing mice with cells of the human erythroleukemic cell line K562 is shown to define a 105 kilodalton (kd) membrane antigen on K562 cells that is restricted within the hematopoietic system to the erythroid lineage and to a minor population of CD3, CD4 positive T lymphocytes. Cocapping studies and immunoprecipitation experiments performed with GA3 and L10, an anti-sialophorin monoclonal antibody reacting with leukosialin (Gp105) on K562 cells, demonstrate that the antigen detected by GA3 on K562 cells is identical to leukosialin. Neuraminidase treatment but not tunicamycin treatment of K562 cells abolishes the expression of the GA3-epitope without affecting the L10-epitope thus providing evidence that terminal sialic acid present on O-linked oligosaccharide chains on Gp105 is essential for the expression of the GA3-epitope. Further analysis by flow cytometry and immune panning experiments performed on bone marrow cells with GA3 or L10 demonstrate that, in contrast to L10, which reacts with all types of hematopoietic progenitors, the epitope recognized by GA3 is restricted to the erythroid lineage, and appears during erythroid differentiation before glycophorin A on the earliest morphologically recognizable erythroid precursor, the proerythroblast. Our results therefore suggest that O-linked oligosaccharides on leukosialin express lineage restricted and even maturation restricted antigenic structures that might serve as cell lineage specific markers.

The surface structure of human erythroid cells has been largely studied, and sequential membrane changes associated with the expression of surface glycoproteins during the process of erythroid maturation have been reported. Thus, band 3 and band 4.5, two major glycoproteins of the red cell membrane, appear only at a late stage of erythroid differentiation, whereas Gp 105, a very early marker of the erythroid lineage, declines during maturation. Erythroblasts possess only small amounts of Gp105 on their cell surface and erythrocytes are completely lacking this glycoprotein. In contrast, glycophorin A, the major sialoglycoprotein of the red cell membrane is present throughout the erythroid cell lineage and its expression increases with the maturation process. However, discrete changes in the O-glycosylation pattern of glycophorin A during maturation have been reported.

An interesting model to study the expression of early erythroid differentiation markers is the human erythroleukemic cell line K562. This cell line expresses glycophorin A and Gp 105 as the major surface glycoproteins and is believed to represent an early erythroid precursor cell. Gp 105 has recently been purified from K562 cells and shown to be a heavily glycosylated single chain sialoglycoprotein. The apparent molecular weight of Gp 105 as calculated from sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) is extremely dependent on the percentage of acrylamide used and varies from 105 kilodaltons (kd) (8% gel) to 116 kd (7% gel). The nonglycosylated apoprotein has a mol wt of 52,000 and an early precursor form that is endo-H sensitive has a mol wt of 54,000. Neuraminidase treatment of Gp 105 results in a characteristic decrease of its electrophoretic mobility on SDS-polyacrylamide gels.

Recently, Carlsson and Fukuda presented evidence that Gp 105 is not restricted to the erythroid lineage but is a major sialoglycoprotein widely distributed among different hematopoietic cell lines belonging to erythroid, myeloid, T and B lymphoid cell lineages. The molecular weight of the mature form of Gp 105, now called leukosialin, varies with the cell types studied. This is due to differences in the glycosylation patterns that appear to be cell lineage specific, whereas a common precursor form of mol wt 54,000 has been detected in all of the cell types. Similar results have also been reported for sialophorin, previously called gpl 115. This sialoglycoprotein, identified by the monoclonal antibody L104 is not exclusively a lymphoid molecule but is also expressed on other blood cells, including monocytes, neutrophils, and platelets, although distinct molecular weights have been observed. Thus, leukosialin and sialophorin have multiple properties in common.

In the present study, we have investigated the reactivity of the monoclonal antibody GA3, which precipitates the 105 kd band from surface-labeled K562 cells. Our results provide evidence that GA3 is directed against leukosialin on K562 cells. The epitope, which is dependent on sialic acid on O-linked oligosaccharides of K562-Gp105, is essentially restricted to the red cell series and is preferentially expressed on immature erythroblasts. Furthermore, we demonstrate that the GA3 epitope appears during erythroid differentiation on the earliest morphologically recognizable erythroid precursor, the proerythroblast. In contrast, leukosialin, iden-
ified by the anti-sialophorin monoclonal antibody (MoAb) L10, is already expressed on erythroid progenitors as well as other hematopoietic progenitors.

MATERIALS AND METHODS

Antibodies
Several murine MoAbs were used. GA3 recently has been obtained with other new MoAbs by immunizing mice with the K562 erythroleukemic cell line (manuscript in preparation). L10, which was kindly donated by Dr E. Remold-O'Donnell, (Center for Blood Research, Boston), reacts with sialophorin previously called gplL115, a membrane glycoprotein present on peripheral blood T lymphocytes and defective in patients with the Wiskott-Aldrich syndrome. This antibody has recently been reported to react with leukosialin (Gp 105) on K562 cells. L1C-LON-R10 is an anti-glycophorin A antibody, B3/25 an anti-human transferrin receptor antibody, and C17 reacts with platelet GP IIIa. In addition, a rabbit anti-von Willebrand factor antisera (Dakopatts, Copenhagen) and a rabbit anti-carbonic anhydrase I (CA 1) IgG were used.

Cells and Cell Culture
Cell lines. Two erythroleukemic cell lines (K562, HEL), two T lymphocytic cell lines (CCRF-CEM, CCRF-HSB2), three B lymphocytic cell lines (Daudi, Raji, Ramos), two myeloid cell lines (HL60, KG1) and a histiocytic cell line (U937) were studied. The cells were routinely cultured in RPMI 1640 medium supplemented with 10% heat-inactivated fetal calf serum and 2 mmol glutamine, except for KG1 cells, which were cultured in minimal essential alpha medium (MEM).

Peripheral blood and bone marrow cells. Normal mononuclear cells from heparinized peripheral blood were separated by Ficoll-metrizoate gradient density centrifugation (Lymphoprep, Nye-gaard, Sweden; d:1.077). Adherent and nonadherent peripheral blood mononuclear cells were separated by a two-hour adherence at 37°C on plastic Petri dishes (Falcon, Oxnard, CA). Adherent cells were detached by incubating the dishes on ice. Granulocytes from the pellet of the Ficoll density gradient centrifugation were separated from RBCs by Dextran-Radioselactan sedimentation. Platelets were obtained by differential centrifugation of whole blood anticoagulated with ACD-A as previously described.

Bone marrow cells were isolated from aspirates obtained from bone marrow transplantation donors. Informed consent was obtained from all bone marrow transplantation donors according to the institutional review board guidelines. The bone marrow aspirates were therefore diluted by blood cells. Light density cells were isolated by Ficoll metrizoate gradient density centrifugation.

Clonogenic assays. Hematopoietic progenitors were cloned in semisolid medium using either the plasma clot or the methylcellulose technique. Stimulating factors were: Epo (1 IU/mL) plus 5% supernatant of the Mo cell line for erythroid progenitor assays, 5% supernatant from the s167 cell line for the CFU-GM assay, and PHA-ALM for the CFU-MK assay. Colonies were scored at day 7 and 14 for CFU-GM, at day 7 for CFU-E, and at day 12 for late BFU-E using an inverted microscope. CFU-MK colonies grown in plasma clot were scored at day 12 of culture by indirect immunofluorescence using the MoAb C17.

Indirect immunofluorescence. Unfixed native or neuraminidase treated cells (0.5 to 1.106 cells in 100 µL PBS) were incubated for 30 minutes at 4°C with 100 µL of an appropriate dilution of the antibody. The cells were washed three times in ice cold phosphate buffered saline (PBS) and reincubated with 100 µL of an appropriately diluted FITC-GAM F(ab)2 or RITC-GAM F(ab)2. After 30 minutes at 4°C, the cells were washed three times and scored for surface immunofluorescence positive cells using a Zeiss microscope equipped with fluorescence epi-illumination or sorted with fluorescence-activated cell sorter. Desialylation was performed by incubating 2.106 washed cells with 0.1 U of neuraminidase from Vibrio cholerae (Behringwerke, Marburg, West Germany). Copping studies were performed with saturating amounts of L10 and GA3, which are both of the IgGl subclass. Briefly, K562 cells were incubated with the first antibody (L10 or GA3), stained with FITC-GAM F(ab)2, then cycled for four hours at 37°C, followed by an incubation with the second antibody and staining with RITC-GAM F(ab)2.

Indirect immunofluorescence on in vitro cultures of hematopoietic colonies was directly performed in Petri dishes as previously described. For the detection of immature cells of the erythroid and megakaryocytic lines, a second intracellular labeling was subsequently performed after methanol fixation using a polyclonal rabbit serum directed against either CA I or von Willebrand factor.

Cell sorting. Cell sorting was performed on a fluorescence activated cell sorter FACS IV (Becton Dickinson, Mountain View, CA). Fresh light density marrow cells were labeled by GA3 or L10 and FITC-GAM (F(ab)2) using the indirect immunofluorescence assay. Cells incubated only with FITC-GAM F(ab)2 or with an irrelevant IgGl MoAb and FITC-GAM F(ab)2 served as a negative control. Positive and negative cells were collected and cultured either by the methylcellulose or the plasma clot technique.

Immune panning. Light density marrow cells were first incubated with an irrelevant MoAb and layered on 100-mm Petri dishes coated with affinity purified goat anti-mouse IgG to deplete the Fc receptor positive cells. The non-adherent cells were recovered, labeled with either GA3 or L10 for 30 minutes at 4°C, and incubated in coated plates as described above. Adherent and nonadherent cells were recovered, counted, and cultured in methylcellulose or plasma clot.

Radiolabeling of cells. Exponentially growing K562 cells were harvested by low speed centrifugation and washed twice in PBS. Cell surface iodination was performed by the lactoperoxidase catalyzed iodination procedure. Galactose and N-acetylgalactosamine residues were labeled by the neuraminidase/galactose oxidase/NaB₃H₄ method and sialic acid residues by the periodate/NaB₃H₄ technique. Metabolic labeling of K562 cells was performed using 35S-methionine (Amersham, United Kingdom; specific activity >800 Ci/mmol) as previously described. Briefly, washed K562 cells were resuspended at 106 cells/mL in methionine-free RPMI medium supplemented with 10% heat-inactivated diazylated fetal calf serum. After 30 minutes at 37°C, 500 µCi/mL 35S-methionine were added to the medium and the cells cultured for three hours at 37°C. Metabolic labeling in the presence of tunicamycin (Boehringer, Mannheim, West Germany) was performed by preincubating the cells for three hours in RPMI medium containing 10 µg/mL tunicamycin. 35S-methionine labeling of the cells was then performed as described with tunicamycin in the culture medium.

Immunoprecipitation. Surface-labeled or metabolically labeled cells were washed three times in PBS and resuspended at 5 x 109 cells/mL in buffer A (10 mM Tris-HCl pH 7.4 containing 1% [vol/vol] Triton X-100, 2 mM PMSF, 1% aprotinin, 1 µmol leupeptin, 3 mM EDTA and 0.15 mol/L NaCl). After 30 minutes on ice, the samples were centrifuged at 1000 g for ten minutes and the supernatant recovered. Cell lysate corresponding to 106 cells was immunoprecipitated with 5 µL of ascites and protein A-Sepharose as described. For sequential immunoprecipitation, labeled K562 cell lysate was first immunoprecipitated with 5 µL of L10 ascites. The supernatant was then recovered, depleted of free L10 IgG with protein A-Sepharose, and further immunoprecipitated with 10 µL of GA3 ascites. The pellets were resuspended in 10...
mmol/L Tris-HCl pH 7.4 containing 2% SDS and 5% (vol/vol) 2-mercaptoethanol. Bound proteins were eluted by heating the suspension at 100°C for five minutes.

**SDS-PAGE.** SDS-PAGE was performed according to Laemmli using 8% gels. After electrophoresis, the gels were fixed, processed for fluorography with Amplify (Amersham, United Kingdom), dried, and exposed for autoradiography at -80°C to Kodak XAR-5 films (Kodak-Pathé, France).

**RESULTS**

**Cocapping of GA3 and L10 Antigen on Unfixed K562 Cells**

We recently obtained a MoAb (GA3), which, in preliminary experiments, precipitated a band of mol wt 105,000 from surface-labeled K562 cells. To determine the identity of the antigen detected by GA3 on K562 cells, we performed indirect immunofluorescence using GA3 and L10, an antisialophorin MoAb previously shown to recognize the 105 kd leukosialin molecule on K562 cells. Preliminary experiments, performed on unfixed K562 cells with L10 and FITC-GAM F(ab')2, showed that almost complete cap formation could be achieved by incubating the stained cells for four hours at 37°C (Fig 1A). Subsequent incubation of the stained cells with GA3 and RITC-GAM F(ab')2 revealed a concordant staining and a colocalization of the cap formed with L10 (Fig 1B). As a control, L10/FITC-GAM F(ab); labeled and capped cells (Fig 1C) were incubated with R10, an antiglycophorin A antibody. In this experiment, no colocalization but only a diffuse peripheral staining of the cells was observed (Fig 1D). Thus, glycophorin A remained uniformly distributed and did not coaggregate with the L10 antigen. Cocapping was also observed when the cells were first incubated with GA3 followed by a second incubation with L10. However, under these conditions, an additional diffuse membrane staining was also observed, suggesting that GA3 did not react with all the molecules identified by L10 (result not shown).

**Immunocchemical Characterization of the Antigens Recognized by GA3 and L10**

Immunoprecipitation experiments were performed with GA3 and L10 on 125I-surface labeled K562 cells. The result is shown in Fig 2. GA3 precipitated a band of apparent mol wt 105,000 (Fig 2, lane 3), which comigrated with leukosialin identified by L10 (Fig 2, lane 2), although this latter band was more intense than that observed with GA3. Further evidence that GA3 reacted with leukosialin was obtained by sequential immunoprecipitation experiments performed on 125I-surface labeled K562 cells. The cell lysate was first immunoprecipitated with L10 and the supernatant obtained from this experiment was then immunoprecipitated with GA3. As shown in lane 4, no band was precipitated after the addition of GA3. In contrast, when the lysate was first reacted with GA3 followed by L10, a weak band was again observed (Fig 2, lane 5). This result confirms the immunofluorescence studies and indicates that L10 and GA3 recognize different epitopes on the same molecule, although not all L10 binding molecules express the GA3 epitope. Furthermore, as shown in Fig 3, immunoprecipitation experiments performed on periodate/NaB3H4 surface-labeled K562 cells lysed under conditions where proteases were not completely

![Fig 1](image1.png)

![Fig 2](image2.png)
Fig 3. Fluorography pattern of an 8% polyacrylamide slab gel of immunoprecipitates obtained with L10, GA3, and R10 from K562 cells labeled by the periodate/NaB₃H₄ technique and solubilized in lysis buffer A in the absence of EDTA and leupeptin. (1) Pattern of ³H-labeled K562 cells (200 µg protein). (2) Immunoprecipitate obtained with L10. (3) Immunoprecipitate obtained with GA3. (4) Immunoprecipitate obtained with R10.

blocked, eg, in the absence of EDTA and leupeptin, revealed two major bands of mol wt 105,000 and 95,000, which were both precipitated by L10 (Fig 3, lane 2) and GA3 (Fig 3, lane 3), providing evidence that the GA3 antigen is a glycoprotein and that its proteolytic cleavage profile is identical to that of leukosialin. Nonspecific entrapment of heavily labeled bands in the protein A-sepharose pellet could be ruled out since a single band of mol wt 40,000 corresponding to glycophorin A was precipitated by the MoAb R10 (Fig 3, lane 4).

Reactivity of GA3 and L10 With Peripheral Blood Cells and Adult Bone Marrow Cells

Since leukosialin has been shown to be a differently glycosylated sialoglycoprotein on leukocytes with diverse functions, we used indirect immunofluorescence to study the interaction of GA3 and L10 with peripheral blood cells and adult bone marrow cells. Interestingly, in contrast to L10, which did not react with red cells, but gave a bright fluorescence with peripheral blood granulocytes, monocytes, and lymphocytes and reacted with 60% of the bone marrow cells (Fig 4, panel 1), GA3 did not react with blood leukocytes except a subpopulation of CD3, CD4 positive T lymphocytes (4%). In addition, agglutination and a faint staining of red cells was observed. When tested on bone marrow cells, GA3 reacted with 9% of the cells (Fig 4, panel 2), which were identified as erythroblasts by double staining with an anti-CA 1 polyclonal antibody. Staining of erythroblasts and K562 cells with GA3 was weaker than that observed with L10, suggesting that the number of GA3 epitopes was lower than that of L10 epitopes. However, the fluorescence inten-

Fig 4. Flow cytometry profiles of bone marrow cells stained with L10 or GA3. Each histogram displays the light scatter (abscissa) plotted against the fluorescence intensity on linear scale (ordinate). (Top) Marrow cells stained with L10 followed by GAM-FITC (Fab')₂. (Bottom) Marrow cells stained with GA3 followed by GAM-FITC (Fab')₂.
finding was the reactivity of L10 with colonies of the megakaryocytic lineage. The antibody reacted strongly with immature megakaryocytes, slightly with mature megakaryocytes, and reacted only very weakly or not with platelet shedding megakaryocytes (Fig 5). This suggests that L10 antigen is predominantly expressed on immature cells and decreases during megakaryocytic maturation.

Reactivity of GA3 and L10 With Hematopoietic Progenitor Cells (BFU-E, CFU-E, CFU-GM, CFU-MK)

Reactivity of GA3 and L10 with hematopoietic progenitors was tested in parallel by immune panning and flow cytometry. Both procedures gave similar results (Tables 1 and 2). GA3 did not bind to hematopoietic progenitors including CFU-E and BFU-E, whereas L10 was reactive with the great majority (70% to 90%) of all hematopoietic progenitors. When the L10 positive fraction was further separated into weakly and brightly positive cells by flow cytometry, most hematopoietic progenitors, especially CFU-E, were found in the most positive fraction. In all these experiments, a higher number of BFU-E than of CFU-E was regularly observed, due to the dilution of the bone marrow sample by blood cells.

Table 1. Recovery of Hematopoietic Progenitors After Immune Panning With L10 and GA3 MoAbs

<table>
<thead>
<tr>
<th>Progenitors</th>
<th>GA 3 MoAb</th>
<th>L10 MoAb</th>
</tr>
</thead>
<tbody>
<tr>
<td>Positive Cells</td>
<td>Negative Cells</td>
<td>Positive Cells</td>
</tr>
<tr>
<td>CFU-E</td>
<td>(14%)</td>
<td>(86%)</td>
</tr>
<tr>
<td>30 ± 1</td>
<td>105 ± 10</td>
<td>107 ± 11</td>
</tr>
<tr>
<td>(4%)</td>
<td>(96%)</td>
<td>(80.5%)</td>
</tr>
<tr>
<td>BFU-E</td>
<td>(0.6%)</td>
<td>(99.4%)</td>
</tr>
<tr>
<td>8 ± 2</td>
<td>205 ± 14</td>
<td>173 ± 8</td>
</tr>
<tr>
<td>(3.4%)</td>
<td>(96.6%)</td>
<td>(65.4%)</td>
</tr>
<tr>
<td>CFU-GM</td>
<td>(Day 7)</td>
<td>(Day 12)</td>
</tr>
<tr>
<td>175 ± 7</td>
<td>834 ± 16</td>
<td>282 ± 42</td>
</tr>
<tr>
<td>(3.4%)</td>
<td>(96.6%)</td>
<td>(65.4%)</td>
</tr>
<tr>
<td>291 ± 23</td>
<td>526 ± 114</td>
<td></td>
</tr>
<tr>
<td>(73.4%)</td>
<td>(26.6%)</td>
<td></td>
</tr>
<tr>
<td>CFU-MK</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>ND</td>
<td>ND</td>
<td>(92%)</td>
</tr>
</tbody>
</table>

Results are expressed as the number of colonies per 10^6 cells in each fraction. The numbers in brackets represent the percentage of cells or progenitors recovered in each fraction. The data here represent the mean of two experiments performed on two different bone marrow samples.

Abbreviation: ND, not determined.

Immunochemical Characterization of the Epitope Recognized by GA3 on K562-Gp 105

Since the leukosialins or sialophorins detected in various cell lines have differences in the structure of O-linked oligosaccharide chains, we wondered whether GA3 reacted with a carbohydrate structure restricted to the leukosialin of the erythroid lineage. To answer this question, immunoprecipitation experiments were performed on K562 cells that had been surface-labeled by either the periodate/NaB₃H₄ method or the neuraminidase/galactose oxidase/NaB₃H₄ procedure. As shown in Fig 6, neuraminidase treatment of the cells and incorporation of ^3H into penultimate galactosyl residues of the O-linked oligosaccharide chains revealed a characteristic shift in the electrophoretic mobility of Gp 105, which now migrated with a mol wt of 140,000 (Fig 6, lane B). Immunoprecipitation experiments performed with L10 and GA3 revealed that L10 precipitated the 140,000 radiolabeled band from desialylated K562 cells (Fig 6, lane C), whereas GA3 did not (Fig 6, lane D). This result was confirmed by indirect immunofluorescence. After treatment of K562 cells with neuraminidase from Vibrio Cholerae, which cleaves terminal α(2-3) as well as α(2-6) linked sialic acid, a bright fluorescence was still observed with L10, whereas GA3 became negative, providing evidence that terminal sialic acid is essential for the expression of the GA3-epitope on leukosialin.

Because of the probable presence on leukosialin of at least one N-linked oligosaccharide chain, we investigated whether the GA3-epitope was dependent on terminal sialic acid present on O-linked or N-linked oligosaccharide chains on Gp 105. This time, immunoprecipitation experiments were performed on K562 cells that had been metabolically labeled by ^35S-methionine in the presence of tunicamycin, an inhibitor of N-linked oligosaccharide chain biosynthesis. The results are shown in Fig 7. Effective inhibition of N-linked oligosaccharide chains after tunicamycin treatment of K562 cells is illustrated in the immunoprecipitation experiment.
Table 2. Flow Cytometry of Adult Bone Marrow Cells Labeled by L10 or GA3 MoAbs

<table>
<thead>
<tr>
<th>Antibody</th>
<th>Fractions</th>
<th>Percentage</th>
<th>CFU-E</th>
<th>BFU-E</th>
<th>CFU-GM</th>
<th>CFU-MK</th>
</tr>
</thead>
<tbody>
<tr>
<td>L10</td>
<td>Unsorted</td>
<td>35%</td>
<td>104.5 ± 7.8</td>
<td>162 ± 3</td>
<td>201.5 ± 0.7</td>
<td>64.3 ± 1.3</td>
</tr>
<tr>
<td></td>
<td>Brightly positive</td>
<td></td>
<td>441.7 ± 40</td>
<td>296.7 ± 13.2</td>
<td>360 ± 0</td>
<td>95 ± 2.4</td>
</tr>
<tr>
<td></td>
<td>Weakly positive</td>
<td></td>
<td>88 ± 12</td>
<td>158 ± 8</td>
<td>426 ± 60</td>
<td>65.3 ± 20</td>
</tr>
<tr>
<td></td>
<td>Negative</td>
<td>30%</td>
<td>10 ± 10</td>
<td>30 ± 14</td>
<td>120 ± 0</td>
<td>6.7 ± 0</td>
</tr>
<tr>
<td>GA3</td>
<td>Positive</td>
<td>10%</td>
<td>82.5 ± 50</td>
<td>13.3 ± 5.8</td>
<td>103.3 ± 50</td>
<td>ND</td>
</tr>
<tr>
<td></td>
<td>Negative</td>
<td>80%</td>
<td>154.3 ± 15</td>
<td>140.0 ± 31.4</td>
<td>261.9 ± 7.2</td>
<td>ND</td>
</tr>
</tbody>
</table>

Three fractions were obtained with L10 MoAb: A brightly positive fraction (over channel 100), a weakly positive fraction, and a negative fraction. Two fractions were sorted with GA3 MoAb: A positive fraction identical in fluorescence intensity to the positive and weakly positive fractions obtained with L10 MoAb and a negative fraction. A window (4 channels) was intercalated between the two fractions. After reanalysis, 5% negative cells were included in the positive fraction. The percentage of progenitors was calculated by multiplying the number of colonies in each fraction by the percentage of cells in the fraction. The recovery was in the range of 70% to 120%. Results are the average of two experiments.

performed with the MoAb B3/25, directed against the transferrin receptor whose molecular weight has been reported to decrease from 90,000 to 80,000 after tunicamycin treatment (Fig 7, lanes 2 and 4). Lanes 3 and 5 show the result obtained with GA3, which precipitated a radiolabeled band from both nontreated and tunicamycin treated cells, thus demonstrating that sialic acid present on a possible complex-type N-linked oligosaccharide on Gp 105 is not involved in the expression of the GA3-epitope. It is interesting to note that tunicamycin treatment of K562 cells had only a very slight effect on the electrophoretic mobility of Gp 105, suggesting that only small N-linked oligosaccharide chains are present on leukosialin.

DISCUSSION

We have presented the characterization of a monoclonal antibody (GA3), which was obtained by immunizing mice with cells of the human erythroleukemic cell line K562. We have demonstrated that the antibody defines a membrane associated antigen restricted within the hematopoietic system to the erythroid lineage and to a minor population of CD3, CD4 positive T lymphocytes. Since GA3 precipitated a

![Fluorograph illustrating the analysis on an 8% polyacrylamide slab gel of immunoprecipitates from K562 cells surface-labeled by the neuraminidase/galactose oxidase/NaB3H4 method.](image1)

![Fluorograph illustrating the analysis on an 8% polyacrylamide slab gel of immunoprecipitates obtained from K562 cells with GA3 and B3/25 MoAbs after metabolic labeling of the cells with 35S-methionine in the presence or absence of tunicamycin.](image2)
band of apparent mol wt 105,000 from both 3H or 125I-
surface labeled and 35S-methionine labeled K562 cells, we
wondered whether the antigen corresponded to leukosialin,
the 105 kd sialoglycoprotein recently characterized by Car-
lsson and Fukuda and recognized by the MoAb L10. Using
L10 and GA3 in immunofluorescence and immunoprecipita-
tion experiments, we have demonstrated that the glycopro-
tins identified by the two antibodies on K562 cells are
indeed identical. First, the glycoproteins precipitated by
GA3 and L10 have the same electrophoretic mobility in 8%
polyacrylamide gels. Second, when immunoprecipitations
were performed on K562 cells lysed under conditions where
proteases were not completely blocked, eg, in the absence of
EDTA and leupeptin, GA3 as well as L10 precipitated two
bands of 105 and 95 kd. The 95 kd band, which has been
observed by several investigators on the fluorogram of 3H-
surface labeled K562 cells, has recently been shown to be a
proteolytic cleavage product of Gp 105. Third, cocapping
experiments performed with L10 and GA3 on unfixed cells
as well as sequential immunoprecipitation experiments
revealed that both antibodies are directed against a common
antigen.

Recently, Carlsson et al showed that leukosialins
belonging to different cell lineages have distinct electropho-
retic mobilities due to lineage specific alterations in the
carbohydrate moiety of their O-linked oligosaccharides.
These authors suggested that the O-linked oligosaccharides
could express unique lineage-restricted and even maturation-
restricted antigenic determinants that might serve as cell
lineage specific markers. The epitope identified by GA3 on
K562-leukosialin seems to correspond to such a structure.
Thus, we have shown that the reactivity of GA3 is essentially
restricted to the erythroid lineage and to a minor subpopula-
tion of T lymphocytes. Furthermore, terminal sialic acid on
O-linked oligosaccharides is essential for the expression of
the GA3 epitope. Finally, expression of the GA3 epitope is
maturation dependent since it is absent from erythroid
progenitors (BFU-E and CFU-E) although these cells are
reactive with L10, but is detected on erythroblasts including
those that are CA I positive/glycophorin A negative. The
phenotype of these latter cells may correspond to a pro-
erthroblast. This conclusion is further indicated by the
presence of proerythroblasts in the positive fraction retained
by GA3 during immune panning experiments (data not
shown). Therefore, our results suggest that erythroid specific
O-glycosylation occurs on leukosialin during terminal ery-
throid differentiation, whereas the leukosialin molecule is
immunologically detectable on all stem cells including the
erythroid ones. A similar but not identical phenomenon has
been described for glycophorin A where O-glycosylation is
maturation dependent and appears only on basophilic ery-
throblasts, whereas the glycophorin A molecule is already
detectable on proerythroblasts.

O-linked oligosaccharides attached to K562-leukosialin
have been characterized in detail and shown to be of the
erythroid type. However, they present some slight differ-
ences with O-linked oligosaccharides found on mature eryth-
rocytes as they are composed of a significant amount of
monosialated trisaccharides in contrast to erythrocytes,
which contain tetrasaccharides with two sialic acid residues
as a major component. Because of the maturation-dependent
expression of the GA3 epitope on erythroid leukosialin, it will
be of interest to determine which O-linked oligosaccharide
chains on K562-leukosialin express the GA3 antigenic deter-
minant. The cocapping studies as well as the sequential
immunoprecipitation experiments performed on surface-
 labeled K562 cells showed that not all leukosialin molecules
identified by L10 are recognized by GA3, suggesting some
heterogeneity in the glycosylation pattern of the K562-
leukosialin population.

An interesting result is the reactivity of L10 with progeni-
tors and precursors of the platelet series, providing evidence
that leukosialin is expressed at early stages of megakaryo-
cytic differentiation and declines during maturation since
mature megakaryocytes and platelets express only very
weakly this glycoprotein. This finding, which is in accor-
dance with the recent results by Remold-O'Donnell et al,
might contribute to the understanding of the physiopathol-
y of the Wiskott-Aldrich syndrome, an X-linked recessive
disorder affecting lymphocytes as well as platelets, which
is characterized by eczema, reduced T cell function and
decreased antibody production in response to carbohydrate
antigens, thrombocytopenia with platelets of reduced size,
and abnormal function. Since the major biochemical
abnormality in this syndrome is the abnormal expression of
sialophorin, the major surface glycoprotein previously called
gpL 115, it was suggested that other glycoprotein defects
could be involved to explain the platelet abnormalities. From
the present study, one might speculate that a unique primary
genetic defect in the expression of leukosialin results in
abnormal functions of the lymphoid as well as the mega-
karyocytic cells.

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