ISOLATION AND IMMUNOLOGICAL CHARACTERIZATION
OF A MAJOR SURFACE GLYCOPROTEIN (gp54)
PREFERENTIALLY EXPRESSED ON CERTAIN HUMAN B CELLS*

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Lymphocytes from many species have been classified into T and B cells according to their differentiation pathways and specialized immune functions. They are distinguishable phenotypically by characteristic molecules that are expressed on their cell surfaces (1). In man, Ia-like antigens, surface immunoglobulins, and complement receptors are expressed preferentially on B lymphocytes, whereas the receptor for sheep erythrocytes is a general marker for T lymphocytes. Using serological methods, subpopulations of human B and T cells have been found recently (2–6). Efforts have begun in a number of laboratories towards the biochemical characterization of specific lymphocyte-surface components (7–9) and the development of serological reagents toward these marker proteins.

In the present study, the identification of molecules, expressed preferentially on the cell surface of cultured human B cells and on normal lymphoid tissue, was initially studied through the use of a B-cell-specific rabbit antiserum. In addition to reacting with Ia-like antigens in immunoprecipitation experiments, this absorbed anti-B-cell serum reacted with several other major surface glycoproteins with mol wt of 54,000, 86,000, and 105,000. These glycoproteins were purified from the B-lymphoid cell line, B35M, and attempts were made to raise rabbit antisera towards each component. This paper describes the purification and immunological characterization of the 54,000 dalton antigen which proved most amenable to isolation and study. Its expression on cultured human cell lines, normal lymphocytes, as well as various types of leukemic cells, was determined. The effect of anti-gp54 serum on DNA synthesis of lymphocytes was also investigated.

Materials and Methods

Cell Preparation. Mononuclear cells were isolated from normal or leukemic peripheral blood by discontinuous Ficoll-Hypaque gradients (Pharmacia Fine Chemicals, Div. of Pharmacia Inc., Piscataway, N. J.). Mononuclear cells were obtained from tonsillar tissues as described previously (10). Monocytes were removed by a carbonyl iron-ingestion method. Preparations of enriched T- and B-lymphocyte populations were made by Ficoll-Hypaque gradient separation of lymphocytes rosetted with neuraminidase-treated sheep erythrocytes.

The following cultured human cell lines were utilized which had been derived from Burkitt's lymphomas: Daudi, B35M, and Raji; from leukemic patients: RPMI 8866, K562, Jurkat, SB, and HSB; or from normal peripheral blood lymphocytes, RPMI 1788. They were maintained

in medium RPMI-1640 (Microbiological Associates, Walkersville, Md.), supplemented with 10% fetal calf serum. 17 B-lymphoblastoid cell lines derived from one tonsil and other cell lines from normal donors were initiated and maintained as previously described (11).

Preparation of Antisera. New Zealand white rabbits, selected for the absence of natural antibodies to lymphocytes, were used for immunization. All the antigen preparations were emulsified with complete Freund's adjuvant, injected intradermally at multiple sites on the back, and followed by four to eight injections given in a similar way, 2 wk apart.

(a) A rabbit anti-human B-lymphocyte serum was raised by repeatedly immunizing a rabbit with 500 μg of glycoprotein fraction of B cell-line plasma membranes (B35M) isolated from a concanavalin A (Con A)[1]-affinity column.

(b) Rabbit anti-human Ia serum was prepared by immunizing a rabbit with 25 μg/injection of a 65,000 dalton gp28, 37 Ia bimolecular complex isolated from a B-lymphoblastoid cell line, B35M, as described previously (12, 13).

(c) Rabbit anti-gp54 serum was produced by immunizing rabbits with 25-50 μg/injection of a 54,000-dalton component isolated from the B cell line, B35M (material identical to that in gel b of Fig. 3).

A rabbit anti-HLA heavy-chain serum was a generous gift from Dr. R. E. Humphreys, University of Massachusettes Medical Center, Worcester, Mass. It was found to have broad HLA reactivity when tested by immunoprecipitation assay on various peripheral blood lymphocyte, leukemias, and tonsil cells. Rabbit antiserum made against Fab fragments of human FrII IgG and sheep antiserum made against F(ab')2 fragments of rabbit immunoglobulin, were produced in similar ways. All sera were heat inactivated at 56°C for 45 min before use.

Surface Immunofluorescence. F(ab')2 fragments, from all the antisera, were prepared and conjugated with tetramethylrhodamine isothiocyanate as previously reported (14). The trace Fc-containing molecules were removed by absorbing the reagents with protein-A Sepharose CL-4B beads (Pharmacia Fine Chemicals, Div. of Pharmacia Inc.). The sheep anti-F(ab')2 of rabbit IgG reagent was absorbed with human Fr.II-Sepharose 4B beads and human erythrocytes before use. The cell-surface antigens were detected by direct immunofluorescence as described before (14). Usually, in the case of gp54 antigen, a second reagent, sheep anti-F(ab')2 of rabbit IgG, was used to intensify the staining. Monocytes were identified by their capacity to ingest latex particles.

[51]Cr-Release Complement-Mediated Cytotoxicity Assays. The ^{51}Cr-release cytotoxicity assay of antisera and the cytotoxicity inhibition assay for antigen was performed in triplicate according to established techniques (15).

Target cells incubated in medium alone, without added complement and antibodies, were included in each experiment to determine the percentage of spontaneous ^{51}Cr-release. Target cell aliquots were lysed with 0.5% Nonidet P-40 (NP-40) detergent solution for determination of the maximum percentage of releasable [51]Cr. Percentage of cytotoxicity was calculated using the following formula:

$$\% \text{ Cytotoxicity} = \frac{\text{Experimental } \% \ ^{51}\text{Cr release} - \% \text{ spontaneous } ^{51}\text{Cr release}}{\text{Maximal } \% \ ^{51}\text{Cr release} - \% \text{ spontaneous } ^{51}\text{Cr release}} \times 100.$$

Quantitative Absorption Analysis. Suspensions containing variable amounts of cells (0.08-250 × 10^5/50 μl) were added to equal volumes of the heat-inactivated antiserum and incubated at room temperature for 1 h with constant shaking. Cells were removed by centrifugation at 15,000 rpm for 3 min, and the remaining reactivity of the antiserum was tested by complement-mediated cytotoxicity and by immunoprecipitation analysis. For Ia-antigen quantitation, a 1:50 dilution of the serum was used for absorption and for gp54 antigen quantitation, a 1:10 dilution of the serum was used.

Iodination of Cells. A modification of the method of Marchalonis et al. (16) was used to iodinate the cell surface. Cells from various cell lines were harvested and washed three times in phosphate-buffered saline (PBS) (pH 7.4); the viability was measured by trypan blue dye exclusion and usually was >95%. To 2 ml of cell suspension (1 × 10^7/ml), 0.5 mCi Na ^{125}I and

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[1] *Abbreviations used in this paper:* Con A, concanavalin A; NP-40, Nonidet P-40; PAS, periodate acid-Schiff; PAGE, polyacrylamide gel electrophoresis; PBS, phosphate-buffered saline; SDS, sodium dodecyl sulfate.

200 μg lactoperoxidase, purified as described, (17) were added, followed by 25 μl of freshly prepared 0.03% H_2O_2 at 5-min intervals. The reaction was terminated by the addition of cold phosphate-buffered saline (PBS). The cells were washed and lysed with 0.5% NP-40 solution at a concentration of 1 $\times$ 10^7 cells/ml. The lysate was dialyzed extensively before further processing.

Immunoprecipitation. For immunoprecipitation experiments, 50 μl of rabbit antiserum known to be in excess was incubated with 100 μl of a 10% (wt/vol) solution of protein-A Sepharose CL-4B beads at 37°C for 30 min. The washed antibody-coated immunoabsorbent was then incubated with an aliquot of the radiolabeled cell lysate at 37°C for another 30 min and followed by three washes in PBS containing 0.1% NP-40. The bound ^{125}I-labeled surface antigens were eluted by boiling the beads with 4 M urea and 2% sodium dodecyl sulfate (SDS) in 0.1 M Tris HCl (pH 8.0) buffer, and followed by SDS-polyacrylamide gel electrophoresis.

SDS-Polyacrylamide Gel Electrophoresis. The discontinuous Tris-glycine system of Laemmli (18) was used for both slab gels and cylindrical gels. Unless especially mentioned, all the gels were electrophoresed under nonreducing conditions. After electrophoresis, gels were stained with Coomassie Brilliant Blue or periodic acid-Schiff (PAS) reagent (20). In the case of immunoprecipitation experiments, the gels were dried onto filter paper under vacuum for subsequent autoradiography on Chrondex x-ray film (DuPont Instruments, Wilmington, Del.). Apparent molecular weights were determined by the method of Weber and Osborn (19) with the following protein standards: human IgG myeloma protein (150,000 daltons), bovine serum albumin (68,000 daltons), ovalbumin (44,000 daltons), α-chymotrypsinogen (25,000 daltons), and cytochrome C (11,000 daltons).

For antigen isolation, guide strips (1-cm wide) of the preparative slab gel were cut from both edges and stained with Coomassie Brilliant Blue. The areas having the antigens were cut from the unstained portion of the gel, and eluted with PBS (pH 7.4) overnight at 37°C.

Results

Serological and Biochemical Analysis of Cell-Surface Components of Human B-Lymphoblastoid Cell Lines. A B-cell antiserum was raised by immunizing a rabbit with glycoproteins isolated by Con A-affinity column chromatography from a B-lymphoblastoid cell line (B35M). This antiserum, after absorption with pooled T cell lines, reacted specifically with isolated peripheral and tonsillar B cells. The surface components, recognized by the primary B-cell antiserum, were identified by immunoprecipitation of the surface radioiodinated B35M cell lysate as illustrated in Fig. 1. At least 10 components with molecular weights ranging from 15,000 to 150,000 were precipitated by this serum (Fig. 1 a). In contrast, rabbit anti-actin serum (Fig. 1 b) did not precipitate any discernible component, and thus served as a control indicating the external nature of the iodinated membrane proteins.

To analyze further these surface membrane components, large quantities of plasma membrane from a B cell line, B35M, were prepared, solubilized by sodium deoxycholate and chromatographed on a Ultrogel AcA 34 (LKB Instruments Inc., Hicksville, N. Y.) column (2.5 $\times$ 40 cm) as described before (15). Fig. 2 illustrates a representative gel-filtration chromatogram and SDS-gel electrophoresis profile of such detergent solubilized B-cell membranes. The major proportion of the membrane components elute in the void volume and they were not studied further. Characterization of every third subsequent fraction by SDS-polyacrylamide gel electrophoresis demonstrated a series of polypeptides. The principal proteins had apparent mol wt of 105,000, 86,000, 54,000, 37,000, and 28,000, as indicated. All five components gave positive PAS staining which suggested their glycoprotein nature.

By cytotoxicity inhibition and immunoprecipitation assays, the Ia alloantigens were found to be present in a detergent micelle eluted in column fractions from 34 to 40
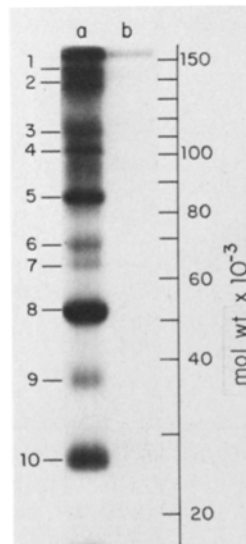
Fig. 1.   Autoradiographs of SDS gels of immunoprecipitates prepared from Nonidet-P40 lysate of a B-lymphoid cell line, B35M. The rabbit antisera used were (a) anti-B-cell glycoprotein serum (b) anti-actin serum.

with elution volumes slightly larger than that of IgG, and as a bimolecular complex with subunit mol wt of 37,000 and 28,000 as described (12, 13, 21). They were precipitated and identified by the anti-B-cell serum as components 9 and 10 shown in Fig. 1 a. The classical HLA antigens were eluted in the same column fractions as the Ia antigens in parallel cell membrane preparations, although in the case of B35M cell line, these antigens were present at relatively low concentrations. In fractions 34 to 46, two polypeptides with mol wt of 86,000 and 105,000 were identified by immunoprecipitation as components 4 and 5 of Fig. 1 a. In fractions 43 to 52, another major protein with approximate mol wt of 54,000 was identified by immunoprecipitation as component 8 of Fig. 1 a which is the subject of the present study. For convenience, it will be referred as gp54 antigen.

Isolation of gp54 Antigen.   The isolation of the gp54 antigen involved plasma membrane preparations of B35M cell lines and solubilization in sodium deoxycholate, followed by gel filtration on a Ultrogel 34 column as described above. The gp54 antigen-enriched column fractions (43-49) were pooled, concentrated, and the membrane proteins were then separated by preparative SDS-polyacrylamide gel electrophoresis (PAGE) as described in Methods. The gp54 antigen was eluted from the gel and re-electrophoresed on a 9% slab gel along with the detergent solubilized membrane preparation as shown in Fig. 3. The isolated gp54 antigen electrophoresed as a single component under both reduced and nonreduced conditions.

Characterization of Antiserum to the gp54 Antigen.   By immunoprecipitation and SDS-PAGE analysis, the gp54 antiserum recognized only one protein component with a mol wt of 54,000. This is shown for one representative cell line (T51) using the whole NP-40 solubilized lysate of cells iodinated with ^{125}I (Fig. 4). This reaction is specific, because preimmune serum does not precipitate any protein components in the whole cell lysate. For comparison, immunoprecipitation of the anti-Ia serum and anti-HLA
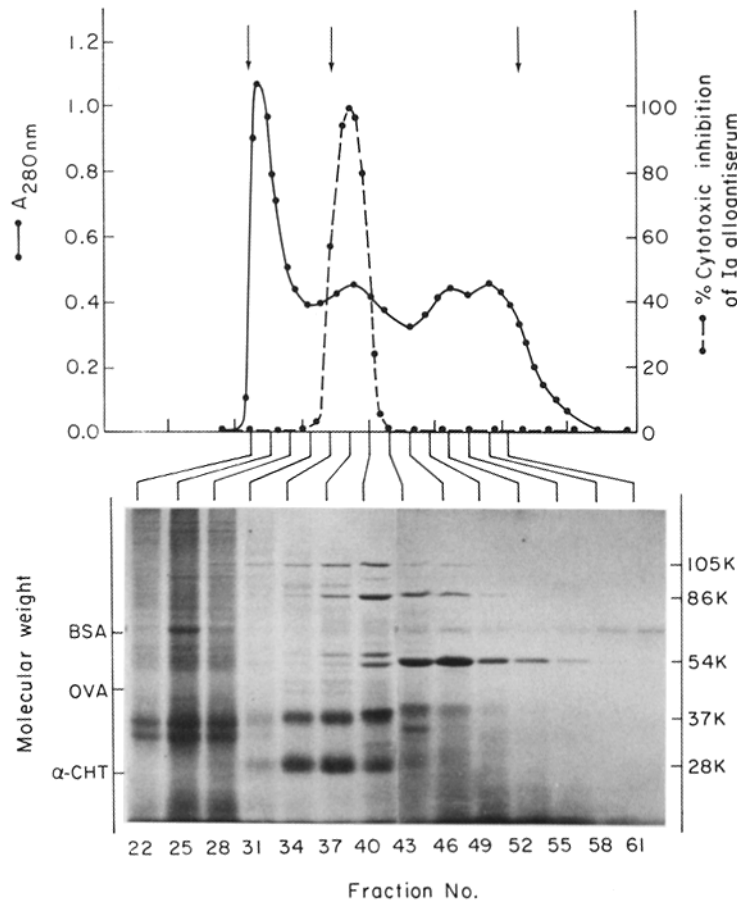
FIG. 2. The filtration profile from a preparative Ultrogel AcA 34 column of the detergent-solubilized B35M cell-line membrane macromolecules (upper portion). The molecular weight markers used for calibration of the column were Dextran blue, IgG, and DNP-glycine, as indicated by arrows from left to right. The Iá-alloantigenic activity was detected by the inhibition of cytotoxicity assay (dashed line curve). Every third column fraction was subjected to SDS-PAGE (10%) analysis under nonreduced condition and stained with Coomassie Brilliant Blue (lower portion). The fractions with peak Ia activity showed heavy protein bands at mol wt 28,000 and 37,000. In fractions 43 to 52, the protein band of mol wt 54,000 is evident as a dominant component.

heavy-chain serum were also analyzed on the same gel. The anti-Ia serum precipitated the typical bimolecular complex with two subunits of mol wt of 28,000 and 37,000. The anti-HLA serum precipitated the 48,000 heavy chain which is associated with β_2-microglobulin (Figs. 4c and d). The gel was analyzed under nonreduced condition to separate the HLA heavy-chain molecule from the gp54 antigen because they comigrate at an apparent mol wt of 45,000 in the presence of reducing agent. The anti-gp54 serum reacted in a similar manner with NP-40 solubilized lysate of cells metabolically labeled with [³H]leucine.

Expression of gp54 Antigen on Various Human Lymphoid Lines. Representative results by cytotoxicity titration of cell lines of B or T origin with anti-gp54 serum are shown in Fig. 5. Lysis of the lines occurred in a plateau fashion with the percentage cell lysis
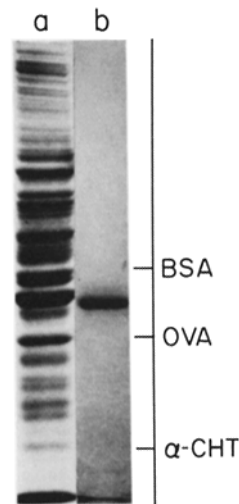
Fig. 3. SDS-PAGE pattern of the isolated gp54 glycoprotein(b). For comparison, the whole plasma membrane preparation solubilized by 0.5% sodium deoxycholate is shown (a). The position of three marker proteins is indicated on the right.

ranging between 5 and 90%. There is a preferential reactivity of the anti-gp54 serum towards certain B cell lines. In comparison, the anti-Ia serum gave close to 100% cytotoxicity against all B cell lines with the exception of the line 616, a T51 variant (22). The results of fluorescent antibody analyses for the gp54 antigen revealed parallel results to the cytotoxicity assay. The surface immunofluorescent staining of gp54 antigen has a characteristic speckled and patchy pattern as shown in Fig. 6. A substantial cell to cell variation in the staining intensity was found in the lines having a high percentage of positive cells. To determine the nature of the restricted reactivity of the anti-gp54 serum, 17 cell lines were derived by Epstein-Barr virus transformation of the tonsillar B cells from one individual. Cells from each line were examined for the expression of three surface markers (gp54, smIg, and Ia antigens). The results of immunofluorescent studies are shown in Fig. 7. Nearly 100% of the cells in all 17 lines expressed Ia antigens. However, the expression of surface immunoglobulins and gp54 antigens among the cell lines showed extreme heterogeneity and no correlation was found between the two markers.

Quantitative Absorption Analyses. Although the T cell lines studied were negative for the gp54 antigen by both direct cytotoxicity and immunofluorescent analyses, evidence was obtained by immunoprecipitation of the surface radioiodinated T cell-line lysate and in absorption experiments for low level expression of the gp54 antigen on these cell lines. In the immunoprecipitation experiments, a relatively small band was usually obtained from the radiolabeled T cell line as shown in Fig. 8. The quantitative difference of the gp54 antigen expression on the B and T cell lines was further determined by absorption experiments. Two positive B-cell lines (B35M and 8866) and the two T cell lines (Jurkat and 1301) were chosen for absorption test. The residual antibody activity in the absorbed serum was tested by both complement dependent cytotoxicity and immunoprecipitation assays on a B cell line, T51. The 50% absorption endpoints for the two positive B cell lines and the negative T cell lines
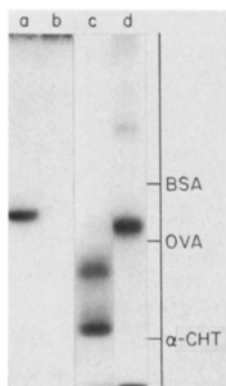
Fig. 4. Autoradiographs of SDS gels of immunoprecipitates prepared from Nonidet P-40 lysates of a surface-radioiodinated B-lymphoid cell line, T51. The rabbit antisera used were: (a) anti-gp54 serum; (b) preimmune control serum; (c) anti-Ia serum; (d) anti-HLA serum (heavy chain).

were: 0.4×10^5 cells/5 μl serum and 50×10^5 cells/5 μl serum, respectively, which indicates that the two positive B cell lines have $\cong 125$-fold more gp54 antigen expressed on the surface than the two negative T cell lines. As for Ia antigens, similar absorption tests indicated that B cell lines expressed 500 times more than the T cell lines did.

After extensive absorption with pooled B cells, the anti-gp54 serum completely lost its lytic activity. However, it still possessed up to 20% of the original precipitating activity as determined by the densitometer scan of the gp54 band in the autoradiogram. This suggests that some antibody activities were directed towards the buried determinants of the gp54 antigen although other explanations for these differences are present.

Expression of gp54 Antigens on Tonsillar and Normal Peripheral Blood B Lymphocytes. The reactivity of the anti-gp54 serum was assayed in the complement dependent cytotoxicity system. In a typical experiment, the antiserum lysed 40% of the unseparated tonsillar mononuclear cells and 60% of the cells in the tonsillar B layer at a dilution of 1:50. Similar results were obtained with less diluted serum. In the case of T cells, no discernible cytotoxicity was observed even when the undiluted anti-gp54 serum was used. An anti-Ia serum however reacted with 60% of the unseparated cells and close to 100% of the isolated B cells with no appreciable reactivity towards T cells. When peripheral blood lymphocytes were tested, a broad spectrum of reactivities towards the isolated B-cell preparations were observed with the anti-gp54 serum. It varied from <5% to 50% according to the individual studied. On the contrary, a consistent high percentage of lysis was detected in every B-cell preparation with the anti-Ia serum. Both antisera showed minimum reactivities towards the T-cell preparations. Surface immunofluorescent studies revealed a similar reaction pattern to that shown by cytotoxicity as indicated in Table I and Fig. 6. There is again a considerable divergence in the number of cells expressing gp54 antigens in the peripheral blood B-cell preparations. Using Ia antigen as a general marker for B lymphocytes, the gp54 antigen defines a subpopulation of cells residing in the B-lymphocyte-enriched preparations.

Expression of gp54 Antigen on Cells from Various Types of Leukemias. Surface expression of the gp54 antigen on various leukemias was investigated by direct-surface immu-
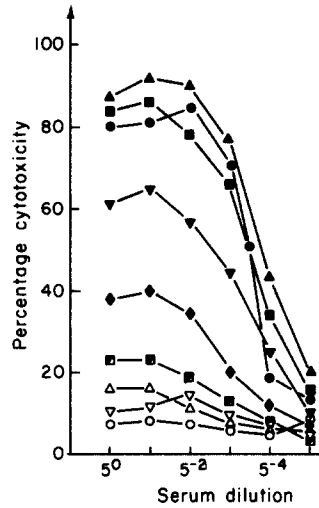
FIG. 5. Lysis by complement-dependent cytotoxicity of various cultured cell lines with anti-gp54 serum. Cell lines used were B type: B35M(▲), 8866(■), T51(●), Raji(▼), MG4(◆), 1788(▨), T type: 1301(△), Jurkat(▽), and the nonlymphoid line K562(○).

nofluorescence. The various leukemias were classified into T-, B-, and non-B-, non-T lymphocytic types according to their surface expression of membrane immunoglobulins or the presence of receptors for the sheep erythrocytes. The expression of gp54 antigen on leukemias of the non-B-, non-T lymphocyte type (e.g. acute lymphatic, acute myelogenous, acute myelomonocytic, chronic myelogenous and erythroid leukemias) was not detectable, although in the majority of these cases Ia antigens were detected. Furthermore, in two of these cases, the leukemic cells were radioiodinated and no discernible component was brought down by the anti-gp54 serum in immunoprecipitation experiments. The expression was also absent on the leukemias of the T-lymphocytic type. The leukemic cells of certain cases of B-type chronic lymphatic leukemias, although 100% positive with the Ia system, were completely negative for the gp54 antigen. Other cases showed a high percentage of gp54 positive cells; however, the percentage of positive cells varied to a great degree. There was also a moderate degree of variability in intensity of staining from cell to cell. The expression of gp54 antigen on certain cells and not on others of the leukemic population in these cases emphasizes the heterogeneous nature of these leukemic cells.

Activation of Human B Lymphocytes by Anti-gp54. The effect of the anti-gp54 serum on DNA synthesis of human lymphocytes obtained from either peripheral blood or tonsils was investigated. As controls, the preimmune serum as well as the anti-Ia serum were used in similar concentrations. The whole anti-gp54 serum as well as its IgG fraction, but not anti-Ia, were mitogenic for B lymphocytes from both sources. This stimulatory effect was further found to be T-cell independent and peak responses were obtained in cultures incubated for 4 d. Divalent and monovalent fragments of gp54 antibodies were prepared and assayed for their stimulatory potential. The results in Fig. 9 show that only the F(ab')₂ fragments can cause the B-cell proliferative response. These results strongly suggest that the activation of B lymphocytes obtained with the anti-gp54 serum is probably a result of the binding of antibodies to the
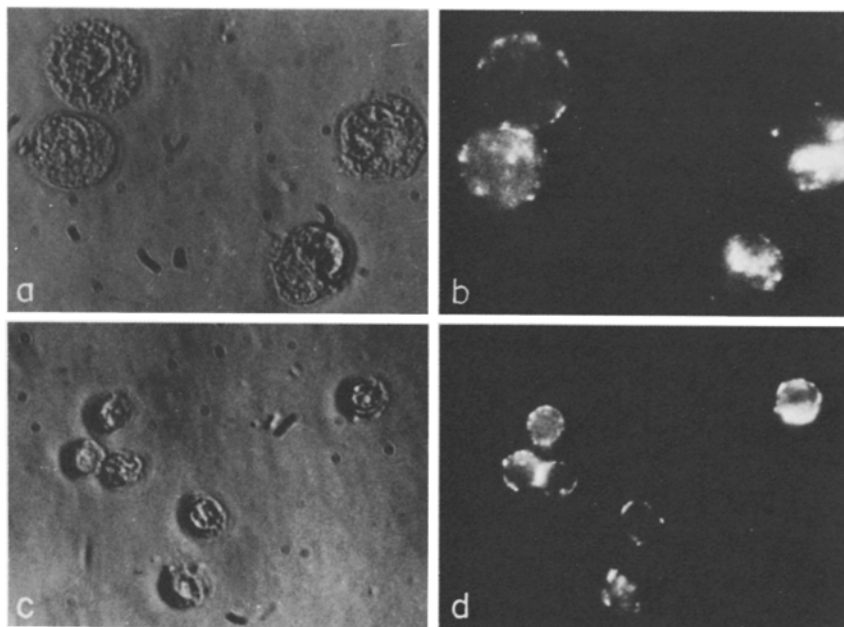
Fig. 6. Fluorescent antibody analysis of B-lymphoid cell line and tonsillar lymphocytes using F(ab')₂ fragments of antibodies raised to the gp54 antigens. (a), (c) are phase-contrast micrographs of (b) and (d). (b) Cells from B-lymphoid cell line(Daudi) showing speckled fluorescent staining cells as well as patchy staining cells. (d) Fluorescent micrograph of a B-cell-enriched preparation of tonsillar lymphocytes showing bright staining cells.

gp54 antigen on the cell surface via the antigen-binding sites and requires crosslinking of the surface antigens. Repeated attempts to induce immunoglobulin synthesis by the activated B cells were unsuccessful. However, preliminary results indicated that the monovalent Fab fragments of the anti-gp54 reagent markedly inhibited the formation of antibody-forming cells in a T-cell-dependent B-cell differentiation system (10).

Discussion

As a result of serological and biochemical analysis of cell-surface components of human B-lymphoblastoid cell lines, a major cell-surface protein with an approximate mol wt of 54,000 was identified and isolated. The biochemical and immunological characterization of this component indicated that this antigen is a membrane glyco-protein exposed on the outer surface of the cell and not associated with β_2-microglob-ulin.

This 54,000 dalton component, termed gp54, is immunochemically different from other structurally known surface components such as the HLA antigens which are associated with β_2-microglobulin, the surface immunoglobulins of IgM and IgD classes, and the recently described I-region associated Ia antigens. By complement-dependent cytotoxicity, surface immunofluorescence, and radioimmunoprecipitation assays, this gp54 antigen was shown to be present on the Daudi cell line, a cell line which does not express either HLA or β_2-microglobulin (23). Also, the 616 cell line, an Ia-negative variant of the T51 cell line which was isolated by cytotoxicity
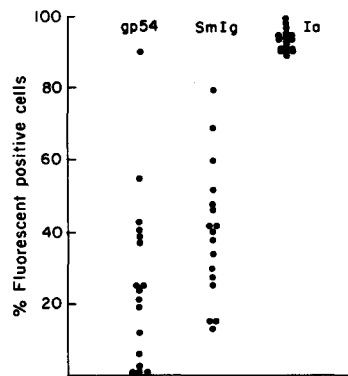
Fig. 7. Antigen expression on 17 lymphoid cell lines derived from the same individual determined by fluorescent antibody analysis using F(ab')₂ fragments of antibodies raised to (a) gp54 antigens, (b) Fab fragment of human IgG, (c) Ia antigens. Each point represents one study on one cell line.

immunoselection using anti-Ia serum and complement (22), expresses the antigen. However, in gel electrophoresis under reducing conditions, it is rather difficult to recognize this component as a distinct molecule from HLA heavy chain, because they comigrate at similar apparent molecular weights between 45,000 and 50,000. Surface membrane components with approximate molecular weights of 45,000 as analyzed by immunoprecipitation and SDS-PAGE under reducing conditions are well known. The antisera used in these studies appeared to be highly specific and various control and preimmune rabbit sera did not precipitate this gp54 component. However, a number of human sera brought down a component of similar molecular weight from the B cell lines. It appears that these sera may contain specific antibodies. Further studies with these human sera are currently underway.

In the mouse, several gene products of chromosome 17 have been reported to have subunits with heavy chains having mol wt of 45,000 and are associated with β_2-microglobulins (24). These antigens include H-2D, H-2K, and TL. More recently, in the mouse, another alloantigen Lyb-2 has been reported (25) which is expressed exclusively on B cells, specified by a locus on chromosome 4, and also has an approximate mol wt of 45,000 yet it is not associated with β_2-microglobulin subunit. In man, other than HLA antigens, there has been no definitive report regarding the identification of any of the above mentioned analogues. Whether the antigen we characterized here is a Lyb-2-like molecule, remains to be determined. In an earlier report, a component with a mol wt of 50,000 was noted on human B cells by Trowbridge and his coworkers (7). This component was not associated with β_2-microglobulin and may be the same as the gp54 antigen of the present report.

More than 50 established human hematopoietic cell lines have been tested for their surface expression of gp54 antigen. Extreme heterogeneity with respect to expression was found both among lines and within a given line. The differential expression of this antigen by different cell lines may reflect different stages of differentiation and maturation of the precursor cells. The quantitative difference of gp54 antigen on cell lines was clearly evident in absorption tests. The gp54 antigen is expressed 100-fold or more in the most positive B cell lines than on the negative B or T cell lines. The gp54 negative cell lines actually express the antigen at a density too low to be detected by immunofluorescence and cytotoxicity assays. A small amount of the gp54 antigen

TABLE I

Immunofluorescent Analysis of Cells Bearing Ia Antigens, Surface Immunoglobulins, and gp54 Antigen in E-RFC* Depleted or Enriched Cell Fractions

	Percentage of Positive Lymphocytes					
	E-RFC depleted			E-RFC enriched		
	Ia	smIg	gp54	Ia	smIg	gp54
PBL 1	78	55	5	2	<1	<1
PBL 2	48	28	18	6	<1	<1
PBL 3	60	37	51	5	<1	<1
Tonsil 1	95	60	78	7	<1	<1
Tonsil 2	96	78	88	2	<1	<1
Tonsil 3	93	72	57	3	<1	<1

* Sheep erythrocyte rosette-forming cells are abbreviated as E-RFC.

could in certain cases be revealed by radioimmunoprecipitation assay even in the T cell lines. Whether the accessibility or conformation of the gp54 antigen at the cell surface is a result of its glycosylation as in the case of T25 antigen expression on certain mouse T-lymphoma cells (26) and the case of glucose-regulated protein on the fibroblast cells (27), or as a "differentiation antigen" which is expressed in varied amounts according to the cellular differentiation stages remains to be answered.

Aside from cultured cell lines, lymphoid cells from peripheral blood and tonsils were also examined for gp54 expression. Anti-gp54 serum identified a subset of human B lymphocytes from both sources which was shown by surface immunofluorescence and cytotoxicity assays. However, tonsil B lymphocytes have much higher reactivities than those of peripheral blood. This phenomenon could reflect differences in degree of maturation between cells in the blood stream and in the tonsil. Expression of gp54 antigen on various mitogen stimulated cultured lymphocytes is currently under investigation.

Among the nonlymphoid cell types studied, this antigen was absent on erythrocytes, platelets, cultured normal fibroblast cells, and nonlymphoid leukemias. However, its presence on certain malignant melanoma cell lines and various carcinoma cell lines was found.

In chronic lymphatic leukemias of the B-cell types, the gp54 antigen was present on the leukemic cells of only some of the cases. In the positive cases, the staining intensity and the percentage of stained cells varied greatly. This heterogeneity was very similar to that found in the peripheral blood B lymphocytes and tonsillar B cells. The question whether this antigen will prove useful in classifying chronic lymphatic leukemia remains to be determined.

The function of the gp54 antigen is not clear. Data were presented showing that F(ab')$_2$ fragments of the anti-gp54 serum were stimulatory when they were added to either peripheral blood B cells or tonsillar B cells in vitro. Parallel studies with anti-Ia serum were completely negative. Preliminary results also indicate that the monovalent Fab fragments of the anti-gp54 reagent markedly inhibit the activation of B-cell differentiation to plasma cells in a T-cell-dependent B-cell differentiation system. These data suggest that the gp54 antigen may in some way relate to the control of B-cell proliferation and differentiation.
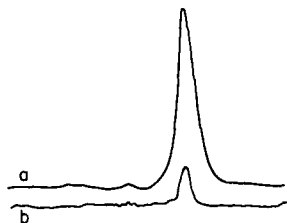
FIG. 8. Densitometer scanning patterns of comparative immunoprecipitates prepared from NP-40 lysate of surface-radioiodinated B cell line Daudi(a) and T cell line-1301(b) using anti-gp54 serum.
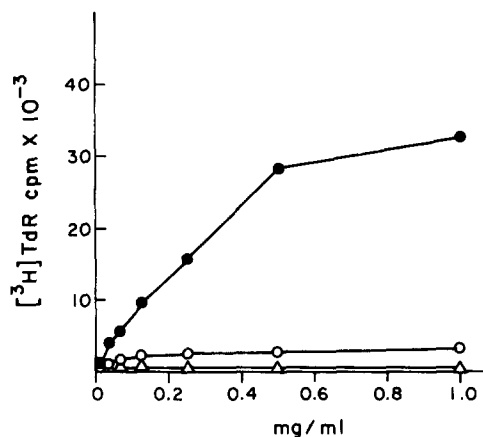

FIG. 9. Stimulation of [^{3}H]thymidine uptake by cultures of isolated human tonsillar B lymphocytes with purified F(ab')$_2$ fragments (●) and Fab fragments (O) of anti-gp54 serum. The control culture with F(ab')$_2$ fragments of preimmune serum is also shown (△).

Summary

A major membrane glycoprotein with mol wt of approximately 54,000 has been isolated from membrane preparations of B-type lymphoid cell lines. Antiserum prepared against the isolated material specifically precipitated this glycoprotein from membranes labeled by surface radioiodination or by metabolic labeling.

This antiserum was shown by complement-mediated cytotoxicity assay, membrane immunofluorescent staining, and by quantitative absorption analysis to react preferentially with certain B-lymphoblastoid cell lines, with a minor population of peripheral blood B lymphocytes, and a major population of tonsillar B lymphocytes. Certain B-cell leukemias also expressed the antigen, whereas others did not. Considerable variability was observed among positive B cells in the intensity of fluorescent staining even among the leukemic cells from a single individual. Although T cells, including T cell lines, were negative by direct immunofluorescent and cytotoxicity assay, evidence for low levels of the antigen on the cells of T cell lines was obtained.

The whole specific antiserum and its F(ab')$_2$ fragments stimulated B lymphocytes to proliferate. This proliferation did not produce differentiation to plasma cells and was T-cell independent. The monovalent Fab fragments had no effect. None of these preparations stimulated T cells. The possibility that this antigen, termed gp54, may play some role in B-cell activation is discussed.

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