

ISOLATION AND PARTIAL CHARACTERIZATION OF THE HUMAN HOMOLOGUE OF THY-1*

BY EDWIN W. ADES,[‡] ROBERT K. ZWERNER,[§] RONALD T. ACTON, AND CHARLES M. BALCH^{||}

From the Cellular Immunobiology Unit of the Tumor Institute, Departments of Surgery and Microbiology, The Veterans Hospital, The Cell Culture Center and the Diabetes Research and Training Center, University of Alabama in Birmingham, Birmingham, Alabama 35294

Expression of the Thy-1 (theta) antigen is one of the most distinguishing characteristics of T cells in mice (1, 2) and rats (3). This differentiation antigen is expressed in large quantities on cells from brain and thymus and to a lesser extent on fibroblast-derived cell lines of both mouse and rat (4). The Thy-1 molecule has been purified from murine T lymphoblastoid cells (5) and murine brain (6, 7), as well as rat thymus (8) and brain (9). It is a glycoprotein of ~25,000–28,000 daltons as determined by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE).¹ We have recently used an antiserum prepared against monkey thymocytes to identify a 25,000-dalton surface membrane antigen expressed on normal human T lymphocytes and human T lymphoblastoid cells (10). These data suggested that a human counterpart of rodent Thy-1 might exist.

In this study, we describe the isolation and preliminary characterization of a 25,000-mol wt membrane protein (p25) from the human T lymphoblastoid cell line MOLT-3. The methodologic approach was essentially the same as previously used for the large-scale purification of mouse Thy-1 antigen (5). Extensive antigenic similarities between purified mouse Thy-1.1 and the p25 protein were demonstrated with both goat anti-Thy-1 and rabbit anti-p25 sera. The results indicate that the p25 antigen is the homologue of Thy-1.

Materials and Methods

The human T-cell antigen was purified from a starting population of 4×10^{10} MOLT-3 cells grown in spinner suspension cultures in RPMI-1640 (Grand Island Biological Co., Grand

* Supported by grants from the Medical Research Service of the Veterans Administration, the Human Cell Biology Section of the National Science Foundation, and the National Institutes of Health (CA13148, CA16673, CA15338, and CA18609).

[‡] Recipient of a Special Fellowship from the Leukemia Society of America. Present address: Department of Basic and Clinical Immunology and Microbiology, Medical University of South Carolina, Charleston, S.C.

[§] Supported in part by an institutional National Research Services Award from the National Institute of General Medical Sciences.

^{||} Recipient of a Faculty Fellowship in Oncology from the American Cancer Society. Reprint requests should be addressed to: Dr. Charles M. Balch, Department of Surgery, 750 Lyons-Harrison Research Building, University of Alabama in Birmingham, Birmingham, Ala.

¹ Abbreviations used in this paper: anti-p Thy-1, goat anti-purified Thy-1.1 molecule; BSA, bovine serum albumin; DOC, deoxycholate; p25, 25,000-mol wt membrane protein from the human T lymphoblastoid cell line MOLT-3; R_f, relative mobility(ies); SDS-PAGE, sodium dodecyl sulfate-polyacrylamide gel electrophoresis; Staph protein A, Staphylococcus aureus Cowan strain I.

Island, N. Y.) plus 10% heat-inactivated horse serum. The cells were collected and disrupted as previously described (11). Nuclei and cell debris were removed by centrifugation at 400 *g* for 15 min. The supernate was centrifuged for 20 min at 4,000 *g*. The resulting supernate was centrifuged at 20,000 *g* for 1 h to harvest a microsomal pellet. This was resuspended in 10 mM Tris-HCl, pH 8.2, and mixed with 10 vol of acetone at -70°C . After centrifugation at 3,000 *g* for 15 min, the pellet was solubilized in 2.5% deoxycholate (DOC) as previously described (5). Nonsolubilized material was removed by centrifugation at 100,000 *g* for 1 h.

The solubilized antigen was then isolated by affinity chromatography followed by gel filtration. *Lens culinaris* lectin coupled to Sepharose 4B (Pharmacia Fine Chemicals, Div. of Pharmacia, Inc., Piscataway, N. J.) was prepared by the method of Allan et al. (12) and used to prepare a column equilibrated with 0.5% DOC, 10 mM Tris-HCl (pH 8.2) buffer. The 100,000 *g* supernate of the DOC-solubilized pellet was passed through this column, the unbound material was removed by washing with buffer, and the glycoproteins were then eluted with 3% alpha-methyl-D-glucoside in the same buffer. The eluted fraction was concentrated by adding 3 vol of absolute ethanol and incubating at -20°C for 48 h. The suspension was centrifuged at 300 *g* for 15 min, the ethanol decanted, and the pellet resuspended in a small volume of buffer. Gel filtration of the resuspended pellet was carried out on a $2.5 \times 100\text{-cm}$ Ultrogel AcA-34 (LKB Instruments, Inc., Rockville, Md.) column in 0.5% DOC, 10 mM Tris-HCl, pH 8.2.

An antiserum was raised in rabbits against the 25,000-dalton fraction isolated from the AcA-34 column. The molecular weight of the protein peak was determined by SDS-PAGE. The first injection of antigen was administered intramuscularly in complete Freund's adjuvant. The second and third injections were given 14 and 28 d later with incomplete Freund's adjuvant. The antiserum (anti-p25) was heat inactivated and used in these experiments without any adsorptions. A goat antiserum raised against purified Thy-1 (goat anti-purified Thy-1.1 molecule [anti-p Thy-1]) isolated from a mouse AKR-derived T lymphoblastoid cell line (BW5147) was prepared as previously described (5).

Surface membrane determinants reactive with these two antisera were examined by solubilizing radiolabeled lymphocyte membrane antigens in 0.5% DOC and then coprecipitating them with antiserum and *Staphylococcus aureus* Cowan strain I (Staph protein A) as previously described (10). They were then analyzed by SDS-PAGE on a 5–12% gradient gel in a discontinuous buffer system using the method of Laemmli (13). The gels were sliced into 1-mm slices and counted on a Beckman Bio-Gamma gamma counter (Beckman Instruments, Inc., Fullerton, Calif.). Relative mobilities (R_f) were expressed as the ratio of the length of migration of the sample and a bromophenol blue dye marker. Known proteins used for estimation of molecular weight were bovine serum albumin (BSA) (68,000), ovalbumin (45,000), chymotrypsinogen A (25,000), and myoglobin (17,000). A standard curve was used to estimate the molecular weights of the antigens.

Results

Solubilized membranes of human lymphocytes were treated with unabsorbed anti-p25 serum raised against purified human T-cell antigen. Anti-p25 precipitated only one protein of 25,000 daltons from E-rosette-positive T cells from human blood (Fig. 1A). No labeled material was precipitated from solubilized membranes of a B-cell leukemia (pre-B-cell type). Cross-reactivity between the human and mouse T-cell antigens was demonstrated in experiments in which anti-p25 was used to precipitate purified radiolabeled mouse Thy-1.1 antigen (Fig. 1A). Neither the prebleed normal rabbit serum and Staph protein A nor Staph protein A alone precipitated Thy-1.1 antigen. Immunodepletion experiments were then performed to determine whether the anti-p25 serum and the anti-p Thy-1 serum were recognizing the same antigenic component. When an excess of anti-p25 serum plus Staph protein A was first used to coprecipitate purified Thy-1.1, no additional material was precipitated from the depleted supernate by a second precipitation step with anti-p Thy-1.1 plus Staph protein A (Fig. 1B). Conversely, rabbit anti-p25 did not precipitate Thy-1 protein

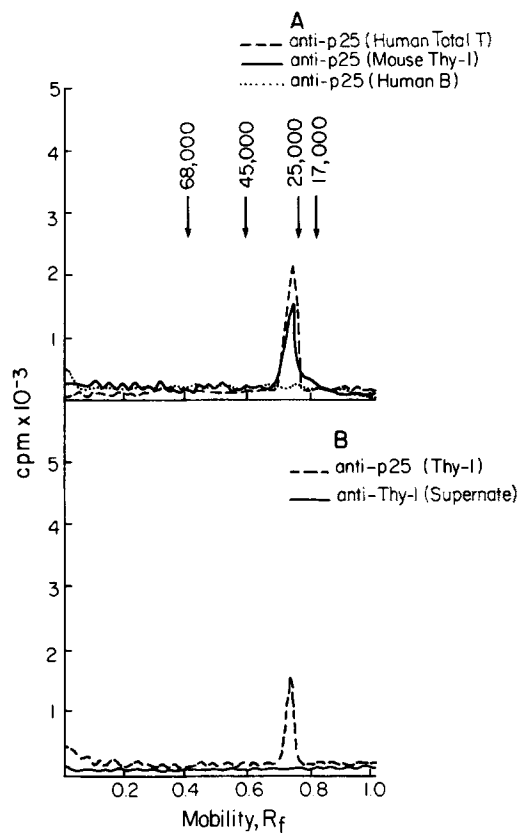


FIG. 1. Migration profile on SDS-PAGE of detergent-solubilized lymphocyte antigens coprecipitated with a rabbit antiserum raised against a purified human T antigen (anti-p25). (A) Anti-p25 recognizes a 25,000-dalton antigen from human blood T cells (E-rosette receptor positive) and cross-reacts with purified mouse Thy-1.1 antigen with the same molecular weight. This reagent was unreactive with membrane proteins from leukemic B cells. (B) Immunodepletion experiments were performed where rabbit anti-p25 was coprecipitated with purified mouse Thy-1.1. After centrifugation, the supernate lacked any antigens that could be coprecipitated with an anti-p Thy-1.1 serum.

from preparations previously reacted with anti-p Thy-1 (data not shown, but identical to Fig. 1 B).

The cross-species reactivity of anti-p25 for mouse T lymphocytes was also determined with a ⁵¹Cr release cytotoxicity assay (5). Anti-p25 serum was cytotoxic for virtually all AKR/Jax (The Jackson Laboratory, Bar Harbor, Maine) mouse thymocytes in the presence of guinea pig complement, but was unreactive with mouse bone marrow cells (Fig. 2). The same results were obtained with goat anti-p Thy-1.1. The 50% cytotoxicity titer of anti-p25 was 1:8 for human T lymphoblastoid target cells (MOLT-3) and 1:64 for cultured mouse T cells (BW5147) in the presence of rabbit serum as a complement source. No lysis of MOLT-3 cells occurred with guinea pig serum as a complement source. Reactivity of anti-p25 serum with MOLT-3 cells was also demonstrated by a direct immunofluorescence assay (10).

Quantitative immunoadsorption studies were performed using purified murine Thy-1.2, AKR/Jax thymocytes, AKR/Jax brain membranes, and human MOLT-3

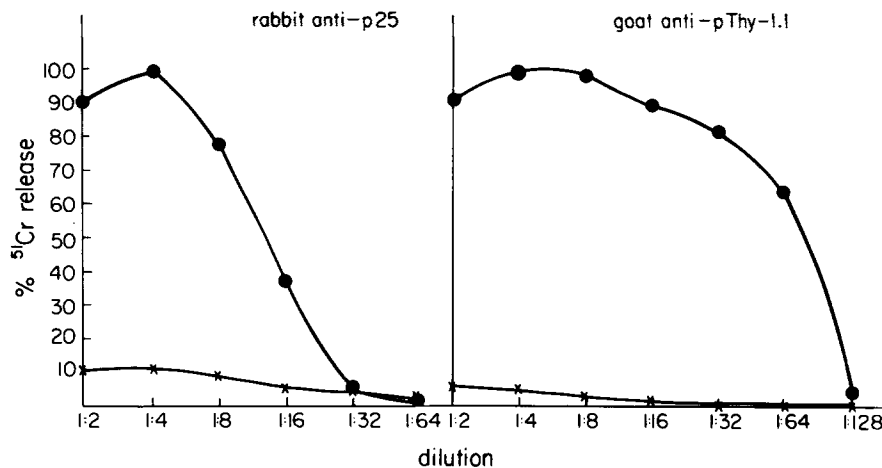


FIG. 2. Cytotoxicity of rabbit anti-p25 and goat anti-pThy-1.1 sera were reacted with ^{51}Cr -labeled AKR/Jax lymphocytes from thymus (●) or bone marrow (×). Percent specific release was calculated with the formula:

$$\% \text{ Release} = \frac{\text{Counts released by antiserum} - \text{counts released in complement control}}{\text{Maximum counts released by acid lysis} - \text{counts released in complement control}} \times 100.$$

This experiment is representative of three individual studies that show that anti-p25 serum cross-reacts with mouse thymocytes but is unreactive with marrow lymphoid cells.

membranes (not detergent solubilized) obtained by differential centrifugation (5). Before adsorptions, each antiserum was diluted to a titer that was not in antibody excess but was still cytotoxic for >50% of AKR/Jax thymocytes as previously described (5). 50 μl of antiserum at the appropriate dilution was incubated with an equal volume of serial dilutions of the adsorbing material for 2 h at 4°C. The membrane protein concentration of each adsorbing material was determined by the method of Lowry et al. (14) with BSA as a standard. The results confirmed again that there was cross-reactivity of the human and murine T antigens, because membranes or purified antigens from either species reciprocally removed the cytotoxic activity of both antisera for AKR thymocytes (Fig. 3). There were quantitative differences in the adsorbing efficiency of the various preparations. Thus, the quantity of MOLT-3 cell membranes required for complete antibody adsorption was 10-fold greater than that of AKR/Jax brain or thymocyte membrane and 100-fold greater than the quantity of purified Thy-1.1 antigen (Fig. 3).

Discussion

We have previously demonstrated cross-reacting T lymphocyte differentiation antigens among mammalian species (15). With few exceptions, no cross-reactivity of lymphocyte antigens was demonstrated between any phylogenetic order. On the other hand, serological cross-reactivity between humans and rodents has been demonstrated for histocompatibility antigens and for immunoglobulin products of B cells (16-18).

A homology between a human T-cell antigen and murine Thy-1 is suggested by the following observations: (a) the antigens have a similar mol wt of ~25,000 as

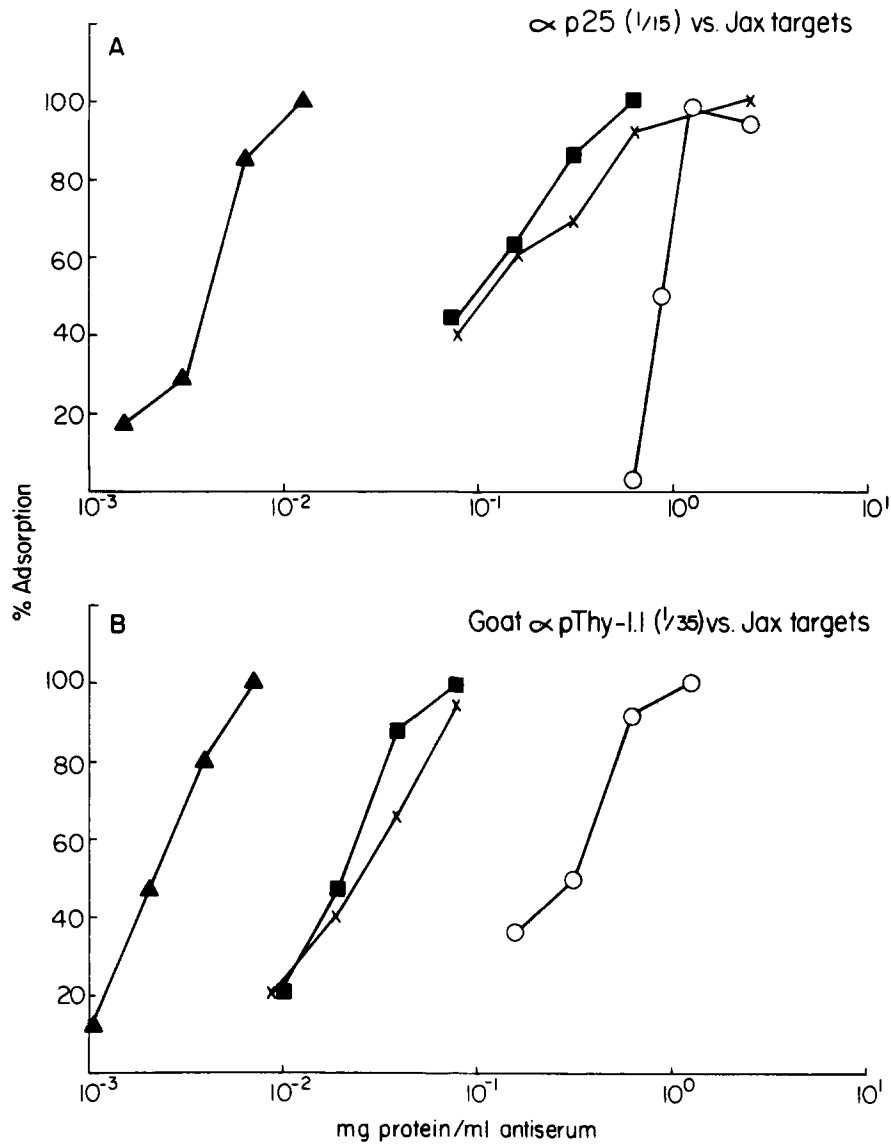


FIG. 3. Quantitative adsorption of rabbit anti-p25 (1/15 dilution) and goat anti-p Thy-1.1 (1/35 dilution) with purified AKR/Jax thymocyte membrane (■), MOLT-3 cell membrane (○), AKR/Jax brain membrane (×), or purified Thy-1 (▲) and tested on AKR/Jax thymocytes as target cells. Activity of both antisera was removed by purified T antigens from the isologous and heterologous species.

determined by SDS-PAGE; (b) they are glycoproteins that bind to *L. culinaris*; (c) the p25 antigen from human MOLT-3 cells elicited an antiserum that cross-reacts with mouse Thy-1 both by radioimmunoprecipitation and by complement-mediated cytotoxicity; and (d) anti-p25 and anti-p Thy-1 reacted with the same antigen as demonstrated by adsorption and immunodepletion experiments. The cytotoxicity and the quantitative adsorption experiments indicate a relatively low density of Thy-1 homologue on cultured human MOLT-3 cells compared with mouse T cells and

brain. This data thus supports previous suggestions (19, 20) that there is a human homologue of rodent Thy-1 analogous to the classical and physicochemical description of the purified Thy-1 molecule. Whether the molecular structure of the human T antigen is identical to the already defined rat and mouse Thy-1 molecule remains to be determined.

Human T-cell membranes are known to contain a multitude of antigenic determinants with different molecular weights (21). The ability to purify and then to immunize with immunochemically defined human T-cell antigens has the advantage of producing monospecific xeno-antisera requiring little if any adsorptions or monoclonal antibodies produced by antigen-directed hybridomas. Owen and Fanger (22) have previously prepared anti-human T-cell sera by immunizing rabbits with purified antigens taken from slices of a polyacrylamide gel. The yield of antigen with such an approach is relatively low. On the other hand, human lymphocyte membrane glycoproteins can be isolated in microgram to milligram quantities as described in this report when employing a system that includes large-scale tissue culture, affinity chromatography, and gel filtration chromatography. This yield and purity should facilitate future studies of human T antigens with respect to their molecular nature and biological properties of T lymphocytes displaying them on their membrane surface.

Summary

A glycoprotein of 25,000 daltons was isolated from a human T lymphoblastoid cell line (MOLT-3). A monovalent antiserum raised against this antigen precipitated mouse Thy-1.1 antigen as well as a 25,000-dalton antigen from human blood T cells. The unabsorbed serum was also cytotoxic for both mouse and human T cells. By multiple criteria, this human T antigen appears to be homologous with mouse Thy-1.

The authors wish to gratefully acknowledge Randy Phillips, Mike Cox, and Judy Williams for their excellent technical assistance.

Received for publication 4 October 1979.

References

1. Reif, A. E., and J. M. V. Allen. 1964. The AKR thymic antigen and its distribution in leukemias and nervous tissue. *J. Exp. Med.* **120**:413.
2. Reif, A. E., and J. M. V. Allen. 1966. Mouse thymic iso-antigens. *Nature (Lond.)*. **209**:521.
3. Douglas, T. C. 1972. Occurrence of a theta-like antigen in rats. *J. Exp. Med.* **136**:1054.
4. Stern, P. L. 1973. θ -alloantigens on mouse and rat fibroblasts. *Nat. New Biol.* **246**:76.
5. Zwerner, R. K., P. A. Barstad, and R. T. Acton. 1977. Isolation and characterization of murine cell surface components. I. Purification of milligram quantities of Thy-1.1. *J. Exp. Med.* **146**:986.
6. McClain, L. D., M. Tomana, and R. T. Acton. 1978. Purification and characterization of mouse brain Thy-1.2 differentiation alloantigen. *Brain Res.* **159**:161.
7. Letarte, M., and G. Maghji. 1978. Purification and characterization of mouse brain Thy-1 glycoprotein antigens. *J. Immunol.* **121**:1718.
8. Letarte-Muirhead, M., A. N. Barclay, and A. F. Williams. 1975. Purification of the Thy-1 molecule - a major cell surface glycoprotein of rat thymocytes. *Biochem. J.* **151**:685.
9. Barclay, A. N., M. Letarte-Muirhead, and A. F. Williams. 1975. Purification of the Thy-1 molecule from rat brain. *Biochem. J.* **151**:699.

10. Ades, E. W., A. Bukacek, R. K. Zwerner, P. A. Dougherty, and C. M. Balch. 1978. Expression of two differentiation antigens on normal and cultured human T-cells. *J. Immunol.* **121**:513.
11. Zwerner, R. K., K. Wise, and R. T. Acton, 1979. Harvesting the products of cell growth. *Methods Enzymol.* **56**:221.
12. Allan, D., J. Auger, and M. J. Crumpton. 1972. Glycoprotein receptors for concanavalin A isolated from pig lymphocyte plasma membrane by affinity chromatography in sodium deoxycholate. *Nat. New Biol.* **236**:23.
13. Laemmli, V. K. 1970. Cleavage of structural proteins during the assembly of the head of bacteriophage T4. *Nature (Lond.)*. **227**:680.
14. Lowry, O. H., N. J., Rosebrough, A. L. Farr, and R. J. Randall. 1951. Protein measurement with Folin phenol reagent. *J. Biol. Chem.* **193**:265.
15. Balch, C. M., M. K. Dagg, and M. D. Cooper. 1976. Cross-reactive T-cell antigens among mammalian species. *J. Immunol.* **117**:447.
16. Hood, L., and J. Prahl. 1971. The immune system: a model for differentiation in higher organisms. *Adv. Immunol.* **14**:291.
17. Neoh, S. H., D. M. Jahoda, D. S. Rowe, and A. Voller. 1973. Immunoglobulin classes in mammalian species identified by cross-reactivity with antisera to human immunoglobulin. *Immunochemistry.* **10**:805.
18. Vaerman, J. P., K. Kobayashi, and J. F. Heremans. 1974. Precipitin cross-reactions between human and animal J-chains. *Adv. Exp. Med. Biol.* **45**:251.
19. Arndt, R., R. Stark, P. Klein, A. Müller, and H. G. Thiele. 1976. Solubilization and molecular characterization of membrane-bound antigens shared by thymocytes and brain. *Eur. J. Immunol.* **6**:333.
20. Dalchau, R., and J. W. Fabre. 1979. Identification and unusual tissue distribution of the canine and human homologues of Thy-1. *J. Exp. Med.* **149**:576.
21. Balch, C. M., and E. W. Ades. 1979. Heterogeneity of cell surface xenoantigens on human T cells. *J. Reticuloendothel. Soc.* **25**:635.
22. Owen, F. L., and M. W. Fanger. 1976. Studies of the human T-lymphocyte population. IV. The isolation of T lymphocyte antigens from peripheral lymphocytes. *Immunochemistry.* **13**:121.