Surface Glycoproteins of Human White Blood Cells. Analysis by Surface Labeling

By Leif C. Andersson and Carl G. Gahmberg

We labeled surface glycoproteins of normal human blood platelets, granulocytes, monocytes, T and B lymphocytes, and null cells by the galactose oxidase-NaB\textsubscript{3}H\textsubscript{4} and periodate-NaB\textsubscript{3}H\textsubscript{4} labeling techniques. The labeled glycoproteins were observed by fluorography after separation by polyacrylamide slab gel electrophoresis. All major types of human leukocytes showed different and characteristic surface glycoprotein patterns. These patterns evidently also include common components.

During the past few years a variety of surface markers characteristic for different populations of leukocytes have been recognized. Analysis of the marker profiles has, in addition to conventional morphology and enzyme histochemistry, received a wide application for identification and classification of normal and malignant blood leukocytes. Comparatively little, however, is known at the molecular level about the surface structures of different types of white blood cells (WBC).

Most, if not all, external proteins of mammalian cells are glycoproteins.\textsuperscript{1} These comprise both various receptors and antigenic determinants.\textsuperscript{2} The establishment of the surface glycoprotein patterns for the different types of human leukocytes would provide the basis for understanding the structural/functional relationships of the surface molecules. Comparison of the surface glycoprotein patterns of the normal leukocyte populations with those of cells in various disorders might give valuable information about the molecular changes that occur in different dysfunctional states and malignancies.

We have been studying the surface glycoproteins of different mouse and human lymphoid cells by the use of the galactose oxidase-tritiated borohydride (GO-NaB\textsubscript{3}H\textsubscript{4}) labeling method.\textsuperscript{3,4} The galactosyl residues of the exposed glycoproteins are oxidized by GO and thereafter reduced with NaB\textsubscript{3}H\textsubscript{4}. The radioactive proteins are separated on polyacrylamide slab gels and observed by autoradiography.\textsuperscript{5}

Another method of selective surface glycoprotein labeling was also recently described. Sialic acid residues of surface glycoproteins were labeled with NaB\textsubscript{3}H\textsubscript{4} after oxidation with sodium periodate under conditions where the periodate did not penetrate the intact cell membrane.\textsuperscript{6}

By employing various methods of cell fractionation we purified the main
populations of human WBC. We report the surface glycoprotein patterns of different types of human blood leukocytes.

MATERIALS AND METHODS

Chemicals and enzymes. Sodium metaperiodate was obtained from Merck, Darmstadt, Germany, acrylamide and N, N’-methylenebisacrylamide from Eastman Kodak, Rochester, N.Y., 2,5-diphenyl oxazole (PPO) and p-bis-2(5-phenyloxazolyl)-benzene (POPOP) from New England Nuclear, Boston, Mass., phenylmethylsulfonylfluoride from Sigma, St. Louis, Mo., Triton X-100 from British Drug Houses, Poole, England, and NaB\textsubscript{3}H\textsubscript{4} (8.6 Ci/mmole) and \textsuperscript{14}C-formaldehyde (4.54 mCi/mmole) from the Radiochemical Centre, Amersham, England. The NaB\textsubscript{3}H\textsubscript{4} preparation was handled as described previously.\textsuperscript{7}

Galactose oxidase with a specific activity of 130 U/mg protein was purchased from Kabi, Stockholm, Sweden. It displayed no protease or neuraminidase activities when measured as described.\textsuperscript{3} Vibrio cholerae neuraminidase (NE) (500 U/ml) was obtained from Behringwerke, Marburg-Lahn, Germany. It was free of protease activity.

Isolation of blood cells. The following main populations of human blood leukocytes were isolated: granulocytes, platelets, monocytes, T and B lymphocytes, and null cells. These cell populations were purified from the buffy coats of blood units of 400 ml each obtained from the Finnish Red Cross Blood Transfusion Service. Theuffy coats were diluted with 2 vol 0.1 M NaCl-0.01 M sodium phosphate pH 7.4 (PBS), and the platelets and the mononuclear cells were separated from the granulocytes and erythrocytes by one-step Ficoll-Isopaque (density 1.077; Pharmacia, Uppsala, Sweden) gradient centrifugation at 400 g for 40 min at 22°C.

Purification of granulocytes. The pellets obtained after Ficoll-Isopaque centrifugation that contained erythrocytes and granulocytes were suspended in 20 ml PBS. Then 10 ml saline containing 6%, dextran was added (Macrodes 6\%, Leiras, Turku, Finland) and the cell suspension kept for 40 min at 37°C. The granulocyte-richuffy coat was then collected, and contaminating erythrocytes were lysed by incubation with a Tris-buffered 0.84%, aqueous solution of ammonium chloride. The leukocytes were then washed three times with PBS. This procedure yielded a cell population that contained more than 97%, of granulocytes as judged from May-Grunwald-Giemsa (MOO) stained cytocentrifuged cell smears. The cell viability was close to 100%, as seen in the trypan blue exclusion test.

Purification of platelets. The platelets were purified from the cell population obtained from the interphase after Ficoll-Isopaque centrifugation. This cell population was suspended in PBS and centrifuged for 10 min at 200 g, and the platelet-rich supernatant was recovered. This procedure was repeated once, and the platelets were then centrifuged at 400 g for 20 min. The purity of the platelet preparation thus obtained approached 100%, and the contamination by other blood cells was always less than 0.002%.

Purification of monocytes. The blood monocytes were separated from the cell population recovered at the interphase after Ficoll-Isopaque centrifugation. The platelets were depleted by three washes with PBS at 200 g for 10 min. The mononuclear cell population was mixed at the ratio of 1:50 with 2-aminoethylisothiouronium bromide (AET) (Sigma) treated sheep erythrocytes (SRBC).\textsuperscript{5} After incubation for 15 min at 37°C the mixture was centrifuged for 10 min at 200 g and the T cells allowed to rosette with the AET-SRBC for 1 hr on ice. The pellet was then gently suspended in cold PBS containing 30%, fetal calf serum (FCS) and the rosette-forming cells were separated from the non-rosette-forming cells by a one-step Ficoll-Isopaque density gradient centrifugation. The cell population recovered from the interphase mainly contained monocytes and non-T lymphocytes. The monocytes were then purified from the contaminating lymphocytes by a 1-g velocity sedimentation.\textsuperscript{9} In this procedure cells are fractionated according to the cell size. The cells were suspended in PBS containing 5% FCS and layered onto a linear gradient of 15%,–30%, FCS in PBS. After sedimentation for 4 hr at 4°C the gradient was drained into fractions of 20 ml and the cell content of each fraction was analyzed from MGG-stained smears. The early fractions, which mainly contained monocytes, were pooled. The cell preparation thus obtained contained 90%,–95%, of monocytes as judged by conventional morphologic criteria. The viability always exceeded 98%. Slight contamination was caused by occasional lymphoblasts, myeloid precursor cells, and some granulocytes.
Purification of T lymphocytes. The platelet-depleted mononuclear cell population recovered from the Ficoll-Isopaque gradient centrifugation was passed over a human Ig-rabbit anti-human Ig column as described by Wigzell et al.10 More than 95% of the column-passed lymphocyte population formed rosettes with AET-SRBC and contained less than 1% of surface immunoglobulin (SIg)-bearing cells as judged by staining with fluorescein isothiocynate-conjugated polyvalent sheep anti-human Ig (obtained from Professor Astrid Fagraeus, Stockholm, Sweden). The viability of the T cell population was always more than 98%.

Purification of non-T lymphocytes, null cells, and B lymphocytes. The mononuclear cells obtained after Ficoll-Isopaque centrifugation were suspended in RPMI-1640 culture medium supplemented with 10% normal human AB plasma. Carbonyl iron was added and the suspension incubated for 1 hr at 37°C. Most of the phagocytic cells were then removed with a magnet. The phagocyte-depleted cell population was allowed to rosette with AET-SRBC as described above. The T cells were depleted by centrifugation of the RFC-containing cell suspension on a Ficoll-Isopaque gradient. The cell suspension recovered from the interphase contained mainly lymphocytes and larger, monocyterlike cells. The non-T lymphocytes were further purified from this population by velocity sedimentation as described above. The later fractions obtained after sedimentation for 4 hr contained mainly small lymphocytes as judged from MOO smears. These fractions were pooled, and the cell population thus obtained was contaminated by less than 3%, nonlymphocytic cells. This cell population was further characterized with regard to surface markers: 2% formed rosettes with AET-SRBC and 61% were SIg positive.

This lymphocyte suspension was further fractionated by passage over an Ig/anti-Ig column at 4°C to minimize Fc binding. The passed cells, of which more than 95%, had the morphology of small lymphocytes, did not bind AET-SRBC (<3%), and lacked Slg (<1%). These were designated null cells. The lymphocytes retained by the column were mechanically eluted by shaking the glass beads in PBS; 86% of the eluted lymphocytes stained positively for Slg and less than 2%, bound AET-SRBC. This population was designated blood B lymphocytes.

Labeling of Cell Surface Glycoproteins with ^3H

Pretreatment with NE and/or GO. About 50 x 10^6 purified leukocytes, 150 μl packed platelets, or 0.5 ml packed erythrocytes were washed in PBS and divided into three equal lots. The cells were suspended in 1 ml Dulbecco's PBS (containing Ca^{2+} and Mg^{2+}), and either (1) 25 units NE plus 5 units GO or (2) GO only were added to the tubes. The third tube served as control, receiving no enzyme treatment. The tubes were incubated at 37°C for 30 min with gentle shaking. The cells were then washed twice with PBS and suspended in 0.5 ml PBS.

Pretreatment with periodate. 20-40 x 10^6 washed lymphocytes, 50 μl packed platelets, or 0.5 ml packed erythrocytes were suspended in 1 ml ice-cold PBS, and 10 μl of 0.1 M sodium metaperiodate was added to give a final concentration of 1 mM (pH 7.4). After incubation on ice for 10 min in the dark the cells were washed twice with PBS and suspended in 0.5 ml PBS.

After treatment with either enzymes or periodate, 0.5 mCi of NaB^3H_4 was added to each tube. The cells were kept for 30 min at room temperature and then washed three times with cold PBS. Then 0.2 ml PBS containing 1%, Triton X-100 and 2 mM phenylmethylsulfonylfluoride (as protease inhibitor) was added on ice. After incubation for 5 min the tubes were centrifuged at 3000 rpm for 10 min and the supernatants recovered.

Polyacrylamide gel electrophoresis (PAGE) in the presence of sodium dodecyl sulfate (SDS) was performed according to the method of Laemmli11 as described previously7 using an acrylamide concentration of 8%. The treatment of the slab gels for fluorography has also been described.5 Cylindrical gels were sliced and the radioactivities counted as described previously.3 The apparent molecular weights of the labeled proteins were calculated according to the method of Weber and Osborn.13 The ^3H-labeled standard proteins were described previously.

Study of possible proteolytic degradation of surface glycoproteins during the labeling procedure. Erythrocytes (0.5 ml packed cells) labeled after NE + GO or periodate treatment were divided into two equal aliquots. To one of these were added 100 x 10^6 purified granulocytes, whereas the other remained without added cells. The mixtures were incubated at 37°C for 30 min and the erythrocyte membranes isolated.3 Aliquots were then run on cylindrical gels to get a more quantitative estimation of possible proteolytic degradation.
RESULTS

Surface glycoprotein patterns of platelets. When platelets were labeled with NaB\textsubscript{3}H\textsubscript{4} after treatment with NE + GO the following major glycoproteins were seen after slab gel electrophoresis: GP210, GP155, GP130, GP120 (the major band), GP105, GP97, GP80, GP68, GP54, GP42 (Fig. 1 A, Table 1). Selective labeling of surface sialic acid residues after periodate treatment using the same amount of platelets and the same amount of radioactivity yielded the following major labeled surface sialoglycoproteins: GP225, GP205, GP155, GP130, GP110 (major band), GP105, GP97, GP85, GP80, GP54, GP42 (Fig. 1 B, Table 1). The galactosyl-N-acetyl and galactosyl aminyl residues of the surface glycoproteins of the platelets apparently were heavily coated by sialic acids, since no labeling could be introduced after treatment with GO only (Fig. 2 A). The abundance of sialic acid residues on the surface proteins of platelets is further indicated by the clear reduction of the electrophoretic mobility of most of the major proteins after NE treatment (cf. Fig. 1 A and B).

Surface glycoprotein pattern of granulocytes. The following major surface glycoproteins were labeled in granulocytes after treatment with NE and GO: GP245, GP230, GP165, GP155, GP130 (major band), GP105, GP97, GP85, GP80, GP62, GP50, GP42 (Fig. 1 C). When the surface sialic acid residues were labeled after periodate treatment a fluorography pattern rather similar to that seen after NE + GO treatment was obtained. The main differences were seen in the area of GP155–GP105; GP130 was not seen after periodate treatment, while GP105 was relatively more strongly labeled than after NE + GO treatment (Fig. 1 D).

In contrast to the situation in platelets, the galactosyl and galactosylaminyl...
residues of the granulocyte surface glycoproteins were relatively available to the GO directly without NE treatment. Most of the major surface proteins were labeled after treatment with GO only (Fig. 2 C). This finding and the similarities between the patterns obtained after NE + GO treatment and periodate treatment indicated that most of the surface glycoproteins with the exception of GP130 (NE + GO)/GP105 (periodate) of granulocytes are relatively poorly substituted with sialic acid residues.

**Surface glycoprotein patterns of monocytes.** The following major surface glycoproteins were labeled in monocytes after treatment with NE + GO: GP210, GP205, GP200, GP170, GP165, GP155, GP130 (major band), GP95, GP85, GP80, GP42 (Fig. 1 E). Labeling of the sialic acid residues after periodate treatment of the same amount of isolated monocytes yielded a pattern similar to that seen after NE + GO treatment. The main difference in the patterns were recorded in the areas of GP165-GP110; GP110 was strongly labeled after periodate treatment, while apparently the same protein showed the mobility of GP130 when labeled after NE + GO treatment (Fig. 1 E and F, Table 1).

Several glycoproteins of monocytes were labeled after treatment with GO only (Fig. 2 E). This shows that these proteins expose galactosyl/galactosaminyl residues available for the GO. The labeling intensity, however, is relatively weaker than that seen in granulocytes after treatment with GO only.
Fig. 2. Fluorography patterns of surface glycoproteins labeled after treatment with galactose oxidase only or without enzyme treatment and separated by polyacrylamide slab gel electrophoresis. (A) Platelets + GO; (B) platelets, no enzyme treatment; (C) granulocytes + GO; (D) granulocytes, no enzyme treatment; (E) monocytes + GO; (F) monocytes, no enzyme treatment; (G) T lymphocytes + GO; (H) T lymphocytes, no enzyme treatment; (I) 14C-labeled standard proteins as in Fig. 1. The same amount of cells and serum borotritiate was used as for the cells in Fig. 1 and the slab exposed for the same time as that of Fig. 1.

Surface glycoprotein patterns of non-T lymphocytes. The following major glycoproteins were labeled on non-T lymphocytes after treatment with NE + GO: GP210, GP205, GP180, GP170, GP155, GP130, GP120, GP95, GP85, GP50, GP42, GP31, GP24 (Fig. 1 G). Labeling after treatment with periodate yielded a rather similar pattern. The major differences were GPIO5 and GP87, which were strongly labeled in the periodate-treated cells while the apparently corresponding proteins showed the mobility of GP130 and 120 in the pattern obtained after NE + GO treatment (Fig. 1 H).

Surface glycoproteins of T lymphocytes. GP200, GP180, GP165, GP160, GP130, GP120, GP97, GP95, GP85, GP50, GP42 were the major labeled glycoproteins in T cells labeled after treatment with NE + GO (Fig. 1 I, Fig. 3 A). Labeling after treatment with periodate gave a pattern similar to that seen after NE + GO treatment (Fig. 1 J). There was again the typical change in the mobilities of two major bands. After NE + GO treatment they were GP130 and GP120 but were GP105 and GP97 after periodate treatment. GP31 was labeled after periodate treatment but was not seen after NE + GO treatment.
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Fig. 3. Fluorography patterns of surface glycoproteins of T lymphocytes, B lymphocytes, and null cells labeled after treatment with neuraminidase and galactose oxidase and separated by polyacrylamide slab gel electrophoresis. (A) T lymphocytes; (B) B lymphocytes; (C) null cells; (D) 14C-labeled standard proteins as in Fig. 1.

Surface glycoproteins of blood B lymphocytes. Figure 3 B shows the surface glycoprotein pattern of normal blood B lymphocytes labeled after NE + GO treatment. The dominating bands are GP210, GP120, and GP110. SIg, which should migrate in the 50,000-dalton region, was not seen. GP31 and GP24 were seen only after prolonged exposure.

Surface glycoproteins of blood null lymphocytes. The most strongly labeled band in null cells after NE + GO treatment was GP210. GP120 was more weakly labeled than in T or B lymphocytes (Fig. 2 C).

Absence of detectable proteolytic degradation of surface glycoproteins during the labeling procedures. When intact labeled erythrocytes were incubated with granulocytes no changes in the labeled surface glycoprotein patterns were observed (Fig. 4).

DISCUSSION

The surface glycoprotein patterns of the main populations of human blood leukocytes were analyzed by using two different methods of radiolabeling with tritium. The use of 3H as a label gave an excellent resolution on the autoradiographs.

The GO-NaB3H4 method has received wide application in the study of surface molecules of different cells.347 This labeling method is specific for the cell surface, since GO has a molecular weight of 75,000 daltons and apparently does not penetrate the plasma membrane of viable cells.3 When combined with NE pretreatment, surface proteins with galactose residues covered by sialic acid may tentatively be identified. However, removal of sialic acid may cause artifactual changes in the physical or chemical properties of the sialoglycoproteins. This might affect their organization at the cell surface and is sometimes reflected by marked reductions in their electrophoretic mobilities.
Periodate in low concentrations specifically oxidizes sialic acids and after reduction with NaB\textsubscript{3}H\textsubscript{4} tritium-labeled 5-acetamido-3,5-dideoxy-L-arabino-2-heptulosonic acid is the main product formed\textsuperscript{14,15}. We recently showed this radiolabeling method to be specific for surface sialic acids under the conditions used in this study\textsuperscript{6}. When the oxidation is carried out at 0°C with low concentrations of periodate and short reaction times the cell membrane is "frozen" and the transport of anions is low or absent.

By using these two methods of selective surface labeling we analyzed the exposed glycoproteins of the main populations of human blood leukocytes by running them on parallel slots on the same SDS-polyacrylamide slab gel. This enabled us to accurately compare the glycoprotein patterns of different cell populations and also gave information about the presence of glycoproteins of similar apparent molecular weights. Because of the anomalous electrophoretic behavior of glycoproteins on different gel systems, it should be emphasized that the figures given for the molecular weights represent only apparent weights.

In comparisons of the glycoprotein patterns obtained by the two labeling methods, all cells contained a major labeled glycoprotein with an apparent molecular weight of 100,000–130,000 daltons. Although it obviously was different in the various cells, it showed some common characteristics: it was a major labeled component, and it was obviously rich in sialic acids because it was weakly labeled with GO alone and it shifted electrophoretic mobility after...
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After treatment of platelets with NE + GO, nine major labeled glycoproteins were identified. The general glycoprotein pattern seen was very similar to that recently published by Phillips and Agin\textsuperscript{16} using the GO labeling method, although the apparent molecular weights of different components are higher throughout in their report. Labeling of the sialic acid residues after periodate treatment of platelets yielded a somewhat different electrophoretic profile. A “new” band, GP225, was seen by this technique. This finding indicates that GP225, which apparently is rich in sialic acid, either has very few galactosyl residues or has them “deeply” located and not available to the GO. The GP210 of the NE + GO profile apparently corresponds to the GP205 of the periodate profile and the GP120 to the GP110, which shows that the removal of sialic acids increases the apparent molecular weights. GP120 probably corresponds to glycoproteins,\textsuperscript{17} and the GP68 seen after NE + GO treatment was recently showed to be the substrate for thrombin.\textsuperscript{16}

The most strongly labeled band of granulocytes had an apparent molecular weight of 130,000 daltons (GP130). This protein is apparently rich in sialic acid residues, since its mobility was considerably higher after labeling with periodate, now recorded as GP105. Except for this protein, the general glycoprotein patterns obtained after NE + GO labeling and periodate labeling were strikingly similar. The granulocytes did not contain glycoproteins in the 160,000–200,000-dalton region. Such proteins are characteristic for lymphocytes but are also found in monocytes. The absence of glycoproteins in this region was apparently not due to proteolytic degradation, since granulocytes do not cleave labeled sialoglycoproteins of erythrocytes, which are known to be very protease sensitive (Fig. 4). In contrast to the surface proteins of platelets, the galactosyl residues on the membrane proteins of granulocytes are only partially covered by sialic acid since most of the surface proteins are labeled after treatment with GO alone.

The general surface glycoprotein patterns of monocytes showed similarities to those of granulocytes but also to those of lymphocytes. After treatment with NE + GO the most strongly labeled protein was GP130. When the protein was labeled after periodate treatment it was recorded as GP110. Most of the surface molecules seen after NE + GO treatment were also labeled after periodate treatment. The surface glycoproteins of monocytes apparently carry some galactosyl residues not covered by sialic acid, since several of the major components were labeled after treatment with GO alone.

We earlier reported that according to the GO-NaB\textsubscript{3}H\textsubscript{4} labeling method human blood T and B lymphocytes have different surface glycoprotein patterns.\textsuperscript{18} Null cells resemble both T and B lymphocytes. They contain the GP210, which probably corresponds to the high molecular weight proteins of B cells. In contrast to both T and B lymphocytes, the GP120 is weak. As in granulocytes and monocytes, the major components in both T and B lymphocytes, GP120 apparently shows a higher electrophoretic mobility in the pattern obtained of surface proteins labeled after periodate treatment.

At present very few surface glycoproteins have been identified and characterized more extensively. The GP31 and GP24 of B cells are the human Ia
antigens, and GP42 is the heavy chain of the HLA antigens. Obviously, many of the other surface proteins are important for the physiologic functions of the white blood cells. Knowledge of the membrane glycoprotein patterns on normal blood leukocytes is fundamental for the discovery of possible molecular defects associated with various dysfunctional states. Moreover, the normal patterns also provide a basis for the search for surface molecules specifically associated with malignant (leukemic) leukocytes.

ACKNOWLEDGMENT

We thank Anneli Asikainen and Liisa Räisänen for technical assistance and Leena Saraste for secretarial work.

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LC Andersson and CG Gahmberg