CHARACTERIZATION OF SURFACE GLYCOPROTEINS OF MOUSE LYMPHOID CELLS

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ABSTRACT

We have labeled exposed surface glycoproteins of mouse lymphoid cells by the galactose oxidase-tritated sodium borohydride technique. The labeled glycoproteins were separated by polyacrylamide slab gel electrophoresis and visualized by autoradiography (fluorography). The major thymocyte surface proteins have molecular weights of 170,000 and 125,000. Thymocytes from TL antigen-positive mouse strains showed an additional band with a molecular weight of 27,000. Highly purified T lymphocytes contain two major surface glycoproteins with molecular weights of 180,000 and 125,000. Purified B lymphocytes have one major surface glycoprotein with a molecular weight of 210,000.

When T lymphocytes are stimulated in vitro by concanavalin A or phytohemagglutinin, the major proteins characteristic of T cells are relatively weakly labeled, but new components of lower molecular weights appear on the cell surface. A similar change is seen in B lymphocytes stimulated by *Escherichia coli* lipopolysaccharide. T lymphoblasts isolated from mixed lymphocyte cultures show a slightly different surface glycoprotein pattern.

A polypeptide with a molecular weight of 57,000, which was labeled without enzymatic treatment by tritiated sodium borohydride alone, is strongly labeled in proliferating cells.

The immunobiological activities of the thymus-dependent (T)[1] and bone marrowed-derived (B) lymphocytes have been extensively studied under different in vivo and in vitro conditions. A variety of antigenic determinants and surface structures with different receptor activities have been identified. Very little, however, is known about the functional correlations between the composition of the plasma membrane and the immunological activities expressed by the lymphocyte.

The majority of the exposed plasma membrane proteins are glycoproteins (10, 11, 36). There is increasing evidence that these glycoproteins are of major importance in the maturation and regulation of the functions displayed by the lymphoid cells, since (*a*) carbohydrate-binding proteins, lectins, selectively stimulate different cells to divide (1), (*b*) mild periodate treatment, known to specifically oxidize sialic acids, or treatment with neuraminidase plus galactose oxidase, exclusively triggers T lymphocytes (29, 38), and (*c*) at least

[1] *Abbreviations used in this paper:* B blast, B lymphocyte stimulated to divide; B lymphocyte, B cell, thymus-independent, bone marrow-derived lymphocyte; Con A, concanavalin A; LPS, *E. coli* lipopolysaccharide; MLC, mixed lymphocyte culture; PBS, phosphate-buffered saline, pH 7.4; PHA, phytohemagglutinin; PPO, 2,5-diphenyloxazole; T blast, T lymphocyte stimulated to divide; T lymphocyte, T cell, thymus-dependent lymphocyte.

some of the cell-surface antigens are molecules that contain carbohydrate (33).

In order to understand the molecular basis of the lymphocyte response, it is obviously necessary to characterize the structures involved and their molecular anatomy in the plasma membrane.

One approach is selectively to label surface-exposed glycoproteins by the galactose oxidase technique (12, 37). Galactose oxidase does not penetrate the cell membrane and it oxidizes terminal galactosyl and *N*-acetyl galactosaminyl residues to the corresponding C6 aldehydes. These aldehydes are then reduced by tritiated sodium borohydride. The labeled surface glycoproteins were separated on polyacrylamide slab gels in the presence of sodium dodecyl sulfate, and the radioactive bands were visualized by modified autoradiography. By using this very sensitive technique we here show that different populations of mouse lymphoid cells have specific and characteristic surface glycoprotein patterns.

MATERIALS AND METHODS

Chemicals and Enzymes

Galactose oxidase with a specific activity of 130 U/mg protein was purchased from Kabi AB, Stockholm, Sweden. It displayed no protease or neuraminidase activity when measured as described previously (12). Neuraminidase (*Vibrio cholerae*, 500 U/ml) was obtained from Behringwerke AG, Marburg-Lahn, Germany, and was found to be free of protease activity. Concanavalin A (Con A) was kindly donated by Professor H. Wigzell, University of Uppsala, Sweden and used at the optimal concentration of 10 μg/ml. Phytohemagglutinin (PHA) was obtained from Difco Pharmaceuticals, Kalamazoo, Mich., and used at a final dilution of 1:150 of the reconstituted stock. *Escherichia coli* lipopolysaccharide (LPS) kindly given by Professor G. Möller, Stockholm, Sweden, was used at the optimal concentration of 10 μg/ml.

Tritiated sodium borohydride (8.2 Ci/mmol) was obtained from the Radiochemical Centre Ltd., Amersham, England. 250 mCi were dissolved in 0.5 ml of 0.01 N NaOH, divided among five tubes and immediately frozen at −70°C. The contents of one tube at a time were diluted with 2 ml of 0.01 NaOH and divided into 20 equal portions which were immediately frozen. Usually, one of these tubes was used for the labeling experiments. When handled in this way the isotope remained active for at least 12 mo.

Acrylamide and *N,N'*-methylenebisacrylamide were obtained from the Eastman Kodak Company, Rochester, N.Y. 2,5-diphenyloxazole (PPO) and *p*-bis/2-(5-phenyloxazolyl)/benzene (POPOP) were obtained from New England Nuclear, Boston, Mass. All other chemicals were of highest possible purity.

Mice

3–5-mo old mice of the following inbred strains were used: BALB/c, A/J, and CBA-H/T6T6. The strains originated from the Jackson Laboratories, Bar Harbor, Me., and were carried in our colony.

Preparation of Cells

Thymuses, lymph nodes (axillary, inguinal, popliteal, and mesenteric), and spleens were teased apart in cold RPMI 1640 culture medium. Clumps were removed by filtration through a loose cotton wool plug. Erythrocytes were lysed with 0.83% aqueous solution of NH_4Cl, and phagocytic cells were removed after incubation with carbonyl iron by treatment with a magnet (16). The thymus preparations contained more than 95% thymocytes. The lymph node and spleen cell preparations contained more than 98% and 80% lymphocytes, respectively, as judged morphologically from May-Gruenwald-stained smears. These cells will be referred to in the text as thymocytes, and lymph node and spleen "lymphocytes."

Cell Fractionation Procedures

PREPARATION OF T LYMPHOCYTES: Spleen cells or lymph node lymphocytes were prepurified by iron powder plus magnetic treatment (16) and passed through a Fenwall-Leukopak (Fenwall Laboratories, Division of Travenol Laboratories, Amsterdam, The Netherlands) nylon wool column as described by Julius et al. (21). The passed cell suspension contained less than 3% cells carrying surface immunoglobulin (B cells) as demonstrated by staining with polyvalent fluorescein isothiocyanate-conjugated goat anti-mouse immunoglobulin (a gift from Professor A. Fagraeus, State Bacteriological Laboratory, Stockholm, Sweden).

FRACTIONATION OF T AND B CELLS BY PREPARATIVE CELL ELECTROPHORESIS: Spleen lymphocytes of nonimmunized mice purified by iron powder plus magnet treatment were fractionated by preparative free-flow cell electrophoresis under conditions already described (3). In brief, the cells were transferred into a low ionic strength electrophoresis buffer (0.04 M potassium acetate, 0.015 M triethanolamine, 0.24 M glycine, pH 7.35, made isotonic with 0.011 M glucose and 0.03 M sucrose (3), and fractionated in the free-flow cell electrophoresis apparatus (Type FF4, Desaga GmbH, Heidelberg, Germany) at 6°C, and collected on ice. Each cell remained in the electric field (100 V/cm) for about 300 s. The cells were collected in tubes containing 10% calf serum in Eagle's minimal essential medium (Orion Pharmaceuticals, Helsinki, Finland) to minimize cell damage. After fractionation, more than 90% of the cells were viable as judged by trypan blue exclusion tests. By employing proper cuts in the electrophoresis profile

(Fig. 1) the T cell fraction was contaminated by less than 2% of surface immunoglobulin-carrying cells (B cells), and the B cell fraction by less than 2% of theta antigen-carrying (T) cells (3).

PURIFICATION OF BLASTS FROM CELL CULTURES: The blast cells were purified from the cultures by 1 *g* velocity sedimentation on a linear gradient of 15–30% fetal calf serum, phosphate-buffered saline (PBS) as described (26). The large-size blasts which sedimented rapidly were recovered from the first fractions. The cells were washed three times in PBS to remove the fetal calf serum. These fractions, containing more than 95% blast cells as judged morphologically from May-Gruenwald-Giemsa-stained smears, were used for surface labeling of activated cells.

Cell Cultures

Cultures with 20–30 × 10^6 cells were set up in 50-ml round-bottom glass tubes in 20 ml of Eagle's minimal essential medium supplemented with 5% fetal calf serum. The cultures were incubated at 37°C in a humidified atmosphere of 10% CO_2 in air. Spleen lymphocytes that had passed a nylon wool column were used as responder cells in the T cell mitogen (Con A and PHA)-stimulated cultures, whereas the B cell mitogen (LPS)-stimulated cultures were set up with unfractionated spleen lymphocytes. For the one-way mixed lymphocyte cultures (MLC), 60 × 10^6 CBA-H/T6T6 spleen T lymphocytes that had passed through a nylon wool column were stimulated with 90 × 10^6 mitomycin C-treated DBA/2 spleen cells (16). The mitogen-stimulated cultures were harvested on the 3rd day of culture and the MLC on the 6th day, at the time of the peaks of the corresponding blast responses. More than 90% of the cells in the PHA and Con A cultures and 50% in MLC were morphologically blasts. Pure (>95%) blast cell populations were recovered from the respective cultures by 1 *g* velocity sedimentation (2, 26).

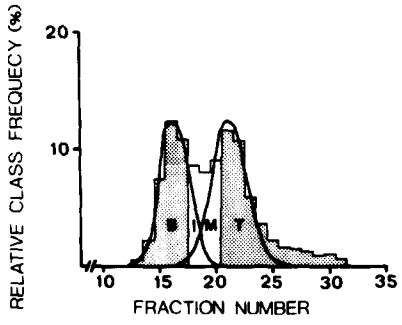

FIGURE 1 Electrophoretic distribution profile of CBA/H-T6T6 spleen lymphocytes. Higher fraction number indicates higher anodal mobility. The Gaussian distributions of the T and B cell populations are shown. Cells from the shaded fractions were used for the experiments.

Labeling Procedure

The labeling procedure was slightly modified from that previously described (12). To be able to do accurate comparisons between different cells, the labeling conditions were strictly standardized. We used 5 U of galactose oxidase with or without 12.5 U of neuraminidase for 1 h at 37°C in 1 ml of Dulbecco's PBS. 20 × 10^6 cells per tube were labeled. In control experiments the enzymes were omitted. After incubation with the enzymes the cells were washed three times by centrifugation at 4,000 rpm for 5 min in a table centrifuge in PBS, and 0.5 ml PBS finally added. Thereafter, 0.5 mCi NaB^3H_4 was added and the cells were left at room temperature for 30 min. Then the cells were washed four times in PBS, suspended in 200 μl of PBS, and an aliquot was counted for radioactivity in a dioxane-based scintillation fluid (8), with Wallac liquid scintillation counter 81000. The efficiency for tritium was 37%. The final yield of cells was >90%, and >90% were alive as shown by the trypan blue dye exclusion test.

To label mouse serum proteins, 100 μl mouse serum were diluted with 0.4 ml Dulbecco's PBS and either 12.5 U of neuraminidase plus 5 U of galactose oxidase, 5 U of galactose oxidase only, or no enzyme were added. The preparations were incubated at 37°C for 1 h, sodium dodecyl sulfate was added to a final concentration of 1%, and the samples were boiled for 1 min. 0.5 mCi NaB^3H_4 was added to each tube, and after incubation at room temperature for 30 min the samples were passed through 1 x 30-cm columns of Sephadex G25 in PBS. The fractions containing serum proteins were pooled, and samples corresponding to 10 μl serum were subjected to electrophoresis.

Polyacrylamide Slab Gel Electrophoresis

Electrophoresis was performed according to Laemmli (23) in 8% acrylamide gels. 5 × 10^6 cells (10^4–10^5 cpm) were solubilized and electrophoresed with [^{14}C]formaldehyde-labeled marker proteins in the peripheral slots (31). The molecular weights of the marker polypeptides were thyroglobulin 210,000, transferrin 78,000, human albumin 68,000, ovalbumin 44,000, and hemoglobin 17,000. The gels were fixed overnight in 20% sulfosalicylic acid, stained with Coomassie brilliant blue, and destained (43). They were then treated with dimethyl sulfoxide-PPO according to Bonner and Laskey (6) and vacuum dried. The dried gels were covered with Kodak RP X-Omat film, wrapped in aluminum foil, and kept at −70°C in a Revco freezer for 1–10 days until developed. When different gels were compared, they were kept for identical times in the deep freeze. The apparent molecular weights of the polypeptides were determined according to Weber and Osborn (43).

Periodate Oxidation

Cells were suspended in PBS and sodium metaperiodate was added to a final concentration of 2 mM. After

TABLE I

Label in Nontreated, Galactose Oxidase-Treated, Neuraminidase Plus Galactose Oxidase-Treated, and Periodate-Treated Mouse Lymphoid Cells

	Neuraminidase + galactose oxidase	Galactose oxidase	No enzymes	Periodate
		cpm/10⁶ cells		
Thymocytes	4,076 (3.46)*	2,320 (1.97)	1,178 (1.00)	—
Lymph node cells	11,214 (9.52)	5,289 (4.49)	2,803 (2.38)	—
T cells	6,778 (5.72)	4,370 (3.71)	3,333 (2.83)	6,255 (5.31)‡
B cells	6,502 (5.52)	3,604 (3.06)	2,391 (2.03).	6,361 (5.40)‡
T blasts				
(Con A)	47,332 (40.18)	25,515 (21.66)	17,811 (15.12)	—
(PHA)	30,698 (26.06)§	14,913 (12.66)§	12,168 (10.33)§	—
B blasts				
(LPS)	41,489 (35.22)	21,427 (18.19)	17,128 (14.54)	—
MLC blasts	29,768 (25.27)§	13,982 (11.87)	9,341 (7.93)	—

Average of two to four determinations.

* Relative amount of label in parentheses.

‡ Specific counts over background by NaB^3H_4 alone.

§ One determination only.

incubation for 10 min in the dark, the cells were washed three times in PBS, reduced with NaB^3H_4 as for the galactose oxidase-treated cells, and washed four times in PBS (see references 24 and 37). Spleen cells were activated to divide by the addition of 2 mM sodium metaperiodate to the medium (29).

RESULTS

Incorporation of Label

The incorporation of ^3H from NaB^3H_4 depends on galactose oxidase and is enhanced by neuraminidase treatment (Table I). Though the total label is appreciable without enzyme treatment, the gels show that very little label actually is incorporated into protein (see below). The label per cell in thymocytes is lower than that of T or B lymphocytes, whereas that of T or B blasts is considerably higher. Without enzyme treatment, some label is incorporated and is distributed among both proteins and lipids. Periodate treatment gives a higher labeling than does neuraminidase plus galactose oxidase, but the total amount of label in T or B lymphocytes is of equal magnitude.[2]

Fluorography Patterns of Labeled Glycoproteins

All experiments have been performed two to five times with very reproducible results.

[2] The molecular weights of the major labeled proteins are listed in Table II.

THYMOCYTES: In these cells we can distinguish two major surface glycoproteins with apparent molecular weights of 170,000 and 125,000 (Figs. 2 A and 3 A and B). The protein with a molecular weight of 125,000 (GP9) is not labeled without treatment with neuraminidase. GP6 can be labeled by galactose oxidase only, though more weakly (Figs. 2 B and 4 A and B). In the thymocytes derived from mouse strains A and CBA-H/T6T6 which have the TL antigen (7) expressed on their surface, an additional glycoprotein, GP15, was found (Fig. 2 D). This band was lacking from the thymocyte glycoprotein pattern of the TL-negative strain BALB/c (Fig. 2 A).

T LYMPHOCYTES: Unstimulated T lymphocytes contain two major surface glycoproteins, GP5 and GP9, and some quantitatively less-labeled glycoproteins. Both of the major components are labeled only after treatment with both neuraminidase and galactose oxidase (Fig. 3 C). Very little label is found in the glycoproteins if oxidized by galactose oxidase alone (Fig. 4 C).

B LYMPHOCYTES: Unstimulated B lymphocytes contain one major labeled surface glycoprotein, GP1 (Fig. 3 D). It is weakly labeled by galactose oxidase alone (Fig. 4 D).

MITOGEN-INDUCED T BLASTS: T blasts obtained after stimulation with Con A or PHA show an altered surface glycoprotein pattern when compared to nonstimulated T cells. The T cell-characteristic glycoproteins GP5 and GP9 are relatively less prominent, and glycoproteins with

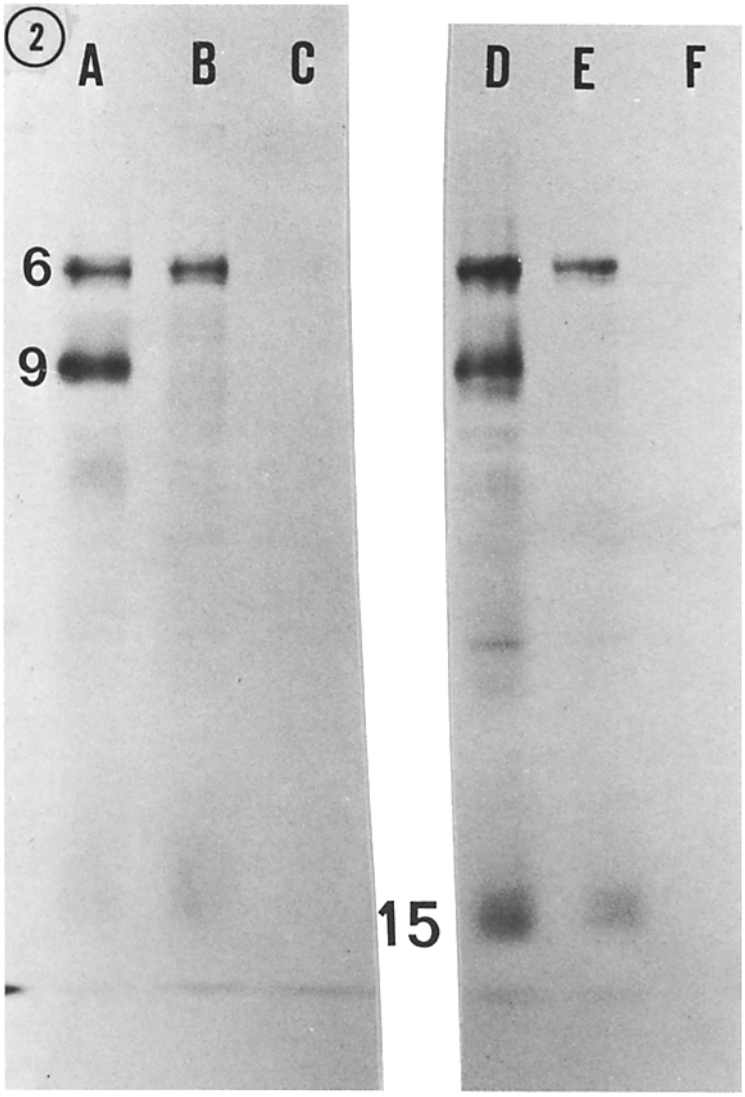

FIGURE 2 Polyacrylamide slab gel electrophoresis of mouse thymocyte glycoproteins. A, BALB/c thymocytes labeled with NaB^3H_4 after neuraminidase and galactose oxidase treatment: B, BALB/c thymocytes labeled after galactose oxidase treatment; C, BALB/c thymocytes labeled without enzyme treatment; D, A/J thymocytes labeled after neuraminidase and galactose oxidase treatment; E, A/J thymocytes labeled after galactose oxidase treatment; F, A/J thymocytes labeled without enzyme treatment.

smaller molecular weights appear instead (Figs. 3 E and F, 4 E and F).

MITOGEN-INDUCED B BLASTS: In B blasts obtained by velocity sedimentation from LPS-stimulated cultures, the B cell-specific band with a molecular weight of 210,000 was relatively weak. Instead, we observed glycoproteins similar to those stimulated T cells (Figs. 3G and 4G).

T BLASTS FROM MLC: The MLC blast population purified by velocity sedimentation showed additional bands: GP7, GP11, GP12, GP13, and GP14. GP7, GP13, and GP14 are not clearly seen in mitogen-stimulated T or B blasts.

Nonspecific Label

All cells incorporate some label without treatment with enzymes (see also reference 12). Therefore, it was always necessary to include controls in

TABLE II

Apparent Molecular Weights of Surface Glycoproteins of Mouse Lymphoid Cells

	Thymocytes	T cells	B cells	T blasts	B blasts	MLC blasts
GP 1	—	210,000	210,000		210,000	210,000
2	200,000					
3	—	190,000	—	—	—	—
4	—	—	185,000	—	—	—
5	—	180,000	—	180,000	180,000	180,000
6	170,000	170,000	—	—	—	—
7	—	—	—	—	—	165,000
8	160,000	—	—	—	—	—
9	125,000	125,000	—	125,000	125,000	125,000
10	110,000	—	—	—	—	—
11	—	—	—	86,000	86,000	86,000
12	—	—	—	77,000	77,000	77,000
13	—	—	—	—	—	72,000
14	—	—	—	—	—	58,000
15	27,000	—	—	—	—	—

Underlined values are major components.

the labeling experiments where no enzymes (but only NaB^3H_4) were used. With one exception, proteins were not appreciably labeled. This labeled protein (NS) with a molecular weight of 57,000 is weakly labeled in T or B lymphocytes, more label is observed in thymocytes, and a strong label is seen in all blast cells.

Proteins Labeled after Periodate Treatment

Fig. 5 shows the electrophoretic pattern of T and B lymphocytes reduced with NaB^3H_4 after periodate treatment. In T cells the proteins GP5 and GP9 are labeled, and in B cells GP1.

Does Neuraminidase Plus Galactose Oxidase Treatment or Periodate Treatment Change the Native Glycoprotein Pattern of T Cells?

Because neuraminidase plus galactose oxidase or periodate treatment per se has a mitogenic effect on T cells, the following experiment was done. Spleen lymphocytes were reduced with NaB^3H_4 after treatment with neuraminidase and galactose oxidase (Fig. 6 A), after periodate treatment (Fig. 6 B), kept for 2 h in culture, and then labeled after neuraminidase plus galactose oxidase (Fig. 6 C), incubated with PHA for 2 h in culture, then labeled after neuraminidase plus galactose oxidase (Fig. 6 D), or treated with periodate, cultured for 2 h, and then labeled after neuraminidase plus galactose oxidase (Fig. 6 E). The only observable effect is on GP9 which, after neuraminidase plus galctose oxidase, has a higher apparent molecular weight than if first treated with periodate. The rest of the characteristic proteins do not change. This indicates that in 2 h, which corresponds to the time it takes to label cells, no extensive change of the surface membrane glycoprotein pattern occurs. Fig. 6 F shows the control without enzyme treatment.

Labeled Mouse Serum Proteins

These proteins are shown as follows: mouse serum proteins labeled after neuraminidase plus galactose oxidase treatment (Fig. 6 G), after galactose oxidase only (Fig. 6 H), and without enzyme treatment (Fig. 6 I). Efficient glycoprotein label is only obtained after neuraminidase treatment. The major labeled proteins do not seem to correspond in electrophoretic mobility to the labeled cellular glycoproteins.

DISCUSSION

By the use of the galactose oxidase-tritiated sodium borohydride technique, we have shown that different resting as well as activated highly purified subpopulations of mouse lymphoid cells expose characteristic surface glycoproteins.

The large size of the galactose oxidase molecule inhibits its penetration into red cells (12) and fibroblasts (13), and therefore only surface-exposed terminal galactosyl and *N*-acetyl galac-

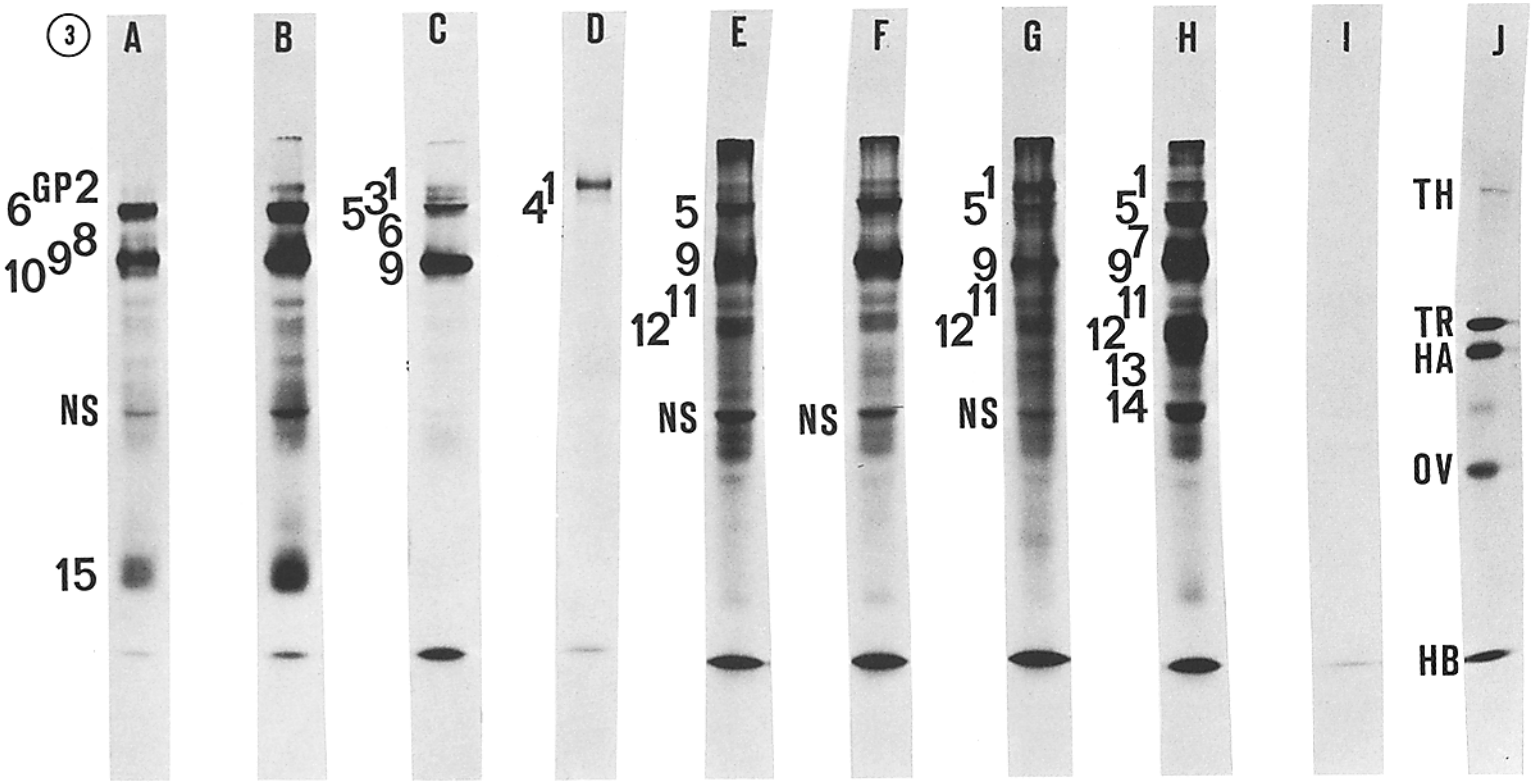

FIGURE 3 Polyacrylamide slab gel electrophoresis of surface glycoproteins of mouse lymphoid cells labeled with NaB^3H_4 after neuraminidase and galactose oxidase treatment. A, thymocytes (A/J); B, thymocytes (CBA/T6T6); C, electrophoretically purified spleen T lymphocytes; D, electrophoretically purified spleen B lymphocytes; E, T blasts from Con A-stimulated cultures; F, T blasts from PHA-stimulated cultures; G, B blasts from LPS-stimulated cultures; H, T blasts from MLC; I, T lymphocytes without enzyme treatment; J, ^{14}C-labeled standard proteins: TH, thyroglobulin; TR, transferrin; HA, human albumin; OV, ovalbumin; HB, hemoglobin. The major protein bands are numbered according to decreasing molecular weights.

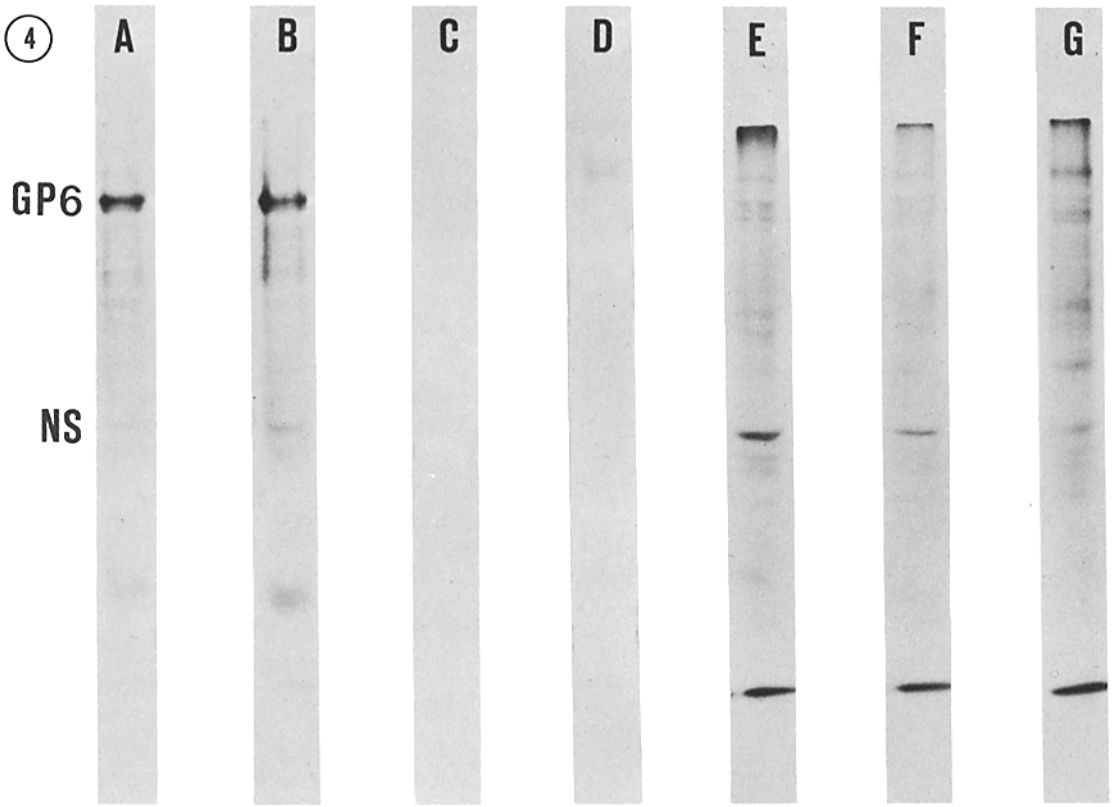

FIGURE 4 Polyacrylamide slab gel electrophoresis of surface glycoproteins of mouse lymphoid cells labeled with NaB^3H_4 after treatment with galactose oxidase only. A, thymocytes (A/J); B, thymocytes (CBA/T6T6); C, electrophoretically purified T lymphocytes; D, electrophoretically purified B lymphocytes; E, T blasts from Con A-stimulated cultures; F, T blasts from PHA-stimulated cultures; G, B blasts from LPS-stimulated cultures. The same numbers of cells were labeled, identical aliquots were electrophoresed, and the same exposure time was used as for Fig. 3.

tosaminyl residues are oxidized to the corresponding C6 aldehydes (5). We have treated intact labeled lymphocytes and thymocytes with pronase and trypsin, to verify the surface distribution of the labeled glycoproteins (not shown). Of the major labeled surface glycoproteins, GP1, GP6, and GP9 are easily degraded by the enzymes, but GP5 is degraded to a smaller extent. One could then argue that GP5 is not a surface glycoprotein. However, we think that it is, but it is for unknown reasons, resistant to the proteases. The situation is similar in hamster fibroblasts where one of the major surface glycoproteins is resistant to proteases (15). The oxidized glycoproteins are subsequently labeled by reduction with tritiated sodium borohydride. Sialic acids are often linked to penultimate galactosyl/*N*-acetyl galactosaminyl residues and, therefore, more efficient labeling is achieved by treating the cells with neuraminidase.

The resolution of autoradiography is superior to the gel slicing and counting techniques. Previously, tritium-labeled proteins could not be autoradiographically visualized from polyacrylamide gels, but recently this has become possible by the introduction of a scintillator (PPO) into the gel. This technique now enables us to define reproducibly the labeled surface glycoproteins. Because glycoproteins (9, 34) and possibly also intrinsic membrane proteins in general behave anomalously on polyacrylamide gels, the molecular weights of these proteins are only approximate.

A number of surface-labeling techniques have recently been introduced, the most popular being lactoperoxidase-catalyzed iodination (18, 25, 30).

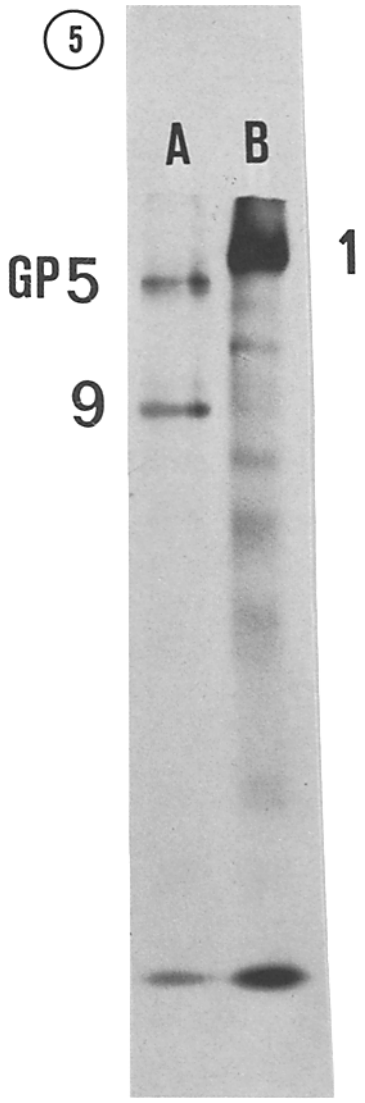

FIGURE 5 Polyacrylamide slab gel electrophoresis of electrophoretically fractionated mouse spleen T and B lymphocytes labeled with NaB^3H_4 after periodate treatment. A, T lymphocytes; B, B lymphocytes.

All labeling techniques have their drawbacks, but the galactose oxidase method enables the specific identification of glycoproteins. When the galactose oxidase method is combined with neuraminidase treatment, molecules containing sialic acid are also visualized.

The protein with a molecular weight of 170,000 from thymocytes is labeled well by galactose oxidase alone. This means that it contains a relatively large proportion of terminal galactosyl/*N*-acetyl galactosaminyl residues. This is in contrast to the glycoproteins of the lymphocytes which are labeled appreciably only if treated with neuraminidase. GP15, which is found only in strains CBA-H/T6T6 and A/J, and not in BALB/c, could correspond to the TL antigen (7).

Using the lactoperoxidase method, Trowbridge et al. (39) obtained results similar to ours and showed that mouse lymphoid cells enriched in T or B lymphocytes have characteristic surface proteins. They also found two T cell-specific proteins and one B cell-specific protein, which may well correspond to those found by us.

The B cell-specific protein with an apparent molecular weight of 210,000 shares characteristics with the major surface glycoprotein of normal fibroblasts which has a similar molecular weight (13, 17, 19, 40). This protein is absent from transformed fibroblasts, and, in mutants temperature-sensitive for transformation, it is present only at the nonpermissive temperature (14, 20, 32). Trowbridge et al. (39) also showed that the B cell-specific protein was absent from a myeloma cell line secreting immunoglobulin A, whereas the T cell-specific proteins were retained on lymphomas of T-cell origin.

Novogrodsky (29) and Thurman et al. (38) have recently shown that periodate treatment or neuraminidase plus galactose oxidase treatment selectively triggers T lymphocytes. It is reasonable to assume that the T lymphocyte-specific surface proteins that we detect by either treatment are involved in this phenomenon.

The presence of surface immunoglobulin on B lymphocytes is well established. Vitetta et al. (42) have characterized such molecules from lactoperoxidase-labeled lymphocytes. We have not definitely observed any labeled proteins corresponding to these. Quantitatively, however, these proteins constitute a rather small proportion of the total surface proteins. It is also possible that the carbohydrate of surface immunoglobulins is relatively deeply imbedded in the cell surface and therefore not available to galactose oxidase.

Both T and B blasts have an altered surface glycoprotein pattern as compared to T and B lymphocytes. This shows that major surface changes occur after triggering to proliferation. The higher label per cell is obviously partially due to the increased cellular size. The surface glycoprotein pattern from one-way MLC seems to be different from that of mitogen-stimulated cells. Quantitatively, the lymphocytes reacting to a

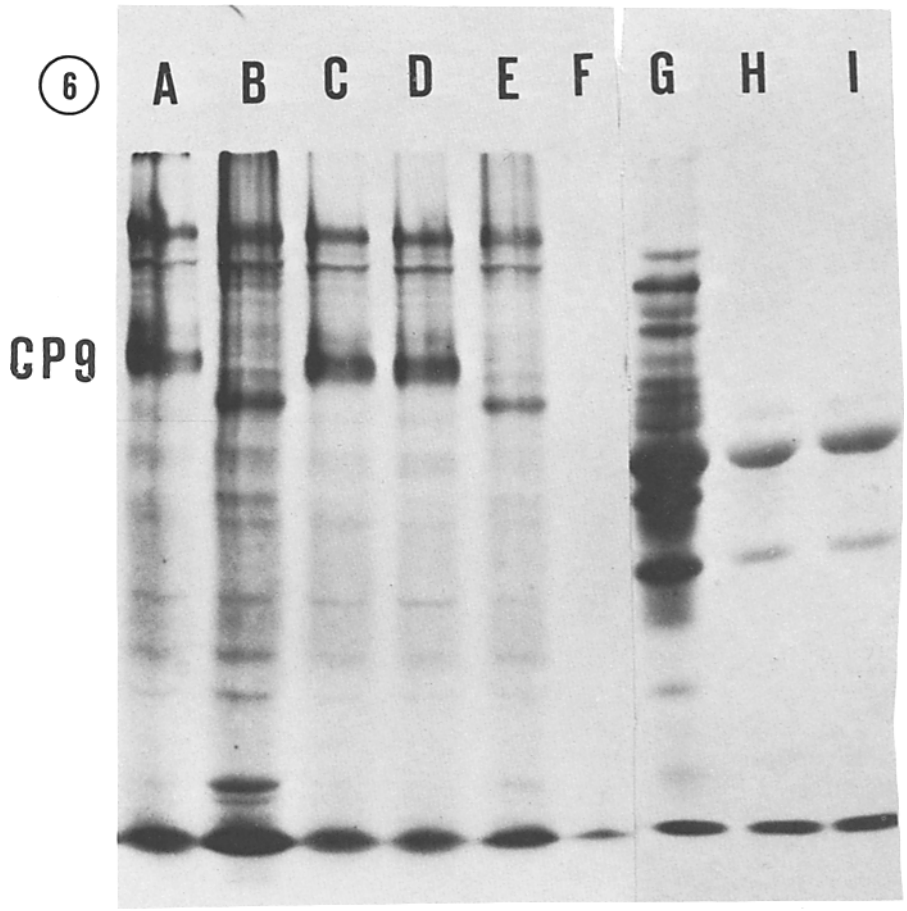

FIGURE 6 Polyacrylamide slab gel electrophoresis of labeled glycoproteins from nonstimulated and activated spleen cells, and of labeled mouse serum. A, spleen cells labeled after neuraminidase and galactose oxidase treatment; B, spleen cells labeled after periodate treatment; C, spleen cells left for 2 h in culture medium then labeled after neuraminidase and galactose oxidase treatment; D, spleen cells treated with PHA and then labeled after neuraminidase and galactose oxidase treatment; E, spleen cells treated with periodate, left for 2 h in culture, and then labeled after neuraminidase plus galactose oxidase treatment; F, control cells labeled without enzyme treatment; G, mouse serum labeled after neuraminidase plus galactose oxidase treatment; H, mouse serum labeled after galactose oxidase treatment; I, mouse serum labeled without enzyme treatment.

certain set of histocompatibility antigens constitute only a small per cent of the total T cell population (35). Whether these lymphocytes also before alloactivation in MLC have surface proteins which differ from those of the major T cell population, or whether they get their surface pattern after stimulation, is not known. An interesting possibility is that some of the additional proteins seen on MLC blasts are involved in target cell recognition. Kimura has been able to raise an antiserum reacting specifically with alloagressive killer T cells, which indicates that they express antigenic determinants not present on resting or mitogen-activated T cells (22).

The blast cells also contain a protein with an apparent molecular weight of 57,000, which is very weakly labeled in nongrowing cells. This protein is labeled without enzyme treatment by tritiated sodium borohydride alone. The nature of the reducible group is not known, but could be due to Schiff base formation between the aldehyde group and an amino group of an enzyme containing pyridoxal phosphate. Enzymes such as ornithine decarboxylase are known to be strongly activated

in growing cells (27). A similar protein is also strongly labeled in transformed fibroblasts (13) and human chronic lymphocytic leukemia cells (4).

The basis for the differences in electrophoretic mobilities of mouse T and B lymphocytes is unclear, but surface sialic acids are obviously involved since the characteristic, biphasic electrophoretic profile of T and B lymphocytes is lost after neuraminidase treatment (28). It has been difficult to determine the absolute amount of sialic acid in T and B lymphocytes because of the limited number of highly purified cells available. Therefore, we have used an indirect approach. Mild periodate treatment oxidized predominantly sialic acids, and 5-acetamido-3,5-dideoxy-L-arabino-2-heptusolonic acid was formed (24, 41). This may be reduced with sodium borohydride. Because we used a very low amount of NaB^3H_4 (about 2.5 μg) per experiment, the modified sialic acids were only partially reduced and the absolute amount of sialic acids could not be determined. Higher concentrations of NaB^3H_4 result in lower specific activities and in electrophoretic patterns resembling those of proteolysis (37).

In both T and B cells the major periodate-oxidizable proteins are similar to those labeled after neuraminidase and galactose oxidase treatments. This suggests that in B lymphocytes which have a slower electrophoretic mobility, cell surface sialic acids are less efficiently exposed at the electrophoretic plane of shear. The major sialic acid-containing surface glycoprotein (GP1) could also be covered by other surface macromolecules, such as immunoglobulin.

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