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Major O-Glycosylated Sialoglycoproteins of Human Hematopoietic Cells: Differentiation Antigens With Poorly Understood Functions

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All human hematopoietic cells seem to contain a major, heavily O-glycosylated sialoglycoprotein. Glycophorin A is specific for the erythroid lineage of cells, and leukocytes have a major sialoglycoprotein, also called leukosialin or sialophorin. Cell differentiation results in patterns of O-glycosylation in these proteins, which reflect the stage of differentiation within a cell lineage as well as lineage specificity. The altered carbohydrate compositions may influence the interactions of the cells with external ligands. Healthy individuals lacking glycophorin A in their red cells are known, whereas a deficiency of the leukocyte sialoglycoprotein may result in immunological disease. Although little is known about the physiological functions of these proteins, they form interesting models for studies on regulation of glycosylation, biosynthesis of O-glycosylated glyoproteins, and function of cell surface receptors.

Key words: membrane glycoproteins, O-glycosylation

Through a complicated set of events, which largely are poorly understood, the hematopoietic cells differentiate from a common pluripotent stem cell to form the various cell lineages. During recent years our knowledge about the factors, hormones, and inducers involved in this differentiation has substantially increased [1]. In parallel much has been learnt about the cell surface components of the human hematopoietic cells, notably their glycoproteins and glycolipids. It has now become apparent that all human hematopoietic cells contain major glycoproteins rich in sialic acid, containing a large number of O-linked carbohydrate chains. This review deals with two of them: the major red cell sialoglycoprotein, glycophorin A, and the major leukocyte sialoglycoprotein with an apparent molecular weight between 100,000 and 170,000, which recently has been named leukosialin [2] or sialophorin [3].

Glycophorin A is a "classical" membrane protein. It was the first membrane protein that was sequenced [4], and it has become a model for structural characteristics

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of one type of integral membrane proteins and for biosynthesis of plasma membrane glycoproteins [5–6]. Its usefulness and characteristics as an erythroid marker have recently been reviewed [7].

The major leukocyte sialoglycoprotein was found much later, when studying plasma membrane glycoproteins of normal human leukocytes and cell lines by radioactive labeling techniques specific for cell-surface-exposed carbohydrate [8–10].

Although much is known about the structures of these proteins, their physiological functions have essentially remained obscure. However, much useful information has been obtained from studies of these proteins. This includes changes in the glycosylation of the proteins during cellular differentiation, resulting in altered reactivities with antibodies, microbes, and other ligands. The emphasis in this review is put on these aspects.

DIFFERENTIATION IN THE HUMAN HEMATOPOIETIC SYSTEM

A schematic drawing of the scheme of differentiation of human hematopoietic cells is shown in Figure 1. Early during differentiation myeloid and lymphoid lineages can be distinguished. Through the action of various inducers, growth and differentiation factors, and hormones the immature cells differentiate further along the different lineages to end up with erythrocytes, platelets, granulocytes, monocytes, and the T and B lymphocytes.

When these cells are surface-labeled by using either the neuraminidase-galactose oxidase/NaB $[^3H]_4$ or periodate/NaB $[^3H]_4$ labeling techniques [11,12] specific for galactose/N-acetylgalactosamine and sialic acids, respectively, glycophorin A in red cells and the major sialoglycoprotein in leukocytes are among the major labeled components in most cells (Fig. 2). Only B lymphocytes and non-T, non-B lymphocytes contain little of the sialoglycoprotein.

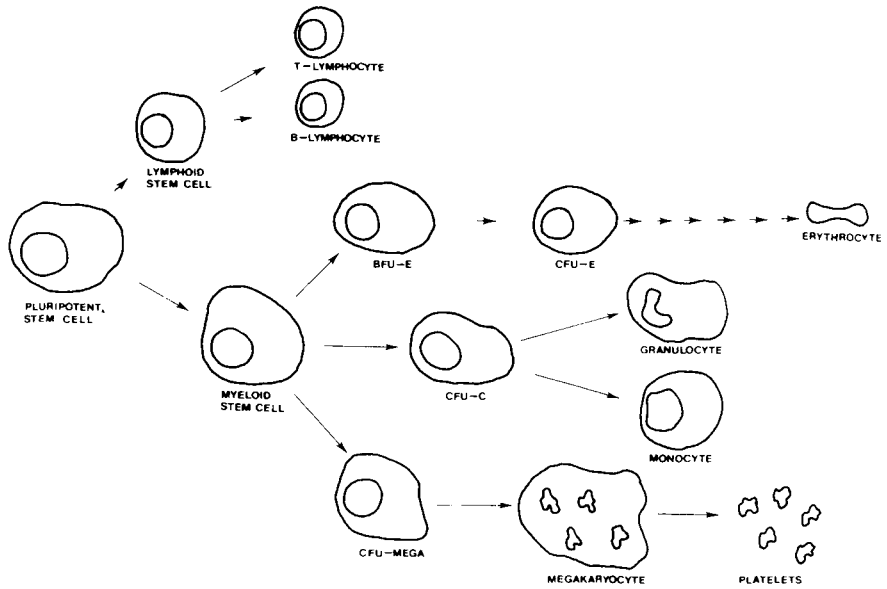

Fig. 1. A schematic representation of human hematopoietic cell differentiation.

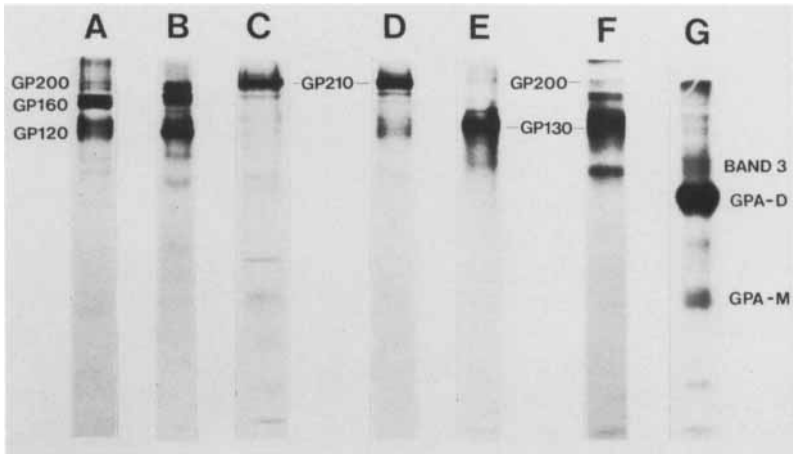

Fig. 2. Fluorogram of a polyacrylamide slab gel of neuraminidase-galactose oxidase/NaB[^{3}H]$_4$-labeled normal hematopoietic cells. **A:** Pattern of thymocytes. **B:** Pattern of T lymphocytes. **C:** Pattern of B lymphocytes. **D:** Pattern of non-T, non-B lymphocytes. **E:** Pattern of granulocytes. **F:** Pattern of monocytes. **G:** Pattern of erythrocytes. The apparent molecular weights of the major surface glycoproteins are indicated: GP200, glycoprotein with an apparent molecular weight of 200,000, etc. GP120-GP130, major leukocyte sialoglycoprotein; GPA-D, glycophorin A dimer; GPA-M, glycophorin A monomer.

GLYCOPHORIN A

Structure

Glycophorin A contains 131 amino acids distributed in three distinct structural domains [4]. The NH_2-terminal is located outside at the erythroid cell surface, and the COOH-terminal is located in the cytoplasm (Fig. 3). The membrane-spanning region is composed of about 20 hydrophobic amino acids. Both the external and cytoplasmic parts are hydrophilic. Figure 4 shows a hydrophilicity-hydrophobicity plot of glycophorin A without taking into account the contribution of the carbohydrate. The cytoplasmic portion contains a cluster of basic amino acids near the lipid bilayer. Such basic sequences are

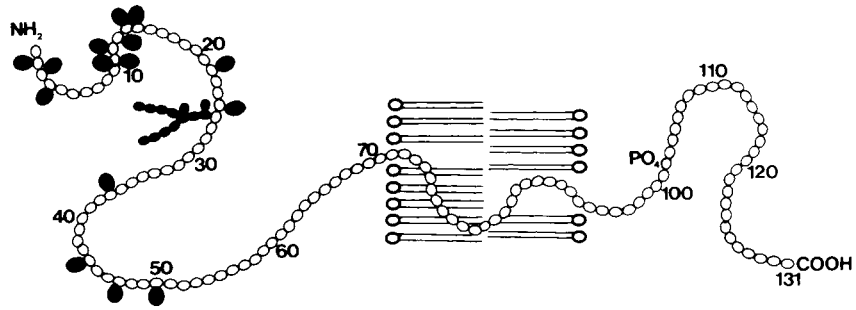

Fig. 3. Schematic structure of glycophorin A. The positions of the oligosaccharides are indicated. The NH_2-terminal is externally located and the COOH-terminal is in the cytoplasm. PO_4 = the position of the phosphate group.

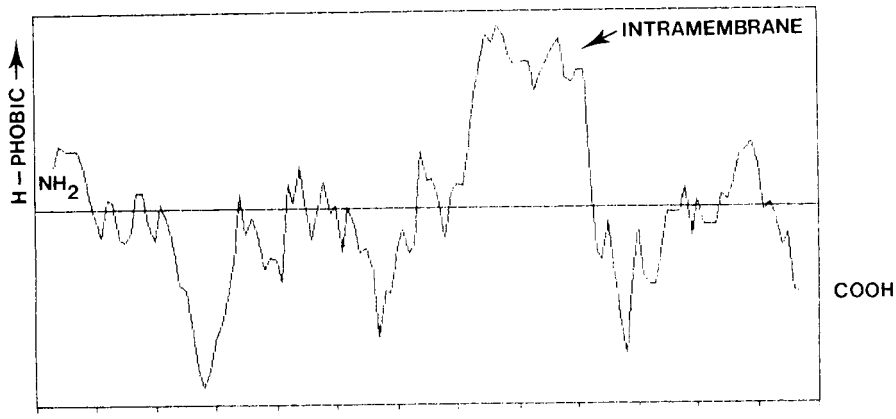

Fig. 4. Hydrophilicity-hydrophobicity plot of the glycophorin A polypeptide. A window of seven amino acids was used. The contribution of carbohydrate is not included.

found in several membrane proteins belonging to the group of proteins, which span the lipid biplayer once. They may function as an extra stop-transfer sequence to prevent the polypeptide from sliding through the membrane. Furthermore, the protein is phosphorylated evidently at serine-102 [13]. The protein partially migrates as a dimer on polyacrylamide gels in the presence of sodium dodecyl sulfate (Fig. 2G). This fact indicates that it exists as a dimer already in the membrane.

Glycophorin A from mature red cells contains one N-glycosidic oligosaccharide located at asparagine-26 and 15 O-glycosidic oligosaccharides distributed in the NH_2-terminal portion of the molecule [4]. The structures of the major oligosaccharides are shown in Figure 5. The heavy O-glycosylation makes the NH_2-terminal portion more hydrophilic and charged than is apparent from Figure 4.

Glycophorin A carries the MN-blood group activities. Most anti-MN antisera need the NH_2-terminal sialic acids for activity. Therefore neuraminidase treatment ususally inactivates the antigens [14,15]. On the other hand, the MN-*specificity* is due to the NH_2-terminal amino acid sequence. Thus serine and glycine at positions 1 and 5 of glycophorin A^M are replaced by leucine and glutamic acid in glycophorin A^N [4,7].

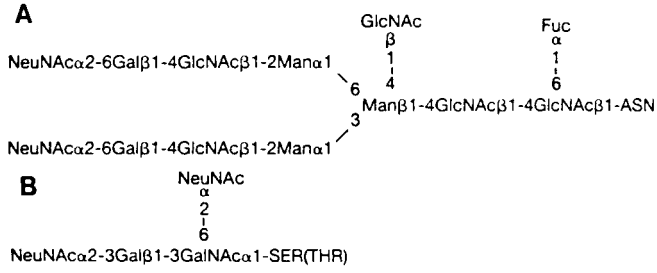

Fig. 5. The major oligosaccharides of glycophorin A from erythrocytes. **A:** Structure of the N-glycosidic oligosaccharide. **B:** Structure of the O-glycosidic tetrasaccharide.

Expression of Glycophorin A During Erythroid Differentiation

Using a specific rabbit antiglycophorin A antiserum we showed in 1978 that glycophorin A is already expressed on early erythroid precursor cells in bone marrow [16]. Furthermore, we showed that the protein is specific for the erythroid lineage of cells.

The expression of glycophorin A in bone marrow cells was studied with monoclonal anti-M- or anti-N-specific antibodies. It became apparent that early erythroid precursor cells reacted poorly, whereas rabbit antisera showed a strong reaction already with proerythroblasts [17] (Fig. 6). M and N activities began to appear at the basophilic normoblast stage of differentation and substantially increased at the polychromatophilic stage. These results show that the polypeptides are present already at an early stage of differentiation, but they do not then have the final structure needed for M/N antigenicity.

Expression of Glycophorin A in Cell Lines With Erythroid Features

The K562 cell line was established in the early 70s from a patient with the diagnosis of chronic myeloid leukemia [18]. The cells were Philadelphia-chromosome positive and lacked the common lymphocytic markers used at that time. We later showed that K562 cells express glycophorin A, which we at that time knew to be specific for the erythroid lineage [19]. Further proof for the erythroid characteristics of the cell line was obtained when it could be induced to erythroid differentiation by sodium butyrate, resulting in the synthesis of hemoglobin [20]. Subsequently, Rutherford et al. [21] showed

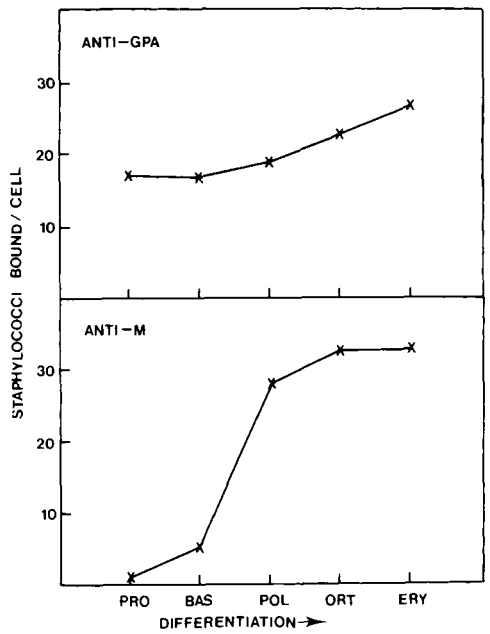

Fig. 6. Expression of glycophorin A and M antigen in bone marrow cells. The expression of glycophorin A (above) was determined with rabbit antiglycophorin A antiserum and protein A containing staphylococci. The expression of M-antigen was determined by using a monoclonal antibody [17]. PRO = proerythroblast; BAS = basophilic normoblast; POL = polychromatophilic normoblast; ORT = orthochromatic normoblast; ERY = erythrocyte.

that hemin was a potent inducer of differentiation and that the hemoglobins synthesized were of embryonic and fetal types.

The K562 cell line has proved especially useful for studies on the biosynthesis of glycophorin [5–7]. The O-glycosylation has been studied extensively and shown to begin relatively early during biosynthesis, before the arrival of the polypeptide to the medial Golgi cisternae [6].

From K562 cells the glycophorin A cDNA was obtained and sequenced. The sequence analysis showed almost complete identity with that reported by Marchesi and co-workers and showed the presence of a leader peptide 19 amino acids long [22]. The sequence corresponded to that of glycophorin A^M, which was expected because the patient from which the cells originated had blood group MM.

The HEL (human erythroleukemia) cell line was reported in 1982 [23]. In many respects it is similar to the K562 line, but it expresses relatively more platelet antigens [24–26]. Therefore it probably originates from a cell transformed at a stage just prior to the divergation of the erythroid and megakaryocytic lineages.

Change in Glycophorin A During Erythroid Differentiation

The poor reactivity of glycophorin A from early erythroid precursor cells in the bone marrow with monoclonal anti-M and anti-N antibodies indicated that it had a structure different from glycophorin A of erythrocytes. Similar results were obtained with other monoclonal antiglycophorin A antibodies. The glycophorin A molecules from K562 cells behaved in a similar way (see also Rearden et al [27]).

This phenomenon was explored further. We surface-labeled bone marrow erythroid cells representing various stages of differentiation and K562 and HEL cells using the periodate/NaB$[^3H]_4$ technique. The glycophorin A molecules were isolated by immune precipitation, digested with pronase, and subject to mild alkaline-borohydride treatment. The liberated glycopeptides/oligosaccharides were then analyzed by Biogel P-6 gel filtration [28]. As shown in Figure 7 relatively less labeled O-glycosidic tetrasaccharides (peak 2) and more trisaccharides (peak 3) were obtained from immature cells. Whether this is due to less O-glycosidic oligosaccharides or hyposialylation of glycophorin A is not certainly known.

Glycophorin A as a Receptor

Influenza virus recognizes sialic acids on glycophorin A as its receptor. Subsequently sialic acids are cleaved by the viral envelope neuraminidase.

Glycophorin A also seems to function as receptor for *Plasmodium falciparum* malaria merozoites [29,30]. Soluble glycophorin A competes for binding, and En(a−) red cells lacking glycophorin A [31–33] are not efficiently infected. Friedman and co-workers have, however, shown that the polylactosamine type of oligosaccharides, found primarily on band 3 and band 4.5 in red cells [31,34,35], inhibit the binding of the malaria merozoites [36]. It is not excluded, however, that a minor part of the glycophorin A molecules contain polylactosamine types of oligosaccharides.

Interestingly, K562 cells and bone marrow precursor cells are not infected by the malaria merozoites. This may be due to the fact that the glycophorin A molecules in these cells are incompletely O-glycosylated, and it indicates an important role for O-glycosidic oligosaccharides in this receptor function.

A few years ago we found that a certain *E. coli* strain, IH 11165, specifically agglutinated erythrocytes of blood groups M and MN. NN cells were not agglutinated

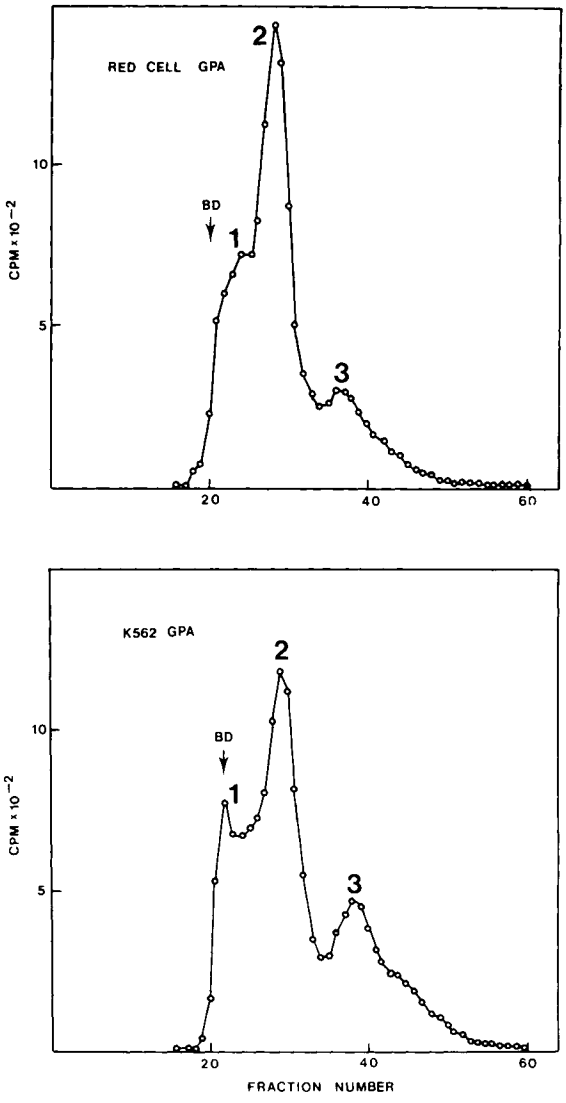

Fig. 7. Biogel P-6 gel filtration patterns of periodate/NaB $[^3H]_4$-labeled glycophorin A glycopeptides/oligosaccharides. Pattern obtained from erythrocyte glycophorin A (above) and pattern obtained from K562-glycophorin A (below). 1 = position of N-glycosidic glycopeptide; 2 = position of O-glycosidic tetrasaccharide; 3 = position of O-glycosidic trisaccharides. BD = position of Blue Dextran at the void volume.

[37]. We then studied the erythrocyte receptor in more detail [38]. As expected, detergent-solubilized glycophorin A^M bound to the bacteria. If glycophorin A was treated with 1 mM periodate it did not bind. Such a periodate treatment primarily oxidizes sialic acids, but in this case the NH_2-terminal of glycophorin A^M, which happens to be serine, was also oxidized. The loss of binding was due to this serine residue, because removal of sialic acids by neuraminidase had no effect on the binding. Isolated O-glycosidic oligosaccharides from glycophorin A^M showed a weak competitive inhibition of binding

of glycophorin A^M to the bacteria. We then chemically synthesized the NH_2-terminal pentapeptide of glycophorin A^M containing the M-specific sequence. This peptide showed no inhibition of binding. These findings indicated that the receptor structure involved both carbohydrate and the NH_2-terminal peptide portion of glycophorin A^M. This hypothesis was finally proved when it was shown that the isolated NH_2-terminal octaglycopeptide of glycophorin A^M very efficiently inhibited binding [38].

The detailed studies on the structural requirements needed for the binding of this *E. coli* strain, which obviously is a biological curiosity, may draw some light on other biological receptors, which may need a combination of the backbone peptide *and* oligosaccharides for full activity.

Glycophorin A as a Marker for Erythroleukemia

Leukemias with obvious erythroid phenotypes, i.e., leukemic cells containing hemoglobin, are rare. From the proportion of red cell precursors in the normal bone marrow, and assuming a random hit of the leukemogenic agent(s), many more cases would be expected. When we knew that glycophorin A is specific for the erythroid lineage of bone marrow cells, we screened clinically diagnosed myeloid leukemias for the presence of glycophorin A in the malignant cells. In fact, we found that in 5–10% of the M 1 group of leukemias, glycophorin A was found on the malignant blast cell surfaces by using rabbit antiglycophorin A antisera [39,40]. In some cases the erythroid nature of the cells was proven by induction of hemoglobin synthesis.

Monoclonal antiglycophorin A antibodies reacted poorly with such cells [41]. The reason is probably the same as with normal immature bone marrow cells and K562 cells: the glycophorin A molecules in these cells are less O-glycosylated. The low degree of glycosylation may alter the conformation of the molecule, and the antigenic sites recognized by monoclonal antibodies may not be seen anymore.

THE MAJOR SIALOGLYCOPROTEIN OF HUMAN LEUKOCYTES

Ten years ago we observed by using the neuraminidase-galactose oxidase/NaB$[^3H]_4$ surface labeling method a major heavily labeled sialoglycoprotein in all major groups of human leukocytes (Fig. 2) [42]. Furthermore, different T lymphocytic and myeloid cell lines contained the protein. Later we were able to immune precipitate the protein with specific antiserum from various blood cells and cell lines [43,44]. Interestingly, the protein had different apparent molecular weights in different leukocytes [see also reference 45]. A further peculiar feature of this protein was its smaller electrophoretic mobility on polyacrylamide gels in the presence of sodium dodecyl sulfate after treatment with neuraminidase [42]. This is typical of proteins rich in O-glycosidic carbohydrate. Interestingly non-T acute lymphocytic and myeloid leukemias strongly expressed the protein, and often two closely spaced bands were observed [46,47].

Carlsson and Fukuda [2] and Remold-O'Donnell et al [48] have determined the amino acid composition of the protein. As expected the protein contained a high content of serine and threonine residues reflecting its high degree of O-glycosylation. Both groups have studied its biosynthesis. With the aid of pulse-chase experiments a precursor was observed with an apparent molecular weight of 54,000 [2] or 62,000 [49]. Synthesis in the presence of tunicamycin or treatment with endo-N-acetylglucosaminidases H and F indicated the presence of 1–2 N-glycosidic oligosaccharides. The number of O-glycosidic oligosaccharides was calculated to be 90–100 [2,49].

The sequence of the sialoglycoprotein polypeptide has not been determined. Several groups have now, however, good polyclonal antisera for screening of expression libraries and therefore it may be expected that the protein will be sequenced through its cDNA in the near future.

Differentiation-Associated Changes in the Leukocyte Sialoglycoprotein

When T lymphocytes were stimulated to blast cell formation using mitogens such as phytohemagglutinin or concanavalin A or in mixed lymphocyte culture, the major sialoglycoproteins showed differences in apparent molecular weights [42] (Fig. 8). This change was not due to sialylation because a similar difference was observed after neuraminidase treatment. The apparently corresponding protein from murine cells was characterized further by Kimura and Wigzell [50]. They showed that the protein from cytotoxic T cells specifically bound to the lectin from *Vicia villosa*. This lectin is relatively specific for terminal N-acetylgalactosamine residues. In fact, Conzelmann and Kornfeld [51] found β-linked N-acetylgalactosamine in a cytotoxic murine T cell line.

Another obvious change in a major surface sialoglycoprotein was seen in the HL-60 promyelocytic cell line after induction to differentiation [52]. Whereas the sialoglycoprotein of uninduced cells had an apparent molecular weight of 160,000 after neuraminidase treatment, the apparent molecular weight of the protein of differentiated cells was 130,000. This corresponded to that of the protein from mature granulocytes [8]. Whether these proteins have the same polypeptide chains is not certainly known. Also retinoic acid or phorbol-12-myristate-13-acetate treatments changed the apparent molecular weight of the protein, but in different ways [53]. When different leukocytes were studied it was found that the apparent molecular weight of the sialoglycoprotein differed appreciably [43,44] (Fig. 9).

Axelsson et al [54] purified the protein from human T cells and determined its carbohydrate composition. This was compatible with the presence of a large number of O-glycosidic oligosaccharides. Carlsson et al purified the sialoglycoprotein from K562 cells, HL-60 cells, and HSB-2 lymphoid cells [55] and determined the structures of the O-glycosidic oligosaccharides (Fig. 10). It was clearly shown that the K562 or erythroid form of the protein contained oligosaccharides similar to those found in glycophorin A, whereas the HL-60 and HSB-2 cell protein had a substantial amount of N-acetylglucosamine-containing oligosaccharides.

We recently established B lymphocytic cell lines from an individual with the blood group Tn by infection of his leukocytes with Epstein-Barr virus [56]. A proportion of

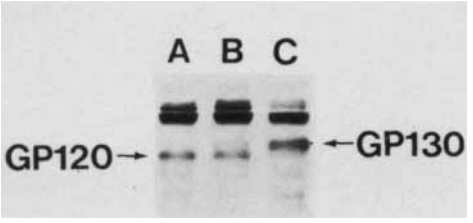

Fig. 8. Fluorogram of a polyacrylamide slab gel of neuraminidase-galactose oxidase/NaB $[^3H]_4$-labeled T lymphocytes. **A:** Pattern of T lymphoblasts obtained after activation with phytohemagglutinin. **B:** Pattern obtained after activation with concanavalin A. **C:** Pattern obtained after activation in mixed lymphocyte culture.

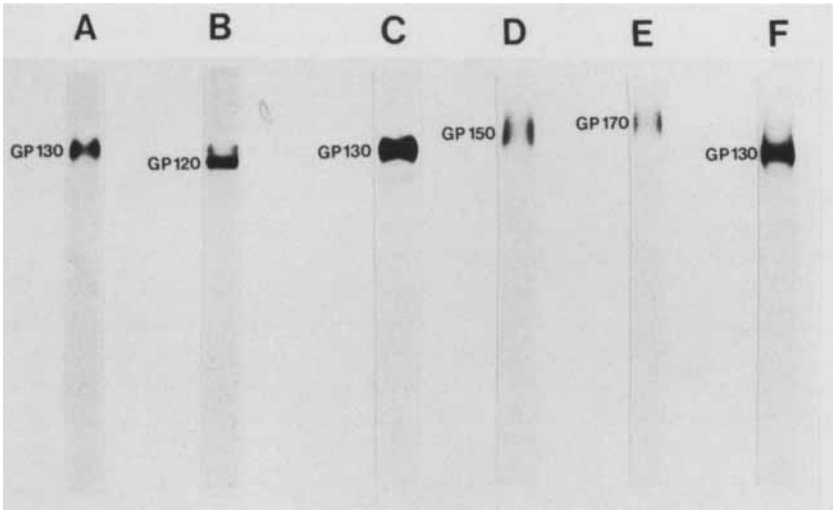

Fig. 9. Fluorogram of a polyacrylamide slab gel of immune precipitate obtained with antileukocyte sialoglycoprotein antiserum from neuraminidase-galactose oxidase/NaB $[^3H]_4$-labeled cells. **A:** Pattern from T lymphocytes. **B:** Pattern from platelets. **C:** Pattern from uninduced HL-60 cells. **D:** Pattern from uninduced ML-2 cells. **E:** Pattern from uninduced RC2A cells. **F:** Pattern from phorbol-12-myristate-13-acetate-induced RC2A cells.

the red cells of such individuals are known to be polyagglutinable due to the presence of terminal O-glycosidically linked N-acetylgalactosamine residues. The mixture of B lymphoblastoid cells was treated with fluorescent *Helix pomatia* lectin, which preferentially reacts with terminal N-acetylgalactosamine [57]. Using a fluorescence-activated cell sorter the lectin-binding and nonbinding cells were isolated and cultivated as pure Tn-positive and -negative lines. The Tn-positive cells were found to lack UDP-galactose/N-acetylgalactosamine transferase. With the aid of the L10 monoclonal antibody specific for the major sialoglycoprotein [58], this was isolated and partially characterized. The surface-labeled oligosaccharides of this protein from Tn-positive and -negative cells were clearly different with terminal N-acetylgalactosamine only in the protein from Tn-positive cells. Otherwise no obvious structural changes were observed in the cells. The Tn-positive and Tn-negative cells have, however, now been cultivated for 2 yr and it seems obvious that the Tn-positive cells are more difficult to grow. The reason is not yet known.

The Major Sialoglycoprotein of Lymphocytes From Patients With the Wiskott-Aldrich Syndrome is Lacking or Modified

Remold-O'Donnell and co-workers have shown that the major sialoglycoprotein is absent or shows aberrant apparent molecular weights in lymphocytes from patients with the rare Wiskott-Aldrich syndrome [58]. These individuals have a generalized T lymphocyte defect resulting in decreased antibody production especially to carbohydrate antigens. In addition they have thrombocytopenia. The affected lymphocytes had less of the normally seen slender microvilli at the surface; instead they were blunted [59].

The reason for the sialoglycoprotein abnormalities is not known but could possibly in some cases be due to lack or malfunctioning of glycosyl transferases involved in O-glycosylation.

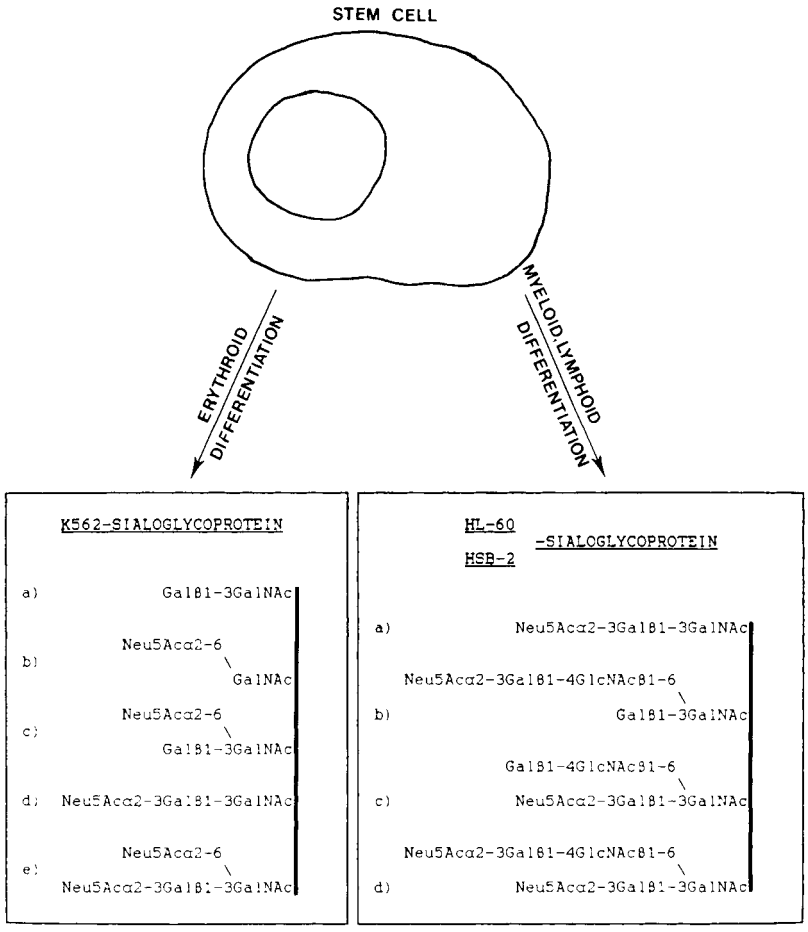

Fig. 10. O-glycosidic oligosaccharides found in the major leukocyte sialoglycoprotein from K562 cells (**left**) and HL-60 and HSB-2 cells (**right**). The pattern to the left represents that of erythroid differentiation and that to the right myeloid-lymphoid differentiation [adapted from reference 55].

DISCUSSION

This review deals with the two major sialoglycoproteins of human hematopoietic cells—that of erythroid cells or glycophorin A, and that of leukocytes also called leukosialin [2] or leukophorin [3]. The rat W13/W13 antigen is apparently very similar to the leukocyte sialoglycoprotein in having a large number of O-glycosidic oligosaccharides [60]. The two human proteins have several common characteristics: (1) they are major carriers of cell surface sialic acids; (2) they are both heavily O-glycosylated with 1–2 N-glycosidic chains; (3) they are differently glycosylated in precursor cells and in cells of different cell lineages; (4) they are both phosphorylated at serine residues; and (5) they show decreased electrophoretic mobility on polyacrylamide gels in the presence of sodium dodecyl sulfate after treatment with neuraminidase. Red cell glycophorin A is evidently phosphorylated at serine-102, and the K562 molecule also contains phosphate

[61]. The major leukocyte sialoglycoprotein is phosphorylated and the phosphate is mainly serine-bound (Fig. 11). No function is known for the phosphate.

The mechanism for the decreased electrophoretic mobility on polyacrylamide gel electrophoresis in the presence of sodium dodecyl sulfate has been explored in detail for glycophorin A [62]. We found that treatment of intact red cells or Triton X-100 extracts of red cell membranes with *Vibrio cholerae* neuraminidase preferentially removed the peripheral 2-3-galactose-linked sialic acids, but not the 2-6-N-acetylgalactosamine-linked sialic acids. This shows that the galactose-bound sialic acids contribute on a electrophoresis relatively much to the negative charge of the molecule. After denaturation followed by neuraminidase treatment, glycophorin A moved faster than the intact molecule on electrophoresis and then also the N-acetylgalactosamine-linked sialic acids had been removed. Thus the N-acetylgalactosamine-linked sialic acids contribute relatively much to the apparent mass of the polypeptide whereas their negative charges do not seem to be readily available at the surface of the sodium dodecyl sulfate-polypeptide complexes.

Most probably the mechanism for this peculiar behavior is similar for the leukocyte sialoglycoprotein (Fig. 12).

The physiological roles of the sialoglycoproteins have remained poorly understood. The highly negative cell surface charge attributable to them could be important for circulating blood cells. Against this argues, however, the fact that red cells of the En(a−) blood group, which completely lack glycophorin A, seem to function normally. The affected individuals do not show hematologic disease. The band 3 molecule in En(a−) cells is, however, overglycosylated with a longer than normal polylactosamine type of oligosaccharide [31]. This finding indicates a sort of compensation for the lack of glycophorin A carbohydrate, although the carbohydrate structure of band 3 is completely different.

In contrast, as discussed above, the absence or abnormality of the major leukocyte

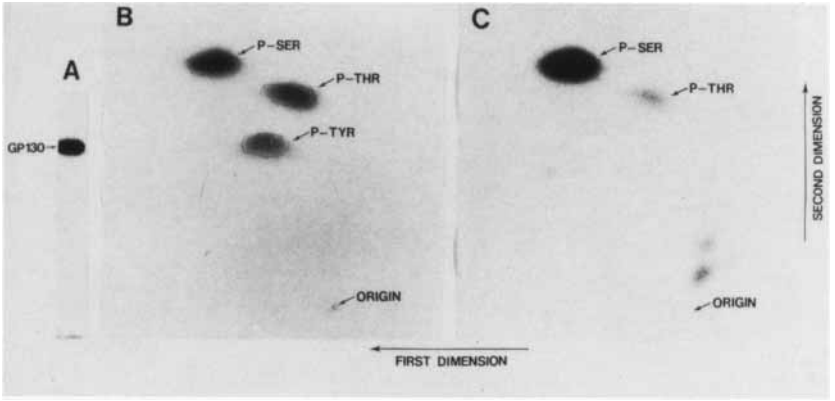

Fig. 11. Phosphoamino acid analysis of the major sialoglycoprotein of HL-60 cells. The protein was isolated from ^{32}P-labeled HL-60 cells (**A**) and hydrolysed in 6 N HCl for 2 hr at 110°C in a vacuum-sealed tube. The hydrolysate was analyzed by using two-dimensional thin-layer electrophoresis (pH 3.5 in the first dimension and pH 1.8 in the second dimension). Nonradioactive phosphoserine (P-SER), phosphothreonine (P-THR), and phosphotyrosine (P-TYR) were used as internal standards. After electrophoresis the plate was stained with ninhydrin (**B**) and subjected to autoradiography (**C**).

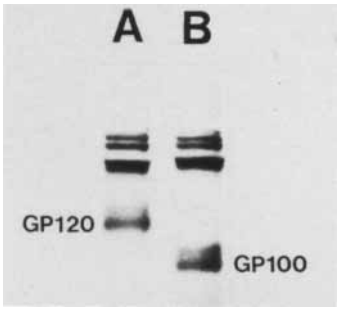

Fig. 12. Effect of sialic acids on the electrophoretic mobility of the major leukocyte sialoglycoprotein. **A**: Pattern obtained after pretreatment with neuraminidase and galactose oxidase followed by NaB $[^3H]_4$. **B:** Pattern obtained after pretreatment with periodate followed by NaB$[^3H]_4$.

sialoglycoprotein is associated with the Wiskott-Aldrich syndrome, which is a severe immunological disorder.

Mentzer et al. [3] have recently shown that the L10 monoclonal antibody reacting with an epitope on the major leukocyte sialoglycoprotein triggers the proliferation of blood T lymphocytes. The activation was dependent on the presence of monocytes. This may be an important finding indicating that the defective proliferative capacity of the T lymphocytes is important in the pathogenesis of the Wiskott-Aldrich syndrome.

It is plausible to assume that the sialoglycoprotein normally functions as a receptor for growth or differentiation factors. It is quite possible that the O-glycosidic carbohydrate plays an important role for the putative receptor functions, as is the case for glycophorin A. In fact several well-characterized receptors at the cell surface contain a large number of O-glycosidically linked oligosaccharides. For example, the low-density lipoprotein receptor contains a cluster of O-glycosidic oligosaccharides near the membrane [63], and the interleukin-2 receptor is O-glycosylated [64,65]. The role of such sugars in cell surface protein functions will continue to be an important subject of study.

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REFERENCES

1. Metcalf D: In Andersson LC, Gahmberg CG, Ekblom P (eds): "Gene Expression During Normal and Malignant Differentiation." London: Academic Press, pp 85–93, 1985.
2. Carlsson SR, Fukuda M: J Biol Chem 261:12779, 1986.
3. Mentzer SJ, Remold-O'Donnell E, Crimmins MAV, Bierer BE, Rosen FS, Burakoff SJ: J Exp Med 165:1383, 1987.
4. Tomita M, Marchesi VT: Proc Natl Acad Sci USA 72:2964, 1975.
5. Jokinen M, Gahmberg CG, Andersson LC: Nature 279:605, 1979.
6. Jokinen M, Andersson LC, Gahmberg CG: J Biol Chem 260:11314, 1985.
7. Gahmberg CG, Ekblom M, Andersson LC: In Daar AS (ed): "Tumour Markers in Clinical Practice: Concepts, Applications. London: Blackwell Scientific Publications 1987, pp 377–427.
8. Andersson LC, Gahmberg CG: Blood 52:57, 1978.

9. Andersson LC, Gahmberg CG, Nilsson K, Wigzell H: Int J Cancer 20:702, 1977.
10. Nilsson K, Andersson LC, Gahmberg CG, Wigzell H: Int J Cancer 20:708, 1977.
11. Gahmberg CG, Hakomori S: J Biol Chem 248:4311, 1973.
12. Gahmberg CG, Andersson LC: J Biol Chem 252:5888, 1977.
13. Shapiro DL, Marchesi VT: J Biol Chem 252:508, 1977.
14. Mäkelä O, Cantell K: Ann Med Exp Biol Fenn 36:366, 1958.
15. Springer GF, Ansell NJ: Proc Natl Acad Sci USA 44:182, 1958.
16. Gahmberg CG, Jokinen M, Andersson LC: Blood 52:379, 1978.
17. Ekblom M, Gahmberg CG, Andersson LC: Blood 66:233, 1985.
18. Lozzio CB, Lozzio BB: Blood 45:321, 1975.
19. Gahmberg CG, Jokinen M, Andersson LC: J Biol Chem 254:7442, 1979.
20. Andersson LC, Jokinen M, Gahmberg CG: Nature 278:364, 1979.
21. Rutherford TR, Clegg JB, Weatherall DJ: Nature 280:164, 1979.
22. Siebert PD, Fukuda M: PRoc Natl Acad Sci USA 83:1665, 1986.
23. Martin P, Papyannopoulou T: Science 216:1233, 1982.
24. Gerwitz AM, Burger D, Rado TA, Benz EJ, Hoffman R: Blood 60:785, 1982.
25. Vainchenker W, Testa U, Guichard J, Titeux M, Breton-Gorius J: Blood Cells 7:357, 1981.
26. Kieffer N, Debili N, Wicki A, Titeux M, Henri A, Mishal Z, Breton-Gorius J, Vainchenker W, Clemetson KJ: J Biol Chem 261:15854, 1986.
27. Rearden A, Taetle R, Elmajian DA, Majda JA, Baird SM: Mol Immunol 22:369, 1985.
28. Gahmberg CG, Ekblom M, Andersson LC: Proc Natl Acad Sci USA 81:6752, 1984.
29. Perkins M: J Cell Biol 90:563, 1981.
30. Pasvol G, Wainscoat JS, Wheatherall DJ: Nature 297:64, 1982.
31. Gahmberg CG, Myllylä G, Leikola J, Pirkola A, Nordling S: J Biol Chem 251:6108, 1976.
32. Tanner MJA, Anstee DJ: Biochem J 155:701, 1976.
33. Dahr W, Uhlenbruck G, Leikola J, Wagstaff W, Landfried K: J Immunogenet 3:329, 1976.
34. Järnefelt J, Rush J, Li Y-T, Laine RA: J Biol Chem 253:8006, 1978.
35. Krusius T, Finne J, Rauvala H: Eur J Biochem 92:289, 1978.
36. Friedman MJ, Fukuda M, Laine RA: Science 228:75, 1985.
37. Väisänen V, Korhonen TK, Jokinen M, Gahmberg CG, Ehnholm C: Lancet i:1192, 1982.
38. Jokinen M, Ehnholm C, Väisänen-Rhen V, Korhonen TK, Pipkorn R, Kalkkinen N, Gahmberg CG: Eur J Biochem 147:47, 1985.
39. Andersson LC, Gahmberg CG, Teerenhovi L, Vuopio P: Int J Cancer 24:717, 1979.
40. Ekblom M, Borström G, von Willebrand E, Gahmberg CG, Vuopio P, Andersson LC: Blood 62:591, 1983.
41. Greaves MF, Sieff C, Edwards PAW: Blood 61:645, 1983.
42. Andersson LC, Gahmberg CG, Kimura AK, Wigzell H: Proc Natl Acad Sci USA 75:3455, 1978.
43. Gahmberg, CG, Andersson LC, Karhi KK, Rodt H: Biochem Soc Trans 12:549, 1984.
44. Gahmberg CG, Karhi KK, Ekblom M, Stöckbauer P, Andersson LC: In "Gene Expression During Normal and Malignant Differentiation." London: Academic Press, Andersson LC, Ekblom P, Gahmberg CG (eds): pp 107, 1985.
45. Remold-O'Donnell E, Zimmerman C, Kenney D, Rosen FS: Blood 70:104, 1987.
46. Andersson LC, Gahmberg CG, Siimes MA, Teerenhovi L, Vuopio P: Int J Cancer 23:306, 1979.
47. Gahmberg CG, Andersson LC: Biochim Biophys Acta 651:65, 1982.
48. Remold-O'Donnell E, Davis AE III, Kenney D, Bhaskar K, Rosen FS: J Biol Chem 261:7526, 1986.
49. Remold-O'Donnell E, Kenney D, Rosen FS: Biochemistry 26:3908, 1987.
50. Kimura AK, Wigzell H: J Exp Med 147:1418, 1979.
51. Conzelmann A, Kornfeld S: J Biol Chem 259:12528, 1984.
52. Gahmberg CG, Nilsson K, Andersson LC: Proc Natl Acad Sci USA 76:4087, 1979.
53. Stöckbauer P, Gahmberg CG, Andersson LC: Cancer Res 45:2821, 1985.
54. Axelsson B, Hammarström S, Finne J, Perlmann P: Eur J Immunol 15:427, 1985.
55. Carlsson SR, Sasaki H, Fukuda M: J Biol Chem 261:12787, 1986.
56. Gahmberg CG, Peltokorpi L, Andersson LC: Blood 67:973, 1986.
57. Hammarström S: Methods Enzymol 28:368, 1972.
58. Remold-O'Donnell E, Kenney DM, Parkman R, Cairns L, Savage B, Rosen FS: J Exp Med 159:1705, 1984.

59. Kenney D, Cairns L, Remold-O'Donnell E, Peterson J, Rosen FS, Parkman R: Blood 68:1329, 1986.
60. Brown WRA, Barclay AN, Sunderland CA, Williams AF: Nature 289:456, 1981.
61. Gahmberg CG, Jokinen M, Karhi KK, Kämpe O, Peterson PA, Andersson LC: Methods Enzymol 96:281, 1983.
62. Gahmberg CG, Andersson LC: Eur J Biochem 122:581, 1982.
63. Cummings RD, Kornfeld S, Schneider WJ, Hobgood KK, Tolleshaug H, Brown MS, Goldstein JL: J Biol Chem 258:15261, 1983.
64. Leonard WJ, Depper JM, Crabtree GR, Rudikoff S, Pumphrey J, Robb RJ, Krönke M, Svetlik PB, Peffer NJ, Waldmann TA, Greene WC: Nature 311:626, 1984.
65. Nikaido T, Shimizu A, Ishida N, Sabe H, Techigawana K, Maeda M, Uchiyama T, Yodoi J, Honjo T: Nature 311:631, 1984.