#171-01

To appear in Differentiation Antigens of Lymphocytes (Ed) N. Warner Academic Press

# HOMOLOGOUS MOUSE AND HUMAN T CELL ANTIGENS

Jeffrey A. Ledbetter[1] and Leonard A. Herzenberg

Department of Genetics

Stanford University School of Medicine

Stanford, California, 94305

1

Recipient of National Research Service Award (CA-06207)

*This work supported, in part, by grants from the National Institutes of Health (GM-17367, CA-04681, AI-08917)

# TABLE OF CONTENTS

## I. INTRODUCTION

Antibodies directed against polymorphic determinants on mouse T cells and T cell subsets have been used as the primary tools in studies of murine T cell differentiaton and function. For example, antibodies to the Lyt-1, Lyt-2, Lyt-3, and Thy-1 antigens allowed the separation of T cell subsets with distinct function (Cantor and Boyse, 1977) and the understanding of the helper/inducer T cells versus the cytotoxic/suppressor T cells as separate lineages emerging from the thymus (Scollay et al., 1978). Further, each of these surface markers undergoes characteristic changes in surface density expression as T cells mature in the thymus (Ledbetter et al., 1980), reflecting specific patterns of control of these gene products during differentiation.

In man, the helper/inducer and the cytotoxic/suppressor subpopulations of T cells also have been distinguished as separate lineages by their selective expression of surface antigens (Evans et al., 1977; Evans et al., 1978). Monoclonal antibodies to these T cell antigens have been prepared in several laboratories (McMichael et al., 1979; Evans et al., 1980; Reinherz and Schlossman, 1980) and used in immunofluorescence and biochemical experiments to characterize human T cell differentiaton. The specificities of the monoclonal anti-mouse and anti-human T cell antibodies used in our studies are summarized in Table I.

In our recent comparative studies of human and mouse T cell differentiation we presented evidence that some of the cell surface glycoprotein antigens that distinguish T cell subsets in both species are homologs (Ledbetter et al., 1981). The apparent evolutionary conservation of surface molecules in the two species means that these antigens probably play a valuable role in cellular function. Futher, the conserved surface molecules must have developed in an evolutionary ancester common to both mouse and man.

In this report we review and extend the evidence that the human Leu-1, Leu-2a and Leu-2b antigens are homologs of the mouse Lyt-1, Lyt-2 and Lyt-3 antigens, respectively. Studies presented here indicate that the molecules carrying the human Leu antigens approximate the size and subunit composition of the corresponding molecules carrying the mouse Lyt antigens. Moreover, each of these structurally homologous antigen systems has similar sensitivities to proteases and show similar quantitative changes in surface density expression during differentiation in the thymus.

## II. MATERIALS AND METHODS

Source of lymphoid cells. Mouse thymus or lymph node cells were from 8-10 week BALB/cNHz females. Human peripheral blood cells were from volunteers in the Medical Center at Stanford University. Human thymuses were from children undergoing cardiac surgery, kindly provided by Dr. R. Rouse, Stanford University.

Monoclonal antibodies. The mouse (BALB/c) anti-human T cell monoclonal antibodies produced by Evans et al. (1980) were obtained from Becton Dickinson FACS Systems, Monoclonal Antibody Center, Sunnyvale, California. The rat anti-mouse T cell monoclonal antibodies produced by Ledbetter and Herzenberg (1979) were purified from hybridoma culture supernates as previously described (Ledbetter et al., 1980). The antibodies used in these studies are described in Table I.

Immunofluorescence of cell suspensions. Direct immunofluorescence staining of mouse lymphoid cells with rat monoclonal antibodies and human lymphoid cells with mouse monoclonal antibodies was performed using fluorescein-conjugated monoclonal antibodies. Fluorescein conjugations were with fluorescein isothiocyanate according to Goding (1976). For staining mouse or human cells, reagents were centrifuged for 20 min at 100,000 x g before use, and $10^6$ target lymphoid cells per well in microtiter plates were reacted with saturation levels of antibody. Cells were stained on ice in the presence of 0.1% $NaN_3$ as previously described (Herzenberg and Herzenberg, 1978).

Quantitative fluorescence measurements were made on a modified fluorescence-activated cell sorter (FACS) (Becton Dickinson FACS Systems, Mountain View, California) fitted with a logarithmic amplifier that showed the fluorescence intensity distribution over a 1-10^4 range. The geometric mean fluorescence of particular cell populations was calculated. After calibration of the FACS system with free fluorescein (by Dr. D. R. Parks of this lab), the mean fluorescence per cell obtained by direct staining with fluorescein-conjugated purified monoclonal antibodies was converted to fluorescein equivalents (FE) per cell. In this way mean numbers of antibody molecules bound to positive cells in thymus, spleen, and lymph node were determined by the following calculation: [mean FE (positive cells) - mean FE (unstained cells)]/fluorescein:protein (F/P) ratio of purified antibody.

<u>Cell surface labeling and detergent extraction.</u> Human or mouse thymocytes or lymph node cells were labeled with $^{125}$-I by a modification of the lactoperoxidase technique. To 1 x 10^8 cells in 1 ml phosphate buffered saline (PBS) at 20$^{o}$C were added 1.0 mCi $^{125}$I (Amersham Corp., Arlington Heights, Illinois), 25 ug lactoperoxidase (B grade, Calbiochem-Behring Corp., La Jolla, California) and successive 10 ul pulses of $H_2O_2$ (0.3 mM, 1 mM, 3 mM, 9 mM) at five minute intervals. Cells were washed twice in PBS and extracted in lysis buffer (0.5% Nonidet-P40, Particle Data Laboratories, Ltd., Elmhurst, Illinois; 50 mM Tris; 150 mM NaCl; 0.02% $NaN_3$; 5 mM ethylenediamine-tetraacetic acid; 50 mM phenylmethylsulfonyl fluoride; 0.2 trypsin inhibiting units (TIU)/ml Aprotinin, Sigma Chemical Co., St. Louis, Missouri; 1 ug/ml pepstatin A (Sigma Chemical Co.); and 50 mM iodoacetamide, pH 8.0) for 30 min at 4$^{o}$C. Nuclei were removed by centrifugation at 5,000 x g for 20 min. Human thymocytes or peripheral blood lymphocytes (isolated on Ficoll-Paque) were similarly labeled with $^{125}$I and lactoperoxidase in balanced

salt solution.

Immunoprecipitation and gel electrophoresis. Immunoprecipitations were with ~5 ug antibody per extract from 2 x 10^7 cells. After a 1 h incubation on ice, antigen-antibody complexes were collectd by the addition of 10 ul of 10% fixed staph A-Cowan I strain (Tufts New England Enzyme Center, Boston, Massachusetts). For the rat antibodies that do not bind to protein A directly, the staph A was precoated with affinity-purified mouse (SJL/J) anti-rat Ig and washed twice before addition to the cell extract. Similarly, for the mouse antibodies that do not bind to protein A directly, the staph A was precoated with affinity-purified rabbit anti-mouse Ig. After 45 min on ice, the staph A-antibody-antigen complexes were washed 3 times in washing buffer (0.5% NP-40, 0.45 M NaCl, 50 mM Tris, 5 mM KI, 0.02% $NaN_3$, pH 8.3) and then extracted with sample buffer for gel electrophoresis. Immunoprecipitates were analyzed by 10% SDS polyacrylamide one-dimensonal gels (Laemmli, 1970). Molecular weight markers (Pharmacia Fine Chemicals, Piscataway, New Jesey) run on each gel were visualized by staining with Coomassie brilliant blue (Bio-Rad Laboratories, Richmond, California). Autoradiography was with intensifying screens (Cronex lightning plus screens, DuPont, Wilmington, Delaware) using Kodak X-Omat R film at -70 $^{o}$C.

Two-dimensional gels with a nonequilibrium charge separation using a 4:1 ratio of pH 3.5-10 and pH 7-9 ampholines (LKB Instruments Inc., Rockville, Maryland) in the first dimension and a size separation on 10% SDS polyacrylamide gels in the second dimension were run as previously described (O'Farrell et al., 1977).

# III. RESULTS

## A. Lyt-1 and Leu-1 molecules appear to be evolutionary homologs

Both Leu-1 and Lyt-1 antigens increase in density during T cell differentiation. We used monoclonal antibodies directly conjugated with fluorescein and immunofluorescence with a FACS to examine the quantitative levels of antigen on thymoctyes and peripheral T cells in mouse and man. Fig. 1 shows the comparison of mouse Lyt-1 verusus human Leu-1 antigens. In each species, the antigen detected is expressed on all thymocytes in a bimodal fashion. Approximately 20-30% of thymocytes have a four to six-fold higher antigen density. Our studies in the mouse using cortisone to deplete the cortical thymocytes have shown that the brighter Lyt-1 bearing cells are derived from the medullary (cortisone-resistent) population. Similar experiments using human thymocytes from a patient undergoing cortisone treatment showed that the medullary (cortisone-resistent) population is composed of the brighter Leu-1 bearing cells (Ledbetter, Frankel and Herzenberg, unpublished). In both species, peripheral T cells show the higher antigen density characteristic of medullary thymocytes (Fig. 1). Thus the Lyt-1 antigen in mouse and the Leu-1 antigen in man are expressed on all thymoctyes and increase in antigen density as thymoctyes mature from the cortical to the medullary and peripheral T cell populations.

The Leu-1 and Lyt-1 antigens are found on essentially all peripheral T cells. However, the two lineages of mature T cells (helper/inducer and cytotoxic/suppressor) exhibit distinctively different levels of membrane antigen. The helper/inducer cells in both species have higher Leu-1 or Lyt-1 antigen levels than the cytotoxic/suppressor cells (Ledbetter et al., 1981). I mouse, cytotoxic depletion studies originally indicated that the cytotoxic/suppressor T cells were Lyt-1 negative (Cantor and Boyse, 1975). We used monoclonal antibodies and sensitive immunofluorescence techniques to show that cytotoxic/suppressor cells have low levels of Lyt-1 and are often difficult to kill with anti-Lyt-1 and complement (Ledbetter et al., 1980).

The Lyt-1 and Leu-1 antigens show similarities on SDS polyacrylamide gels after surface $^{125}$-I-labeling of thymocytes and immunoprecipitation with monoclonal antibodies (Fig. 2). Both antigens are approximately 67,000 Mr when run under either reducing or non-reducing gel conditions, indicating that the two antigens are carried on single polypeptide chains of very similar size (Fig. 3).

Leu-1 and Lyt-1 are found on some B cell leukemias. Although Leu-1 is found exclusively on T cells in normal individuals, B cell (Ig+) chronic lymphocytic leukemias (CLLs) frequently express this antigen (Wang et al., 1980). In the mouse, Lyt-1 is found on T cells and has long been thought to be a specific marker of T cells. However, we recently found Lyt-1+,Thy-1- cells in B cell areas of lymph node and spleen (Ledbetter et al., 1980). Lyt-1 also is expressed by some murine B cell leukemias (Lanier et al., 1981). Thus far, the Leu-1 and Lyt-1 antigens are unique in their exprssion on malignant B cells; none of the other characteristic T cell markers (Thy-1 and Lyt-2 in mouse and Leu-2, Leu-3, Leu-4 and Leu-5 in man) have been detected on these Ig+ leukemias.

Leu-1 and Lyt-1 show similar sensitivities to proteases. The structural relationships of surface molecules can be studied by digestion of viable cells with specific proteases followed by measurement of remaining antigen with monoclonal antibodies and quantitative immunofluorescence. The comparison of protease sensitivity for Leu-1 and Lyt-1 is shown in Table III. Both antigens are cleaved by trypsin and pronase when the enzymes are present in high levels. In comparison to other T cell antigens, Leu-1 and Lyt-1 are more resistent to digestion. At enzyme levels that effectively remove Lyt-2, Lyt-3, Leu-2a, and Leu-2b, the Leu-1 and Lyt-1 antigens are only slightly reduced (Table III). This probably means that Leu-1 and Lyt-1 are less accessible to proteases on the cell membrane than most other surface proteins.

B. Lyt-2,Lyt-3 and Leu-2a, Leu-2b molecules appear to be evolutionary homologs

The mouse Lyt-2 and Lyt-3 antigens and the human Leu-2a and Leu-2b antigens are selective markers of the cytotoxic/suppressor lineage of mature T cells. In both species, these antigens are found on essentially all immature (cortical) thymocytes, but are found on only 30-50% of medullary (cortisone-resistent) thymocytes and 30-50% of peripheral T cells. The fluorescence (FACS) distribution patterns of the human and mouse antigens are very similar (Fig. 1). In the mouse, the Lyt-2 and Lyt-3 antigen densities of thymocytes and peripheral T cells are almost equal, whereas the human Leu-2a and Leu-2b antigens are expressed in higher density on peripheral T cells than on immature thymocytes (Table II).

There are structural similarities in the human and mouse molecules, since both are composed of at least two nonidentical subunits (Fig. 2) that are associated through disulfide bonds into a variety of multimeric combinations (Ledbetter et al., 1981). In the mouse, we know more details of the Lyt-2,Lyt-3 molecular structure. The Lyt-2 antigens are found on subunits of 38,000 and 34,000 Mr whereas the Lyt-3 antigens are found on a subunit of 30,000 Mr (Fig. 4). These three subunits are associated in non-random combinations to form dimers, tetramers and hexamers (Ledbetter et al., 1981).

The Leu-2a,Leu-2b and Lyt-2,Lyt-3 antigens show similar sensitivities to protease digestion from viable cells (Table III). Trypsin cleaves the Leu-2b antigen faster than the Leu-2a antigen, even though both are susceptible to trypsin digestion. Similarly, the Lyt-3 antigen is more sensitive to trypsin digestion than the Lyt-2 antigen. In both cases, the differential sensitivity to trypsin reflects digestion at arginine residues, since the arginine-specific enzyme, clostridium protease, produces the same differential cleavage of Leu-2a versus Leu-2b and Lyt-2 versus Lyt-3 (Table III). Other proteases such as chymotrypsin and pronase effectively cleave the human and mouse antigens and do not show a differential rate of cleavage for the separate determinants on each molecule (e.g., Leu-2a and Leu-2b, or Lyt-2 and Lyt-3; see Table III). These data suggest that the human and mouse molecules bearing these antigens have conserved some protease digestion sites and are both accesible on the cell membrane to cleavage with a variety of proteases.

## IV. DISCUSSION

We recently proposed homologies between human and mouse T cell antigens (Ledbetter et al., 1981). Our evidence on homologies derives from comparing the mouse and human antigens by determination of their density distributions on thymocytes and T cells and T cell subsets by FACS analysis, their size and subunit compositions, their functional activity and their sensitivites to cleavage by proteolytic enzymes. This evidence strongly supports the idea tha the Leu-1 and Leu-2a,Leu-2b molecules are the human homologs of the mouse Lyt-1 and Lyt-2,Lyt-3 molecules. Definitive proof of these homologies will require comparative amino acid sequencing of the purified antigens or nucleotide sequencing of the genes.

The conservation of the molecular structure and subpopulation denstiy distributions for the Leu and Lyt antigens suggests that these molecules perform essential functions for the cells on which they are found. There is as yet no information on a potential function for Lyt-1 or Leu-1. For Lyt-2,Lyt-3 and Leu-2a, Leu-2b, however, monoclonal antibody blocking studies suggest that these molecules are involved in antigen recognition by cytotoxic effector cells (Nakayama et al., 1979; Shinohara and Sachs, 1979). The close linkage of the Lyt-2 and Lyt-3 genes to the k chain region on chromosome 6 (Gottlieb, 1974; Gibsen et al., 1978) suggests that Vk gene products may have a functional association with the Lyt-2,Lyt-3 molecule. If the Leu-2a and Leu-2b genes are found to be linked to k in man, the linkage may be evolutionarily maintained to permit functional interaction between these genes.

The Leu-1 antign is unique among human T lymphocyte antigens thus far detected by monoclonal antibodies since it is found on B cell (Ig+) leukemias from most patients with CLL but has not yet been found on any normal B cells (Wang et al., 1980). Although it is possible that Leu-1 is induced on CLL cells as a consequence of leukemogenesis, the presence of this antigen on a small subpopulation of normal B cells cannot be excluded. The mouse Lyt-1 antigen is similar to Leu-1 in its expression on B cell leukemias, since Lyt-1 is found in high levels on some Ig+ leukemias (Lanier et al., 1981). In each species, the molecular form of the antigen on B cell leukemias is very similar or identical to the molecular form found on normal T cells.

Perhaps the strongest evidence that the Leu-2a,Leu-2b molecule is the human homolog of the mouse Lyt-2,Lyt-3 molecule is that in both species, cytotoxic effector cells are blocked by antibodies to Leu-2a and Leu-2b (human (Evans et al., 1981) or Lyt-2 and Lyt-3 (mouse) (Nakayama et al., 1979). The functional studies in the mouse so far suggest that the Lyt-2,Lyt-3 molecule is involved in the recognition phase of T cell killing. Lyt-2,Lyt-3 negative variants of a cytotoxic T cell line lose the ability to bind to target cells, but can still kill when they are artificially linked to their targets (Dialynas et al., 1981). Other studies have shown that anti-Lyt-2 and anti-Lyt-3 block stimulation in mixed lymphocyte cultures (MLC) (Hollander et al., 1980). The Lyt-2,Lyt-3 molecule may be identical to or closely associated with an antigen receptor of cytotoxic T cells. Further structural studies of the human and mouse moleules will help reveal how they work.

There are practical advantages in identifying homologous surface antigens between man and mouse. The potential direct application of anti-lymphocyte monoclonal antibodies in the treatment of human leukemias and other diseases of the immune system, and to achieve immunosuppression, will benefit from the convenient mouse model. That is, the effects of injecting monoclonal antibodies directly into patients on the normal immune system and on leukemia and autoimmunity will be aided by similar studies of the homologous antigen systems in the mouse. The goal of understanding the function of the antigens will be aided by comparative structural studies of the molecules and their genes in both species.

## V. REFERENCES

Cantor, H., and Boyse, E. A. (1975). Functional subclasses of T lymphocytes bearing different Ly antigens. I. The generation of functionally distinct T cell subclasses in a differentiative process independent of antigen. J. Exp. Med. 141, 1376-1389.

Cantor, H., and Boyse, E. A. (1977). Lymphocytes as models for the study of mammalian cellular differentiation. Immunol. Rev. 33, 105-

Dialynas, D. P., Loken, M. R., Glasebrook, A. L., and Fitch, F. W. Fitch. (1981) Lyt-1^-/Lyt-3^3 variants of a cloned cytolytic T cell line lack an antigen receptor functional in cytolysis. J. Exp. Med. (in press).

Engleman, E. G., Warnke, R., Fox, R. I., and Levy, R. (1980). Studies of a human T lymphocyte antigen recognized by a monoclonal antibody. Proc. Natl. Acad. Sci. USA (in press)

Evans, R. L., Breard, J. M., Lazarus, H., Schlossman, S. F., and Chess, L. (1977). Detection, isolation and function characterization of two human T cell subclasses bearing unique differentiation antigens. J. Exp. Med. 145, 221-

Evans, R. L., Lazarus, H., Pentta, A. C., and Schlossman, S. F. (1978). Two functionally distinct subpopulations of human T cells that collaborate in the generation of cytotoxic cells responsible for cell-mediated lympholysis. J. Immunol. 129, 1423-

Evans, R. L., Wall, D. W., Platsoucas, C. P., Siegal, F. P., Fikrig, S. M., Testa, C. M., and Good., R. A. (1981) Thymus dependent membrane antigens in man: Inhibition of cell-mediated lympholysis by monoclonal antibodies to the TH2 antigens. Proc. Nat. Acad. Sci. USA 78, 544-548.

Gibsen, D. M., Taylor, B. A. and Cherry, M. (1978) Evidence for close linkage of a mouse light chain marker with the Ly-2,3 locus. J. Immunol. 121: 1585-

Goding, J. W. (1976). Conjugations of antibodies with fluorochromes: modificatons to the standard methods. J. Immunol. Methods 13, 215-

Gottlieb, P. D. (1974) Genetic correlation of a mouse light chain variable region marker with a thymocyte surface antigen. J. Exp. Med. 140: 1432-

Hansen, J. A., Martin, P. J., and Nowinski, R. C. (1980) Monoclonal antibodies identifying a novel T cell antigen and Ia antigens of human lymphocytes. Immunogenetics 10, 247-

Haynes, B. F., Mann, D. L., Hemler, M. E., Schroder, J. A., Shelhamer, J. H., Eisenbarth, G. S., Strominger, J. L., Thomas, C. A., Mostowski, H. S. and Fauci, A. S. (1980). Characterization of a monoclonal antibody that defines an immunoregulatory T cell subset for immunogloblin synthesis in humans. Proc. Natl. Acad. Sci. USA 77, 2914-

Herzenberg, L. A. and Herzenberg, L. A. (1978) Analysis and separation using the fluorescence activated cell sorter (FACS). In: Handbook of Experimental Immunology, 3rd Edition (D. M. Weir, ed.), pp. 22.1-22.21. Blackwell Scientific Publications, Oxford, England.

Laemmli, U. K. (1970). Cleavage of structural protein during the assembly of the head of bacterophage T4. Nature 227, 680-

Lanier, L. L., Warner, N. L., Ledbetter, J. A., and Herzenberg, L. A. (1981) Expression of Lyt-1 antigen on certain murine B cell lymphomas. J. Exp. Med. (in press)

Ledbetter, J. A., and Herzenberg, L. A. (1979). Xenogeneic monoclonal antibodies to mouse lymphoid differentiation antigens. Immunol. Rev. 47, 63-90.

Ledbetter, J. A., Rouse, R. V., Micklem, H. S. and Herzenberg, L. A. (1980) T cell subsets defined by expression of Lyt-1,2,3 and Thy-1 antigens. J. Exp. Med. 152: 280-295.

Ledbetter, J. A., Evans, R. L., Lipinski, M., Cunningham-Rundles, C., Good, R. A., and Herzenberg, L. A. (1981). Evolutionary conservation of surface molecules that distinguish T lymphocyte helper/inducer and cytotoxic/suppressor subpopulations in mouse and man. J. Exp. Med. 153: 310-323.

Ledbetter, J. A., Seaman, W. E., Tsu, T. T., and Herzenberg, L. A. (1981) Lyt-2 and Lyt-3 antigens are on two different subunits linked by disulfide bonds: Relationship of subunits to T cell cytolytic activity.

(submitted)

McMichael, A.J., Pilch, J. R., Galfre, G., Mason, D. Y., Fabre, J.W., and Milstein, C. (1979). A human thymoctye antigen defined by a hybrid myeloma monoclonal antibody. Eur. J. Immunol. 9, 205-

Nakayama, E., Shiku, H., Stockert, E., Oettgen, H. F. and Old, L. J. (1979)Cytotoxic T cells: Lyt phenotype and blocking of killing activity by Lyt antisera. Proc. Natl. Acad. Sci. USA 76: 1977

O'Farrell, P. Z., Goodman, H. M. and O'Farrell, P. H. (1977) High resolution two dimensional electrophoresis of basic as well as acidic proteins. Cell 12: 1133-1142.

Reinherz, E. L., Kung, P. C., Goldstein, G., and Schlossman, S. F. (1979). Separation of functional subsets of human T cells by a monoclonal antibody. Proc. Natl. Acad. Sci. USA 76, 4061-

Reinherz, E. L., and Schlossman, S. F. (1980). The differentiation and function of human T lymphocytes. Cell 19, 821-

Scollay, R., Kochen, M., Butcher, E., and Weissman, I. (1978) Lyt markers on thymus cell migrants. Nature (London) 276: 79-

Shinohara, N. and Sachs, D. H. (1979) Mouse alloantibodies capable of blocking cytotoxic T-cell function. I. Relationship between the antigen reactive with blocking antibodies and the Lyt-2 locus. J. Exp. Med. 150: 432-

Wang, C., Good, R. A., Ammirati, P., Dymbort, G. and Evans, R. L. (1980) Identification of a p69,71 complex expressed on human T cells sharing determinants with B-type chronic lymphatic leukemia cells. J. Exp. Med. 151: 1539-

TABLE I

| Monoclonal Antibody | | | | |
| --- | --- | --- | --- | --- |
| Name | Species | Isotype | Antigen | Reference |
| 53-7.3 | Rat | IgG2a | Lyt-1 | Ledbetter and Herzenberg, 1979 |
| 53-6.7 | Rat | IgG2a | Lyt-2 | " |
| 53-5 | Rat | IgG1 | Lyt-3.2 | Ledbetter et al., 1980 |
| SK7 | Mouse | IgG2a | Leu-1 | Wang et al., 1980 |
| 17F12 | Mouse | IgG2a | Leu-1 | Engleman et al., 1981 |
| SK1 | Mouse | IgG1 | Leu-2a | Evans et al., 1981 |
| SK2 | Mouse | IgG2a | Leu-2b | " |

TABLE II

QUANTITATIVE CELLULAR EXPRESSION AND DENSITY OF HUMAN AND MOUSE T CELL ANTIGENS BY FACS ANALYSIS*

| Antibody | Thymus | | Lymph Node (mouse) or PBL (human) | |
|---|---|---|---|---|
| | Positive Cells (%) | Antigen Density† | Positive Cells (%) | Antigen Density† |
| Anti-Lyt-2 | 90 | 5.7 | 24 | 5.6 |
| Anti-Lyt-3 | 89 | 6.0 | 22 | 5.8 |
| Anti-Lyt-1 | >95 | 1.5 | 72 | 4.2 |
| Anti-Leu-2a | 84 | 3.4 | 29 | 12.5 |
| Anti-Leu-2b | 83 | 2.8 | 28 | 12.5 |
| Anti-Leu-1 | >95 | 1.5 | 78 | 5.1 |

*BALB/cNHz mouse cells and human cells were stained with directly fluorescein-conjugated antibodies and analyzed on a FACS-II.

†Antigen densities of positive cells are expressed as mean number of antibody molecules bound per cell ($\times 10^4$), calculated as described in Materials and Methods.

TABLE III

SENSITIVITY OF Leu AND Lyt ANTIGENS TO PROTEOLYSIS FROM VIABLE CELLS*

| Enzyme | (μg)† | Antigen Density (% of maximum)§ | | | | | |
|---|---|---|---|---|---|---|---|
| | | Leu-1 | Leu-2a | Leu-2b | Lyt-1 | Lyt-2 | Lyt-3 |
| -- | | 100 | 100 | 100 | 100 | 100 | 100 |
| Trypsin | 5 | 100 | 97 | 39 | 100 | 72 | 5 |
| | 25 | 93 | 43 | 0 | 96 | 43 | 0 |
| | 100 | 82 | 9.5 | 0 | 88 | 17 | 0 |
| Chymotrypsin | 10 | 85 | 68 | 60 | 100 | 34 | 35 |
| | 25 | 80 | 43 | 35 | 100 | 7.5 | 16 |
| Pronase | 10 | 97 | 24 | 36 | 96 | 9 | 12 |
| | 25 | 92 | 15 | 27 | 90 | 0 | 0 |
| Clostridium protease | 400 | 99 | 84 | 42 | 97 | 70 | 38 |

*Antigens were detected by immunofluorescence staining and analyzed on a FACS-II.

† $2 \times 10^7$ cells were treated with the indicated enzyme for 10 min in 0.5 ml PBS at 37°C.

§ Mean fluorescence intensity of positive cells was measured and percentage of antigen remaining was calculated by:

$$\frac{\text{(mean intensity after enzyme treatment)} - \text{(background)}}{\text{(maximum intensity)} - \text{(background)}} \times 100$$

## LEGENDS TO THE FIGURES

---

Fisure 1. Immunofluorescence comparison of human Leu-1 and Leu-2a antisens with mouse Lyt-1 and Lyt-2 antisens on thymocytes and lymph node cells (mouse) or peripheral blood lymphocytes (PBL) (human). Cells were stained with directly fluroescein-conjusated antibodies and analyzed on a modified FACS-II. In each panel, autofluorescence of unstained cells is shown by a dotted line. The fluorescence distributions are displayed on a los(10) scale.

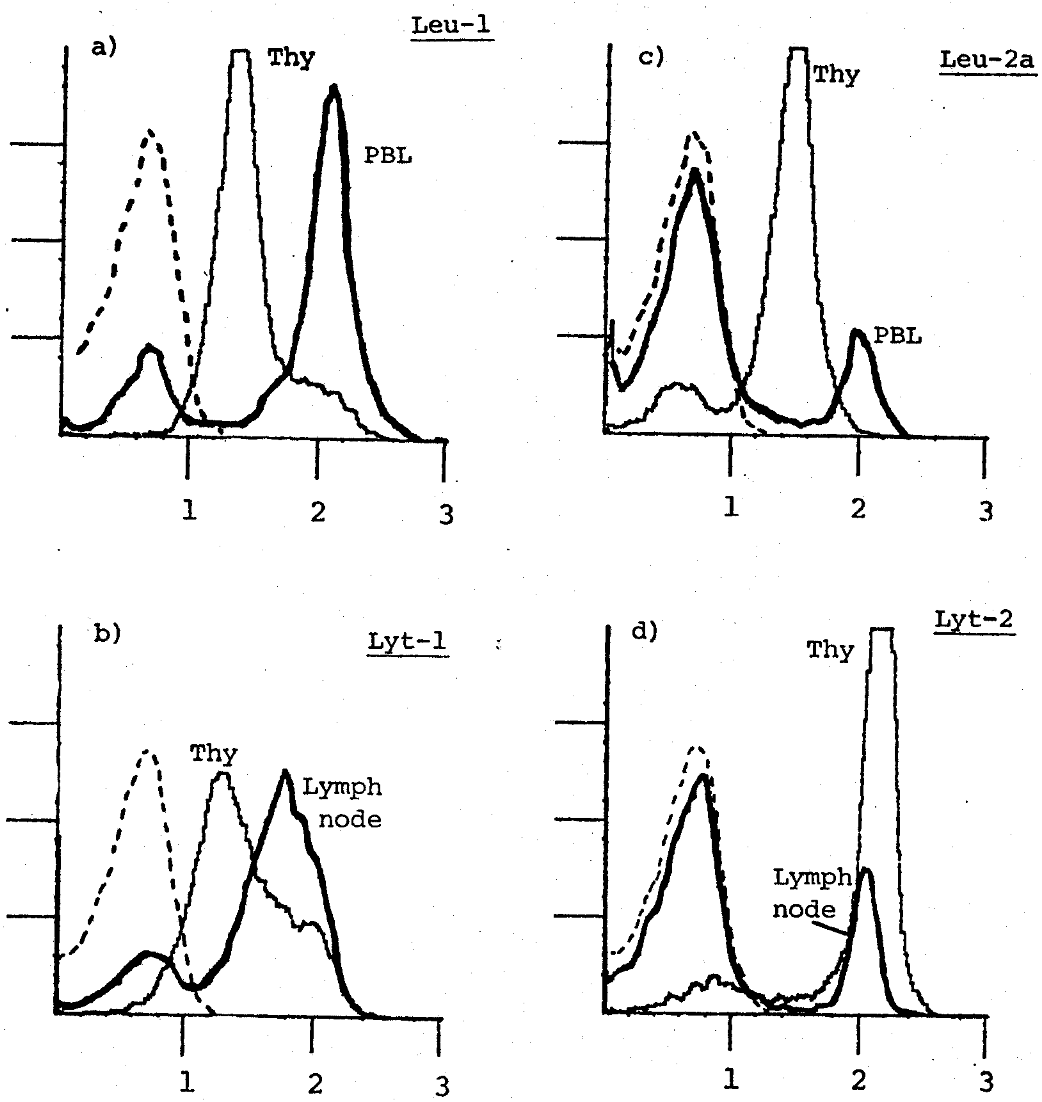
Fisure 2. Comparison of human Leu-1 and Leu-2a with mouse Lyt-1 and Lyt-2 molecules immunoprecipitated from detersent extracts of $^{125}$I lactoperoxidase-labeled thymocytes and electrophoresed on 10% SDS polyacrylamide sels under reducins conditions. Immunoprecipitations were with (1) anti-Leu-1; (2) anti-Leu-2a; (3) anti-Lyt-1; (4) anti-Lyt-2.

Fisure 3. Comparison of mouse and human Lyt-1,Leu-1 and Lyt-2,Leu-2a molecules. Mouse antisens were immunoprecipitated from $^{125}$I-labeled BALB/cNHz thymocytes and human antisnes were immunoprecipitated from 125-I-labeled human thymocytes. Isolated antisens were analyzed on 10% SDS polyacrylamide sels run under either reducins or non-reducins conditions. Immunoprecipitations were with (1) anti-Lyt-1; (2) anti-Leu-1; (3) anti-Lyt-2; (4) anti-Leu-2a; (5) backsround (staph A alone) from mouse thymocytes; and (6) backsround (staph A alone) from human thymocytes.

Fisure 4. Two-dimensional sel electrophoresis of the Lyt-2,3 macromolecule.

A detergent lysate from BALB/cNHz surface-$^{125}$I-labeled thymocytes was immunoprecipitated with anti-Lyt-2. The first dimension was a charge separation with the acidic side on the right and the basic side on the left. The second dimension, from top to bottom, was a 10% SDS-polyacrylamide slab gel. (A) non-reducing gel conditions; (B) reducing gel conditions.

Fig. I



a)
Leu-1
Thy
PBL
1
2
3
c)
Leu-2a
Thy
PBL
1
2
3
b)
Lyt-1
Thy
Lymph node
1
2
3
d)
Lyt-2
Thy
Lymph node
1
2
3

Figure 3

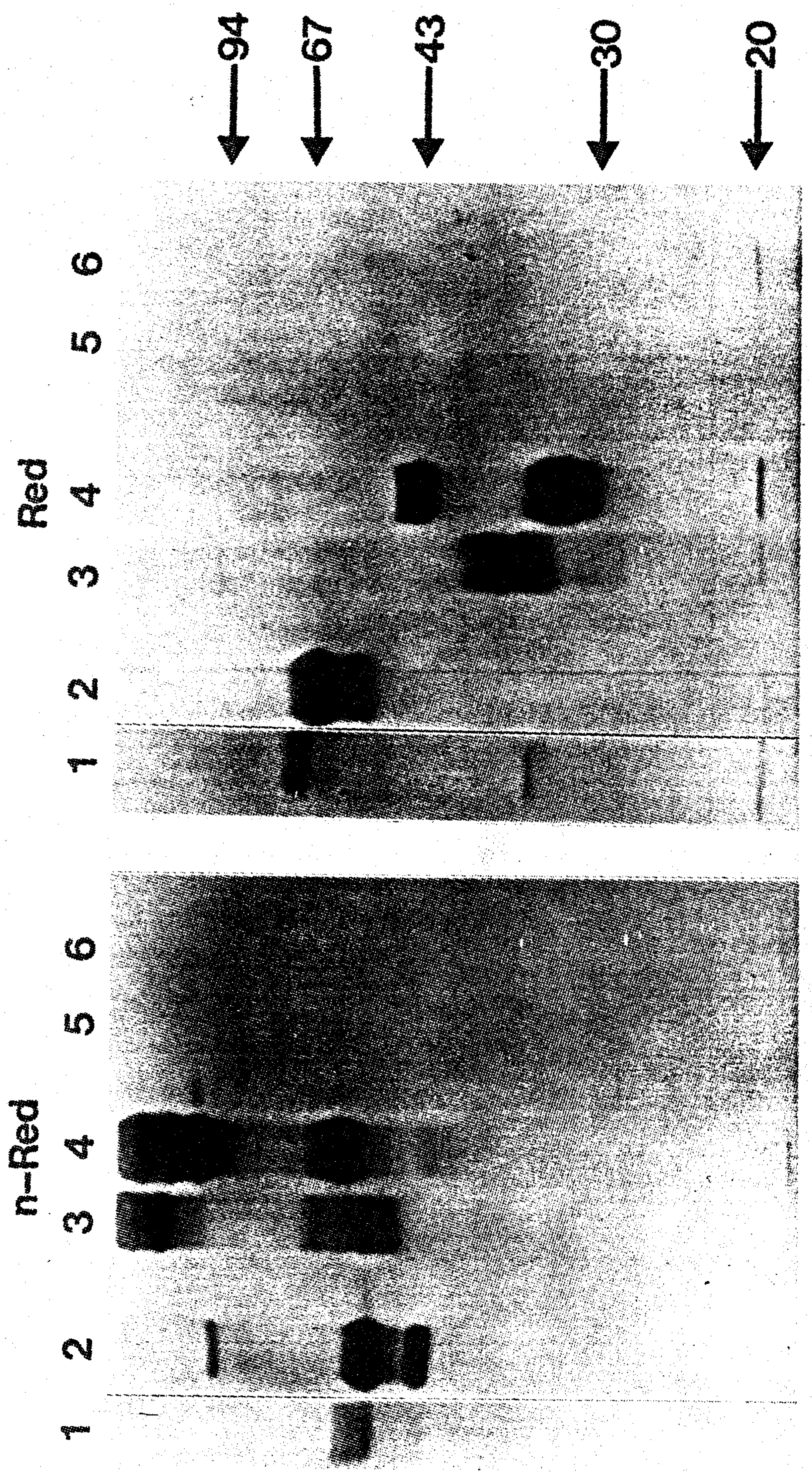


Fig. 4

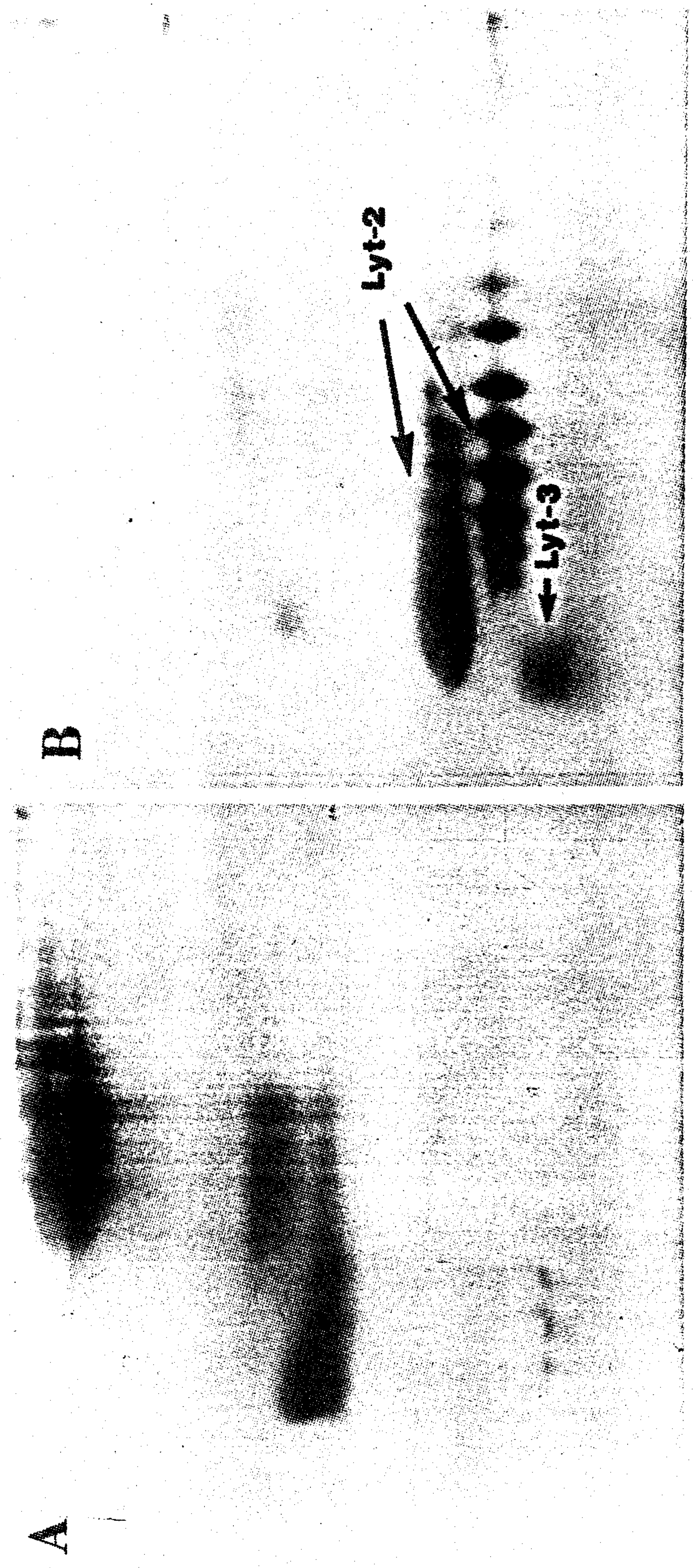


Fig. 2