

Comparison of Physical and Immunological Properties of Plasma Membranes of Two Mouse Leukemia Cell Lines, P388 and L1210¹

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SUMMARY

A method for the isolation of the plasma membranes of the mouse leukemic cell P388 is described. The method includes surface labeling of the cell membrane with ¹²⁵I. The ¹²⁵I specific activity of the plasma membrane preparation is 10- to 13-fold higher than that of the cell homogenate; enzyme assays and electron microscopy further corroborate the purity of the P388 plasma membrane preparation. The predominant iodinated membrane proteins have molecular weights of 85,000, 66,000, and 13,000 daltons. Antibodies prepared against P388 and L1210 plasma membranes inhibit the growth of the corresponding cells in the absence of complement. They show cross-reactivity to each other but do not affect the growth of HeLa cells.

INTRODUCTION

As discussed in great detail in many recent reviews (9, 13, 27), it is recognized that there is no general method for the isolation of plasma membranes and that careful control of the homogenization conditions is more important for the isolation of plasma membranes than for the isolation of other cell components.

Recently, we have established a general method that defines the optimal conditions for labeling with ¹²⁵I the surface proteins of cells (14, 26). Consequently, we have a convenient assay for the purification of the plasma membranes, since the increase in the specific activity of ¹²⁵I parallels the increased content in plasma membranes.

We present the results of such a study and also discuss the growth-inhibitory effects of antisera prepared against such purified membranes. Although the cytotoxic effect of antibody in the presence of complement has been well studied, the effect of antibody on cell growth in the absence of complement is not well documented. Carey *et al.* (6) reported that the anticellular antibody of HeLa cells inhibited phagocytosis, Yang and Vas (30) showed that heat-inactivated antisera prepared against whole L5178YR cells inhibited cell growth, while Huang *et al.* (14) showed

that antiserum made against HeLa cell membrane inhibited the growth of HeLa cells.

MATERIALS AND METHODS

Radioactive ¹²⁵I, [methyl-¹⁴C]thymidine, [6-³H]uridine, and [2-¹⁴C]glycine were purchased from New England Nuclear, Boston, Mass. Glucose oxidase (EC 1.1.3.4), PMSF,⁴ 2,4-dinitrophenyl phosphate, lactoperoxidase (EC 1.11.1.7), and disodium phenyl phosphate were obtained from Sigma Chemical Co., St. Louis, Mo. The complete Freund's adjuvant (Perrine's modification) was from Calbiochem, Los Angeles, Calif. Ficoll was from Pharmacia Fine Chemicals, Uppsala, Sweden. Fischer's medium and horse serum were from Grand Island Biological Co., Grand Island, N. Y. All other chemical reagents were of reagent grade.

Cell Harvest. P388 cells were grown in suspension culture in Fischer's medium supplemented with 10% horse serum (37°, 5% CO₂-95% air). The doubling time of P388 cells is about 14 hr. Log-phase cells were harvested at the density of 6 to 8 × 10⁵ cells/ml, centrifuged at 300 × g for 5 min, and washed twice with ice-cold Fischer's medium.

Iodination Procedure. P388 cells should not be kept in isotonic phosphate at pH 7.0 or higher because a portion of these cells tends to lyse and clump. The iodination was performed in 0.25 M sucrose (buffered with 10 mM phosphate, pH 7.4). About 8 × 10⁸ cells were washed with 40 ml cold 0.9% NaCl solution and suspended in 5 ml iodination solution (isotonic sucrose with 10 mM phosphate at pH 7.4, 10 μM lactoperoxidase, 10 mM glucose, 5 milliunits glucose oxidase, and 50 to 100 μCi carrier-free ¹²⁵I). Iodination was performed for 15 to 20 min at 4°. After the reaction, the cells were washed 3 times with cold Fischer's medium and once with cold 0.9% NaCl solution. More than 95% of these iodinated and washed cells remained trypan blue negative.

Isolation of Plasma Membranes. All operations were done at 0-4°, packed cells, 1 ml, (8 × 10⁸ cells) were resuspended in 9 ml homogenization medium that was specifically designed for the P388 cells. The medium contained 1 mM CaCl₂, 1 mM MgCl₂, 1 mM PMSF, and 1.6% Ficoll in 10 mM Tris-HCl, pH 7.4. A 5-ml B-D syringe fitted with a 25G 5/8-inch needle was used to disrupt 3-ml portions of the cells.

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⁴ The abbreviations used are: PMSF, phenylmethylsulfonyl fluoride; TCA, trichloroacetic acid; SDS, sodium dodecyl sulfate.

Three forceful passes through the syringe against the side of a vial were fully adequate to disrupt each portion of cells. The suspension was then made isotonic by the addition of NaCl solution to a final concentration of 0.9% in order to maintain the nuclei intact. The nuclei were removed ($1000 \times g$ for 1 min) and the pellet was washed with 5 ml homogenization medium. The combined supernatant solutions (14 ml) were mixed with 4 ml 45% sucrose to yield a 10% sucrose solution. This was divided into 2 portions; each portion was applied on the discontinuous gradient (10 ml 45% sucrose-20 ml 3 sucrose) and centrifuged at $23,300 \times g$ (swinging bucket) for 25 min. The 30%-10% interface layer was diluted with 0.9% NaCl solution and pelleted ($32,800 \times g$ for 25 min); this was designated as the I-30 fraction. The total I-30 fraction was suspended in about 5 ml 15% sucrose-0.9% NaCl solution, applied on a 2nd discontinuous gradient (7 ml 45% sucrose, 14 ml 3 sucrose), and centrifuged for 25 min at $23,300 \times g$. The 30%-15% interface was diluted with 0.9% NaCl solution and pelleted ($32,800 \times g$ for 25 min). This pellet was suspended in 0.9% NaCl solution and designated the II-30 membrane fraction. Early experiments indicated that plasma membranes of leukemic cells tended to degrade in homogenates or while standing in sucrose gradients. This degradation could be inhibited by PMSF, and it has been routinely incorporated in the isolation procedures.

Enzyme Assays

5'-Nucleotidase (5-Ribonucleotide Phosphohydrolase, EC 3.1.3.5). Activity was measured by a modification of the method published by Chen *et al.* (8). A 20- to 100- μ l sample (either homogenate or membrane fraction) was added to 1 ml glycine buffer that contained 0.1 M glycine (pH 8.5), 10 mM $MgCl_2$ -5 mM 5'-AMP. After incubation at 37° for 15 min, 1 ml 10% TCA was added, the mixture was centrifuged at $1000 \times g$, and 1 ml supernatant was mixed with 3 ml 0.4 N sodium acetate to bring the pH to about 4; 0.3 ml 1% ascorbic acid in 0.3 ml 1% ammonium molybdate solution was added to measure the P_i released (18). The absorbance was read at 700 nm after 20 min.

Alkaline Phosphatase (Orthophosphoric Monoester Phosphohydrolase, EC 3.1.3.1). Activity was measured by the method described in the Worthington manual (28) by following the increase in absorbance of nitrophenol at 410 nm.

Succinic Dehydrogenase (Succinate-Cytochrome *c* Reductase, EC 1.3.99.1). Activity was measured by the increase in absorbance at 550 nm. A 20- to 50- μ l sample was added to 1.2 ml phosphate buffer (20 mM phosphate at pH 7.0 with 1% bovine serum albumin) at room temperature. In addition, 0.1 ml of 1% cytochrome *c* was added and mixed. The substrate solution, 0.4 ml (50 mM succinate plus 10 mM KCN), was then added and mixed quickly, and the absorbance at 550 nm was read immediately (12).

NADPH-Cytochrome *c* Reductase (EC 1.6.2.3). A 10- to 50- μ l sample was added to 0.9 ml phosphate buffer (0.1 M phosphate, pH 7.4). One % cytochrome *c*, 0.1 ml, was added

and mixed. The reaction was initiated by addition of 0.1 ml 1 mM NADPH and the absorbance change at 550 nm was read. The activity of enzyme was expressed as nmoles cytochrome *c* reduced per min per mg protein.

Assay of Cytochrome P-450. A 50- to 100- μ l sample was added to 1 ml phosphate buffer (0.1 M, pH 7.5, with 1 mM KCN) in the cuvet. The baseline was taken with an Aminco-Chance dual wavelength spectrophotometer. Dithionite was added, the spectrum was taken, carbon monoxide was bubbled for 20 sec, and the spectrum was taken again. The absorption difference in the region of 450 nm was taken to measure the amount of P-450 (22).

Glucose 6-Phosphate (EC 3.1.3.9). A 50- μ l sample was mixed with 0.3 ml maleic buffer (0.1 M, pH 6.5) and 0.1 ml of 0.1 M glucose 6-phosphate. The reaction was terminated by addition of 1 ml cold 10% TCA and was centrifuged at 1500 rpm for 5 min to remove the proteins (24). The P_i in 1 ml supernatant was measured by the method described before.

Gel Electrophoresis. The membranes were dissolved in 2 to 3% SDS with 5% 2-mercaptoethanol and were immediately heated at 100° for 3 min. Disc gel electrophoresis was performed as described previously (26). SDS gradient slab gel (7.5 to 15%) was prepared and run essentially according to the procedure of Alvares and Siekevitz (1).

Electron Microscopy. Electron microscopic examination of plasma membranes was performed after fixation in 2.5% glutaraldehyde in 0.1 M cacodylate buffer, pH 7.4. The pellets were then transferred to 1% OsO_4 containing cacodylate buffer (0.1 M, pH 7.4), dehydrated with ethanol, and embedded in Vestopal-W (15).

Preparation of Antiserum against L1210 Plasma Membranes and P388 Plasma Membranes. Purified plasma membranes (3 mg protein per ml 0.9% NaCl solution, well mixed with an equal volume of complete Freund's adjuvant, were injected s.c. into the back of New Zealand White rabbits. Every 2nd week the membrane suspension in 0.9% NaCl solution (0.5 ml, about 2 to 3 mg protein per ml) was injected i.d. into the foot pad of the rabbits. Blood samples were collected 1 week after each injection. The serum titer was determined and the serum was stored in the freezer at -70°.

Determination of the Titer of Antiserum. The serum titer was determined by the passive agglutination method (3). Tanned goat RBC were prepared as described by Mitchell *et al.* (19). The sensitized, tanned RBC for the plasma membranes were prepared by incubating the tanned RBC with plasma membranes that were solubilized in 2% Triton X-100 for 30 min at 22°. They were then used to determine the antibody titer of the rabbit serum with the microtitration plates.

The Effects of Anti-L1210 and Anti-P388 Serum on the Growth of L1210, P388, L5178Y, and HeLa Cells. Rabbit antisera were added to 10 ml cells (4 to 5×10^4 cells/ml); the doubling times of the cells were determined with a Coulter counter. Both the serum supplement used in growth medium as well as the rabbit antisera were heated at 56° for 45 min. The complement titration experiment showed that by the end of this procedure no complement was present. In

addition, the cells had been maintained for more than 6 generations in the complement-free medium before the assays were performed. L1210, P388, and L5178Y cells were maintained in Fischer's growth medium. HeLa cells were maintained in spinner culture in Joklik's modified minimum essential medium supplemented with 10% fetal calf serum and 2 mM glutamine. Rabbit antisera were sterilized by passing through a Millipore filter (20).

Metabolic Studies. Two 100-ml suspension cultures (2×10^5 cells/ml) were prepared; the control contained 5 μ l of normal rabbit serum per ml, and the experiment contained 5 μ l of rabbit antiserum per ml. At intervals, 5-ml cell suspensions were placed in Falcon culture tubes (16 x 125 mm) in the presence of 1 of the following radioactive precursors: [14 C]glycine (specific activity, 0.25 mCi/0.58 mg), [14 C]thymidine (specific activity, 0.25 mCi/12 mg), or [3 H]uridine (specific activity, 1 mCi/0.0078 mg) to yield final concentrations of 1, 4, and 1 μ Ci/ml, respectively, and were incubated for 60 min at 37°. The incorporation was stopped by chilling, viable cell counts were made, and the acid-insoluble radioactivity was determined (4).

RESULTS

Purity of the P388 Plasma Membrane Preparation. Three criteria were used to define the purity of the plasma membrane preparations. These were: (a) the specific activity of covalently labeled 125 I; (b) the specific activity of the enzyme markers; and (c) electron microscopy. Table 1 shows the distribution of 125 I among various subcellular fractions. The membrane preparation has a specific activity of 8- to 13-fold higher than that of the cell homogenate. The protein yield is about 1 to 2% and the yield of the TCA-insoluble radioactivity is about 10 to 2. Table 2 shows the enzyme activities of the P388 membrane preparation. The pattern of enzyme activities is very similar to that of the L1210 plasma membrane preparation, except that the specific activity of the 5'-nucleotidase is only about one-half that of L1210 plasma membrane (7). As shown in Table 2, the increase in the specific activity of alkaline phosphatase

and of the 5'-nucleotidase together with the more than 10-fold increase of the specific activity of 125 I indicate a relatively pure plasma membrane preparation. The lack of succinic dehydrogenase activity in the membrane preparation is a good indication of the absence of mitochondria particles. The total activities of NADPH-cytochrome *c* reductase and glucose-6-phosphate in the membrane were 80 and 60% of the cell homogenate, respectively. The microsomal contamination cannot be excluded; however, Hinton (13) has discussed in detail that the increase of the purity of plasma membrane does not necessarily parallel a proportional decrease of microsomal enzyme activities. Electron microscopy of the P388 plasma membrane (Fig. 1) shows that they form small vesicles and no other cell organelles could be detected.

Comparison of the SDS-Polyacrylamide Gel Electrophoretic Pattern of Plasma Membranes of P388 Cells and of L1210 Cells. Although the SDS-polyacrylamide disc gel electrophoresis system of Lenard (17) resolved the 125 I-labeled proteins of HeLa cells very well, it did not give a good resolution for the plasma membrane proteins of P388 and L1210 cells (Chart 1). The distinctive M.W. 13,000, 125 I-labeled component moved ahead of the bromophenol blue marker. It appeared very clearly on the gradient slab gel (Fig. 2) but always ran past the bromophenol blue marker on a plain slab gel or disc gel as shown in both Chart 1 and Fig. 3. Slab gel electrophoresis has been used successfully in resolving microsomal cytochrome P-450 components (1). We found that both the gradient slab gel system (7.5 to 15% acrylamide) and plain slab gel system (7.5% acrylamide) gave us satisfactory separation of both L1210 and P388 plasma membrane proteins. Fig. 2 shows both the autoradiographs and Coomassie blue-stained protein patterns of P388 and L1210 plasma membranes on the gradient slab gels. The autoradiographs were exposed for a very short period in order to show the most predominantly labeled bands. Both cell lines have similar heavily labeled bands at molecular weights of 85,000 and 13,000 daltons. The resolution in the higher-molecular-weight region of the labeled proteins of P388 and L1210 cell membranes is better on a 7.5% plain slab gel (Fig. 3). Cross-marks show the

Table 1

Distribution of 125 I among subcellular fractions of P388 cells

P388 cells were homogenized in 10 mM tris-HCl buffer (pH 7.4) containing 1.6% Ficoll, 1 mM MgCl_2 , 1 mM CaCl_2 , and 1 mM PMSF. The supernatant fraction was obtained from the homogenate after 1 min centrifugation at $1000 \times g$. II-30 represents the plasma membrane fraction obtained from the 30%-15% interface of the discontinuous sucrose gradient. II-45 represents the 45%-30% interface fraction of the discontinuous sucrose gradient.

	Protein (mg/fraction)	TCA insoluble ($10^{-3} \times \text{cpm/fraction}$)	Specific activity (cpm/mg)	Relative specific activity	Radio- activity yield (%)	Protein yield (%)
Homogenate	80.1	2091.6	26.1	1.0	100	100
Supernatant	74.5	1921.2	25.8	0.99	92	93
II-30	1.78	490.6	275.6	10.6	23.5	2.2
II-45	5.26	124.1	23.6	0.9	5.9	6.6

Table 2

Enzyme activities of the homogenate and plasma membranes of P388 cells

P388 cells were homogenized in 10 mM tris-HCl buffer (pH 7.4) containing 1.6% Ficoll, 1 mM $MgCl_2$, 1 mM $CaCl_2$, and 1 mM PMSF. Membranes were obtained after 2 discontinuous sucrose gradient centrifugations. Enzyme activities were assayed by addition of either homogenate or plasma membranes to the assay mediums. Relative specific activities were calculated, taking the enzyme activities present in the homogenate as 1.

Enzyme	Specific activity	Relative specific activity
5'-Nucleotidase (nmoles P_i /min/mg protein)		
Homogenate	110	1
Membrane fraction	1000	9
Alkaline phosphatase (nmoles P_i /min/mg protein)		
Homogenate	30	1
Membrane fraction	674	22
NADPH-cytochrome <i>c</i> reductase (nmoles cytochrome <i>c</i> reduced/min/mg protein)		
Homogenate	11	1
Membrane fraction	9	0.8
Cytochrome P-450 hydroxylase		
Homogenate	Nondetectable	0
Membrane fraction	Nondetectable	0
Glucose-6-phosphatase (nmoles P_i /min/mg protein)		
Homogenate	5	1
Membrane fraction	3	0.6
Succinic dehydrogenase (nmoles cytochrome <i>c</i> reduced/min/mg protein)		
Homogenate	48	1
Membrane fraction	0	0

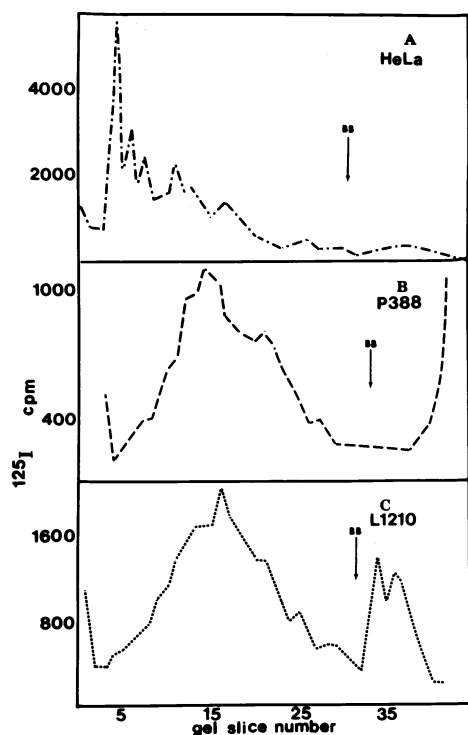


Chart 1. The radioactive profile of SDS-polyacrylamide disc gel electrophoresis of ^{125}I -labeled membrane proteins of HeLa, P388, and L1210 cells (gel concentration, 7.5%, 1.5% cross-linking; 100 ma/gel; 0.1 M phosphate buffer, pH 7.0). BB, bromophenol blue.

location of radioactive bands revealed by autoradiography. Iodinated protein bands common to both L1210 and P388 cells have molecular weights of 175,000, 165,000, 135,000, 105,000, and 85,000. The radioactive band of 13,000 daltons runs off the gel in this system. The Coomassie blue-stained protein patterns for both cell lines are also very similar. A major protein appeared at 50,000 daltons. The amount of this peptide in the membranes fraction has been shown to be proportional to the purity of the membrane preparation of L1210 cells (7); this is also true for P388 cells.

Titer of Antiserum Prepared against L1210 Plasma Membrane. Chart 2 shows the titration pattern of the titer of the antiserum prepared against L1210 plasma membrane by using the passive hemagglutination technique. Since the RBC were sensitized with solubilized membrane proteins, this method detects specifically the antibodies against the membranes. In Chart 2, we can see that the highest titer was obtained at 256 after a 2nd intradermal boost.

The Effect of Anti-L1210 Serum on the Growth of L1210, P388, L5178Y, and HeLa Cells. In order to study the effect of antibody on the cell growth, all the experiments were carried out in the absence of complement. Chart 3 shows the effect of anti-L1210 on the growth of L1210 cells. Antiserum at 5×10^{-3} dilution completely inhibits the cell growth. Anti-L1210 serum was similarly inhibitory to the growth of P388 as shown in Chart 4. However, anti-L1210 serum was less potent to L5178Y cells and had almost no effect on the growth of HeLa cells (Chart 5). In these experiments, we used both normal rabbit serum and anti-

Fig. 1. Electron microscopic picture of P388 plasma membranes stained with 1% OsO_4 . The relative specific activity of labeled ^{125}I of this membrane preparation is $11. \times 99,000$.



bovine serum albumin serum as our control. We found that neither control serum has any effect on the growth of L1210, P388, and HeLa cells whether in the presence or in the absence of complement. Similar results were obtained with anti-P388 serum (Chart 6).

Metabolic Studies. Anti-L1210 serum at 2×10^{-3} dilution partially inhibits the incorporation of glycine, uridine, and thymidine into the L1210 cells as shown in Charts 7 to 9; it has similar effects on P388 cells. Corresponding inhibitions were obtained with anti-P388 for these cells.

DISCUSSION

The P388 and L1210 cells are originally both derived from DBA mice with the same carcinogen, methylchol-

threne (11). Both cell lines can be carried either in ascites form in DBA mice or in suspension culture. Both cell lines have the same doubling time and very similar membrane protein patterns; consequently, we may expect that the membrane isolation procedure for L1210 cells (25) should also apply to P388 cells, but this is not the case. The presence of Ficoll, which has been used in other cellular fractionation work (2), is critical to the isolation of the P388 cell membranes and, in addition, a different homogenization medium was required for the isolation of the P388 cells. This result clearly shows that, despite many similarities between these 2 cell lines, some delicate and important differences must exist as defined by this criterion.

The membrane preparations that we used for enzyme assay work have relative specific activity values of 10. The

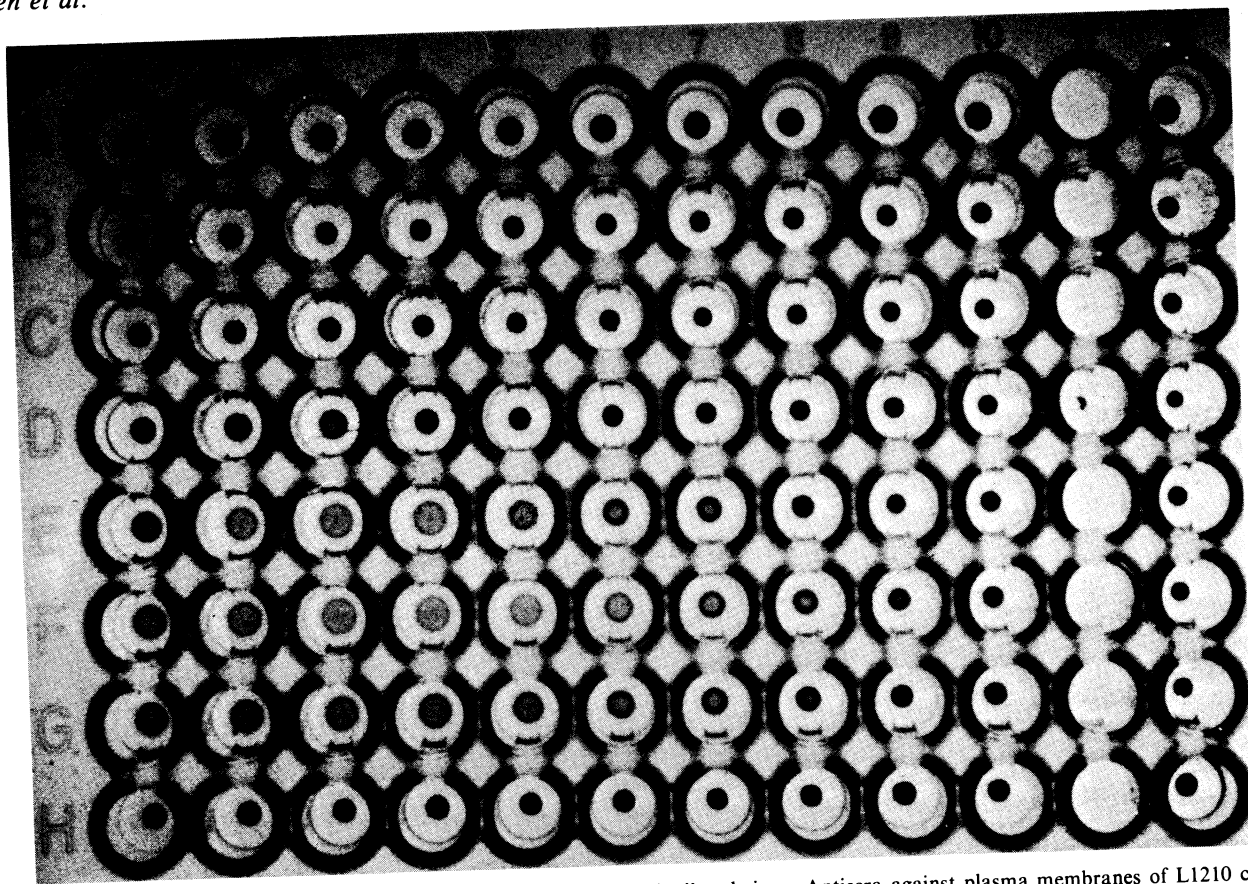


Chart 2. Typical titration patterns observed with the "passive agglutination" technique. Antisera against plasma membranes of L1210 cells from rabbits obtained at various periods after injections were titrated in Rows A to H. Wells 1 to 10 contain 2-fold dilutions of antiserum; Well 12 contains only diluent and RBC. A, 2.5% tanned unsensitized RBC with normal rabbit serum; B, 1.25% tanned sensitized RBC with rabbit serum before injection; C, 1.25% tanned sensitized RBC with rabbit antiserum 2 weeks after 1st injection. End point, Well 1. D, 1.25% tanned sensitized RBC with rabbit antiserum 1 week after 2nd boost. End point, Well 8. F, 1.25% tanned sensitized RBC with rabbit antiserum 1 week after 3rd boost. Each point, Well 9. G, 1.25% tanned sensitized RBC with rabbit antiserum against bovine serum albumin (after 4 injections).

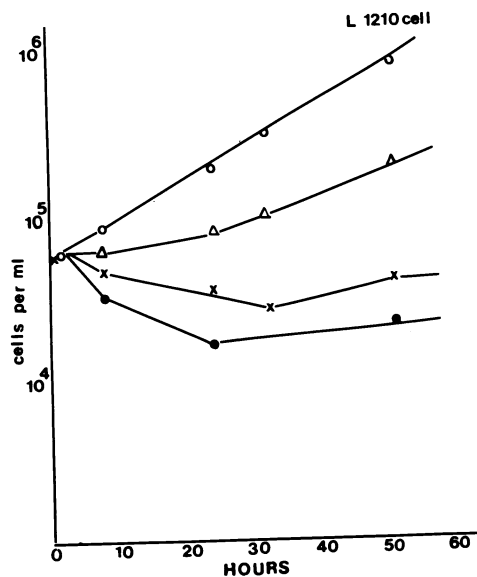


Chart 3. Effect of antiserum made against L1210 cell plasma membranes on the cell growth of L1210 cells in the complement-free medium. O, control, anti-bovine serum albumin at 2×10^{-2} dilution; Δ , antiserum

increase of 5'-nucleotidase activity in the membrane fraction is about 10-fold compared to a corresponding 20-fold increase for the L1210 membranes. However, another cell surface enzyme marker, alkaline phosphatase, showed a 22-fold increase of activity for the P388 plasma membranes. As discussed by DePierre and Karnovsky (9), the enzymatic markers can be used only supplementarily and not conclusively as the criteria for the membrane purity, because the enzyme activities are subject to many "environmental" factors such as pH, buffers, and ionic strength.

The acrylamide gradient slab gel system has much greater resolving power than did the disc gel system (22). The gradient slab gel system shows more than 30 Coomassie blue staining bands for both P388 and L1210 cells. The most prominent protein band (50,000 daltons) appears in proportion to the degree of purity of the plasma membrane preparations (7); however, it was only very slightly labeled by ^{125}I . Neville and Glossmann (21) indicated that a protein of 48,000 daltons was a major component for the rat liver,

at 1×10^{-3} dilution; \times , antiserum at 5×10^{-3} dilution; \bullet , antiserum at 2×10^{-2} dilution.

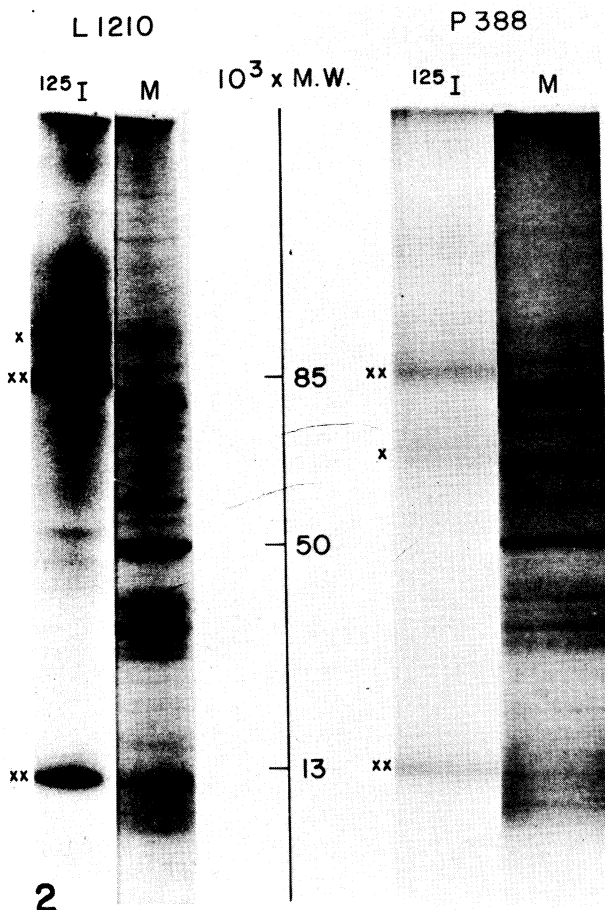


Fig. 2. Autoradiographs (^{125}I) and Coomassie blue-stained protein profiles (m) of gradient slab gel electrophoresis of the plasma membranes of P388 and L1210 cells (a gradient of 7.5% to 15% gel, 2.7% cross-linking). X, location of radioactive bands; XX, major radioactive band.

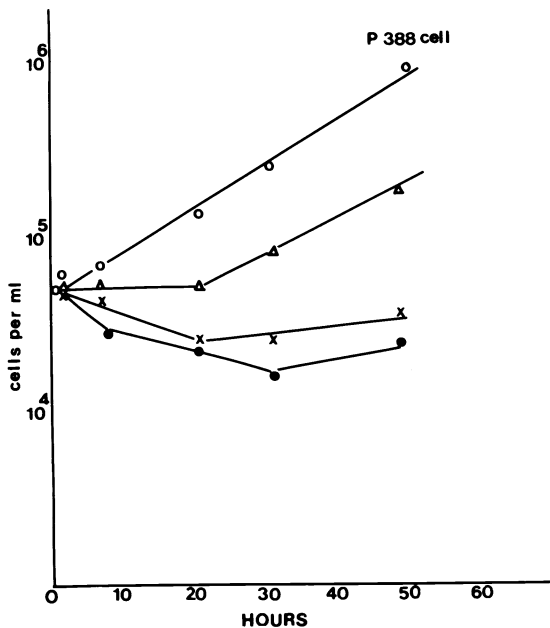


Chart 4. Effect of antiserum made against L1210 cell plasma membranes on the cell growth of P388 cells in the complement-free medium. O,

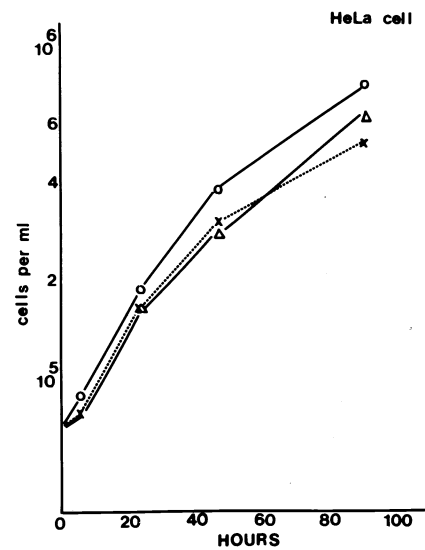


Chart 5. Effect of antiserum made against L1210 cell plasma membranes on the cell growth of HeLa cells in the complement-free medium. C control, anti-bovine serum albumin at 2×10^{-2} dilution; X, antiserum at 1×10^{-2} dilution; Δ, antiserum at 2×10^{-2} dilution.

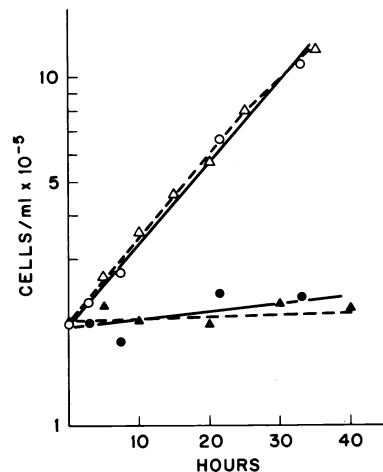


Chart 6. Effect of antiserum made against P388 cell plasma membranes on the cell growth of P388 cells and L1210 cells in the absence of complement. O, P388 cells with normal rabbit serum; ●, P388 cells with antiserum at 1×10^{-2} dilution; Δ, L1210 cells with normal rabbit serum; ▲, L1210 cells with antiserum at 1×10^{-2} dilution.

kidney, and erythrocyte membranes. This may coincide with our 50,000-dalton protein; however, it cannot be an exposed protein, as indicated by the lack of ^{125}I labeling. The iodinated protein bands for both cell lines are strikingly similar except for minor differences in the region between 20,000 and 70,000 daltons; however, the similarity is only qualitatively shown in Figs. 2 and 3. The antisera prepared against either cell plasma membranes showed cross-reactivity toward each other. If the exposed iodinated proteins possess potent antigenicity, then even the minor differences

control, anti-bovine serum albumin at 2×10^{-2} dilution; Δ, antiserum at 1×10^{-3} dilution; X, antiserum at 5×10^{-3} dilution; ●, antiserum at 2×10^{-2} dilution.

Fig. 3. The radioactive (^{125}I) and protein profiles (m) of the slab polyacrylamide gel electrophoresis of P388 and L1210 plasma membranes (7.5% gel, 2.7% cross-linking). Numbers on the right, molecular weights using human α (94,000), bovine serum albumin (67,000), ovalbumin (45,000), and chymotrypsinogen A (25,000) as markers. X, correspondent radioactive bands.

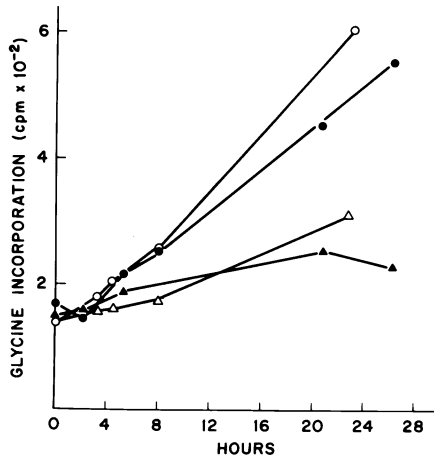
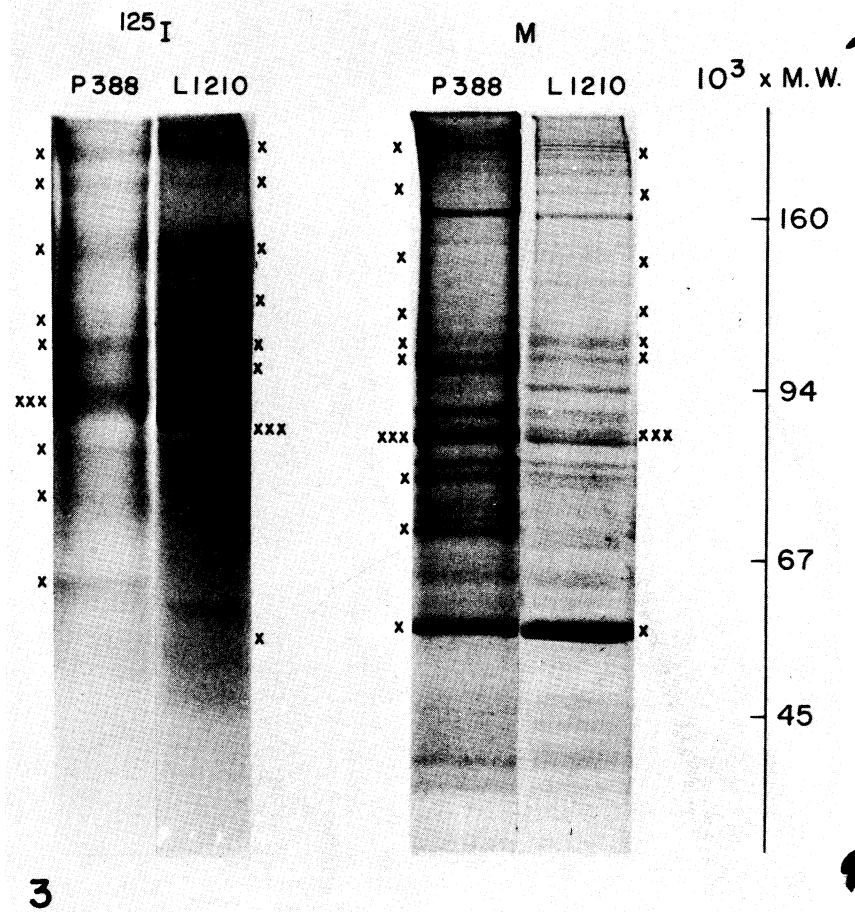


Chart 7. Glycine incorporation study, O, L1210 cells with normal rabbit serum; Δ , L1210 cells with anti-L1210 serum at 2×10^{-3} dilution; \bullet , P388 cells with normal rabbit serum; \blacktriangle , P388 cells with anti-L1210 serum at 2×10^{-3} dilution.

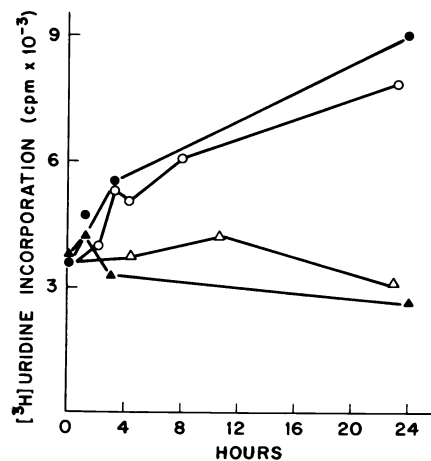


Chart 8. Uridine incorporation study, O, L1210 cells with normal rabbit serum; Δ , L1210 cells with anti-L1210 serum at 2×10^{-3} dilution; \bullet , P388 cells with normal rabbit serum; \blacktriangle , P388 cells with anti-L1210 serum at 2×10^{-3} dilution.

between the ^{125}I -labeled profiles of P388 and L1210 will be significant.

The inhibitory effect of the heat-inactivated antiserum on cell growth has been reported by Yang and Vas (29, 30) for the L5178YR cells and by Huang *et al.* (14) for the HeLa cells. Yang and Vas prepared their antiserum by immuniz-

ing the rabbit with whole intact cells, whereas we immunized the rabbit with preparations of plasma membranes, and our antisera have at least a 20-fold higher titer. The mechanism of inhibition of cell growth in the absence of complement is unknown. This inhibitory effect became prominent only 10 to 12 hr after adding the antiserum.

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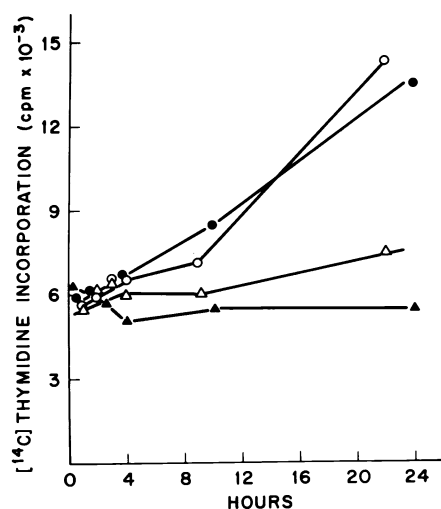


Chart 9. Thymidine incorporation study. ○, L1210 cells with normal rabbit serum; △, L1210 cells with anti-L1210 serum at 2×10^{-3} dilution; ●, P388 cells with normal rabbit serum; ▲, P388 cells with anti-L1210 serum at 2×10^{-3} dilution.

Preliminary studies showed that the incorporation of thymidine into DNA, uridine into RNA, and glycine into proteins was partially inhibited. However, there was still a residual incorporation by cells in the presence of antiserum and most cells are still viable, as judged by trypan blue exclusion test. Recently, many groups have studied the interactions of lectins, mitogens, and hormones with the membrane receptors as well as the correlation between such interactions and the regulation of cell growth (10, 16). For example, concanavalin A has been reported to be mitogenic at lower concentrations (5) but cytotoxic at higher concentrations (23). The intracellular cyclic adenosine 3':5'-monophosphate level can be modulated by concanavalin A (5). Although we do not know the exact molecular mechanisms of these phenomena, there is no doubt that the interaction between the membrane receptor and ligands is the primary requirement which then triggers various cell activities. In view of this, we believe that the antiserum made against the plasma membrane proteins or a single antibody made against a specific membrane protein constitutes a very good system to study the cytotoxic mechanism and the regulation of cell growth by its plasma membrane.

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