Isolation and Characterization of Membrane Receptors for Pokeweed Mitogens from Mouse Lymphocytes

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The major glycoproteins that bind pokeweed B-cell mitogen (Pa-1) and pokeweed T-cell mitogen (Pa-2) were isolated and identified from bone-marrow-derived lymphocytes (B-cells) and thymus-derived lymphocytes (T-cells) of C3H/He mice. The surfaces of the cells were ^{125}I -labelled by using the enzyme lactoperoxidase, and the plasma membranes were isolated from the ^{125}I -labelled cells. These membranes were solubilized with Triton X-100 and subjected to affinity chromatography on the affinity adsorbent prepared by coupling mitogen Pa-1 or Pa-2 to activated Sepharose 4B. The glycoproteins specifically eluted with di-*N*-acetylchitobiose from the affinity adsorbents were analysed according to their mobility on polyacrylamide-gel electrophoresis in sodium dodecyl sulphate. These glycoproteins were further identified by immunoprecipitation with specific antisera. Immunoglobulins, possibly immunoglobulins M and D, were identified in the eluate from the B-cell membranes, but they were not detected in the eluate from the T-cell membranes. The histocompatibility-2-complex proteins (H-2D, H-2K and Ia antigens) were found to be major receptor sites for the pokeweed mitogens on both B-cells and T-cells. However, mitogen Pa-1 (B-cell) has a stronger affinity to Ia antigens than does mitogen Pa-2 (T-cell).

The transformation of lymphocytes into blast cells by various lectins serves as an interesting model for the study of the mechanism of cell transformation from resting state to growing state. Since various sugars and glycopeptides have been found to show inhibitory activity against the transformation of lymphocytes by lectins, this phenomenon appears to stem from the initial binding of the lectins to certain receptor sites of a glycoprotein nature on the lymphocyte cell surface (Lis & Sharon, 1973). Recent investigation has indicated that the lectins that preferentially bind to the sugar chains of the type found in serum glycoproteins, containing *O*- β -D-galactopyranosyl – *O*-(2-acetamido-2-deoxy- β -D-glucopyranosyl)-D-mannose sugar sequence, show strong mitogenic activity against lymphocytes (Irimura *et al.*, 1975). This suggests that the receptor glycoproteins for mitogenic lectins on the lymphocyte cell surface may contain sugar chains of this type. At present, however, information on the actual receptor sites for mitogenic lectins on the lymphocyte cell surface is limited. Cell-surface immunoglobulins of B-cells and cell-surface H-2-complex proteins of T- and B-cells were identified by Nilsson & Waxdal (1976) as the major concanavalin A-binding glycoproteins of mouse lymphocytes.

We have reported (Yokoyama *et al.*, 1976) the specific purification of five mitogens from roots of

pokeweed (*Phytolacca americana*) by use of affinity chromatography. Of these mitogens, only Pa-1 is mitogenic for both mouse B- and T-cells, and the others are T-cell mitogens, of which mitogen Pa-2 exhibits the strongest mitogenic activity.

In the present paper, we describe the isolation and characterization of membrane glycoproteins which serve as receptors for pokeweed mitogens (Pa-1 and Pa-2) from mouse B- and T-cells.

Materials and Methods

Animals

C3H mice were obtained from the Institute of Medical Science, University of Tokyo.

Mitogens

Pa-1 and Pa-2 mitogens were purified from pokeweed roots by the method described previously (Yokoyama *et al.*, 1976).

Preparation of lectin–Sepharose 4B affinity adsorbents

Pokeweed mitogens (Pa-1 and Pa-2) were coupled to activated Sepharose 4B by the method described by Matsumoto & Osawa (1972).

Preparation of B- and T-cells

(a) *B-cells*. Spleens of C3H/He mice were teased

apart at 4°C in Hanks balanced-salt solution (Hanks & Wallace, 1949; Nissui Co., Tokyo, Japan). After removal of clumps by sedimentation for 10 min, cells were collected by centrifugation (400g for 5 min), washed twice with the same medium and suspended in foetal calf serum (Grand Island Biological Co., Madison, WI, U.S.A.). Purified lymphocytes were obtained by subjecting the cell suspension to Ficoll (Pharmacia Fine Chemical, Uppsala, Sweden)–Urografin (Schering, Berlin, Germany)–density-gradient centrifugation as described by Kawaguchi *et al.* (1974). The purified lymphocytes were then treated with anti- θ (C3H) serum prepared by the method of Golub (1971) and complement (Toshiba Chemical, Tokyo, Japan). After washing twice with Hanks balanced-salt solution, the cells were purified again by Ficoll–Urografin density-gradient centrifugation as described by Kawaguchi *et al.* (1974). In some experiments, B-cells were prepared from thymectomized mice (C3H/He) by the method described previously (Yokoyama *et al.*, 1976). The average proportion of lymphocytes in the B-cell preparation obtained as described above was more than 98%, and their viability was found to be 98–100% by the Trypan Blue-exclusion test. Further, when B-cells were enumerated by direct immunofluorescence (Möller, 1961; Lubaroff & Waksman, 1968), by using fluorescein-conjugated goat anti-(mouse immunoglobulin) serum (Hyland, Los Angeles, CA, U.S.A.), the average proportion of cells positive to membrane immunoglobulin was more than 95%.

(b) *T-cells (corticosteroid-resistant thymocytes)*. C3H/He mice were injected intraperitoneally with 2.5 mg of cortisone acetate (Banyu Pharmaceutical Co., Tokyo, Japan), and 2 days later the animals were killed and the cells obtained by teasing their thymus glands were purified by Ficoll–Urografin density-gradient centrifugation.

Labelling of lymphocytes

¹²⁵I-labelling of lymphocytes was performed by virtually the same method as that described by Phillips & Morrison (1971). To the suspension of lymphocytes (1×10^8 cells) in 10 mM-sodium phosphate buffer containing 0.15 M-NaCl, pH 7.4, were added 3.6 munits (3600 μ mol/min) of lactoperoxidase (Boehringer Mannheim, Mannheim, Germany) and 500 μ Ci of Na¹²⁵I (New England Nuclear Corp., Boston, MA, U.S.A.). Then 3.6 munits of glucose oxidase (Boehringer) and 5 μ mol of glucose were added to the mixture. After incubation for 30 min at 20°C, 10 ml of ice-cold 10 mM-sodium phosphate-buffered saline, pH 7.4, containing 10 mM-NaI was added to stop the reaction. The labelled cells were then washed several times with ice-cold 10 mM-sodium phosphate-buffered saline, pH 7.4.

Isolation of plasma membranes

Plasma membranes of the lymphocytes were isolated by the methods of Kornfeld & Siemers (1974) and Misra *et al.* (1975) with a few modifications. The lymphocytes (5×10^8 cells) were suspended in 1 ml of ice-cold 10 mM-Tris/HCl buffer/0.15 M-NaCl, pH 7.4, containing 1 mM-EDTA and 0.01 μ M-di-isopropyl phosphorofluoridate. To this cell suspension, 2 vol. of 10 mM-Tris/HCl buffer, pH 7.4, containing 1 mM-EDTA and 0.01 μ M-di-isopropyl phosphorofluoridate were added slowly while the cell suspension was being stirred. The mixture was left at 0°C for 5 min, and then 10 mM-Tris/HCl buffer/1.5 M-NaCl, pH 7.4, containing 1 mM-EDTA and 0.01 μ M-di-isopropyl phosphorofluoridate was added to restore the NaCl concentration to 0.15 M. Then the cells were homogenized in a loosely fitting glass Potter–Elvehjem homogenizer, by using five to seven gentle strokes. The homogenate was centrifuged at 980g for 10 min to remove the cell nuclei. The supernatant was collected and centrifuged again as above. The volume of this supernatant, free of nuclei, was measured, and an amount of 3 M-LiCl to give a final concentration of 0.1 M-LiCl was added to the supernatant, which was then centrifuged for 45 min at 45000g in a Hitachi 65P ultracentrifuge. The crude membranes thus obtained were suspended in 5 ml of 10 mM-Tris/HCl buffer/0.15 M-NaCl containing 1 mM-EDTA and 0.01 μ M-di-isopropyl phosphorofluoridate, and then homogenized to a fine suspension by three passes in a Potter–Elvehjem homogenizer. These resuspended crude membranes (microsomal fraction) were again centrifuged at 45000g for 45 min and the pellet was resuspended in 1.25 ml of 10 mM-Tris/HCl buffer, pH 7.4, and then mixed with an equal volume of 80% (w/v) sucrose in 10 mM-Tris/HCl buffer, pH 7.4, to give a 40% (w/v) sucrose solution. The microsomal fraction was then fractionated by centrifugation through a 40–30% (w/v) sucrose step gradient similar to that described by Allan & Crumpton (1970); 2.5 ml of a sucrose solution (30%, w/v) in 10 mM-Tris/HCl buffer, pH 7.4, were put on the sample in each tube, which was then centrifuged for 4 h at 55000g in a Hitachi 65P ultracentrifuge. After centrifugation each tube contained a band of milky-white plasma membranes at the interface between the 30 and 40% (w/v) sucrose layers.

Isolation of membrane receptors for pokeweed mitogens

The radioactively labelled microsomal fraction obtained from 5×10^8 cells was suspended in 1 ml of 56 mM-sodium borate buffer, pH 8.0. Triton X-100 (Wako Chemical, Tokyo, Japan) was added to a final concentration of 0.2%. The cell suspension was mixed vigorously and incubated at 4°C overnight.

Then 10 μ l of 1M-phenylmethanesulphonyl fluoride (Sigma Chemical Co., St. Louis, MO, U.S.A.) was added to inhibit enzymic breakdown of labelled proteins. After centrifugation at 15000g for 15min at 4°C, the Triton X-100 extract was applied to an affinity column (1cm $\times$ 15cm; Pa-1-Sephrose 4B or Pa-2-Sephrose 4B) equilibrated with 56mM-sodium borate buffer, pH8.0, containing 0.1% Triton X-100. Elution was carried out with the same buffer and, after the first peak had been eluted completely, the active fraction was eluted with 0.1M-di-N-acetylchitobiose solution containing 0.1% Triton X-100. Fractions (0.8ml) were collected at 5ml/h at 4°C. Radioactivity was tested for on a 100 μ l sample of each fraction in an Aloka autogamma counter. The specifically eluted fractions were pooled and dialysed against 50% (v/v) ethanol to remove the detergent. After extensive dialysis, this fraction was concentrated and analysed by sodium dodecyl sulphate/polyacrylamide-gel electrophoresis.

Sodium dodecyl sulphate/polyacrylamide-gel electrophoresis

The eluate from the affinity column and the immunoprecipitates were dissolved by boiling in an equal volume of 2% sodium dodecyl sulphate for 2min. 2-Mercaptoethanol was added to a final concentration of 5% (v/v) to reduce disulphide bonds. The discontinuous sodium dodecyl sulphate/polyacrylamide-gel electrophoresis system was used as described by Laemmli (1970). After electrophoresis, the gels were immersed in 10% (w/v) trichloroacetic acid for 12h. The fixed radioactivity in each slice was determined in an Aloka well-type autogamma counter. The polyacrylamide gel concentration was 10%. Mouse immunoglobulin μ -, γ - and light chains were used as reference proteins. The following proteins, obtained from Mann Research Laboratories (Orangeburg, NY, U.S.A.), were used as standards for the estimation of molecular weights (mol.wts. shown in parentheses): horse heart cytochrome *c* (12400), ox pancreas chymotrypsinogen A (25000), ovalbumin (45000), bovine serum albumin (67000) and human γ -globulin (160000). The distance of migration of the various standard proteins relative to the distance of migration of Bromophenol Blue are a linear function of the logarithm of the molecular weight of the standard proteins (Laemmli, 1970). Radioactive membrane proteins were mixed with unlabelled standard proteins before electrophoresis. The distance of migration of the membrane proteins was determined by measuring the radioactivity and those of the standard proteins by staining the gels with Coomassie Brilliant Blue.

Indirect precipitation of cell-membrane proteins

To the membrane proteins of B-cells specifically eluted from the Pa-1-Sephrose 4B column was

added 1% (v/v) Nonidet P-40 solution in 10mM-sodium phosphate-buffered saline, pH7.4, to a final concentration of 0.5%. Then 10 μ l of 1M-phenylmethanesulphonyl fluoride was added to inhibit enzymic breakdown of labelled proteins, and the mixture incubated for 30min at 4°C. The following specific immunoprecipitation experiments were always preceded by three precipitations by adding goat anti-(human IgG*) serum (Miles Laboratories, Kankakee, IL, U.S.A.) and an equivalent amount of human IgG (Miles). After centrifugation at 15000g for 15min, 0.1ml of rabbit anti-(mouse immunoglobulin) serum (Miles) was added and the mixture incubated at 37°C for 15min. Precipitation was caused by adding an equivalent amount of C3H/He mouse serum to provide a carrier protein. Incubation was allowed to proceed for 15min at 37°C and then for 10min at 4°C. The precipitates were centrifuged at 1700g for 10min, washed with 10mM-sodium phosphate-buffered saline, pH7.4, and subjected to sodium dodecyl sulphate/polyacrylamide-gel electrophoresis by the method of Laemmli (1970). The supernatants were then treated with a mouse alloantiserum specific for the H-2D antigen and rabbit anti-(mouse immunoglobulin) serum. The precipitates obtained were analysed by sodium dodecyl sulphate/polyacrylamide-gel electrophoresis. The same sandwich technique was also used to precipitate the H-2K antigen and I-region-associated antigens (Ia antigens) from the supernatant by using an antiserum specific for the H-2K antigen and Ia antigens and rabbit anti-(mouse immunoglobulin) serum.

The membrane proteins of T-cells specifically eluted from the affinity column were likewise analysed by using antisera specific for the H-2-complex proteins in the presence of 0.1% Nonidet P-40.

The alloantiserum specific for the H-2D antigen, (A $\times$ BALB $\cdot$ G)_F₁ anti-BALB $\cdot$ K, and the alloantiserum against the H-2K antigen and the Ia antigens, C3HOL anti-C3H, were kindly provided by Dr. S. G. Nathenson (Albert Einstein College of Medicine, Bronx, NY, U.S.A.).

Results and Discussion

The plasma-membrane fraction of radioactively labelled T-cells solubilized in 0.2% Triton X-100 was subjected to affinity chromatography on a column of Sepharose 4B coupled with Pa-2 mitogen, which is a specific mitogen for T-cells. Similarly, the labelled B-cell membrane solubilized with Triton X-100 were applied to a column of Sepharose 4B coupled with Pa-1 mitogen, which is strongly mitogenic towards B-cells. In both cases, approx. 10%

* Abbreviations: IgG, immunoglobulin G; IgM, immunoglobulin M (macroglobulin); IgD, immunoglobulin D; Ia antigens, I-region-associated antigens.

of the Triton X-100-extracted radioactivity remained bound to the column, even after extensive washing with 56mM-sodium borate buffer, pH 8.0, containing 0.1% Triton X-100. Since di-*N*-acetylchitobiose had been found to be a specific inhibitor of haem-agglutinating and mitogenic activities of Pa-1 and Pa-2 mitogens (K. Yokoyama, T. Terao & T. Osawa, unpublished work), the fractions of cell-membrane glycoproteins that were bound to the affinity adsorbents were eluted with 0.1M-di-*N*-acetylchitobiose in 56mM-sodium borate buffer, pH 8.0, containing 0.1% Triton X-100. The recovery of the retained radioactive glycoproteins by elution with sugar varied between 75 and 85%. A typical example of this chromatography is shown in Fig. 1. The specifically eluted fractions were then analysed by sodium dodecyl sulphate/polyacrylamide-gel electrophoresis after treatment with 2-mercaptoethanol. By comparison with the migration of the standard proteins, the apparent mol.wt. of the T-cell-membrane glycoprotein bound to the column of Pa-2-Sepharose 4B was estimated to be 42000, and those of the B-cell-membrane glycoproteins bound to the column of Pa-1-Sepharose 4B were 73000, 43000, 32000, 24000 and 23000 (Fig. 2a and Fig. 3). However, when the T-cell-membrane glycoprotein specifically eluted from the affinity column was subjected to sodium dodecyl sulphate/polyacrylamide-gel electrophoresis without treatment with 2-mercaptoethanol, the estimated mol.wt. for the radioactive peak observed was 98000 (Fig. 2b).

To correlate these radioactive peaks with the cell-surface components of T- and B-cells, immunoprecipitation with specific antisera was carried out. The specifically eluted fraction from the affinity chromatography of solubilized B-cell membranes was first treated with rabbit anti-(mouse immuno-

Fig. 1. Affinity chromatography of solubilized B-cell membranes on a Pa-1-Sepharose 4B column

Experimental details are described in the text. After a large peak was eluted, the column was further eluted (at the arrow) with 0.1 M-di-*N*-acetylchitobiose solution.

globulin) serum. Precipitation was effected by adding an equivalent amount of C3H/He mouse serum to provide a carrier protein. When the precipitates were subjected to sodium dodecyl sulphate/

Fig. 2. Sodium dodecyl sulphate/polyacrylamide-gel electrophoresis of T-cell-membrane proteins eluted with di-*N*-acetylchitobiose from Pa-2-Sepharose 4B

Experimental details are described in the text. (a) With 2-mercaptoethanol treatment, (b) without 2-mercaptoethanol treatment. The numbers in the Figure are the estimated molecular weights of the protein peaks indicated by arrows. μ , γ and L refer to positions of standard mouse immunoglobulin chains. The direction of protein migration is from left to right.

Fig. 3. Sodium dodecyl sulphate/polyacrylamide-gel electrophoresis of B-cell-membrane proteins eluted with di-*N*-acetylchitobiose from Pa-1-Sepharose 4B

The same symbols are used as described in the legend to Fig. 2. The experimental conditions are described in the text.

polyacrylamide-gel electrophoresis, three peaks were observed as shown in Fig. 4. On the basis of the approximate molecular weights estimated and the migration of mouse immunoglobulin μ -, γ - and light chains, the component of mol.wt. 72000 was tentatively identified as the μ -chain from cell-surface IgM, and the component of mol.wt. 63000 as the δ -chain from cell-surface IgD. These estimated molecular weights agree well with the values reported by other investigators (Melchers *et al.*, 1974; Finkelman *et al.*, 1975). However, the lack of specific antisera precludes the final characterization of these immunoglobulins. The protein peak with mol.wt. about 24000 is possibly the light chain of the cell-surface immunoglobulins. A controversy has existed as to the presence of cell-surface immunoglobulin on T-cells (Crone *et al.*, 1972; Vitetta *et al.*, 1972; Marchalonis & Cone, 1973; Cone & Marchalonis, 1974). In the present study we could not detect the immunoglobulins that were precipitable with rabbit anti-(mouse immunoglobulin) serum on T-cells. This cannot be explained by the presence of a steric barrier which hinders the ^{125}I -labelling of surface immunoglobulins on T-cells, because both of the affinity adsorbents used (Pa-1-Sepharose 4B and Pa-2-Sepharose 4B) failed to adsorb immunoglobulins from the solubilized plasma membranes of T-cells that had been radioactively labelled by culturing in media containing D-[^3H]glucosamine hydrochloride (results not shown).

The supernatant, which was obtained from the specifically eluted fraction from the affinity chromatography of solubilized B-cell membranes after the precipitation of the ^{125}I -labelled cell-surface immuno-

Fig. 4. Sodium dodecyl sulphate/polyacrylamide-gel electrophoresis of B-cell-membrane proteins eluted with di-N-acetylchitobiose from Pa-1-Sepharose 4B after immunoprecipitation by adding rabbit anti-(mouse immunoglobulin) serum and an equivalent amount of C3H/He mouse serum

The same symbols are used as described in the legend to Fig. 2. The experimental conditions are described in the text.

globulins with rabbit anti-(mouse immunoglobulin) serum, was further treated with an alloantiserum specific for the H-2D antigen or an alloantiserum specific for the H-2K and Ia antigens. Precipitation was caused by the sandwich technique by adding an equivalent amount of rabbit anti-(mouse immunoglobulin) serum. When the precipitates obtained by using the anti-H-2D serum were subjected to sodium dodecyl sulphate/polyacrylamide-gel electro-

Fig. 5. Sodium dodecyl sulphate/polyacrylamide-gel electrophoresis of the specifically eluted B-cell-membrane proteins from Pa-1-Sepharose which remained after immunoprecipitation with rabbit anti-(mouse immunoglobulin) serum

(a) Immunoprecipitation of H-2D antigen with an alloantiserum specific for the H-2D antigen. (b) Immunoprecipitation of H-2K and Ia antigens with an alloantiserum specific for the H-2K and Ia antigens. Precipitates were subjected to electrophoresis without 2-mercaptoethanol treatment. (c) Immunoprecipitated H-2K and Ia antigens subjected to electrophoresis after treatment with 2-mercaptoethanol. The same symbols are used as described in the legend to Fig. 2. The experimental conditions are described in the text.

phoresis, only one peak (approx. mol.wt. 42000) was observed (Fig. 5a). The precipitates with the alloantiserum specific for the H-2K and Ia antigens were likewise analysed by sodium dodecyl sulphate/polyacrylamide-gel electrophoresis with and without pretreatment with 2-mercaptoethanol. Without the 2-mercaptoethanol pretreatment, two peaks with mol.wts. of about 98000 and 58000 were observed, whereas three distinct peaks with mol.wts. about 42000, 31000 and 25000 were seen after 2-mercaptoethanol pretreatment (Figs. 5b and 5c). From these results and from the estimated molecular weights of the peaks, the protein of approx. mol.wt. 42000 could be the H-2D of H-2K antigen and the proteins of approx. mol.wts. 31000 and 25000 are possibly Ia antigens. These estimated molecular weights of H-2 and Ia antigens correspond closely to those reported by other workers (Schwartz & Nathenson, 1971; Cullen *et al.*, 1974; Rask *et al.*, 1974; Finkelman *et al.*, 1975).

In the same fashion, T-cell-membrane glycoproteins eluted with di-*N*-acetylchitobiose from a Pa-2-Sepharose 4B column were treated with the alloantiserum specific for the H-2D antigen or the alloantiserum specific for the H-2K and Ia antigens.

Fig. 6. Sodium dodecyl sulphate/polyacrylamide-gel electrophoresis of T-cell-membrane proteins eluted with di-*N*-acetylchitobiose from Pa-2-Sepharose 4B

(a) Immunoprecipitation with an alloantiserum specific for the H-2D antigen. (b) Immunoprecipitation with an alloantiserum specific for the H-2K and Ia antigens. The same symbols are used as described in the legend to Fig. 2. The experimental conditions are described in the text.

When the precipitates obtained by treatment with the alloantiserum specific for the H-2D antigen were subjected to sodium dodecyl sulphate/polyacrylamide-gel electrophoresis, only one peak with a mol.wt. about 42000 was observed (Fig. 6a). The alloantiserum specific for the H-2K and Ia antigens gave precipitates that showed three distinct peaks with approx. mol.wts. 42000, 31000 and 24000 on sodium dodecyl sulphate/polyacrylamide-gel electrophoresis (Fig. 6b). Thus the Ia antigens of T-cell membranes could be detected only after immunoprecipitation with the specific alloantiserum. The apparent absence of Ia antigens in Fig. 2(a) may be due to their low concentration on T-cells. The presence of Ia antigens on T-cells has also been indicated by Nilsson & Waxdal (1976) by analysing the con-

Fig. 7. Sodium dodecyl sulphate/polyacrylamide-gel electrophoresis of B-cell-membrane proteins eluted with di-*N*-acetylchitobiose from Pa-2-Sepharose 4B

The same symbols are used as described in the legend to Fig. 2. The experimental conditions are described in the text.

Fig. 8. Sodium dodecyl sulphate/polyacrylamide-gel electrophoresis of T-cell-membrane proteins eluted with di-*N*-acetylchitobiose from Pa-1-Sepharose 4B

The same symbols are used as described in the legend to Fig. 2. The experimental conditions are described in the text.

canavalin A-binding glycoproteins from purified T-cells.

To reveal the difference between the affinities of the two mitogens (Pa-1 and Pa-2) to these cell-surface antigens, the solubilized B-cell membranes were subjected to affinity chromatography on a column of Sepharose 4B coupled with the T-cell-specific Pa-2 mitogen. The di-*N*-acetylchitobiose eluate was analysed by sodium dodecyl sulphate/polyacrylamide-gel electrophoresis. As shown in Fig. 7, two distinct peaks with approx. mol.wts. 73000 and 42000 were observed, but the protein peaks corresponding to the Ia antigens could not be seen clearly, in spite of the fact that these antigens were observable in the electrophoresis of the di-*N*-acetylchitobiose eluate from Pa-1-Sepharose 4B as shown in Fig. 3. On the contrary, when the di-*N*-acetylchitobiose eluate from the affinity chromatography of the solubilized T-cell membranes on a column of Pa-1-Sepharose 4B was subjected to sodium dodecyl sulphate/polyacrylamide-gel electrophoresis, the protein peaks, which correspond to the Ia antigens having mol.wts. about 32000 and 23000, were seen clearly, in addition to a peak that corresponds to the H-2 antigen with a mol.wt. about 42000 (Fig. 8). These findings indicate that the B-cell mitogen, Pa-1, has a stronger affinity to Ia antigens than does the T-cell mitogen, Pa-2. The elucidation of the possible relationship of this fact to the different mechanisms of activation between B-cells and T-cells must await further investigation of cell-surface receptors of several other B-cell mitogens and T-cell mitogens.

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