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Glycophorin A as an Erythroid Marker in Normal and Malignant Hematopoiesis

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A. Molecular Structure of Glycophorin A

Glycophorin A (GpA), which is the major sialoglycoprotein on human red cells, is one of the best characterized mammalian integral membrane proteins (Marchesi et al. 1972; Tomita and Marchesi 1975). Its amino acid sequence is known. The protein molecule contains three distinct domains. A large hydrophilic portion, carrying the NH_2-terminal, is located on the external surface of the red cell, and the COOH-terminal is located in the cytoplasm (Bretscher 1975) and probably interacts with peripheral proteins on the inner aspect of the membrane. These two hydrophilic sequences are connected by a hydrophobic segment of 23 amino acids which must be embedded within the lipid bilayer.

An unusually large proportion (about 60%) of the GpA is made up of carbohydrates. The molecule contains 15 O-glycosidic oligosaccharides with the structure of N-acetyl neuraminyl α(2–3)-galactosyl β(1–3) |N-acetyl neuraminyl α(2–6)| N-acetyl galactosamine (Thomas and Winzler 1969) and one N-glycosidic oligosaccharide located at asn-26. The carbohydrate is located outside the lipid bilayer in the NH_2-terminal portion of the molecule (Fig. 1).

Glycophorin A carries the MN blood group activity of red cells (Marchesi et al. 1972; Tomita and Marchesi 1975). This is most probably due to an interaction between amino acids and O-glycosidic oligosaccharides, since either treatment with neuraminidase (Mähelä and Cantell 1958) or modifications of amino acids (Lisowska and Duk 1975) abolish the activity. GpAs from M and N cells also show different amino acid sequences in the NH_2-terminal portions (Dahr et al. 1977; Tomita and Marchesi 1975).

B. Biosynthesis of Glycophorin A

Most of our knowledge of the molecular mechanisms operating in biosynthesis of integral membrane proteins of mammalian cells derives from studies of the glycoproteins of enveloped viruses. In these systems only a few membrane glycoproteins are made, which makes it relatively easy to follow their biosynthesis. The situation is, however, much more complex in normal cells, where a multitude of membrane proteins are simultaneously synthesized, and makes informative experiments difficult to perform.

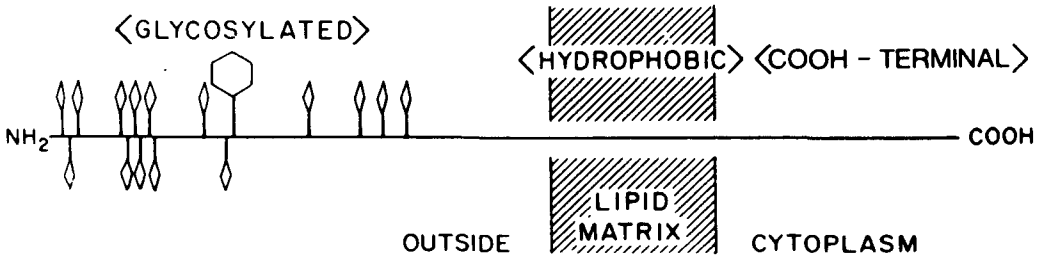

Fig. 1. Schematic drawing of glycophorin A structure. ◇ O-glycosidic chains. ⬡ N-glycosidic oligosaccharide

The detailed information available on the structure of GpA makes it an attractive candidate for biosynthetic studies. Such studies recently became possible with our finding that the human leukemia cell line K562, previously thought to be myeloid (Lozzio and Lozzio 1975), in fact shows erythroid characteristics, including the expression of GpA (Andersson et al. 1979b,c; Gahmberg et al. 1979). The reader is referred to our original reports for technical details (Gahmberg et al. 1980; Jokinen et al. 1979).

The biosynthesis of GpA in the K562 leukemia cell line was followed by pulse-chase labeling with [^{35}S] methionine. A precursor of GpA was visualized by appropriate lectin-Sepharose affinity chromatography and immune precipitation with specific anti-GpA serum (see below) and followed by polyacrylamide slab gel electrophoresis. This had an apparent molecular weight of 37,000 and contained an incompleted N-glycosidic oligosaccharide and unfinished O-glycosidic oligosaccharides. After chase for 10 min, the completed GpA with an apparent molecular weight of 39,000 was seen and it appeared at the cell surface in about 30 min. Addition of tunicamycin inhibited the N-glycosylation but not the O-glycosylation. The absence of the N-glycosidic oligosaccharide did not significantly affect the migration of the protein to the cell surface but the expression of glycophorin A was lower.

The cell-free biosynthesis of GpA was achieved by translation of glycophorin A messenger RNA which had been isolated from K562 cells in a rabbit reticulocyte system. This yielded a nonglycosylated protein with an apparent molecular weight of 19,500, which exceeded that of the glycophorin A apoprotein by about 5000. This indicates the presence of a "signal sequence" in the preprotein. When the translation was performed in the presence of microsomal membranes from dog pancreas the GpA apoprotein was both N- and O-glycosylated and the apparent molecular weight (37,000) of the synthesized protein was identical to that of the GpA precursor obtained from K562 cells.

C. Glycophorin A in Normal Hematopoiesis

To study the appearance of GpA on bone marrow cells during normal hematopoiesis we made an antiserum to GpA. This was possible because erythrocytes from a person with the rare blood group En(a-) which lack GpA were made available (Gahmberg et al. 1976). Rabbits were immunized with isolated GpA and the antiserum was adsorbed with En(a-) red cell membranes. The specificity of the antiserum was assessed by immune precipitations from Triton X-100 lysates of normal erythrocyte membranes and bone marrow cells which were surface radiolabeled by the galactose oxidase-NaB^3H_4 method (Gahmberg and Hakomori 1973). Analysis of the precipitates by polyacrylamide gel electrophoresis (PAGE) under reducing conditions revealed that the antiserum reacted exclusively with surface molecules corresponding to the monomer (PAS2) and dimer (PAS1) of GpA (Gahmberg et al. 1978).

GpA-expressing cells in normal bone marrow were identified by the staphylococcus rosetting technique (Gahmberg et al. 1978). Suspensions of bone marrow cells were treated with antiserum followed by Staphylococcus aureus strain Cowan I, which carry protein A on their surface and therefore strongly attach to the Fc portion of IgG. The cells which bound staphylococci were identified by morphology from cytocentrifuged smears stained with May-Grünwald-Giemsa in combination with the Lephenes benzidine reaction to detect the presence of hemoglobin.

Only cells of the erythroid lineage formed rosettes with staphylococci after treatment with anti-GpA serum (Fig. 2). Weak reactivity was seen with the pronormoblasts, while the basophilic normoblasts and later stages of the erythropoiesis made strong rosettes. This indicates that the surface expression of GpA occurs slightly earlier than the onset of hemoglobin synthesis and the appearance of the ABO blood group antigens (Karhi et al. 1981) during normal red cell differentiation (Fig. 3).

D. Glycophorin A in Malignant Hematopoiesis

After our initial observations on the strong surface expression of GpA by the human leukemia cell line K562 (Andersson et al. 1979c) we have tested freshly isolated cells from leukemic patients for the presence of GpA.

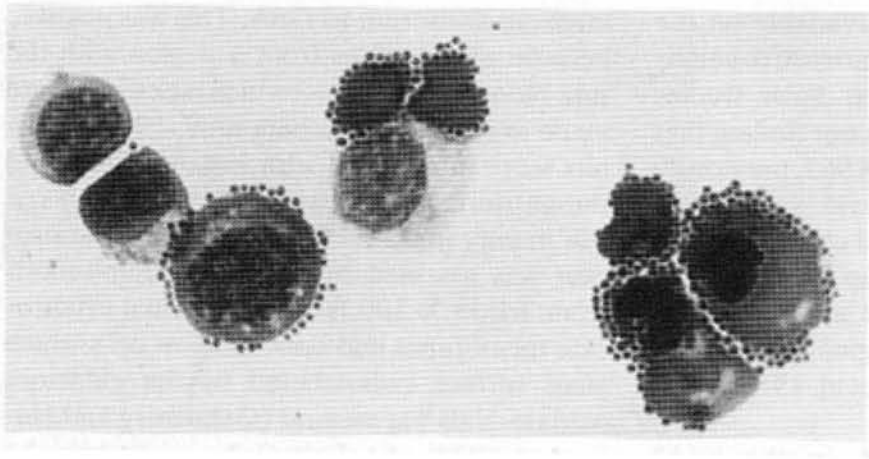

Fig. 2. Cytocentrifuged smears of bone marrow cells treated with anti-GpA serum and Staphylococcus aureus Cowan I. Benzidine-May-Grünwald-Giemsa staining

The leukemic cells were analyzed by indirect immunofluorescence using a $F(ab)_2$ preparation of the anti-GpA serum followed by a fluorescein isothiocynate-conjugated IgG preparation of sheep anti-rabbit Ig (Andersson et al. 1979a).

In 6 of 51 (12%) subsequent adult patients diagnosed as having acute myeloid or acute lymphoid leukemia we found positive staining in a large proportion (50%) of the leukemic cells. The specificity of the staining was further established by immunoprecipitation with anti-GpA serum from surface labeled leukemic cells followed by PAGE. Occassionally only the dimeric form of GpA was precipitated, while in most cases a surface protein corresponding to the monomeric form of GpA could be seen (Andersson et al. 1979a).

The GpA-expressing cells from acute leukemias were usually morphologically classified as poorly differentiated. Usually they have a basophilic, rather abundant cytoplasm lacking granulae but occasionally with azurophilic granulae and a kidney-shaped nucleus.

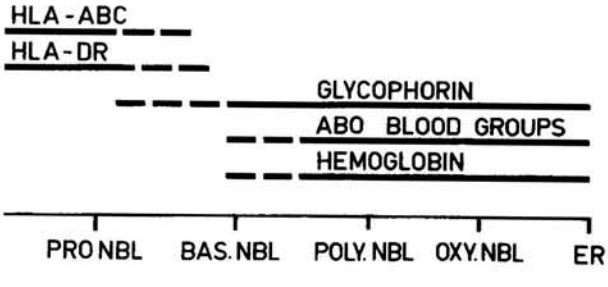

Fig. 3. Tentative sequence of appearance and disappearance of cell surface markers during erythropoiesis. *PRO NBL*, pronormoblast; *BAS.NBL*, basophilic normoblast; *POLY.NBL*, polychromatic normoblast; *OXY.NBL*, oxyphilic normoblast; *ER*, erythrocyte

Different proportions of GpA positive precursor cells can be observed during blast crisis of chronic myeloid leukemia (CML). This indicates that the arrest in maturation also involves to various degrees the erythroid lineage.

We have recently characterized the phenotype of the leukemic cells of relapsing childhood acute lymphocytic leukemia (ALL) (Andersson et al. 1980). So far we have found four cases (of a total of 30) which according to conventional surface marker analysis at initial diagnosis were classified as non-T, non-B ALL but where the majority of the leukemic blasts during relapse expressed surface GpA. In three of these the intracytoplasmic presence of fetal hemoglobin could be demonstrated in 30%–50% of the blast cells by direct staining of cold acetone fixed smears with FITC-conjugated rabbit antiserum to fetal hemoglobin. These cells, however, did not show a positive benzidine reaction, indicating low levels of hemoglobin or incomplete assembly of the hemoglobin molecule.

The phenotypic change from "lymphoid" to erythroid in relapsing childhood ALL is surprising. It is possible that in these cases the malignant transformation involves stem cells endowed with a pluripotential differentiation capacity. The anti-ALL treatment might have selected for relatively threapy-resistant clones showing early erythroid features.

These findings indicate that glycophorin A is also a useful marker of early erythroid derivation in malignant hematopoiesis. Moreover, leukemias with features of early erythroid differentiation are obviously more common than previously reported. These are, however, not identified by conventional means, since the malignant erythroid cells apparently only rarely maturate to synthesize adult hemoglobin.

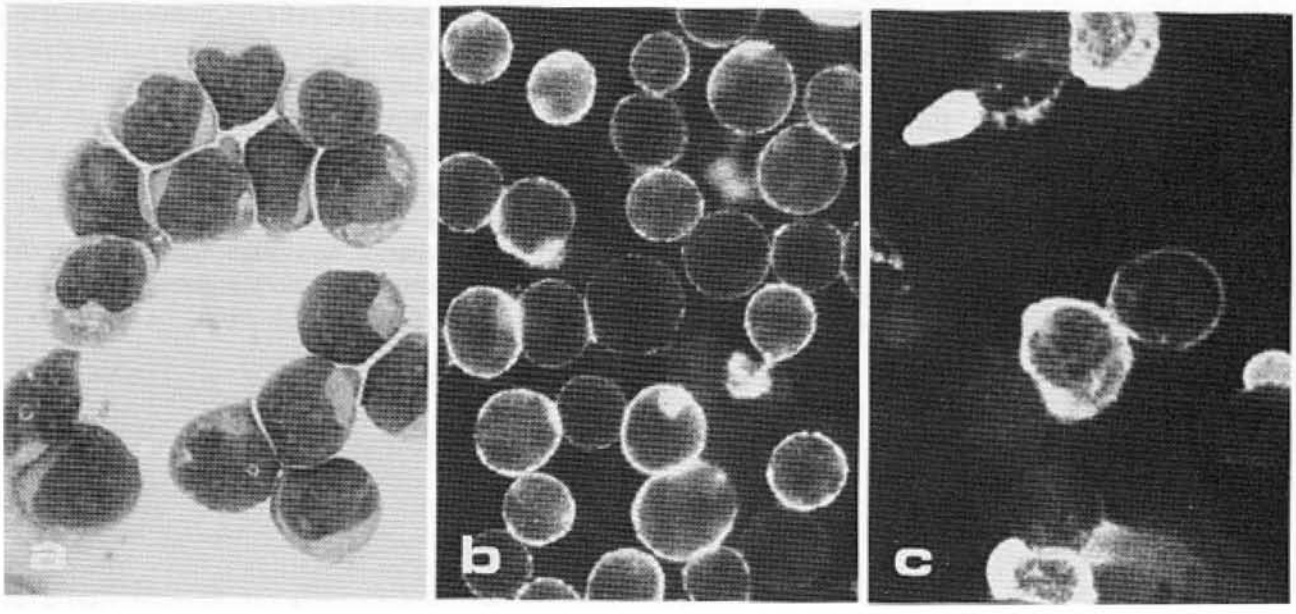

Fig. 4. a Photomicrograph of blasts from an adult patient with AML. **b** Positive membrane immunofluorescence of the same cells with a $F(ab)_2$ preparation of rabbit anti-GpA serum followed by FITC-conjugated sheep anti-rabbit IgG. **c** Direct immunofluorescence staining of acetone-fixed smears with FITC-conjugated rabbit anti-fetal hemoglobin

The recognition of leukemic cells with markers of erythroid derivation might be of clinical importance, since at least in adult acute leukemia the presence of surface GpA seems to imply poor prognosis.

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