CYTOCHEMICAL GLUCOSE-6-PHOSPHATASE ACTIVITY IN HUMAN BLOOD CELLS

TAKASHI KIMURA

*The First Department of Internal Medicine,
Kansai Medical University, Moriguchi 570*

Received for publication August 7, 1986 and in revised form January 5, 1987

The cytochemical localization of glucose-6-phosphatase (G6Pase) activity was examined in human peripheral blood cells and in hematopoietic cells in bone marrow. The reaction product for G6Pase activity was present in the endoplasmic reticulum and nuclear envelope in all types of blood cells except for orthochromatic erythroblasts and mature erythrocytes. In erythroblasts, the reaction product decreased during development from erythroblasts into polychromatophilic erythroblasts. In neutrophil precursors, the reaction product decreased with maturation. Therefore, the maturational stage of hematopoietic cells can be determined according to the relative amount of reaction product for G6Pase activity. The high activity in immature cells may be related to their powerful proliferation. The physiological role of G6Pase in blood cells may be to regulate the concentration of glucose-6-phosphate in the cells as in other cell types.

Cytochemical procedures have been utilized to determine the origin and maturational stage of hematopoietic cells. Myeloperoxidase activity in the endoplasmic reticulum and nuclear envelope is a marker for identifying promyelocytes and promonocytes (5). Platelet peroxidase activity in the endoplasmic reticulum and nuclear envelope is used for identifying promegakaryoblasts (6). Acetylcholinesterase activity is shown to be present in the endoplasmic reticulum and nuclear envelope in erythroblasts and thymic lymphocytes (27, 32). Thus, enzyme activities in the endoplasmic reticulum and nuclear envelope are markers in these cytochemical procedures.

Glucose-6-phosphatase (G6Pase) activity is known to be exclusively localized in the endoplasmic reticulum and nuclear envelope of the cells (3, 9, 11, 13, 15, 18, 25, 26, 30, 31, 34). Therefore, as a marker for endoplasmic reticulum and nuclear envelope of blood cells, it appears more reasonable to use G6Pase than peroxidase and acetylcholinesterase, because the two enzymes are also present in cell organelles other than the endoplasmic reticulum and nuclear envelope. Cytochemical G6Pase activity in blood cells has been reported. Rosen observed the activity in rat heterophilic leukocytes and erythroblasts (25). Monneron reported the activity in calf thymocytes (19). Kanamura found the activity in polychromatophilic erythroblasts in hematopoietic islets of the newborn mouse (14). Nichols and Setzer observed the activity in rabbit promonocytes and monocytes (20). Nichols and co-workers described the enzyme activity in neutrophils, basophils, eosinophils,

monocytes, lymphocytes and platelets of human peripheral blood (21). We found the enzyme activity in human megakaryocytes (16) and human malignant blood cells (17). However, there have been no systematic reports concerning cytochemical G6Pase activity in human hematopoietic cells.

In the present study, cytochemical localization of G6Pase activity was examined in human peripheral blood cells and hematopoietic cells in red bone marrow. The purpose was to study whether G6Pase activity is useful as a marker for determining the origin and maturational stage of hematopoietic cells.

MATERIALS AND METHODS

Preparation of blood cells

The peripheral blood samples were drawn from healthy persons into plastic syringes with citrate. Buffy coats were isolated by the dextran sedimentation method (28). The normal bone marrow samples which we used in the present study were obtained from patients with malignant disorders, but without bone marrow involvement before chemotherapy. The aspirated bone marrow fragments from the sterna were used in the present study.

Ultrastructural demonstration of G6Pase activity

Buffy coats in their free states and bone marrow fragments were immediately fixed in 2% glutaraldehyde in 0.1 M cacodylate buffer (pH 7.4) with 1.6% sucrose at 0–4°C for 10 min. The fixed samples were rinsed three times in 0.1 M cacodylate buffer (pH 7.4) containing 6.8% sucrose at 0–4°C for 1 hr or overnight. The fixed bone marrow fragments were embedded in a mixture of 2% agar and 1% gelatin at 45–50°C, chilled at 0–4°C for 30 min and cut on a Vibratome (Lancer Co. Ltd) at 30–40 μ m in thickness. The samples were incubated in a modified Wachstein and Meisel's medium (11, 33) for demonstration of G6Pase activity at 37°C for 30–60 min. The samples were rinsed twice in 0.1 M cacodylate buffer (pH 7.4) containing 6.8% sucrose at 0–4°C for 20 min. After postfixation in 1% osmium tetroxide in 1/35 M veronal acetate buffer (pH 7.4) with 4.5% sucrose, the samples were dehydrated in graded ethanols, and embedded in Spurr's medium (29). Ultrathin sections were prepared by an Ultratome (LKB) and examined under an Hitachi-500 electron microscope without staining, with lead nitrate staining or with uranyl acetate and lead nitrate staining.

Cytochemical controls

As the cytochemical control for the demonstration of G6Pase, the following experiments were carried out (12, 31, 34). 1) Incubation in a substrate free medium. 2) Preincubation in a 0.05 M acetate buffer (pH 5.0) at 0–4°C for 15 min. 3) Incubation in a medium containing equal moles of β -glycerophosphate, fructose-6-phosphate, fructose-1,6-diphosphate, glucose-1-phosphate, or ribose-5-phosphate (Sigma Chemicals Co.) in place of glucose-6-phosphate. 4) Preincubation in a buffer containing 5 mM levamisole and incubation in a complete medium containing 5 mM levamisole.

FIG. 1. Bone marrow erythroblasts incubated in G6Pase medium. The reaction product for G6Pase activity (arrow) is scarce in the nuclear envelope in polychromatophilic erythroblast (P-Ebl), however, is not present in orthochromatic erythroblast (O-Ebl). Stained with uranyl acetate. $\times 27,800$

RESULTS

The reaction product for G6Pase activity was present in the endoplasmic reticulum and nuclear envelope in all types of blood cells examined except for orthochromatic erythroblasts and mature erythrocytes (Fig. 1). In the present study, however, eosinophil and basophil precursors were not examined.

In erythroblasts, the amount of reaction product appeared generally medium in proerythroblasts, medium to scarce in basophilic erythroblasts, and scarce in polychromatophilic erythroblasts (Figs. 1, 2). In neutrophil precursors, the reaction product was abundant in myeloblasts and promyelocytes, medium in myelocytes, and scarce in metamyelocytes and mature neutrophils (Fig. 3). Thus, the amount of the reaction product decreased with maturation of the cells both in erythroblasts and neutrophil precursors. The reaction product was abundant in megakaryocytes and scarce in plasma cells.

In peripheral blood cells, the reaction product was abundant in monocytes and platelets, and scarce in neutrophils and lymphocytes (Fig. 4).

Mitochondria, Golgi apparatus, lysosomes (azurophilic granules in neutrophils, lymphocytes and monocytes) and other cell organelles, and plasma membrane in all types of blood cells examined showed no reaction product (Fig. 4).

FIG. 2. Bone marrow erythroblasts incubated in G6Pase medium. The reaction product for G6Pase activity is medium in endoplasmic reticulum and nuclear envelope in proerythroblast (Pro-Ebl) and scarce in nuclear envelope in polychromatophilic erythroblast (P-Ebl). Not stained. $\times 15,300$

FIG. 3. Bone marrow neutrophils incubated in G6Pase medium. The reaction product for G6Pase activity is abundant in promyelocyte (Prom) and scarce in mature neutrophil (MN). Not stained. $\times 10,400$

Omission of glucose-6-phosphate from the incubation medium resulted in a complete absence of the reaction product. Immersion of the fixed materials in the acetate buffer and use of β -glycerophosphate in place of glucose-6-phosphate in the reaction medium caused loss of the reaction in the endoplasmic reticulum and nuclear envelope. Incubation in a medium containing equal moles of β -glycerophosphate, fructose-6-phosphate, fructose-1,6-diphosphate, glucose-1-phosphate, or ribose-5-phosphate in place of glucose-6-phosphate resulted in a complete absence of the reaction product. Preincubation with levamisole and incubation in the reaction medium containing levamisole did not affect the amount of reaction

FIG. 4. Peripheral blood cells incubated in G6Pase medium. The reaction product is present in endoplasmic reticulum and nuclear envelope, however, is not present in Golgi apparatus, granules and mitochondria. The amount of reaction product for G6Pase activity is abundant in monocyte (Mo) and scarce in neutrophils (N). Not stained. $\times 18,100$

product in the endoplasmic reticulum and nuclear envelope. The results indicate that the reaction product in the endoplasmic reticulum and nuclear envelope is due to G6Pase activity.

DISCUSSION

As revealed in the present results, G6Pase activity was localized in the endoplasmic reticulum and nuclear envelope in all types of blood cells except for orthochromatic erythroblasts and mature erythrocytes. The enzyme activity in the endoplasmic reticulum and nuclear envelope decreased or disappeared with maturation of myeloid or erythroid precursors. Therefore, the maturational stage of hematopoietic cells can be determined according to the relative amount of reaction product for G6Pase activity.

The myeloperoxidase method has been used to determine the maturational stage of neutrophils (5). The use of G6Pase method in addition to the myeloperoxidase method probably results in more a reliable determination of the maturational stage of neutrophil precursors. Further, because no specific cytochemical marker, by which the maturational stage of erythroid precursors is determined, is yet known, the ultrastructural demonstration of G6Pase is useful for presuming the maturational stage of erythroid precursors.

G6Pase activity in the endoplasmic reticulum and nuclear envelope was high in immature blood cell precursors. Proerythroblasts and myeloblasts are proliferating cells. Sawaragi and Wynn observed strong G6Pase activity in epithelial cells of the endometrium at the early secretory phase (26). Boti and coworkers reported the high activity in regenerating tubular epithelium of the kidney (4). The high activity in proerythroblasts and myeloblasts may be related to their powerful proliferation. Basophilic and polychromatophilic erythroblasts, and promyelocytes and myelocytes are functionally active, producing hemoglobin, and azurophilic and specific granules. G6Pase activity appears high in the endoplasmic reticulum and nuclear envelope of such functionally active cells.

G6Pase activity was high in monocytes, megakaryocytes and platelets, medium in neutrophils, and low in lymphocytes and plasma cells. The activity in hematopoietic cells decreased with their maturation. These differences in G6Pase activity may relate to the difference in their oxygen consumption, which is high in monocytes, medium in neutrophils, low in lymphocytes, and higher in immature hematopoietic cells than mature cells (2, 8, 24). Thus, high G6Pase activity in monocytes and immature cells may indicate an increase in oxygen consumption, active carbohydrate metabolism and active aerobic metabolism.

Rosen found glucose-6-phosphate hydrolyzing enzyme in polychromatophilic erythroblasts and myelocytes in hematopoietic islets of newborn rat liver (25). Kanamura reported G6Pase activity in polychromatophilic erythroblasts in hematopoietic islets of newborn mouse liver (14). Nichols and Setzer observed G6Pase activity in rabbit bone marrow promonocytes and monocytes (20). In these studies, however, such a decrease in G6Pase activity with maturation of blood cell precursors as found in the present study was not described.

At present, G6Pase activity is shown to be in a variety of cell types of various organs, such as hepatocytes (31), pancreatic B cells (18), endometrial epithelial cells (26), jejunal epithelial cells (9), proximal convoluted tubule cells of the kidney (13), myocardial cells, endothelial cells, smooth muscle cells and adventitial fibroblasts of the coronary artery (3), tracheal epithelial cells (15), cells of the epididymis (11) and submandibular acinar cells (34). In all of these cell types as well as all

types of blood cells except for orthochromatic erythroblasts and mature erythrocytes, as revealed in the present results, G6Pase activity is exclusively localized in the endoplasmic reticulum and nuclear envelope.

The role of the enzyme in the liver is to release glucose into the blood (2, 7, 22). The enzyme in the kidney may be related to the reabsorption of glucose (23), and the enzyme in jejunal epithelial cells may be concerned with absorption of nutrients (10). Kanai and co-workers postulated that the enzyme in the ductus epididymidis releases glucose in the epididymal fluid for use by spermatozoa (11). However, the functional role of the relatively low enzyme activity in various cell types of other organs is unknown. Kanamura supposed that the role of the enzyme in the cells having low activity is to regulate the concentration of glucose-6-phosphate in the cells, hydrolysing any excess (15). The physiological role of G6Pase in blood cells may be also to regulate the concentration of glucose-6-phosphate in the cells.

ACKNOWLEDGMENT

The author wishes to thank Professor Shinsuke Kanamura (the First Department of Anatomy, Kansai Medical University) and Professor Kojiro Yasunaga (the First Department of Internal Medicine, Kansai Medical University) for their critical review of the manuscript and helpful comments, and Dr. Mitsuaki Yamauchi (the First Department of Internal Medicine, Kansai Medical University) for his technical advice.

REFERENCES

1. Ashmore, J. and Weber, G.: The role of hepatic glucose-6-phosphatase in the regulation of carbohydrate metabolism. *Vitam. Horm.* 17; 91-132, 1959.
2. Baehner, R. L., Johnston, R. B. Jr.: Monocyte function in children with neutropenia and chronic infections. *Blood* 40; 31-41, 1972.
3. Borgers, M., Schaper, J. and Schaper, W.: Localization of specific phosphatase activities in canine coronary blood vessels and heart muscle. *J. Histochem. Cytochem.* 19; 526-539, 1971.
4. Boti, Z. S., Kobor, J. and Ormos, J.: Activity of glucose-6-phosphatase in regenerating tubular epithelium in rat kidney after necrosis induced with mercuric chloride: A light and electron-microscopical study. *Br. J. Exp. Pathol.* 63; 615-624, 1982.
5. Breton-Gorius, J. and Guichard, J.: Etude au microscope electronique de la localisation des peroxydases dans les cellules de la moelle osseuse humaine. *Nouv. Rev. Fr. Hematol.* 9; 678-687, 1969.
6. Breton-Gorius, J. and Guichard, J.: Ultrastructural localization peroxidase activity in human platelets and megakaryocytes. *Am. J. Pathol.* 66; 277-293, 1972.
7. Cahill, G. F. Jr., Ashmore, J., Renold, A. E. and Hastings, A. B.: Blood glucose and the liver. *Am. J. Med.* 26; 264-282, 1959.
8. Frei, J., Borel, C. L., Horvath, G., Cullity, B. and Vannotti, A.: Enzymatic studies in the different types of normal and leukemic human white cells. *Blood* 18; 317-327, 1961.
9. Hugon, J. S., Borgers, M. and Maestracci, D.: Glucose 6-phosphatase and thiamine pyrophosphatase activities in the jejunal epithelium of the mouse. *J. Histochem. Cytochem.* 18; 361-364, 1970.
10. Hugon, J. S., Maestracci, D. and Menard, D.: Glucose 6-phosphatase activity in the intestinal epithelium of the mouse. *J. Histochem. Cytochem.* 19; 515-525, 1971.

11. Kanai, K., Asada-Kubota, M. and Kanamura, S.: Ultrastructural localization of glucose 6-phosphatase activity in the cells of the epididymis of the mouse. *Experientia* 37; 509-511, 1981.
12. Kanamura, S.: Fine structural demonstration of hepatic glucose 6-phosphatase activity after prefixation of fresh frozen sections in glutaraldehyde. *J. Histochem. Cytochem.* 19; 320-321, 1971.
13. Kanamura, S.: Ultrastructural localization of glucose-6-phosphatase activity in proximal convoluted tubule cells of rat kidney. *Histochemie* 28; 288-295, 1971.
14. Kanamura, S.: Postnatal changes in the localization of glucose 6-phosphatase activity within the liver lobule of the mouse. *Anat. Rec.* 181; 635-640, 1975.
15. Kanamura, S.: Ultrastructural localization of glucose 6-phosphatase activity in tracheal epithelium of the rat. *J. Anat.* 119; 499-504, 1975.
16. Kimura, T., Yamauchi, M. and Yasunaga, K.: A study of ultrastructural cytochemistry in megakaryocyte. Detection of glucose-6-phosphatase activity. *Ketsueki To Myakukan* 14; 367-369, 1983 (in Japanese).
17. Kimura, T., Yamauchi, M. and Yasunaga, K.: Ultrastructural cytochemistry of blood cells. II. The cytochemical detection of glucose-6-phosphatase activity in malignant blood cells. *J. Clin. Electron Microscopy* 16; 653-654, 1983.
18. Lazarus, S. S. and Barden, H.: Specificity and ultrastructural localization of pancreatic B cell glucose-6-phosphatase. *Diabetes* 14; 146-156, 1965.
19. Monneron, A.: Ultrastructural study of membrane-bound enzymes in thymocytes. *J. Histochem. Cytochem.* 22; 1128-1134, 1974.
20. Nichols, B. A. and Setzer, P. Y.: Cytochemical localization of glucose-6-phosphatase in rabbit mononuclear phagocytes during differentiation. *J. Histochem. Cytochem.* 29; 317-320, 1981.
21. Nichols, B. A., Setzer, P. Y. and Bainton, D. F.: Glucose-6-phosphatase as a cytochemical marker of endoplasmic reticulum in human leukocytes and platelets. *J. Histochem. Cytochem.* 32; 165-171, 1984.
22. Nordlie, R. C.: Some properties and possible physiological functions of phosphotransferase activities of microsomal glucose-6-phosphatase. *Ann. NY Acad. Sci.* 166; 699-718, 1969.
23. Nordlie, R. C. and Soodsma, J. F.: Phosphotransferase activities of kidney glucose 6-phosphatase. *J. Biol. Chem.* 241; 1719-1924, 1966.
24. Pachman, L. M.: The carbohydrate metabolism and respiration of isolated small lymphocytes. In vitro studies of normal and phytohemagglutinin stimulated cells. *Blood* 30; 691-706, 1967.
25. Rosen, S. I.: The localization of glucose-6-phosphate hydrolysing enzyme in hepatocytes, red blood cells and leukocytes in the liver of the newborn rat. *J. Anat.* 105; 579-584, 1969.
26. Sawaragi, I. and Wynn, R. M.: Ultrastructural localization of metabolic enzymes during the human endometrial cycle. *Obstet. Gynecol.* 34; 50-61, 1969.
27. Skaer, R. J.: Acetylcholinesterase in human erythroid cells. *J. Cell Sci.* 12; 911-923, 1973.
28. Skoog, W. A. and Beck, W. S.: Studies on the fibrinogen, dextran and phytohemagglutinin methods of isolating leukocytes. *Blood* 11; 436-454, 1956.
29. Spurr, A. R.: A low-viscosity epoxy resin embedding medium for electron microscopy. *J. Ultrastruct. Res.* 26; 31-43, 1969.
30. Stephens, H. R. and Sandborn, E. B.: Cytochemical localization of glucose-6-phosphatase activity in the central nervous system of the rat. *Brain Res.* 113; 127-146, 1976.
31. Tice, L. W. and Barnett, R. J.: The fine structural localization of glucose-6-phosphatase in rat liver. *J. Histochem. Cytochem.* 10; 754-762, 1962.
32. Topilko, A. and Caillou, B.: Acetylcholinesterase in human thymus cells. *Blood* 66; 891-762, 1985.
33. Wachstein, M. and Meisel, E.: On the histochemical demonstration of glucose-6-phosphatase. *J. Histochem. Cytochem.* 4; 592, 1956.
34. Watanabe, J., Kanamura, S., Kanai, K., Yoshikawa, M. and Asada-Kubota, M.: Cytochemical glucose-6-phosphatase activity in the cells of mouse pancreas and submandibular gland. *Histochem. J.* 15; 999-1009, 1983.