EVOLUTIONARY CONSERVATION OF SURFACE MOLECULES THAT DISTINGUISH T LYMPHOCYTE HELPER/INDUCER AND CYTOTOXIC/SUPPRESSOR SUBPOPULATIONS IN MOUSE AND MAN*

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The mouse Lyt-1, Lyt-2, and Lyt-3 lymphocyte surface antigens have been used for some years now as immunogenetic markers whose selective expression distinguishes maturational and functional T cell subpopulations (1, 2). Lyt-2 and Lyt-3, for example, are selectively expressed on cytotoxic and suppressor T cells but are not found on helper T cells (3, 4). Similarly, cytotoxic depletion studies originally indicated that Lyt-1 is selectively expressed on helper T cells but not on suppressor or cytotoxic T cells (5, 6). More sensitive immunofluorescence techniques, however, have not shown that Lyt-1 is found on all T cells (7–10) but is present in higher levels on helper T cells than on suppressor or cytotoxic T cells (10). In addition, each of the Lyt-1, Lyt-2, and Lyt-3 antigens undergoes characteristic changes in surface-density expression as T cells mature in the thymus and peripheral lymphoid tissues (9, 10).

Functional subpopulations of human T cells have also been defined by selective expression of certain surface antigens. Two subpopulations of T cells were originally defined by a heteroantiserum termed αTH_2 (11). TH_2^+ cells were shown to mediate suppressor effects in vitro and to be responsible for most of the killing in cell-mediated lympholysis (CML)[1] (12). In contrast, TH_2^- cells had markedly less cytotoxic activity in CML but were found to amplify the functions of other cells and to proliferate in response to specific antigens (mumps and tetanus toxoid) (12).

Recently, both of these subsets have been redefined by monoclonal antibodies (13–15)[2] and analogies drawn between the human antigens and mouse Lyt antigens (15). The analogy is based primarily on the view that Lyt-1 is expressed on the helper/inducer but not the suppressor/cytotoxic subset in the mouse. As this view needs to be

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[1] *Abbreviations used in this paper*: CLL, chronic lymphocytic leukemia; CML, cell-mediated lympholysis, CTL, cytotoxic T lymphocytes; FACS, fluorescence-activated cell sorter; M_r, molecular weight (relative); NP-40, Nonidet P-40; staph A, formalin-fixed, heat-inactivated *Staphylococcus aureus*, Cowan I strain.

[2] Evans, R. L., D. W. Wall, C. D. Platsoucas, F. P. Siegal, S. M. Fikrig, C. M. Testa, and R. A. Good. Thymus-dependent membrane antigens in man: inhibition of cell-mediated lympholysis by monoclonal antibodies to the T_{H2} antigen. Manuscript submitted for publication.

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revised (see above), the human-mouse analogy also needs careful reevaluation based on comparative quantitative flow immunofluorescence, biochemical, developmental, as well as functional analyses.

In this article, we show that the exclusive marker of the human helper/inducer T cell subset has no known mouse counterpart, whereas the human T cell antigens, Leu-1, Leu-2a, and Leu-2b, have strong analogies to the mouse Lyt-1, Lyt-2, and Lyt-3 antigens, respectively. Our studies indicate that molecules carrying these human Leu antigens approximate the size, charge, and subunit composition of the corresponding molecules carrying the mouse Lyt antigens. Moreover, each of these structurally homologous antigen systems has similar sensitivity to trypsin and shows similar quantitative changes during T cell maturation in the thymus.

The molecules carrying the Leu and Lyt antigens have undergone evolutionary change because no cross-reactivity was observed between murine and human T lymphocyte antigens when staining with either anti-Lyt or anti-Leu antibodies. Nevertheless, these molecules appear to have conserved their basic properties such as surface expression patterns and molecular configuration. This finding suggests that such properties may play a sufficiently valuable role to have favored retention through evolution.

Materials and Methods

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

Monoclonal Antibodies. Production of the mouse (BALB/c) anti-human T cell monoclonal antibodies was previously described.[2] Production of the rat (Lou/Ws1/M) anti-mouse T cell monoclonal antibodies also was previously described (8).

Immunofluorescence of Cell Suspensions. Immunofluorescence staining of mouse lymphoid cells with rat monoclonal antibodies was performed using directly fluorescein-conjugated antibodies. For the direct conjugations with fluorescein, antibodies were first purified from supernate on goat anti-rat Ig affinity columns. (The goat anti-rat Ig serum was kindly provided by Dr. R. Wilsnack, Huntingdon Research Center, Brooklandville, Maryland.) Fluorescein conjugations were with fluorescein isothiocyanate according to the method of Goding (16). Immunofluorescence staining of human lymphoid cells with monoclonal mouse antibodies was performed with an indirect system using monoclonal antibody-containing supernate in the first step and fluorescein-conjugated goat anti-mouse IgG_1 or fluorescein-conjugated goat anti-mouse IgG_2 in the second step. For staining mouse or human cells, reagents were first centrifuged at 100,000 *g* before use, and 10^6 target lymphoid cells/well in microtiter plates were reacted with saturation levels of first and second step reagents. Cells were stained on ice in the presence of 0.01% NaN_3 as previously described (17).

Cell Surface Labeling and Detergent Extraction. BALB/cNHz thymocytes or lymph node cells were labeled with ^{125}I by a modification of the lactoperoxidase technique. To 1×10^8 cells in 1 ml phosphate-buffered saline at 20°C were added 1.0 mCi ^{125}I (Amersham Corp., Arlington Heights, Ill.), 25 μg lactoperoxidase (B grade; Calbiochemi-Behring Corp., American Hoechst Corp., San Diego, Calif.) and successive 10 μl pulses of H_2O_2 (0.3 mM, 1 mM, 3 mM, and 9 mM) at 5-min intervals. Cells were washed twice and extracted in lysis buffer (0.5% Nonidet P-40 [NP-40; Particle Data, Inc., Elmhurst, Ill.]; 50 mM Tris, 150 mM NaCl; 0.02% NaN_3; 5 mM EDTA; 50 mM phenylmethylsulfonyl fluoride; 0.2 TIU [tryspin-inhibitor unit]/ml Aprotinin, [Sigma Chemical Co., St Louis, Mo.]; 1 μg/ml pepstatin A, [Sigma Chemical Co.]; and 50 mM iodoacetamide, pH 8.0) for 30 min at 4°C. Nuclei were removed by centrifugation at 5,000 *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 μg antibody/extract from 2×10^7 cells. After a 1-h incubation on ice, antigen-antibody complexes were collected by the addition of 10 μl of 10% formalin-fixed, heat-inactivated *Staphylococcus aureus*, Cowan I strain (staph A; Tufts New England Enzyme Center, Boston, Mass.). 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. For the mouse γ_1 antibodies that do not bind to protein A directly, the staph A was precoated with affinity-purified rabbit anti-mouse Ig and washed twice before addition to the cell extract. After 45 min on ice, the staph A-antibody-antigen complexes were washed three times in washing buffer (0.5% NP-40, 0.45 M NaCl, 50 mM Tris, 5 mM KI, and 0.02% NaN_3; pH 8.3) and then extracted with sample buffer for gel electrophoresis. Immunoprecipitates were analyzed by 10% sodium dodecyl sulfate (SDS)-polyacrylamide one-dimensional gels (18). Molecular weight markers (Pharmacia Fine Chemicals, Div. of Pharmacia, Inc., Piscataway, N. J.) run on each gel were visualized by staining with Coomassie brilliant blue (Bio-Rad Laboratories, Richmond, Calif.). Radioautography was with intensifying screens (Cronex lightning plus screens; E. I. Du Pont de Nemours & Co., Wilmington, Del.) using Kodak X-Omat R film (Eastman Kodak Co., Rochester, N. Y.) at −70°C.

Results

Lyt-2, Lyt-3 and Leu-2a, Leu-2b Show Similar Biochemical Characteristics and Determinant Density Distributions (Fluorescence-activated Cell Sorter [FACS] Profiles). The Leu-2a and Leu-2b antigens in man, like the Lyt-2 and Lyt-3 antigens in mice (10), are lost from the majority of thymocyte cells as they mature. Thus they are found on 80–90% of thymocytes and 20–40% of peripheral T cells (Fig. 1). The distinction between positive and negative cells is quite clear in the FACS log profiles of fluorescence intensity obtained with stained populations. The variations indicated in the size of the populations carrying these determinants derive from clear and reproducible differences between individuals.

The identical staining patterns obtained with the Leu-2a and Leu-2b monoclonal antibodies suggest that the determinants detected by these antibodies, like the Lyt-2 and Lyt-3 determinants (J. A. Ledbetter and L. A. Herzenberg, unpublished observations), are found on the same macromolecule and appear to be expressed to the same extent on the same cells (Fig. 1). Staining with a mixture of anti-Leu-2a and anti-Leu-2b confirms the coincident expression of these determinants in that the frequency of cells stained with both antibodies is the same as the frequency of cells stained with either antibody alone (data not shown).

Biochemical analyses also show that the anti-Leu-2a and anti-Leu-2b antibodies immunoprecipitate the same macromolecule (Fig. 2) which is quite similar to the mouse Lyt-2, Lyt-3 macromolecule. Both the human and the mouse macromolecules are composed of polypeptide subunits that are disulfide-bonded into a variety of multimeric forms (see Fig. 3). The macromolecule immunoprecipitated from mouse thymocytes has subunits of 30,000, 34,000, and 38,000 molecular weight (relative) (M_r) in SDS-polyacrylamide gels run under reducing conditions. The macromolecule from human thymocytes shows two subunits (32,000 and 43,000 M_r). The 32,000-M_r subunit is quite broad, and there is a hint that this band could be resolved into two separate bands. Another possibility is that the apparent absence of the third subunit on human cells could be a result of poor ^{125}I-labeling, because one of the mouse subunits (30,000 M_r), which we have shown carries the Lyt-3 determinant, is also often difficult to detect under the labeling conditions used here (J. A. Ledbetter and L. A. Herzenberg. Unpublished observation.). The subunits of both the mouse and

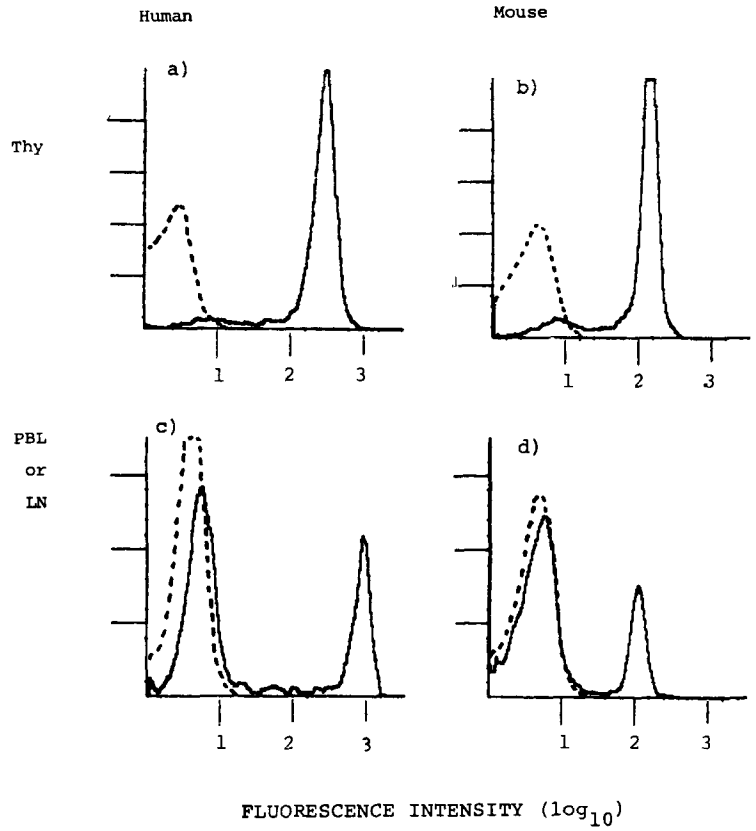

FIG. 1. FACS analysis of Leu-2a and Lyt-2 determinant expression on human and mouse lymphoid cells. The Leu-2a antigen on human thymocytes (Thy) (a) and peripheral blood lymphocytes (PBL) (c) was detected by indirect staining with SK1 monoclonal antibody supernate followed by fluorescein-conjugated goat anti-mouse IgG_1. Lyt-2 antigen on mouse thymocytes (b) and lymph node cells (d) was detected by direct staining with fluorescein-conjugated 53-6.7 antibody. Background (second step alone or unstained cells) is shown as the dotted line in each panel. Fluorescence distributions were analyzed with a FACS-II fitted with a logarithmic amplifier.

human molecules are exceptionally basic among cell surface molecules (isoelectric point >8) as judged by two-dimensional gel analyses (data not shown).

Under nonreducing gel conditions, both the mouse Lyt-2, Lyt-3 and human Leu-2a, Leu-2b molecules from thymocytes appear in several larger forms whose sizes suggest the association of subunits into dimers and tetramers (see Fig. 3). These forms from the thymus differ slightly in size from the molecules immunoprecipitated from peripheral lymphocytes (lymph node in mouse or peripheral blood in human) (Fig. 2). The subunits visible under reducing conditions show corresponding size differences. Thus, the quite similar patterns of subunit size and associations into multimeric forms of the human and mouse macromolecules along with the FACS data presented above, suggests that these molecules are homologous.

Trypsin digestion studies provide further indication of this homology because Leu-2a resembles Lyt-2 and Leu-2b resembles Lyt-3 in trypsin sensitivity. The Lyt-3 and Leu-2b determinants are carried on a relatively trypsin-sensitive segment of each

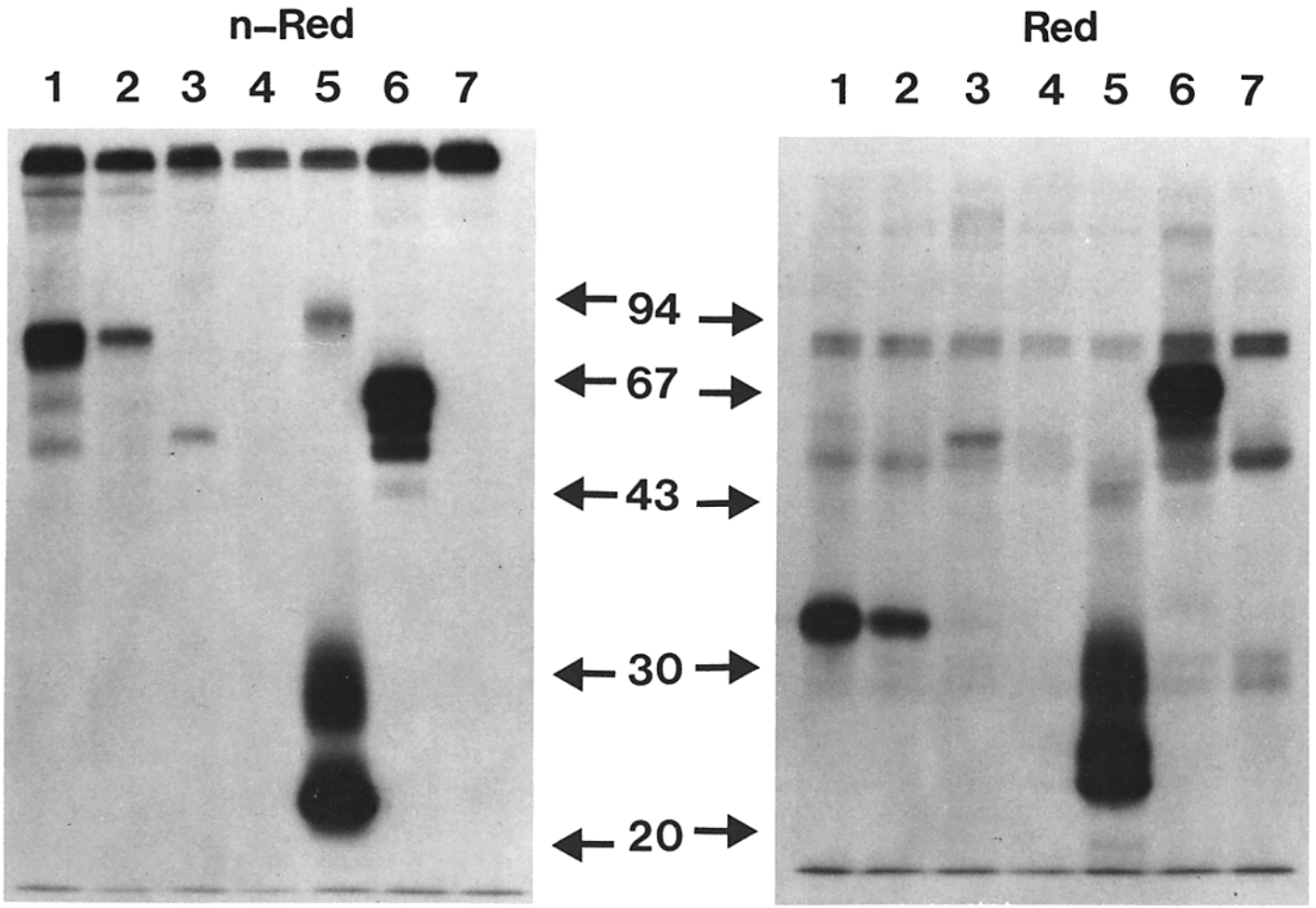

FIG. 2. Immunoprecipitation of human T cell antigens from peripheral blood lymphocytes. Peripheral blood mononuclear cells from one of us (J. A. Ledbetter) were isolated on Ficoll-Paque, labeled with ^{125}I using lactoperoxidase, extracted with detergent, and immunoprecipitated with monoclonal anti-Leu antibodies for analysis for 10% SDS-polyacrylamide gels run under either reducing or nonreducing conditions. Immunoprecipitations were with the following antibodies: (1) anti-Leu-2a (SK1); (2) anti-Leu-2b (SK2); (3) anti-Leu-3a (SK3); (4) anti-Leu-3b (SK4); (5) anti-Leu-4 (SK7); (6) anti-Leu-1 (SK5); and (7) background (staph A alone). Red, reduced; n-Red, nonreduced.

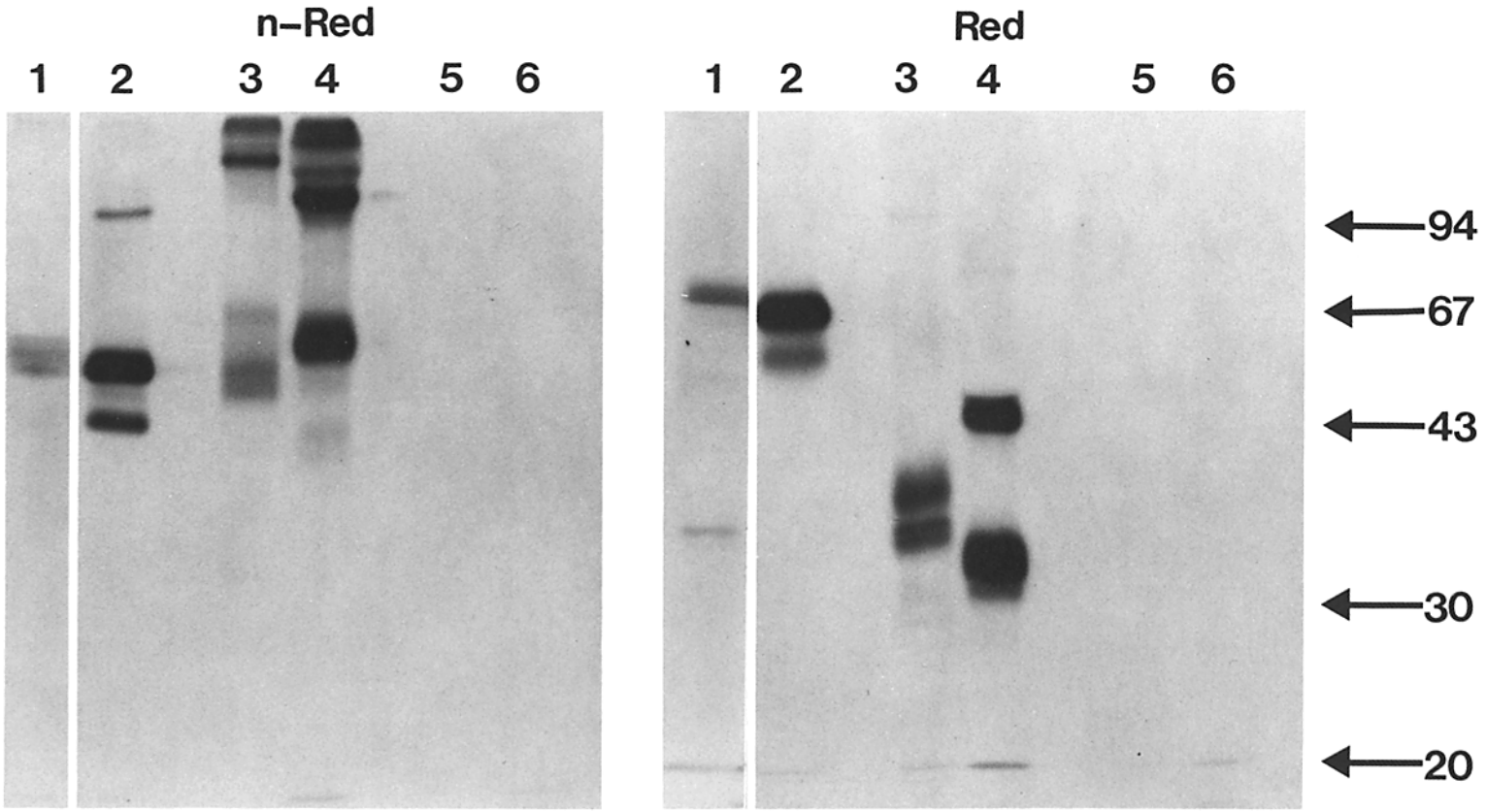

FIG. 3. Comparison of mouse and human Lyt-1, Leu-1 and Lyt-2, Leu-2a molecules. Mouse antigens were immunoprecipitated from ^{125}I-labeled BALB/cNHz thymocytes and human antigens were immunoprecipitated from ^{125}I-labeled human thymocytes. Isolated antigens were analyzed on 10% SDS polyacrylamide gels run under either reducing or nonreducing conditions. Immunoprecipitations were with (1) anti-Lyt-1 (53-7.3); (2) anti-Leu-1 (SK5); (3) anti-Lyt-2 (53-6.7); (4) anti-Leu-2a (SK1); (5) background (staph A alone) from mouse thymocytes; and (6) background (staph A alone) from human thymocytes. The radioautographs were made with image-enhancing screens at −70°C Red, reduced; n-Red, non-reduced.

TABLE I

Trypsin Sensitivity of T Cell Antigens*

Cell type	Antibody	Mean fluorescence intensity‡
Human PBL	Leu-1	0.95
	Leu-2a	0.79
	Leu-2b	0.09
Mouse spleen	Lyt-1	0.98
	Lyt-2	0.76
	Lyt-3	<0.05

* Trypsin treatment: 25 μg trypsin, 2×10^7 cells for 10 min in 0.5-ml wells at 37°C.

‡ After trypsin treatment. Untreated controls = 1.0.

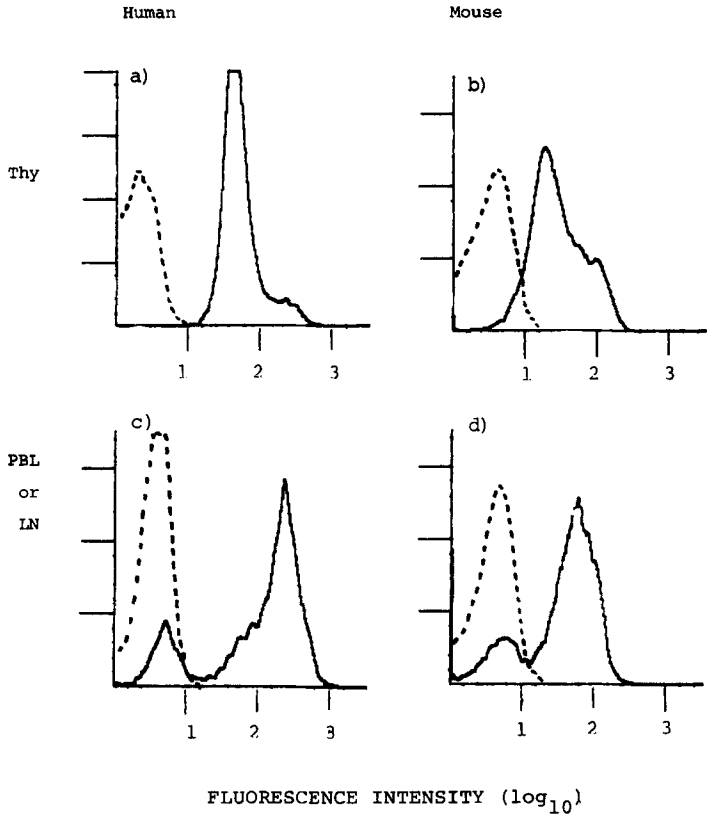

FIG. 4. FACS analysis of Leu-1 and Lyt-1 determinant expression on human and mouse lymphoid cells. The Leu-1 antigen on human thymocytes (Thy) (a) and peripheral blood lymphocytes (PBL) (c) was detected by indirect staining with SK5 monoclonal antibody supernate followed by fluorescein-conjugated goat anti-mouse IgG_2. Lyt-1 antigen on mouse thymocytes (b) and lymph node cells (LN) (d) was detected by direct staining with fluorescein-conjugated 53-7.3 antibody. Background (second step alone or unstained cells) is shown as the dotted line in each panel. Fluorescence distributions were analyzed with a FACS-II fitted with a logarithmic amplifier.

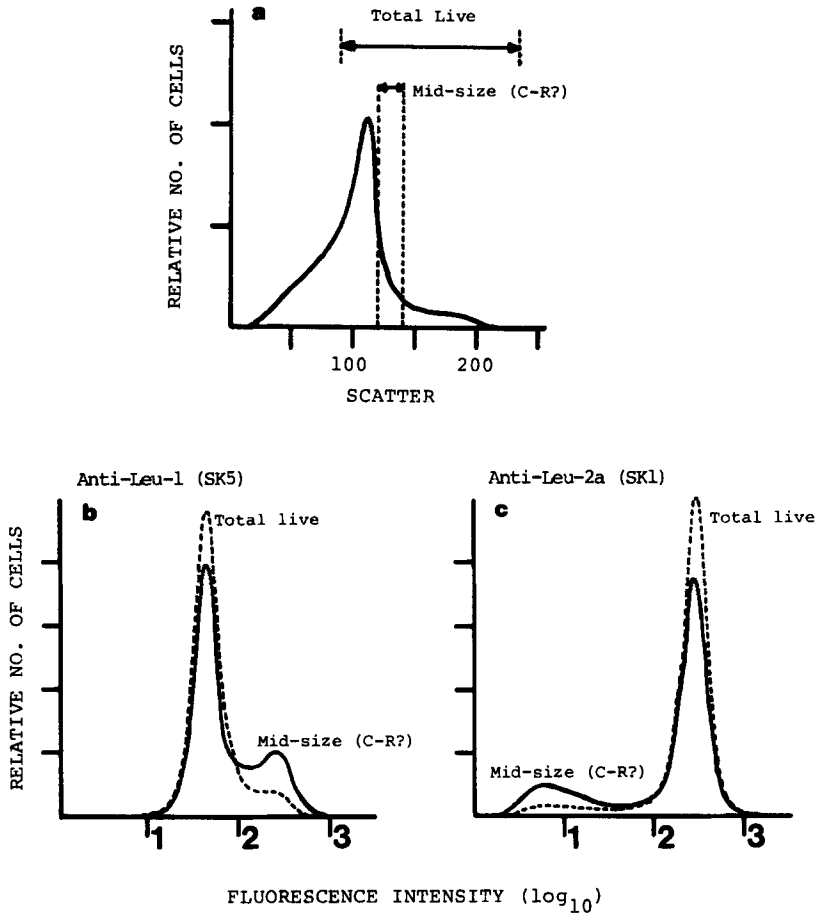

FIG. 5. FACS analysis of Leu-1 and Leu-2a determinant expression on human thymocytes. The scatter profile in (a) shows the gating for total live thymocytes and mid-size thymocytes (cortisone-resistant [C-R?]) used for the fluorescence profiles in (b) and (c). The fluorescence profiles of the mid-size (C-R?) scatter gating is shown as a solid line.

molecule which is removed almost completely from the peripheral lymphocyte surface by exposure to 50 μg/ml of trypsin for 10 min at 37°C (see Table I). Lyt-2 and Leu-2a, in contrast, are poorly removed under these conditions (Table I) and require exposure to substantially more trypsin for complete removal.

Lyt-1 and Leu-1 Show Similar Biochemical Characteristics and Determinant Density Distributions. Fig. 3 shows the biochemical similarities between the Lyt-1 and Leu-1 glycoproteins. Both consist of single polypeptide chains of ~67,000 M_r (i.e., no disulfide-linked subunits) that show extensive charge heterogeneity on two-dimensional gels (data not shown) as well as two or three lower molecular weight derivatives, depending on the lymphoid organ (Fig. 2) or T cell line from which the molecules are derived. Recent internal labeling with [^{35}S]methionine and pulse-chase studies show that the smaller molecular weight form of Leu-1 is a precursor of the large form (data not shown). These results confirm the approximate size of the Leu-1 antigen that was

previously reported (19). Both Lyt-1 and Leu-1 are resistant to trypsin treatment of viable cells (Table I).

Lyt-1 and Leu-1 also show strikingly similar antigen density distributions (FACS fluorescence profiles) when human and mouse thymocytes or peripheral lymphocytes are compared (Fig. 4). The thymocyte profiles both show a distinct shoulder of cells with higher antigen density. This shoulder has been shown in the mouse to derive from a medullary (more mature) subpopulation (7, 9) that contains cells in a narrow size range slightly larger than most thymocytes but smaller than the largest cells located in the subcapsular region (20). FACS profiles of human cells that fall in a

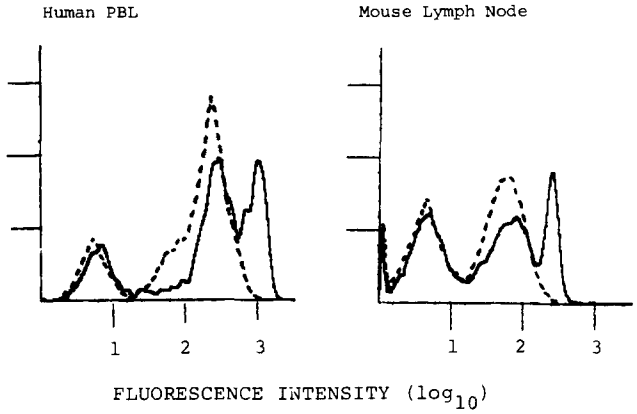

FIG. 6. Immunofluorescence staining of human peripheral blood lymphocytes (PBL) on mouse lymph node cells with anti-Leu-1 (SK5) alone or anti-Lyt-1 alone (53-7.3) (dotted line) compared with anti-Leu-1 plus anti-Leu-2a (SK1) or anti-Lyt-1 plus anti-Lyt-2 (53-6.7) (solid line). Human cells were stained indirectly using monoclonal antibody supernates followed by a fluorescein-conjugated goat anti-mouse IgG_1 second-step antibody. Mouse cells were stained with directly fluorescein-conjugated anti-Lyt-1 (53-7.3) and anti-Lyt-2 (53-6.7) antibodies. Fluorescence distributions were analyzed on a FACS-II fitted with a logarithmic amplifier.

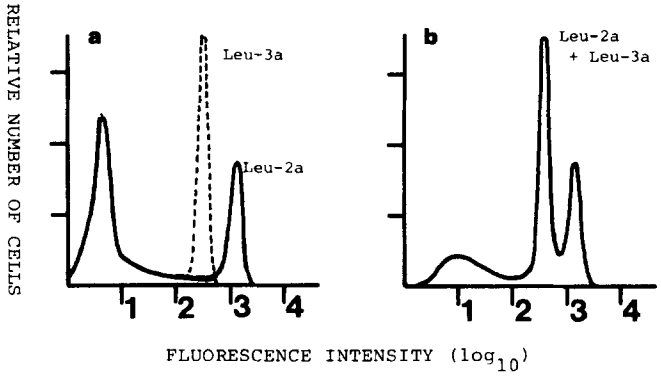

FIG. 7. Immunofluorescence analysis of Leu-2 and Leu-3 peripheral T cell subpopulations. Human peripheral blood lymphocytes were stained indirectly with monoclonal antibody supernate followed by fluorescein-conjugated goat anti-mouse IgG_1. In (a) the Leu-3a- and Leu-2a- (detected by monoclonal antibodies SK1 and SK3) positive cells were stained separately. In (b) the cells were stained with anti-Leu-2a and anti-Leu-3a simultaneously. Fluorescence distributions were analyzed using a FACS-II fitted with a logarithmic amplifier.

similar size range to mouse medullary thymocytes show substantial enrichment of the bright Leu-1 thymocyte subpopulation (Fig. 5); therefore the cells under the bright shoulder in the profile of human thymocytes are probably medullary cells.

In the Leu-1 FACS profile of human peripheral lymphocytes, as in the Lyt-1 profile of mouse peripheral T cells, stained cells display the antigen density of the bright (medullary) thymocyte subpopulation (Fig. 4). Thus both Lyt-1 in mouse and Leu-1 in human appear to increase in density as thymocytes mature and to show corresponding densities on what seems to be the more mature population in thymus and the T cells that are found in the periphery.

The peripheral T cell subpopulations in human and mouse also have similar quantitative Leu-1 and Lyt-1 surface-density patterns. Less Lyt-1 is found on Lyt-2^+ cells than on Lyt-2^- cells, as revealed by two-color FACS analyses (10). This difference is also revealed by additive staining profiles from cells stained with mixtures of Lyt-1 and Lyt-2, which indicates that the cells on the low antigen-density (duller) side of the Lyt-1 profile are selectively increased in staining brightness by inclusion of anti-Lyt-2 in the staining mixture (see Fig. 6). The Leu-1 additive staining profile shows the same shift of the dull Leu-1^+ cells to the brighter side of the curve when anti-Leu-2a is added to the staining mixture. Thus again, the human and mouse antigens show strong similarities in the complex pattern of determinant representation on cell subpopulations.

Human TH_2^- (Helper/Inducer) T Cells Carry Antigens (Leu-3a and Leu-3b) Not Found on TH_2^+ (Cytotoxic/Suppressor) T Cells. Our recent studies (Introduction) have shown that Lyt-1 determinants are found on all T cells, although in smaller amounts on the Lyt-2^+ cells than on Lyt-2^- cells. As shown above, the Leu-1 marker on human cells closely parallels the Lyt-1 marker distribution. Interestingly, unlike the mouse Lyt-1^+, Lyt-2^- population, the human Leu-1^+, Leu-2^- population has a distinctive molecular species, Leu-3 (55,000 M_r; Fig. 3) with two determinants, Leu-3a and Leu-3b, detected by two other monoclonal antibodies, SK3 and SK4.[2] The Leu-3 antigen seems to be distributed similarly to the T4 antigen reported by Reinherz et al. (13, 14).

As reported earlier using cytofluorograph analysis,[2] FACS profiles (Fig. 7) demonstrate that the Leu-2 and Leu-3 antigens are on nonoverlapping subpopulations of peripheral blood T cells. The fluorescence distribution obtained with a mixture of the two antibodies is the composite of the distributions obtained with each of the antibodies alone. The Leu-2 and Leu-3 cells represent >90% of the number of cells stained with anti-Leu-1 or another pan-T cell monoclonal antibody, SK7 (Fig. 2).

Discussion

Homologies or analogies between human and mouse T cell antigens have been proposed (15, 21) but the homologues were not based on direct comparisons nor did they take account of all the data now available. The assignment of the T4 antigen as the Lyt-1 homologue (15, 21) is not correct, in our opinion, as T4 is a unique marker of helper/inducer antigens whereas Lyt-1 is on all T cells. Leu-1 is our candidate for the Lyt-1 homologue, and no mouse equivalent of T4 is known. Our evidence on homologies derives from comparing the mouse and human antigens by determination of their density distributions on thymocytes and T cells by FACS analysis; their size, charge, and subunit compositions; and by their sensitivities to proteolysis by trypsin.

We feel this evidence strongly supports the idea that the Leu-1 and Leu-2a, Leu-2b molecules are the human homologues of the mouse Lyt-1 and Lyt-2, Lyt-3 molecules.

The Leu-1 antigen is unique among human T lymphocyte antigens thus far detected by monoclonal antibodies because it is found on Ig^+ leukemic cells from most patients with chronic lymphocytic leukemia (CLL) but is not found on normal B cells. Although the presence of Leu-1 on a small subpopulation of normal B cells cannot be excluded, it was previously proposed that this antigen is induced on CLL cells as a consequence of leukemogenesis (19). This observation, together with the previously reported molecular weight of Leu-1 (69,000–71,000 dalton), raised the possibility that this antigen is related to the mouse endogenous viral gp70-related antigen GIX, which is also expressed on leukemias derived from GIX^- cells (22).

The Leu-1/Lyt-1 analogy now demonstrated is not consistent with the above view because the size and charge properties of Lyt-1 on two-dimensional gel analyses are distinct from those of gp70. Furthermore, Lyt-1 carries no detectable gp70 determinants because it does not react with a broadly reactive (group-specific) heterologous anti-gp70 antiserum (J. A. Ledbetter and L. A. Herzenberg, unpublished observations). Lyt-1 is similar to Leu-1 in its expression on B cell leukemias, as Lyt-1 also has now been found in high levels on 3 of 15 surface-Ig^+ B cell leukemias (L. Lanier, N. Warner, J. A. Ledbetter, and L. A. Herzenberg, unpublished observation). Although Lyt-1 has not been detected on normal B cells in suspension, it is found on a few $Thy\text{-}1^-$ cells in B cell areas of frozen sections of lymph node and spleen (10).

The apparent conservation of the molecular structure and subpopulation density distributions for the Leu and Lyt antigens suggests that these surface structures are not simply convenient markers that allow the distinction of T cell populations at various stages of maturity or with different functional capabilities. Rather, the maintenance of these structures throughout evolution seems more likely to have occurred because these molecules perform essential functions for the cells on which they are found. Such functions are probably reflected in the absolute changes that occur during differentiation (e.g., $Lyt\text{-}2^+/Leu\text{-}2a^+$ to $Lyt\text{-}2^-/Leu\text{-}2a^-$), the quantitative changes that occur (e.g., Lyt-1/Leu-1 dull to Lyt-1/Leu-1 bright), and the maintenance of selective quantitative expression patterns in different subpopulations (e.g., less Lyt-1/Leu-1 on $Lyt\text{-}2^+/Leu\text{-}2a^+$ cells than on $Lyt\text{-}2^-/Leu\text{-}2a^-$ cells); which, if any, of these changes are directly related to effector functions and which are involved in the ability to send or receive differentiation signals is not clear.

As we have indicated, the forms of the Lyt-2, Lyt-3/Leu-2a, Leu-2b macromolecule present on thymocytes differ from those found on peripheral T cells. Whether these differences are a result of modifications in primary structure or of the carbohydrate moiety of the molecule, and whether they relate to the molecule's functional properties is still unknown. Further biochemical studies on these molecules may help elucidate how they work.

As with Lyt-2, Lyt-3 in the mouse, Leu-2a, Leu-2b is found on virtually all cortical (immature) thymocytes but is selectively expressed on the suppressor subset of peripheral T cells. The mean determinant density of these antigens in both species is higher on the peripheral T cells than in the thymus. A clue to the function of the Lyt-2, Lyt-3 molecule is that cytotoxic T lymphocyte (CTL) killing of target cells is specifically blocked by anti-Lyt-2 or anti-Lyt-3 but not by antibodies to other CTL surface antigens (23, 24). The specific blocking of CTL killing is also seen with anti-

Leu-2a and anti-Leu-2b in man.[2] These findings, properly, have been cautiously interpreted, as the blocking could be a result of a tendency for the Lyt-2, Lyt-3/Leu-2a, Leu-2b molecule to associate with other molecules relevant to the killing activity. This cautious interpretation is supported by recent data (J. A. Ledbetter, W. Seaman, T. Tsu, and L. A. Herzenberg, unpublished observations) which show that trypsin treatment selectively removes most of the Lyt-3 subunit and causes nonparallel losses of cytolytic activity. If the Lyt-3 subunit were directly involved in T cell killing, we should have found parallel losses of the antigen and cytolytic activity.

The loci controlling Lyt-2 and Lyt-3 are closely linked to each other and to the mouse κ-chain region on chromosome 6 (25–28). This close linkage may, of course, be fortuitous. If, however, the homologous human genes Leu-2a, Leu-2b, and κ are also found to be closely linked, then linkage may be evolutionarily maintained to permit functional interaction between these genes. An obvious possibility is that V_K gene products are associated with the Lyt-2, Lyt-3 molecule. However, control of expression of these linked genes seems to be different, as Lyt-2, Lyt-3 are not allelically excluded (J. A. Ledbetter and L. A. Herzenberg, unpublished observations), whereas V_K is allelically excluded on B cells and may also be allelically excluded on T cells.

The definition of the human helper/inducer subset by a specific antigen, Leu-3 or T4 (21), has no known murine counterpart. Such an antigen, if it exists, is presumably not polymorphic because it has not been found by the extensive alloimmunization studies performed in the mouse. However, new hybridomas obtained using xenogeneic immunization may yield a monoclonal antibody revealing this antigen on mouse helper T cells.

Summary

We describe the biochemical properties and cell surface distributions of three human T cell antigens (Leu-1, Leu-2a, and Leu-2b) which we postulate to be the homologues of the Lyt-1, Lyt-2, and Lyt-3 antigens that distinguish functional T cell subsets in the mouse. Leu-1, like Lyt-1, is on all thymocytes and peripheral T cells and is present in greater amounts on the helper/inducer subset than on the cytotoxic/suppressor subset. Both antigens increase in parallel fashion during T cell maturation in the thymus and each antigen is carried on a single 67,000-molecular weight (relative) (M_r) polypeptide chain. Surprisingly, Leu-1 and Lyt-1 each are also expressed in readily detectable amounts on some B cell leukemias but not detectably so on normal B cells.

Leu-2a and Leu-2b are antigens found only on suppressor/cytotoxic cells in the human and are very similar to the murine Lyt-2 and Lyt-3 antigens. In both species, the two antigens are on the same disulfide- linked multimeric molecules. Disulfide-bond reduction in both species yields subunits of similar size and charge. Lyt-3 and Leu-2b are extremely sensitive to trypsin digestion on viable cells whereas Lyt-2 and Leu-2a are much less so.

A different membrane antigen, Leu-3, is an exclusive marker of the helper/inducer subset in man. No mouse homologue for this 55,000-M_r protein is known.

The maintenance of the homologous molecules on functionally distinct T cell subpopulations in two evolutionarily distant species suggests that the Lyt and Leu antigens perform essential functions for the cells on which they are found.

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