Journal of Supramolecular Structure
and Cellular Biochemistry 17:313–324 (1981)
Differentiation and Function of Hematopoietic Cell Surfaces 97–108

Changes in Cell Surface Glycoproteins and Carbohydrate Structures During the Development and Differentiation of Human Erythroid Cells

Minoru Fukuda and Michiko N. Fukuda

Biochemical Oncology, Fred Hutchinson Cancer Research Center and Department of Pathobiology, School of Public Health and Community Medicine, University of Washington, Seattle, Washington 98104

The hematopoietic tissue is one of the few that continuously renews itself even in adults. In this system, pluripotent stem cells differentiate, probably by a multistep process, into mature cells of various functions and structures. The pathogenesis of many hematopoietic disorders can be related to abnormalities either in stem cell proliferation, in their differentiation and/or maturation process, or in cells that regulate these processes. An understanding of the course of differentiation and maturation as well as development is therefore essential to get an insight into the mechanism of some hematopoietic diseases.

Although membrane glycoproteins of human erythrocytes are one of the best characterized systems [1,2], only a few systematic studies have been carried out on the cell surface glycoprotein profile during the course of differentiation and maturation of human erythroid cells as well as the development from fetus to adult. We have developed a technique, cell surface labeling and endo-β-galactosidase digestion, to characterize cell surface glycoproteins [3]. By this technique, we could clearly distinguish two types of membrane glycoproteins, sialoglycoproteins and band 3 and band 4.5 glycoproteins. In addition, we have shown that this technique can distinguish fetal (i) and adult (I) antigens present on cell surfaces [3,4]. By using this newly developed technique as well as immunoprecipitation by specific antibodies, we have then analyzed erythroid cells of earlier stages of differentiation and maturation. To study cell surface glycoproteins in erythroid cells of earlier stages of maturation, we have analyzed erythroblasts generated in vitro (BFUe derived) [5] and K562 leukemic cells [6], which are presumably at the proerythroblast stage. This article summarizes the data from our studies as well as from related studies of others.

Minoru and Michiko Fukuda are now at the La Jolla Cancer Research Foundation, La Jolla, CA 92037.

Received July 31, 1981; accepted August 19, 1981.

0275-3723/81/1704-0313$03.50

CELL SURFACE GLYCOPROTEINS OF MATURE ERYTHROCYTES FROM ADULT BLOOD [5]

Erythrocytes from peripheral adult blood and from fetal blood were first labeled by galactose oxidase/NaB[^{3}H]$_4$ [7] or periodate/NaB[^{3}H$_4$ technique [8]. The former technique labels nonreducing terminal galactose or N-acetyl-galactosamine while the latter technique labels sialic acid. [^{3}H]-labeled cells were then treated with endo-β-galactosidase from Escherichia freundii [9]. After the enzyme treatment, the cell pellet and supernatant were separated and analyzed by SDS-polyacrylamide gel electrophoresis and gel filtration, respectively. As shown in Figure 1, these two cell surface labeling techniques distinguish two distinct groups of glycoproteins, ie, the galactose oxidase/NaB[^{3}H]$_4$ labels band 3 and band 4.5, whereas periodate/NaB[^{3}H]$_4$ labels sialoglycoproteins (PAS-1, -2, -3). Furthermore, the carbohydrate chains labeled by galactose oxidase/NaB[^{3}H]$_4$ were extensively hydrolyzed, whereas those labeled by periodate/NaB[^{3}H]$_4$ were scarcely affected. It has been shown [9–11] that endo-β-galactosidase cleaves the β-galactosidic linkage of polylactosaminyl structure, [Galβ1→4GlcNAcβ1→3]$_n$, which is now called lactosaminoglycan (Fig. 2). The results therefore clearly indicate that lactosaminoglycan is present on band 3 and band 4.5. In fact, we and

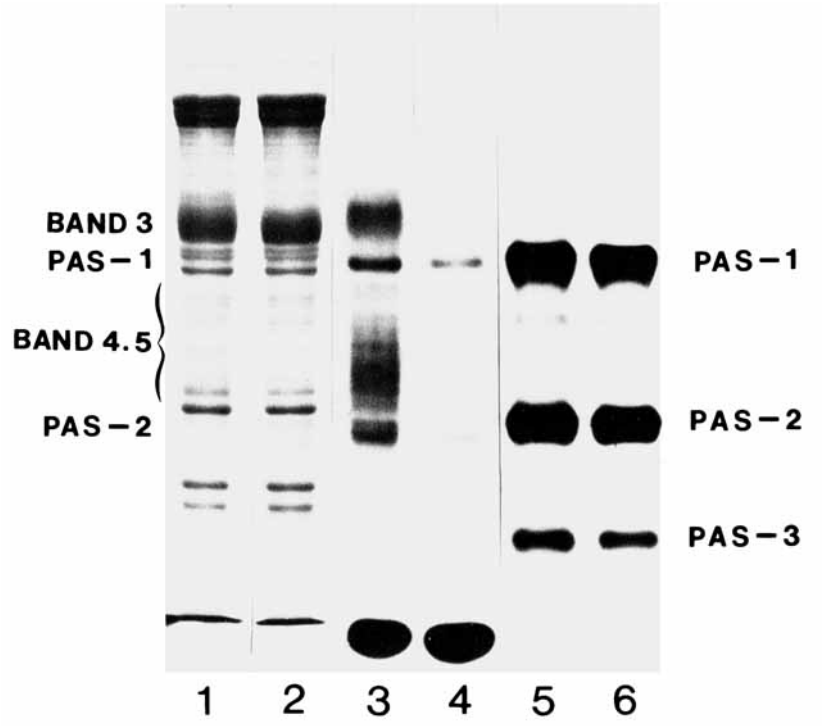

Fig. 1. SDS-polyacrylamide gels of adult erythrocyte membrane before and after endo-β-galactosidase digestion [3]. Cell surface was labeled by galactose oxidase/NaB[^{3}H]$_4$ (Gels 3,4) and by periodate/NaB[^{3}H]$_4$ (Gels 5,6). 1,3,5 are control cells and 2,4,6 are cells incubated with endo-β-galactosidase. Gels 3–6 are fluorogram and Gels 1,2 are protein stain of Gels 3,4 before treated for fluorography. Fetal erythrocytes showed a similar pattern except band 3 was more sharpened after the enzyme treatment.

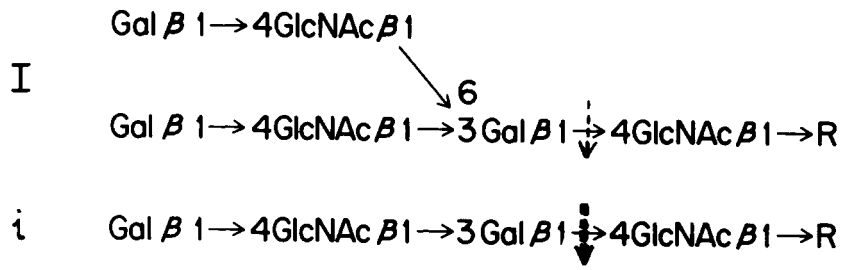

Fig. 2. Structures of antigenic determinants for I and i antigens and their susceptibility to endo-β-galactosidase. I and i antigenic activities were expressed by linear repeating lactosaminyl structure (i) or by branched polylatocamine (I) [from 26,27]. Endo-β-galactosidase cleaves linear chain of polylactosamine extensively but scarcely hydrolyzes the branched galactose [9–11]. See the arrows.

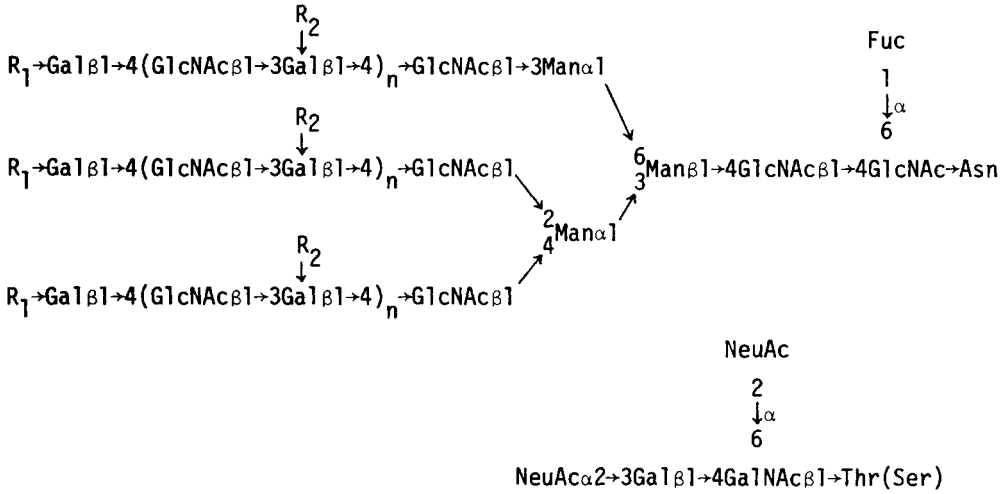

Fig. 3. Two types of carbohydrate chains present on mature erythrocytes. Lactosaminoglycan (upper) is present on band 3 and band 4.5 glycoproteins [3,4], whereas 15 chains of tetrasaccharide units are present on glycophorin [2,14]. R_1, H, Fucα1→2, NANα2→3/6; R_2, R_1→(Galβ1→4GlcNAcβ1→3)$_n$ β1→6.

others found lactosaminoglycan in band 3 [4] and glycopeptides of erythrocyte membranes [12, 13], whereas the major carbohydrate unit of sialoglycoproteins is sialosyltetrasaccharide [14] (Fig. 3). Endo-β-galactosidase can cleave polylactosaminyl structures but not this tetrasaccharide [9–11]. Thus, these two groups of glycoproteins have two different sets of carbohydrate units, and cell surface labeling followed by endo-β-galactosidase digestion can clearly distinguish these two types of glycoproteins.

These results are related to the fact that one of the band 4.5 proteins is derived from band 3 [15], and the isolated glucose transporter, which is one of the band 4.5 glycoproteins, was also susceptible to endo-β-galactosidase [16]. In addi-

tion, it was found that the asparagine-linked oligosaccharide chain in glycophorin A contains a polylactosaminyl structure as a minor component [17], which is consistent with our results that the part of galactose oxidase-labeled carbohydrate chain in PAS-1 and PAS-2 was susceptible to endo-β-galactosidase (see Fig. 1).

Band 3 and glycophorins are thought to be distinctly different in terms of molecular architecture, as shown in Figure 4. The molecular architecture of band 4.5 appears to be similar to band 3 (Fukuda M and Marchesi VT, unpublished data). It is likely, therefore, the proteins that traverse the lipid bilayer only one time have short carbohydrate chains, whereas the proteins that traverse the lipid bilayer multiple times have a long polylactosaminyl structure. Furthermore, the functions of the former proteins are structural, whereas the latter proteins are transporter. It is conceivable that polylactosaminyl long carbohydrate chains are playing certain role(s) during the biosynthesis, folding, and insertion of the proteins to the lipid bilayer.

CHANGES OF STRUCTURE IN LACTOSAMINOGLYCAN OF BAND 3 DURING THE DEVELOPMENT FROM FETUS TO ADULT [4]

During the ontogenic change from fetal to adult human erythrocytes, the cell surface antigen i is converted to I as well as the fetal hemoglobin being replaced by adult hemoglobin [22,23]. We showed that the purified band 3 was found to be a major carrier for Ii antigens on erythrocytes by using anti-I antiserum adsorbent [24]. In addition, we have shown that endo-β-galactosidase abolished Ii antigenic activity of human erythrocytes in concomitant with shortening carbohydrate chains of band 3 and band 4.5 [3]. We therefore compared the carbohydrate

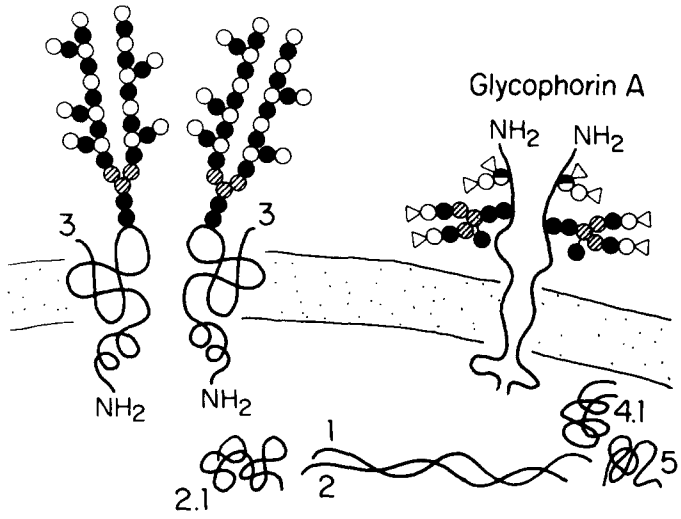

Fig. 4. A schematic version of the organization of human erythrocyte membrane proteins. Band 3 traverses several times lipid bilayer and one long carbohydrate chain is attached to this protein. Glycophorin traverses only one time lipid bilayer and 15 short oligosaccharides and one relatively short asparagine-linked carbohydrate chain is attached to the protein (see also Fig. 3). Band 2.1 is also called ankyrin, PAS-1 is called glycophorin A, 1,2 is spectrin, and band 5 is actin. This version is based on the data in [2, 18, 19, 20, 21, and 44]. It is also suggested that certain populations of band 3 and glycophorin associate with each other [45]. ●, N-methylglucosamine; ⬙, mannose; ○, galactose; ◓, N-acetylgalactosamine; △, sialic acid.

structures of band 3 from newborn blood and adult blood and found that the most critical change from fetus (i) to adult (I) is the conversion of a linear polylactosamine to a branched polylactosamine as shown in Figure 5 [4]. This change was also observed when oligosaccharides released by endo-β-galactosidase from surface-labeled erythrocytes were analyzed. As shown in Figure 6, oligosaccharides of various molecular weights were produced from adult erythrocytes, whereas from

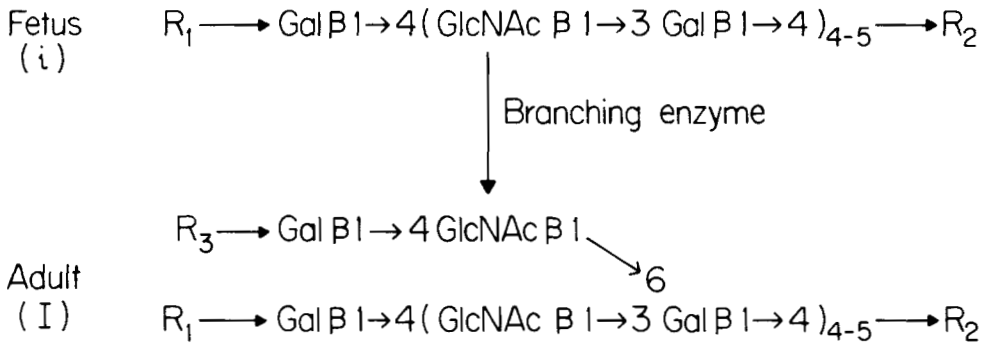

Fig. 5. Proposed structural change of band 3 carbohydrate moiety during development from fetus to adult [4]. R_1, H, Fuc 1→2, NeuNAcα2→3/6; R_2 $(Man)_3(GlcNac)_{4-5}$, exact structure unknown; R_3, the same as R_1, or R_1→$(Gal\beta1\rightarrow4GlcNAc\beta1\rightarrow3)_n$. Two or three chains shown here are linked to the same core pattern (R_2). (see also Fig. 3).

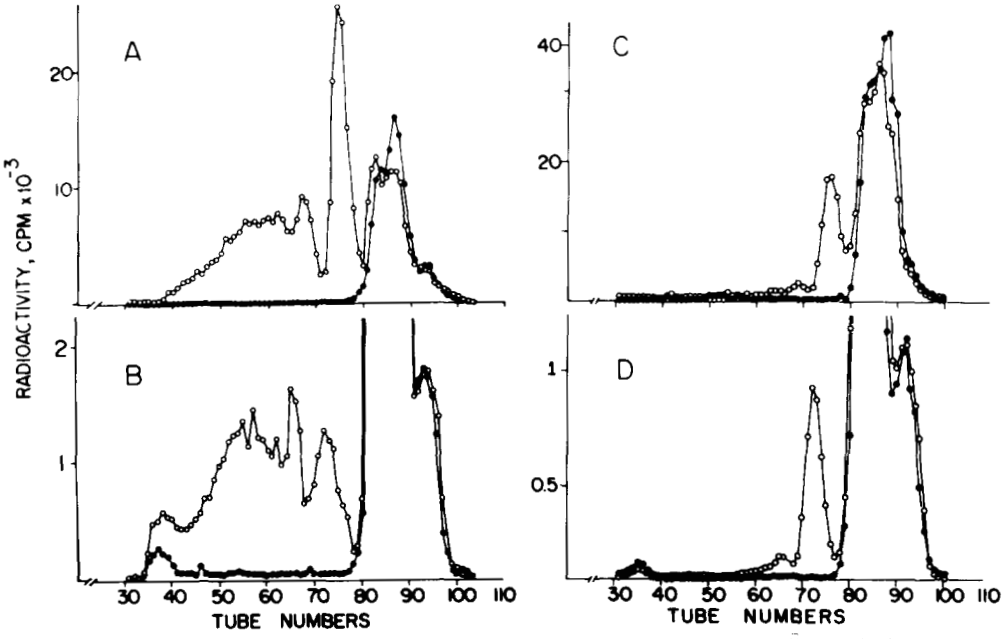

Fig. 6. Gel filtration of released oligosaccharides on a Sephadex G-50 column equilibrated with 0.2 M NaCl. A, oligosaccharides from galactose oxidase-labeled adult I cells; B, oligosaccharide from periodate-labeled adult I cells; C, oligosaccharides from galactose oxidase-labeled cord cells; D, oligosaccharides from periodate-labeled cord cells. Cell supernatant after incubation without endo-β-galactosidase (- ● -) or with endo-β-galactosidase (-○-). The radioactive peak (Tube 80 to 100) are inorganic tritium, salt.

newborn erythrocytes relatively homogenous oligosaccharides of low molecular weights were produced [3]. This result was obtained because adult (I) carbohydrate chains contained branched structure that hinders the complete digestion by endo-β-galactosidase, whereas linear chain structure of fetal (i) antigens could be cleaved extensively (see also Fig. 2). These results are clearly consistent with the changes in glycolipid during the development [25] and antigenic determinants of I and i antigens found by using glycolipids as haptens [26,27]. Therefore, the changes of carbohydrate chains can be schematically depicted as shown in Figure 7.

CELL SURFACE GLYCOPROTEINS OF ERYTHROBLASTS AND K562 LEUKEMIC CELLS [5,6]

As described in previous sections, the analysis of cell surface glycoproteins and glycolipids by cell surface labeling and endo-β-galactosidase digestion enables us to deduce the following [3]: 1) from the change of antigenic activity and carbo-

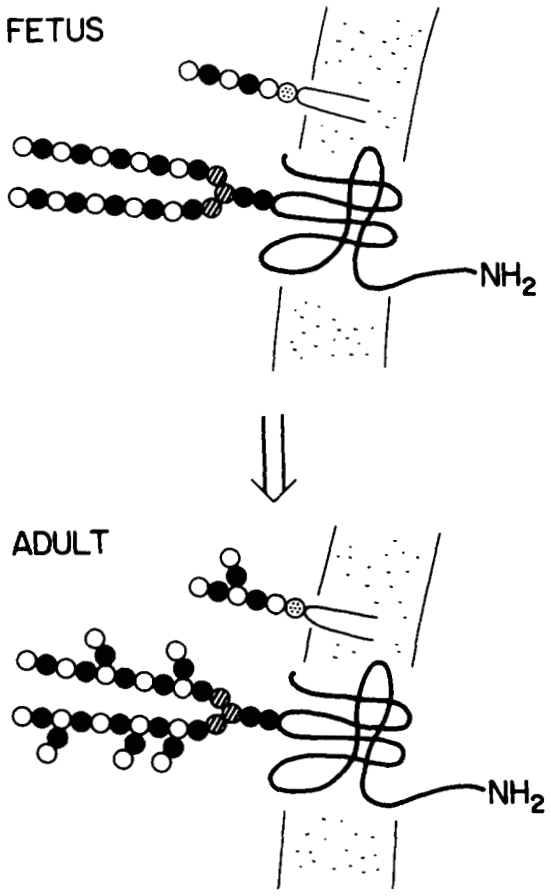

Fig. 7. Idealized version of structural changes of lactosaminoglycan on glycolipid and glycoprotein (Band 3). (○, galactose; ●, N-acetylglucosamine; ⊘, mannose; ⊗, glucose). In fetal erythrocytes, a linear unbranched polylactosamine, $(Gal \rightarrow GlcNAc)_n$ is linked to Gal-Glc-Cer or to $(Man)_3$ $(GlcNAc)_2$ core of band 3. When fetal erythrocytes are changed to adult erythrocytes, the linear chains are converted to those having Gal→Glc NAc branchings.

hydrate chains of the cell surface, it is possible to find out which glycoproteins and glycolipids are carrying such antigenicities; 2) from the analysis before and after endo-β-galactosidase, it can be concluded which glycoproteins have polylactosaminyl structure; and 3) the analysis of oligosaccharides can estimate the degree of branching in lactosaminoglycan on cell surface.

By using this newly developed technique combined with immunoprecipitation by specific antibodies, we have then analyzed erythroid cells of earlier stages of maturation.

Erythroblasts, generated in vitro from precursor cells of adult or neonatal or fetal blood with erythropoietin, were obtained as pooled erythroid bursts [28], and cell surface glycoproteins of the cells were characterized by the following [5] (see Fig. 8): 1) A barely detectable amount of band 3 and band 4.5 glycoproteins; 2) presence of glycophorins (PAS-1, -2, and -3) with PAS-2 as the major component; 3) presence of two glycoproteins with molecular weights of 105,000 and 95,000 (Gp105 and Gp95); 4) a small but detectable quantity of carbohydrate chains susceptible to endo-β-galactosidase ("lactosaminoglycan," Ii antigens). In addition, adult erythroblasts showed detectable amount of branching but not to the same extent as mature erythrocytes, whereas fetal and neonatal erythroblasts showed either no or minimal branching as erythrocytes of the same ontogenic stage. The above results were confirmed by immunoprecipitation using band 3 antiserum [18], glycophorin antiserum, and by anti Ii sera. Parallel controls for these studies were done by using granulocytes and monocytes harvested from the same culture dishes as the erythroid bursts. No common bands between erythroblasts and granulocytes-monocytes were identified (Fig. 8B).

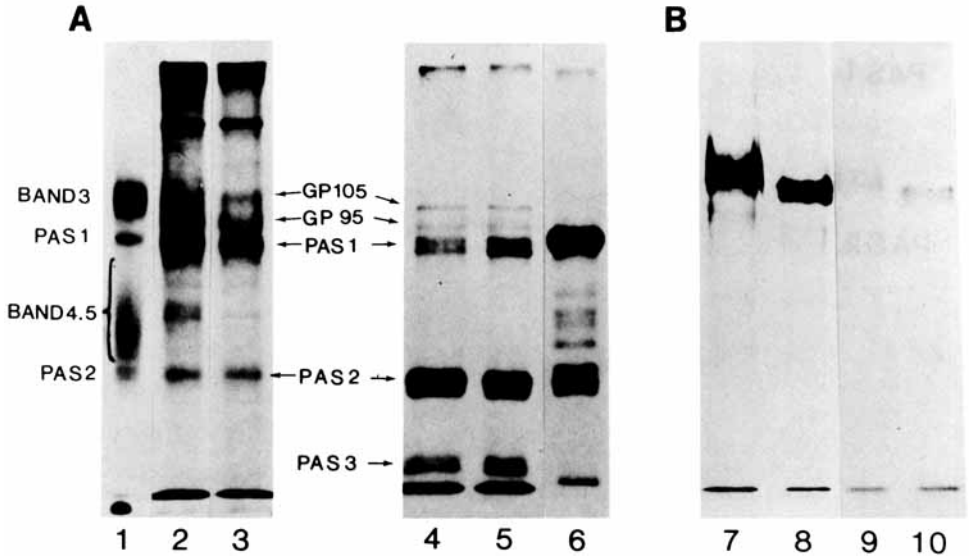

Fig 8 Fluorogram of SDS-polyacrylamide gels of surface-labeled cells 1, galactose oxidase-labeled or 6, periodate-labeled mature erythrocytes, 2,3, galactose oxidase-labeled or 4 5 periodate-labeled erythroblasts, 7,8, periodate-labeled granulocytes, 9,10, periodate-labeled monocytes 1,2,4,7,9 are control cells and 3,5,8,10 are cells incubated with endo-β-galactosidase Cell number used in gel 2,3 were four times more than those in gel 4,5 to obtain comparable amount of incorporated radioactivity

As proerythroblasts, we have used human leukemic cell line K562, originally isolated from a patient at blast crisis of chronic myelocytic leukemia [29]. This cell has been shown by Gahmberg et al to be an "erythroleukemic" cell line by showing the presence and synthesis of glycophorin and the induction of hemoglobin synthesis [30,31]. Since glycophorin A is exclusively expressed on erythroid cell lineage and appears to be absent from undifferentiated precursors [5, 32, 33], we can regard K562 cell as a cell close to a proerythroblast. The cell surface glycoproteins of K562 cells were analyzed by similar techniques, and the following characteristic features were noted [6]: 1) band 3 and band 4.5 are essentially absent and only a few glycoproteins labeled by galactose oxidase/NaB[^{3}H]$_4$ are present; 2) glycophorins are present as minor glycoproteins; 3) Gp105 is present as the major glycoprotein together with Gp95; 4) a minimum amount of lactosaminoglycan is present, and the structure of lactosaminoglycan is unbranched chain, fetal (i) type (see Fig. 9, 10).

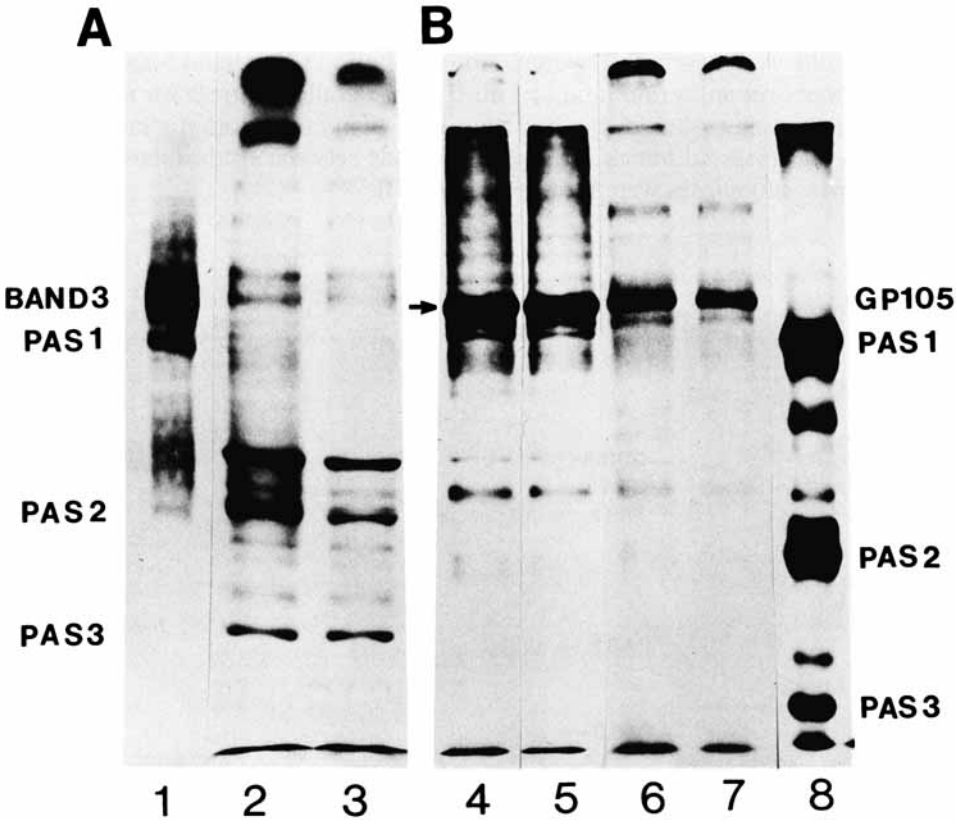

Fig. 9. Fluorogram of SDS-polyacrylamide gels of K562 cells: 1, galactose oxidase-labeled or 8, periodate-labeled adult erythrocytes; 2,3, galactose oxidase-labeled or 4,5, periodate-labeled or 6,7 [^{3}H]-GlcN metabolically labeled K562 cells. Lanes 2,4,6 are control cells, and 3,5,7 are cells incubated with endo-β-galactosidase. The amount of membrane used in gels 2 and 3 is about four times that of gels 4-7.

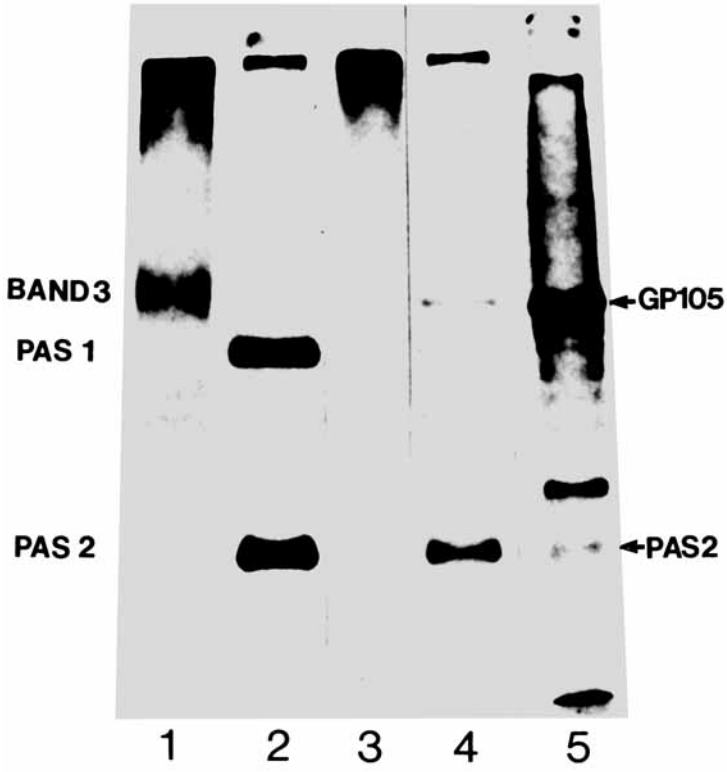

Fig 10 Fluorogram of SDS-polyacrylamide gels of immunoprecipitates with anti-band 3 antiserum (gels 1,3) or anti-glycophorin antiserum (gels 2,4) 1, Immunoprecipitates from galactose oxidase-labeled or 2, periodate-labeled erythrocytes, 3,4 immunoprecipitates from periodate-labeled K562 cells, 5, total membrane of periodate-labeled K562 cells The amount of membrane used in gels 3 and 4 is about four times that of gel 5

CHANGES OF CELL SURFACE GLYCOPROTEINS DURING THE DIFFERENTIATION AND MATURATION OF HUMAN ERYTHROID CELLS

On the basis of the data presented, the following statements can be forwarded: 1) band 3 and band 4.5 are barely present at proerythroblast stage but are evident at the late stages of erythroblast maturation and increase further towards the mature erythrocyte stage; 2) glycophorins are present at the proerythroblast stage before the start of hemoglobin synthesis, but their amount also greatly increases during maturation; 3) glycoproteins of 105K and 95K molecular weights are present as major glycoproteins at proerythroblast stage (K562 cells) but are present as minor glycoproteins at erythroblast stage, and they decline further with continuing maturation; 4) the amount of lactosaminoglycan is minimal on proerythroblasts and is small at erythroblast, then greatly increases as the cells mature and express band 3 and band 4.5; 5) the degree of branching increases significantly during maturation of adult erythroid cells. This view is summarized in Figure 11.

	Proerythroblast	Erythroblast	Erythrocyte
Band 3	-	+	+++
Glycophorin	+	++	+++
Gp105	+++	+	-
Gp95	+++	+	-
Lactosaminoglycan	+ (i)*	+ (Ii)	+++ (I)
Hemoglobin	-	++	+++

Fig. 11. Change of cell surface glycoproteins during maturation of human adult erythroid cells. *The branching of lactosaminoglycan increases during the maturation of adult cells, but no increase of branching is observed during fetal erythroid cell maturation. For details see the text.

Our results concerning the changes in band 3 are consistent with several results reported by others. First, Friend erythroleukemic cells that are considered to be close to proerythroblast stage in mice were found to have significantly different properties in anion transport compared with matured erythrocytes [34]. This difference can be explained by the absence of band 3 in Friend cells as band 3 is an anion transporter for mature erythrocytes. In addition, the significant increased synthesis of band 3 was observed after differentiation of Friend cells were induced by dimethyl sulfoxide [35]. Thirdly, Knauf studied the anion transport properties of K562 cells and found a similar character to Friend cells [36]. More recently, Foxwell and Tanner showed by Staphylococcal rosetting technique [32] that band 3 are much more evident on cell surface at later stages of erythroblasts [37]. Thus, our data and those obtained by others clearly show that band 3 appears at relatively later stages of erythroid maturation. In contrast to this, glycophorin is already present at relatively early stages of maturation before the start of hemoglobin synthesis [6,32], although it is not present at very early stages such as BFUe precursor cells [33]. The appearance of spectrin is similar to that of glycophorin: it is present at proerythroblast stage and increases greatly before hemoglobin synthesis reaches maximum [38-40].

It is interesting that the synthesis of band 3 is slightly later than that of hemoglobin and the amount of band 3 reaches maximum just before erythroid cells are released into peripheral blood. Band 3 is essential for carbonate-chloride exchange of circulating erythrocytes. It is possible that the proteins important for function of matured cells such as band 3 are optimally synthesized.

On the other hand, Gp105 and Gp95 are present primarily in early stages of maturation and almost absent in matured erythrocytes. These proteins may play some roles in such immatured erythroid cells.

Our results showed that the amount of lactosaminoglycan increases during maturation in parallel to the increase of band 3 and band 4.5. This is consistent with the fact that band 3 and band 4.5 are major carriers for this carbohydrate structure in matured erythrocytes [3]. Our results are also consistent with the findin that blood group A antigen is expressed at later than proerythroblast stage [41].

We and others have shown that lactosaminoglycan of band 3 carries ABH antigens as well as Ii antigens [4,12,13]. It is natural therefore that the appearance of blood group A antigen is in parallel to the appearance of lactosaminoglycan and band 3. The increase of branching during maturation provides increase of antibody binding as monogamous bivalency is possible after branched structure is formed [42].

In conclusion, it is clearly shown that differentiation and maturation of human erythroid cells accompany the drastic changes in cell surface glycoproteins as well as carbohydrate structures of cell surface. Similar findings were obtained recently in granulocyte-monocyte differentiation and distinctly different cell surface structures are present between early and late stages of granulocyte maturation [43]. It will be interesting to see how these expressions are regulated during differentiation and maturation and whether such expression is disordered in hematological diseases of benign or malignant etiology.

ACKNOWLEDGMENTS

We thank Dr. S. Hakomori for encouragement and providing facilities and Dr. V.T. Marchesi for providing an opportunity to work on band 3. We also thank Dr. T. Papayannopoulou for providing erythroblasts, Drs. E. Giblett and M.C. Crookston for the donation of anti-Ii sera and Mrs. C.M. Pagni for excellent secretarial assistance. This work was supported in part by the grant RO1 CA29959 awarded by the National Cancer Institute.

REFERENCES

1. Steck TL: J Cell Biol 62:1-19, 1974.
2. Marchesi VT, Furthmayr H, Tomita M: Annu Rev Biochem 45:667-698, 1976.
3. Fukuda MN, Fukuda M, Hakomori S: J Biol Chem 254:5458-5465, 1979.
4. Fukuda M, Fukuda MN, Hakomori S: J Biol Chem 254:3700-3703, 1979.
5. Fukuda M, Fukuda MN, Papayannopoulou T, Hakomori S: Proc Natl Acad Sci USA 77:3474-3478, 1980.
6. Fukuda M: Nature 285:405-407, 1980.
7. Gahmberg C, Hakomori S: J Biol Chem 248:4311-4317, 1973.
8. Gahmberg C, Andersson LC: J Biol Chem 252:5888-5894, 1977.
9. Fukuda MN, Matsumura G: J Biol Chem 251:6218-6225, 1976.
10. Fukuda MN, Watanabe K, Hakomori S: J Biol Chem 253:6814-6819, 1978.
11. Fukuda MN: J Biol Chem 256:3900-3905, 1981.
12. Krusius T, Finne J, Rauvala H: Eur J Biochem 92:289-300, 1978.
13. Järnefelt J, Rush J, Li Y-T, Laine RA: J Biol Chem 253:8006-8009, 1978.
14. Thomas DB, Winzler RJ: J Biol Chem 244:5943-5946, 1969.
15. Tarone G, Hamasaki N, Fukuda M, Marchesi VT: J Membrane Biol 48:1-12, 1979.
16. Gorga FR, Baldwin SA, Lienhard GE: Biochem Biophys Res Commun 91:955-961, 1977.
17. Yoshima H, Furthmayr H, Kobata A: J Biol Chem 255:9713-9718, 1980.
18. Fukuda M, Eshdat Y, Tarone G, Marchesi VT: J Biol Chem 253:2419-2428, 1978.
19. Steck TL, Koziarz JJ, Singh MK, Reddy G, Köhler H: Biochemistry 17:1216-1222, 1978.
20. Bennet V, Stenbuck PJ: Nature 280:468-473, 1979.
21. Tyler JM, Reinhardt BN, Branton D: J Biol Chem 255:7034-7039, 1980.
22. Huhens ER, Shooter EM: J Med Genet 2:1, 1965.
23. Marsh WL: Br J Haematol 7:200-209, 1961.
24. Childs RA, Feizi T, Fukuda M, Hakomori S: Biochem J 173:333-336, 1978.
25. Watanabe K, Hakomori S: J Exp Med 144:644-653, 1976.

26. Watanabe K, Hakomori S, Childs RA, Feizi T: J Biol Chem 254:3221-3228, 1979.
27. Feizi T, Childs RA, Watanabe K, Hakomori S: J Exp Med 149:975-980, 1979.
28. Papayannopoulou T, Nakamoto B, Buckley J, Kurachi S, Stamatoyannopoulos G: Science 199:1349-1350, 1978.
29. Lozzio DB, Lozzio BB: Blood 45:321-334, 1975.
30. Gahmberg CG, Jokinen M, Andersson LC: J Biol Chem 254:7442-7448, 1979.
31. Andersson LC, Jokinen M, Gahmberg C: Nature 278:364-365, 1979.
32. Gahmberg CG, Jokinen M, Andersson LC: Blood 52:379-387, 1978.
33. Robinson J, Sieff C, Delia D, Edwards PAW, Greaves M: Nature 289:68-70, 1981.
34. Harper PA, Knauf PA: J Cell Physiol 95:347-357, 1979.
35. Sabban EL, Sabatini DD, Marchesi VT, Adesnik M: J Cell Physiol 104:261-268, 1980.
36. Knauf PA, Law F-Y: J Supramol Struct Cell Biochem Suppl 5:124, 1981.
37. Foxwell BMJ, Tanner MJA: Biochem J 195:129-137, 1981.
38. Chang H, Langer PJ, Lodish HF: Proc Natl Acad Sci USA 73:3206-3210, 1976.
39. Eisen H, Bach R, Emery R: Proc Natl Acad Sci USA 74:3898-3902, 1977.
40. Geidushek JB, Singer SJ: Cell 16:149-163, 1979.
41. Karhi KK, Andersson LC, Vuopio P, Gahmberg CG: Blood 57:147-151, 1981.
42. Romans DG, Tilley CA, Dorrington KJ: J Immunol 124:2807-2811, 1980.
43. Fukuda M, Koeffler HP, Minowada J: Proc Natl Acad Sci USA 78:6299-6303, 1981.
44. Morrow JS, Speicher DW, Knowles WJ, Hsu CJ, Marchesi VT: Proc Natl Acad Sci USA 77: 6592-6596, 1980.
45. Nigg EA, Bron C, Giradet M, Cherry RJ: Biochemistry 19:1887-1893, 1980.