ISOLATION AND CHARACTERIZATION OF MURINE CELL SURFACE COMPONENTS

I. Purification of Milligram Quantities of Thy-1.1*

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The Thy-1 differentiation alloantigen is expressed as one of two allelic forms, Thy-1.1 (formerly theta-AKR) or Thy-1.2 (formerly theta-C3H) encoded for by Thy-1a or Thy-1b, respectively, in the various strains of mice (1-5). Only the analogue of the Thy-1a allele has been found in rats (6-9). Thy-1 is expressed in large quantities by cells in the brain and thymus of both species (1-3, 6-9). Smaller amounts are found on peripheral thymus-derived (T) lymphocytes and epidermal cells from the mouse (1, 10-12). Although most rat peripheral T lymphocytes do not express Thy-1, approximately 30-40% of bone marrow cells express the molecule (8, 9, 13). Thy-1 has also been found on fibroblast-derived cell lines from mice and rats (14). The rat analogue of Thy-1.1 has been purified and shown to be a glycoprotein with a mol wt of 24,000 daltons for brain Thy-1 (15) and two forms of 25,000 and 27,000 daltons for thymocyte Thy-1 (16). The molecular nature of the murine Thy-1 molecule is still a matter of controversy. Murine Thy-1 has been reported by various investigators as glycolipid (17), ganglioside (18), protein (19-21), or a glycoprotein (22, 23).

Our approach to resolving this matter was to isolate sufficient quantities of Thy-1.1 and Thy-1.2 for a detailed structural and functional analysis. Mouse and rat thymocytes bind more than 500,000 molecules of Thy-1 antibody per cell in direct-binding assays (8, 24). We have taken advantage of the fact that many murine lymphocytic leukemic cell lines express Thy-1 in amounts equal to or greater than that found on normal thymocytes (25-27). In addition these cells can be adapted to grow in suspension culture (28). By using large-scale mammalian cell culture we can now produce a kilogram or more of cells per week. Procedures have been effected for isolating large quantities of membrane from these cells for study (29, 30). In this report the isolation and preliminary

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characterization of the Thy-1.1 molecule from an AKR-derived T-lymphoblastoid cell line BW5147 will be described.

Materials and Methods

Cell Culture. BW5147 cell line was obtained from the Cell Distribution Center of the Salk Institute for Biological Studies, San Diego, Calif. This spontaneously derived AKR T-lymphoblastoid cell line which expresses large amounts of the Thy-1.1 alloantigen and lesser amounts of H-2Kk (30), was adapted to large-scale suspension culture at the Cell Culture Center of the University of Alabama in Birmingham, and grown as previously described (27, 30) using 2% horse or fetal calf serum. 2 x 10^11 cells or more were collected and processed to the membrane stage daily.

Antisera. Congenic anti-Thy-1.1 and anti-Thy-1.2 sera were prepared using AKR/Cum (Cumberland Farms, Clinton, Tenn.) and AKR/Jax (The Jackson Laboratory, Bar Harbor, Maine) mice as previously described (27, 28). Anti-Thy-1.1 was used at an initial dilution of 1:125 for AKR/Jax thymocytes and 1:75 for rat thymocytes. Anti-Thy-1.2 was used at 1:8. Rabbit anti-mouse brain serum was produced according to the method described by Golub (31), and has been shown to be specific for Thy-1 when assayed on rat or mouse thymocytes (32-36).

An antiserum was raised in rabbits against purified Thy-1 preparations from an AcA-34 column. The first two injections administered 10 days apart consisted of 50 µg intravenously (i.v.) and 50 µg subcutaneously in complete Freund's adjuvant. After six weekly bleedings beginning 10 days after the second injection, the titer decreased. The rabbits were then boosted with 50 µg purified Thy-1 i.v. and bled weekly for four additional weeks. All antisera was heat inactivated at 56°C for 30 min and stored at −20°C before use.

Quantitation of Thy-1. Cytotoxicity assay procedures were a modification of the ³¹Cr-release assay (37) described by Williams et al. (38). 50 µl each of the absorbed antiserum, a 1 x 10⁶ cell/ml suspension of ³¹Cr-labeled thymocytes and a 1:8 dilution of guinea pig serum (Texas Biological Labs., Inc., Fort Worth, Texas) as a source of complement were added to each well of a microtiter plate. After incubation at 37°C for 45 min the plates were centrifuged at 1,500 g for 10 min and 100 µl of the supernate was counted in a Beckman Bio-Gamma II gamma counter (Beckman Instruments, Inc., Fullerton, Calif.).

The amount of Thy-1 in a given sample was measured by quantitative absorption of cytotoxic antiserum (27, 28, 39). 50 µl of antiserum at the appropriate dilution was added to an equal volume of serial dilutions of the sample, mixed, and incubated at 4°C for 2 h. All dilutions were carried out in buffer containing 15% bovine serum albumin (BSA)¹ to counteract the effect of deoxycholate (DOC) in the samples. After centrifugation for 10 min at 1,500 g, 50 µl of the supernate was removed and monitored for residual cytotoxic activity. The residual activity in the absorbed antiserum was expressed as:

\[
\text{% absorption} = 100 - \frac{\text{counts released by unabsorbed antiserum} - \text{counts released in complement control}}{\text{counts released by absorbed antiserum} - \text{counts released in complement control}} \times 100
\]

The percent absorption was plotted vs. the amount of protein used for absorption. The amount of protein in micrograms required to reduce a microliter of antiserum to the 50% absorption level is defined as one unit of activity. Data are expressed in units of activity per milligram of protein. Protein concentration was determined according to the method of Lowry et al. (40) using BSA as a standard.

Membrane Isolation. Cells were collected from suspension culture by continuous flow centrifugation using a Sharples T-1P Super Centrifuge equipped with a no. 1-H standard clarifier rotor (Sharples-Stokes Div., Pennwalt Corp., Warminster, Pa.) at a flow rate of approximately 100 liters/h (41). The cells were resuspended to 1 x 10⁶ cells/ml in 10 mM Tris plus 0.15 M NaCl, pH

¹ Abbreviations used in this paper: BSA, bovine serum albumin; DOC, deoxycholate; SDS-PAGE, sodium dodecyl sulfate-polyacrylamide gel electrophoresis.
7.4, and disrupted in a Stansted model AO 612 cell disrupter (Stansted Energy Service Co., Washington, D.C.) equipped with a model 716 disrupting valve (29). A flow rate of 2 liters/h was utilized with a back pressure of 300 lb/in² on the disrupting valve. Nuclei and cell debris were pelleted from the cell homogenate at 400 g for 15 min. This supernate was centrifuged for 20 min at 4,000 g in a Beckman J-21B centrifuge using a JA-14 rotor (Beckman Instruments, Inc.). The resulting supernate was centrifuged at an average of 20,000 g for 1 h to generate a microsomal pellet. This pellet was resuspended in 10 mM Tris-HCl, pH 8, and stored at −70°C. No difference has been detected between membranes that have been frozen at this step or used directly.

**Acetone Precipitation.** Frozen membranes were rapidly thawed and added with mixing to ten volumes of −70°C acetone. The membrane-acetone mixture was mixed several times, centrifuged at 3,000 g for 15 min, and the supernate discarded.

**Solubilization of the Acetone Powder.** The acetone pellet was homogenized in a Dounce homogenizer in 2% sodium DOC (Calbiochem, La Jolla, Calif.) in 10 mM Tris-HCl, pH 8.0, at a final concentration of 5 mg protein/ml. After incubation at 4°C for 2 h with stirring, the insoluble material was removed by centrifugation at 100,000 g for 1 h.

**Lectin Affinity Chromatography.** Lens culinaris lectin coupled to Sepharose 4B was prepared by the method of Allan et al. (42). Between 1 and 2 mg of lectin was coupled/ml of packed Sepharose. The column was equilibrated and run in 0.5% DOC, 10 mM Tris, pH 8.2. The 100,000 g supernate of the DOC-solubilized acetone powder was applied to the column and after washing off the unbound material, the bound material was eluted with 3% alpha-methyl-D-glucoside in the same buffer.

**Ethanol Precipitation.** Three volumes of absolute ethanol and a few drops of saturated sodium acetate were added to each fraction and incubated at −20°C. The ethanol solution was frequently concentrated 10-fold on a PM-10 Amicon filter (Amicon Corp., Lexington, Mass.) before incubation. After 48 h, the suspension was centrifuged at 300 g for 15 min, the ethanol poured off, and the pellet resuspended in the appropriate buffer.

**Gel Filtration.** Gel filtration was carried out on a 2.5 × 100 cm Ultrogel AcA-34 (LKB Instruments, Inc., Rockville, Md.) column in 0.5% DOC 10 mM Tris-HCl, pH 8.2, or a Sepharyl S-200 column (Pharmacia, Inc., Piscataway, N. J.).

**Polyacrylamide Gel Electrophoresis.** Sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) was performed according to the method used by Laemmli et al. (43) using 10% acrylamide gels. The gels were stained with Coomassie Blue (44).

**Results**

**Cytotoxic Assay System.** Our original assay system of congenic anti-Thy-1.1 tested on AKR/Jax target cells gave quantitative results for intact cells and membranes but proved ineffective in detecting Thy-1.1 after DOC solubilization of the membranes (Fig. 1 A). Even at protein concentrations in excess of 1 mg/ml the solubilized material gave less than 20% absorption. Two alternative assay systems were investigated to alleviate this problem. One system which consisted of congenic anti-Thy-1.1 tested on rat thymocyte targets allowed for detection of Thy-1.1 after DOC solubilization but was still less effective than before addition of the detergent (Fig. 1 B). Approximately 10 times as much solubilized material was required as intact membranes for a 50% reduction. The second alternative Thy-1-specific detection system evaluated was rabbit antimouse brain serum tested on rat target cells. As illustrated in Fig. 1 C this system was equally effective in detecting Thy-1 before and after solubilization with detergent. For this reason, the rabbit anti-mouse brain system was used to monitor the Thy-1 activity during the purification procedure.

**Purification of Thy-1.** In effect the procedure was conducted in two stages: (a) isolation of membranes, which were stored at −70°C and (b) solubilization and purification of Thy-1 from the frozen membranes.

The purification scheme for Thy-1 from BW5147 membranes is shown in Fig.
Fig. 1. An evaluation of cytotoxic assay systems for the detection of Thy-1. Plasma membranes of BW5147 cells were prepared according to the text. The membranes were solubilized in 2% DOC and centrifuged at 100,000 g (100 K) for 1 h. The plasma membrane- and DOC-solubilized supernate were used to absorb congenic antiserum and tested on (A) AKR/Jax (1:125 dilution) or (B) rat (1:75 dilution) thymocytes and (C) rabbit anti-mouse brain serum (1:32 dilution) tested on rat thymocytes.

2. A representative total yield and purification for each step is indicated in Table I. A crude membrane preparation was generated by use of differential centrifugation procedures described by Allan and Crumpton (45). The 20,000 g (microsomal) pellet contained 6% of the disrupted cell protein and 27% of the Thy-1
Purification of milligram quantities of Thy-1.1

**Cells**

- Resuspend to 1 x 10⁶ Cells/ml
- **Disrupt**

**Disrupted Cells**

- 400g - 15 min

- **P₁**
- **S₁**
- 4,000g - 20 min
- **P₂**

**Disrupted Cells**

- 20,000g - 1h

**Cold Acetone (10x vol)**

- 2,000g - 15 min

**Acetone Supernate**

- Resuspend in 2% DOC
- Stir at 4°C - 1h

**Acetone Supernate**

- 100,000g - 1 h

**P₃**

**Lectin Column**

- 10mM Tris + 0.5% DOC
- Elute with 3.0% α-methyl-D-glucoside

**Fractions With Thy-1 Activity Concentrated**

**Fig. 2.** Purification scheme for Thy-1.1. Crude membranes were isolated by differential centrifugation. P indicates pellet fractions and S indicates supernates. All operations were performed at 4°C.

Activity of the cells. Although the pellets were washed after the 400g and 4,000g centrifugation steps, P₁ and P₂ still retained some residual specific Thy-1.1 activity as these were negative when tested with anti-Thy-1.2 serum as a control. Only a small amount of Thy-1 activity remaining in the 20,000g supernate could be pelleted by centrifugation at 100,000g for 1h. Routinely 25-50% of Thy-1 activity was recovered in the microsomal pellet which contained 5-7% of the protein of the disrupted cells. The yield of H-2Kk paralleled Thy-1 at all steps indicating that the loss of activity was indeed due to total membrane loss (P. A. Barstad, unpublished observations).

Frozen membranes were rapidly thawed and added to -70°C acetone. This step reduced the amount of lipid and lipoprotein present in the membrane preparation resulting in better solubilization with DOC and more efficient flow through the *Lens culinaris* column. Approximately 33% of the protein was
removed at this step with no loss of Thy-1 activity. The apparent increase in Thy-1 activity was thought to be due to difficulty in obtaining a homogeneous suspension for absorption assays. The acetone pellet was solubilized in 2% DOC at a protein concentration of 5 mg/ml and centrifuged at 100,000 g for 1 h. The Thy-1 activity remained in the supernate which contained approximately 40% of the protein.

The 100,000 g supernate was passed over a **Lens culinaris** lectin affinity column and the bound material eluted with alpha-methyl-D-glucoside (Fig. 3). The fractions were assayed for Thy-1 activity and the active fractions concentrated by ethanol precipitation. Under nonsaturating conditions, all detectable Thy-1 activity bound to the lectin column. Approximately 4-5% of the protein applied to the column was eluted with alpha-methyl-D-glucoside and contained 63% of the Thy-1 activity applied, resulting in a 16-fold purification at this step.

After ethanol precipitation, the alpha-methyl-D-glucoside eluted fraction was chromatographed on an AcA-34 column (Fig. 4). The fractions containing Thy-1 activity eluted after an ovalbumin standard and followed fractions active for H-2Kk. After concentration by ethanol precipitation, 1% of the protein applied to the column and 50% of the Thy-1 activity was recovered in these fractions. This fraction represented greater than a 2,000-fold final purification.

**SDS-PAGE.** Before SDS-PAGE, the sample was chromatographed on a S-200 column and precipitated by ethanol. The purity of the Thy-1 fraction is demonstrated in Fig. 5. The mol wt using this gel system was calculated as approximately 25,000 daltons.

**Antiserum against Purified Thy-1.1.** Rabbit antiserum to the purified Thy-1.1 molecule was prepared and analyzed for activity against mouse and rat thymocytes. The titer utilizing a 50% cytotoxicity end point against AKR/Jax (Fig. 6 A) and AKR/Cum thymocytes (1:512) was essentially identical. The titer of this antiserum was 1:20 against rat thymocytes and 1:128 against BW5147 cells (Fig. 6 B).

No absorption was observed when the antiserum was incubated with AKR/
Purification of milligram quantities of Thy-1.1

Fig. 3. *Lens culinaris* lectin affinity column. The DOC-solubilized 100,000 g supernate was applied to the lectin column and washed with 10 mM tris-HCl, pH 8.2, containing 0.5% DOC. Elution of the glycoprotein fraction was by 3% α-methyl-D-glucoside in the above buffer which was applied beginning at fraction 32. Thy-1 activity was assayed using rabbit anti-mouse brain serum (1:32) and rat thymocytes as target cells.

Jax liver and analyzed against mouse or rat thymocytes. However, AKR/Jax brain and thymus membranes completely absorbed the cytotoxic activity for both target cells. BW5147 cells and AKR/Jax thymocytes were equally effective in removing the rabbit anti-purified Thy-1.1 activity when tested on either AKR/Jax thymocytes (Fig. 6 A) or BW5147 cells (Fig. 6 B).

Since heterologous antiserum was utilized to follow the activity of Thy-1 it was important to ascertain if the purified molecule in the absence of detergent would absorb the mouse congenic anti-Thy-1.1 serum. Purified Thy-1.1 was used to absorb mouse congenic and rabbit anti-purified Thy-1 sera. The purified Thy-1.1 effectively absorbed cytotoxic activity from both antisera (Fig. 7). These results indicate that the purified component is Thy-1.1 and that these two antisera recognize the same molecule. It is interesting to note that a more purified Thy-1.1 was required to absorb congenic antiserum when assayed on AKR/Jax thymocytes as opposed to rat thymocytes. Little difference was evident using the two target cells when purified Thy-1.1 was used to absorb rabbit anti-purified Thy-1.1.

**Discussion**

In our early work on the purification of Thy-1.1 using congenic antisera with mouse target cells, we were unable to detect Thy-1 activity after the solubilization of the membranes with DOC. In contrast, Letarte-Muirhead et al. (46) had demonstrated that DOC did not destroy rat Thy-1 activity. However, these
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Fig. 4. Fractionation on AcA-34 column. The glycoprotein fraction eluted from the affinity column was applied to an AcA-34 column and run in 10 mM Tris-HCl, pH 8.2, containing 0.5% DOC. 5-ml fractions were collected and assayed for Thy-1 activity using rabbit anti-mouse brain serum (1:32 dilution) on rat thymocytes. H-2K^d activity was assayed using (C3H·H-2^d × 129)F^1 anti-C3H spleen (antiserum D-3b obtained from Dr. John Ray at the NIH) tested on AKR/Jax spleen target cells.

Fig. 5. SDS-PAGE. Standards (A) consisting of 10 µg each of BSA (65,000 daltons), ovalbumin (OVA) (45,000 daltons), and chymotrypsinogen (CHY) (25,000 daltons). The Thy-1 fraction from the AcA-34 column (B) ran with the same mobility as chymotrypsinogen. The gels were stained with Coomassie Blue.
investigators were utilizing mouse anti-rat brain serum assayed on AKR mouse thymocytes by use of a radioactive-binding assay. This suggested that either mouse and rat Thy-1 differ extensively or that our assay system was unable to detect detergent-solubilized Thy-1. We therefore evaluated the ability of congeneric mouse antiserum as opposed to rabbit anti-mouse brain serum to recognize solubilized Thy-1 when mouse or rat thymocytes were utilized as target cells. As shown in Fig. 1, these antisera differed extensively in their abilities to detect the Thy-1 molecule after solubilization. Rabbit anti-mouse brain serum when tested on rat thymocytes gave the clearest results and allowed us to detect Thy-1 activity after solubilization. Therefore, this system was utilized to quantitate activity during the purification procedure.

There are several possible explanations why these antisera differ in their ability to detect Thy-1 after detergent solubilization. It should be pointed out that in the purification scheme absorption of cytotoxic activity was effective with whole cells and the various subcellular fractions. After the solubilization in DOC it was not possible to precipitate antigen-antibody complexes in microtiter plates, and the absorption procedure became in effect an inhibition assay. After
the absorption phase soluble antigen-antibody complexes were transferred to the cytotoxic phase which could then dissociate and combine with Thy-1 on the target cells. When enough antibody had attached to the target cell, lysis would occur, giving the same result as if no absorption had taken place. The affinity of the antiserum for Thy-1.1 in the solubilized form as opposed to that for Thy-1.1 on the cell surface determines how much of this exchange occurred and thus influenced the accuracy of detection. One possible explanation for the observed difference in results with antisera analyzed on rat and mouse target cells is that
congenic antiserum has a lower affinity for the rat cell surface Thy-1 as opposed to mouse cell surface Thy-1 and much less antibody exchange takes place after transfer. Such a hypothesis also implies that the antiserum has a low affinity for the solubilized murine Thy-1.

Williams et al. (47) and Morris et al. (35, 36) have suggested that the rat Thy-1 molecule possesses a series of antigenic determinants which may vary in their stability or accessibility in detergent. Congenic antisera detect only the Thy-1.1 or Thy-1.2 determinant. However, the rabbit anti-mouse brain serum recognizes little, if any, Thy-1.1-specific determinants and most of the antibodies are directed toward the mouse-rat cross-reactive determinants on rat cells or mouse-rat cross-reactive in addition to mouse-specific determinants on AKR/Jax and AKR/Cum thymocytes. Therefore, antibodies against two determinants would have to be absorbed out for mouse targets and antibodies against only one determinant for rat target cells. The majority of the antibodies in rabbit anti-mouse brain are for the mouse-specific determinant. Each of these epitopes may differ in their susceptibility to denaturation by detergent depending on their location and whether they are carbohydrate or polypeptide in nature. A second explanation would be a differential effect of DOC binding and structurally hindering these epitopes. We are presently pursuing these questions in an effort to determine which if any of the above explanations is valid.

Esselman and Miller (18) have demonstrated Thy-1-like activity in ganglioside GM1 and Gb1, while Arndt et al. (48) found a lipid moiety was necessary to retain full antigenic activity. In our studies the acetone and ethanol precipitation steps that were used during purification should have removed the majority if not all of the lipid but did not affect the Thy-1 activity. While not empirically conclusive this would indicate that Thy-1 activity resides in a glycoprotein moiety. While Trowbridge and Hyman (22) claim that Thy-1 specificity is related to the carbohydrate moiety others have found loss of activity after protease digestion (20, 47). It will necessitate a thorough study to determine which antigenic moieties are being recognized by the various antisera and what components are essential for the antigenic determinants.

Letarte-Muirhead et al. (16) reported the existence of at least two different types of Thy-1.1 molecules on rat thymocytes. These two forms differed in their molecular weight, carbohydrate composition, and ability to bind to lentil lectin. In contrast all the Thy-1 purified from BW5147 cells binds to the lentil lectin column and gives a single band on SDS-PAGE. This discrepancy may be due to our use of a relatively homogenous T-lymphoblastoid cell line. If two forms do exist in the mouse, however, they must be very similar antigenically as indicated with our serological studies, i.e., the Thy-1.1 purified from BW5147 cells can totally inhibit cytotoxic activity for rat and mouse thymocytes.

In the mouse Thy-1 has been demonstrated on nerve endings (49) in addition to being expressed primarily on T cells. It has been suggested that Thy-1 and other "differentiation alloantigens" may play some role in cell-cell recognition (50). This could be either a general role involving some common anchor or it could be one of specificity, i.e., it could allow only the correct cells to interact resulting in proliferation of the T cells or synaptogenesis in the brain. One indication of the function of a cell surface antigen such as Thy-1 is the existence
of microheterogeneity between different cells. If the specificity function is true for Thy-1, one would expect to find microheterogeneity within the Thy-1 molecules from different populations of T cells analogous to immunoglobulins. To investigate this possibility rabbit antiserum against purified Thy-1.1 from BW5147 cells was absorbed with BW5147 cells or AKR/Jax thymocytes and assayed against either BW5147 cells or AKR/Jax thymocyte targets (Fig. 6). If Thy-1 were a heterogeneous collection of molecules, rabbit anti-purified Thy-1 serum should recognize "variable" portions of the polypeptide chains which differ from cell to cell as well as constant region determinants shared by all Thy-1-positive cells. In such a case normal thymocytes should not remove the "variable" region antibody made against a cell line Thy-1. As shown in Fig. 6A Thy-1.1 from BW5147 has all the major determinants found on normal thymocytes and conversely normal thymocytes totally absorb all the activity against BW5147 cells (Fig. 6B). This indicates that the Thy-1.1 molecule expressed by BW5147 cells has no major antigenic determinants which are not found in comparable amounts of pooled thymus cells. These data indicate that the existence of variable and constant regions in Thy-1.1 are unlikely and suggest that the molecule must play some less specific role, if any, in cell-cell interaction.

As indicated in Table I, only 25% of the Thy-1 activity of the cells was obtained in the microsomal pellet. This is probably due to suboptimal cell disruption conditions resulting in a heterogeneous mixture of membrane fragments. As we gain more experience in processing large volumes of cells we anticipate increasing our membrane yield significantly. As an alternative we have tried Tween-40 solubilization on intact cells following the procedure of Morris et al. (36) but found only a slight increase in yield. Moreover the approach presented difficulties in handling large quantities of cells on a day to day basis. We are presently attempting to modify our isolation and purification scheme to decrease our losses and to be able to purify components present in lesser quantities on cell membranes.

This isolation of Thy-1 is the first report describing the purification of milligram quantities of a lymphoblastoid cell surface component. Our approach to Thy-1 was greatly influenced by the desire to obtain multiple cell surface antigens from a single cell line, more specifically to obtain in addition to Thy-1.1, H-2, and gp69/71 from the BW5147 cell line. Although these molecules are present in much smaller amounts than Thy-1 (reference 30, and Kim Wise, unpublished observations) on BW5147 cells we have adapted conditions allowing us to partially purify them simultaneously with Thy-1. Further purification of these molecules is currently underway in our laboratory.

Summary

The Thy-1.1 molecule was isolated from the BW5147 murine lymphoblastoid cell line. The initial step in purification was the preparation of a crude plasma membrane fraction followed by acetone precipitation. The acetone pellet was solubilized using deoxycholate (DOC) and Thy-1.1 was purified by use of a *Lens culinaris* lectin affinity column and an AcA-34 gel filtration column. The purified glycoprotein with Thy-1.1 activity had a mol wt of approximately 25,000
daltons. The isolation of this molecule was effected by detecting Thy-1 activity utilizing rabbit anti-mouse brain serum tested on rat thymocytes. Congenic anti-Thy-1.1 serum was ineffective in detecting Thy-1.1 after DOC solubilization. An antiserum prepared in rabbits to the purified Thy-1.1 was found to be cytotoxic to mouse and rat thymocytes. The cytotoxic activity of this antiserum could be completely absorbed with AKR/Jax brain and thymus but was not absorbed by liver. In addition, AKR/Jax thymocytes totally absorbed all cytotoxic activity of the rabbit anti-purified Thy-1 serum for BW5147 cells suggesting that the cell line shares identical specificities with normal thymocytes. The purified Thy-1.1 molecule was able to totally absorb the cytotoxic activity of mouse congenic anti-Thy-1. These studies serve as a model for the isolation of other murine lymphoid cell surface components in quantities for detailed structural and functional analysis.

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