#173

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Homologous T-Cell Differentiation Antigens of Mouse and Man

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Introduction

Delighted as I am to participate in this symposium honoring Ray Owen, I must confess that I am an illegitimate participant. I never was formally a student or postdoctoral fellow of Ray's. However, he has played such an important role in my personal and professional development and that I greatly appreciate this opportunity to join with his legitimate "offspring" on this wonderful occasion.

In many ways I do consider myself a student of Ray's. In my graduate student days at Caltech, each of us was required to have a minor thesis and minor subject area and I took Ray's course in immunology. He introduced me to the concepts and facts of immunology and immunogenetics in a marvelous, stimulating and eventually compelling fashion. Most people now consider me more of an immunologist or immunogeneticist than a practitioner in the areas of my pre- and post-doctoral work: biochemical genetics and what has now become known as molecular biology.

Much of the work in our lab and increasingly in cellular immunology generally makes extensive use of the fluorescence-activated cell sorter (FACS) (Bonner et al., 1972; Boyse et al., 1971; Cantor and Boyse, 1975). With hybridoma-produced monoclonal antibodies to cell surface antigens (Cantor et al., 1975; Cantor et al., 1976; Cantor and Boyse, 1977a). providing the opportunity of overcoming the remaining major technical problem for analyzing and sorting distinct lymphoid cells viably, we have focused our efforts on characterization of subpopulations of T and B-cells. Here we present our recent work on T-cells of mice and men (Cantor and Boyse, 1977b; Evans et al., 1977).

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 (Evans et al., 1978; Fatham et al., 1975). Lyt-2 and Lyt-3, for example, are selectively expressed on cytotoxic and suppressor T-cells but are not found on helper T-cells (Herzenberg et al., 1976; Herzenberg and Herzenberg, 1978). Similarly, cytotoxic depletion studies originally indicated that Lyt-1 is selectively expressed on helper T-cells but not on suppressor or cytotoxic T-cells (Jandinski et al., 1976; Köhler and Milstein, 1975). More sensitive immunofluorescence techniques, however, have now shown that Lyt-1 is found on all T-cells (Cantor and Boyse, 1977a; Cantor and Boyse, 1977b; Ledbetter and Herzenberg, 1979; Ledbetter et al., 1980) but is present in higher levels on helper T-cells than on suppressor or cytotoxic T-cells (Cantor and Boyse, 1977b). 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 (Cantor and Boyse, 1977b; Ledbetter et al., 1980) as shown in the following sections.

Lyt-1 is on All T-Cells and Increases During Maturation

All thymocytes and splenic (Thy-1-bearing) T-cells express Lyt-1 antigen; however, there is a clear bimodal distribution of antigen densities that correlates with maturation (Figure 1a). In thymus, approximately 15-20% of the cells stain brightly for Lyt-1. These form a "shoulder" on the brighter side of the main population. The splenic or lymph node Lyt-1$^+$ cells are about four times brighter than the main thymocyte population and correspond in brightness to the smaller number of bright thymus cells. Since this latter is the cortisone-resistant thymocyte population (Cantor and Boyse, 1977a), these data indicate that levels of Lyt-1 antigen increase during T-cell maturation (Table 1).

Table 1. Quantitative Cellular Expression and Density of T-Cell Antigens by FACS Analysis.[a]

	Thymus		Spleen		Lymph node	
Antibody	Positive cells (%)	Antigen density[b]	Positive cells (%)	Antigen density[b]	Positive cells (%)	Antigen density[b]
α-Thy-1.2 (53-2.1)	100	85	30	37	57	39
α-Lyt-1 (53-7.3)	>95	1.5	34	3.8	59	4.4
α-Lyt-2 (53-6.7)	82	5.7	13	6.5	21	6.7
α-Lyt-3 (53-5.1)	82	6.0	11	6.8	20	7.2

[a]C57BL/6.Lyt-2.1 cells were stained with directly fluorescein-conjugated antibodies and analyzed on a FACS-II.

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

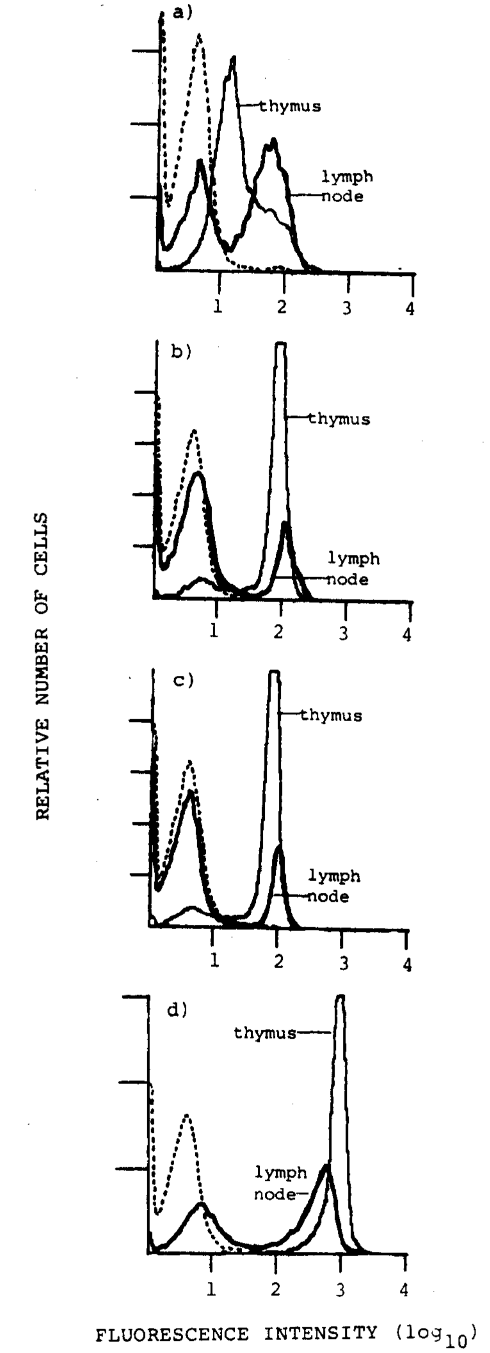


Figure 1. Immunofluorescence staining of C57BL/6J thymocytes and lymph node cells with (a) anti-Lyt-1 (53-7.3): (b) anti-Lyt-2 (53-6.7); (c) anti-Lyt-3 (53-5.1); and (d) anti-Thy-1.2 (53-2.1). Cells were stained with directly fluorescein-conjugated antibodies and analyzed on a FACS-II with a logarithmic amplifier. The dotted line shows the autofluorescence of unstained lymph node cells.

Lyt-2 and Lyt-3 Are Lost From Most T-Cells as Thymocytes Mature and Migrate to the Periphery

Lyt-2 and Lyt-3 bearing cells comprise between 80-90% of thymocytes. The fluorescence distribution patterns are identical for Lyt-2 and Lyt-3 (Figure 1). The antigen densities of Lyt-2 and Lyt-3 are almost identical (Table 1) and these two antigens are expressed on the same cells (Ledbetter et al., 1981 and our unpublished two-color immunofluorescence data). The variation in frequency of Lyt-2^+3^+ cells in thymus which is seen between individual mice is not due to estimation errors which are at most 1 or 2%.

The frequency of Lyt-2^+3^+ cells in spleen and lymph node of normal mice is usually only 27-40% of the Thy-1 cells. Again, the variation is between individual mice and not due to estimation problems. These data indicate the Lyt-2 and Lyt-3 are lost from the majority of T-cells during maturation, and that Lyt-$1^+2^-3^-$ cells already exist in the thymus. Other data (manuscript in preparation) indicate that these cells are all in the hydrocortisone-resistant subpopulation of thymocytes.

The average Lyt-2 and Lyt-3 antigen densities are slightly higher in spleen and lymph node than in thymus (Table 1). However, the peripheral (spleen or lymph node) Lyt-2^+3^+ cells correspond in brightness to the brightest thymocytes, indicating that these antigens do not change substantially in amounts on positive cells during maturation.

Thy-1.2 Decreases in Amount During Maturation

For Thy-1.2, we found the immunofluorescence of thymocytes and peripheral T-cells closely agreed with previous reports using conventional reagents (Evans et al., 1978; Loken et al., 1977; Mathieson et al., 1979). Thy-1.2-stained thymocytes give a very bright peak with a duller shoulder (Figure 1-d). The latter corresponds to the hydrocortisone-resistant subpopulation (manuscript in preparation). In spleen and lymph node, the brighter Thy-1.2 bearing cells were equivalent in brightness to the dullest cells in the thymus.

In summary, the results of the quantitative staining analyses for Thy-1.2, Lyt-1, Lyt-2, and Lyt-3 antigens in thymus, lymph node, and spleen imply the following changes during T-cell maturation: (a) Lyt-1 increases substantially; (b) Thy-1 decreases substantially; and (c) Lyt-2 and Lyt-3 are lost from most cells.

Thus, by comparing thymus, spleen, and lymph node cells on a population basis, Lyt-1 and Thy-1 antigen densities appear to change in opposite directions during T-cell development but frequencies remain essentially the same. In contrast, Lyt-2 and Lyt-3 change in frequencies of positive T-cells but only slightly in density on positive cells.

Two-color Cell Suspension Immunofluorescence for Thy-1.2, Lyt-1, and Lyt-2

The correlations of staining with the monoclonal T antibodies are readily examined by FACS simultaneous detection of fluorescein and rhodamine on individual cells (Micklem et al., 1980). Nylon-purified splenic T-cells were used. Figure 2 shows

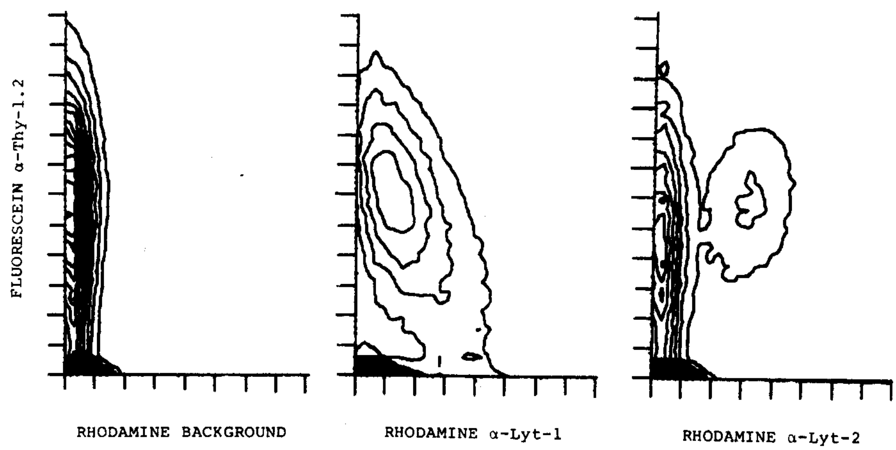


Figure 2. Two-color immunofluorescence analysis of BALB/cNHz nylon-enriched splenic T-cells on a FACS-II with standard linear amplifiers. Staining for Thy-1.2 in each panel was with directly fluorescein-conjugated anti-Thy-1.2 (30-H12). Staining for Lyt-1 or Lyt-2 was with biotin-conjugated antibodies (53-7.3 or 53-6.7) followed by rhodamine-conjugated avidin. The first panel shows the fluorescein anti-Thy-1.2 staining and the rhodamine background (rhodamine-avidin alone). The contour lines represent 75 cells/channel intervals.

the negative correlation between Thy-1 and Lyt-1 antigen densities using fluorescein-conjugated anti-Thy-1.2 in combination with biotin-anti-Lyt-1 followed by rhodamine-conjugated-avidin (panel B). In panel C, the Lyt-2 cells are seen as a discrete population with intermediate to bright levels of Thy-1. The contour plots from these data graphically illustrate the correlations of Thy-1 with the Lyt antigens and agree well with the cytotoxicity results presented above.

Similar two-color staining experiments for Lyt-1 and Lyt-2 depicted on contour plots (Figure 3) show that the brightest Lyt-1 cells are Lyt-2 negative. Conversely, Lyt-2 positive cells stain dully for Lyt-1. Again, quantitative staining analysis rationalizes how selective cytotoxic depletion of the "Lyt-1 subset" or of the "Lyt-2 subset" can occur at certain antibody concentrations. These data also rationalize the failures to get total depletion of the Lyt-2 cells using anti-Lyt-1 and complement, since most of the Lyt-2 cells express low density Lyt-1.

Functional subpopulations of human T-cells selectively express surface antigens analogous to the mouse Lyt-1, Lyt-2 and Lyt-3. Two subpopulations of human T-cells were originally defined by a xenogeneic antiserum termed :gaTH_2 (Oi et al., 1978). TH:i2^+ cells were shown to mediate suppressor effects *in vitro* and to be responsible for most of the killing in cell-mediated lympholysis (CML) (Reinherz et al., 1979a). 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, tetanus toxoid) (Reinherz et al., 1979a).

Recently, both of these subsets have been redefined by monoclonal antibodies

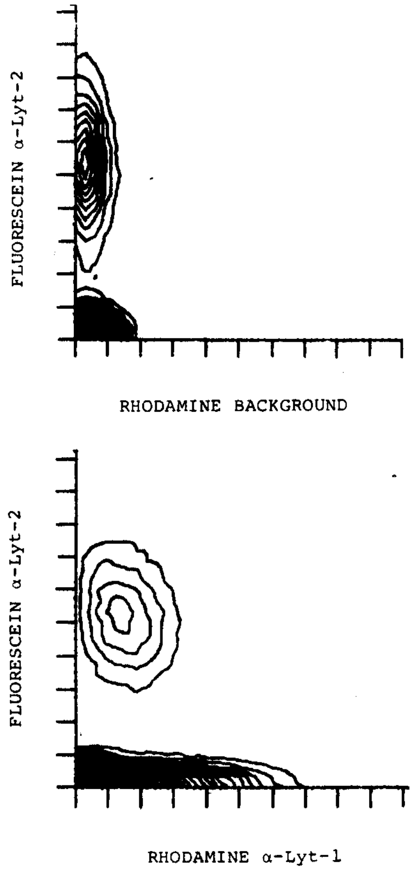


Figure 3. Two-color immunofluorescence analysis of BALB/cNHz nylon-enriched splenic T-cells on a FACS-II with standard linear amplifiers. Directly fluorescein-conjugated anti-Lyt-2 (53-6.7) and biotin-conjugated anti-Lyt-1 (53-7.3) followed by rhodamine-conjugated avidin were used. The first panel shows the fluorescein anti-Lyt-2 staining and the rhodamine background (rhodamine-avidin alone). The contour lines represent 75 cells/channel intervals.

(Reinherz et al., 1979b; Reinherz et al., 1980; Shiku et al., 1975).[1] Two antibodies, SK1 and SK2, defining Leu-2a and Leu-2b antigenic determinants, respectively, appear to react with the same surface antigen identified by the original αTH_2 serum since both of these monoclonal antibodies block the binding of TH_2 antiserum.[1] Two other antibodies, SK3 and SK4 defining Leu-3a and Leu-3b determinants, respectively, react with TH_2^- subsets.[1] These two subsets (Leu-2$^+$ and Leu-3$^+$ T-cells) were shown not to overlap and to comprise subpopulations of ERF-C, pre-

[1]Evans, R.L., Wall, D.C., Platsoucas, C.D., Siegal, F.P., Fikrig, S.M., Testa, C.M., and Good, R.A. Thymus-dependent membrane antigens in man: Inhibition of cell-mediated lympholysis by monoclonal antibodies to the T_{H2} antigen, manuscript in preparation.

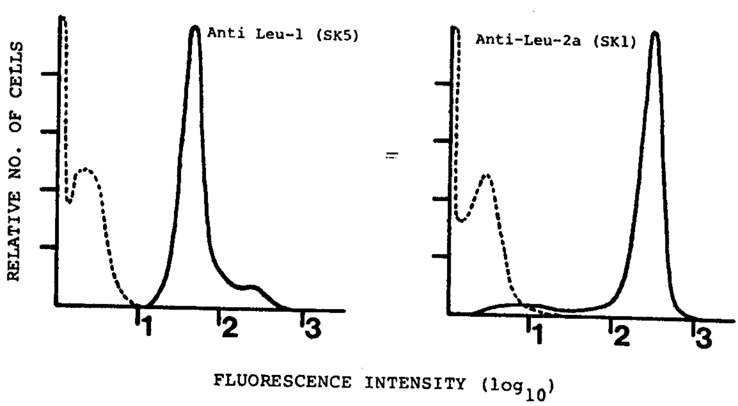
viously defined by a T-cell membrane component (Leu-1) (Wang et al., 1980). The Leu-1 antigen, unlike other human T-cell membrane antigens thus far defined by monoclonal antibodies, is found on Ig⁺ leukemic cells from the majority of patients with chronic lymphocytic leukemia (CLL) (Wang et al., 1980), but is not detected on normal B-cells.

We have recently completed a series of comparative studies showing that 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. Similar studies to those shown above (Ledbetter et al. 1981) indicate that molecules carrying the human Leu antigens approximate the size, charge, and subunit composition of the corresponding molecules carrying the mouse Lyt antigens (data not shown). Moreover, each of these structurally homologous antigen systems have similar sensitivities to trypsin and similar fluorescence distributions on thymocytes and T-cells (see Figure 4).

Analogies between the human and mouse T-cell differentiation antigens are formalized below:

Analogies between Leu-1 on human T-cells and Lyt-1 on mouse cells:
1. Present on all peripheral T cells and thymocytes.
2. Amount per cell varies widely (14-fold range in peripheral T).
3. FACS profiles have similar features. Thymocyte profile has a bright shoulder that derives from the mid-size (C-R) population and coincides with the peak of the peripheral T-cell profile. Peripheral T-cell profile has a dull shoulder that derives from Lyt-2,3$^+$/Leu-2a,2b$^+$ cells.
4. Single chain molecule.

Figure 4. Immunofluorescence staining of human thymocytes with anti-Leu-1 and anti-Leu-2a antibodies. The anti-Leu-1 staining was with monoclonal antibody SK5 followed by a fluorescein-conjugated goat anti-mouse IgG2 second step antibody. The anti-Leu-2a staining was with monoclonal antibody SK1 followed by a fluorescein-conjugated goat anti-mouse IgG1 second step antibody. The background staining with second step alone is shown by a dotted line in each panel.



5. Mr 65-71 K.
6. Found on malignant B-cells from some individuals.

Analogies between Leu-2a,2b molecule on human T-cells and Lyt-2,3 molecule on mouse T-cells:
1. Present on 25-35% of peripheral T-cells and 80-90% of thymocytes.
2. Present on suppressor and cytotoxic T-cells but not on helper T-cells.
3. Amount per cell is relatively constant (4-fold range).
4. Positive cells carry less Lyt-1/Leu-1 than negative cells.
5. Composed of at least two non-identical subunits (30-45 K Mr).
6. Subunits are disulfide-bonded into multimeric forms.
7. More basic than most cell surface molecules.
8. Leu-2a and Lyt-2 relatively stable to trypsin.
9. Leu-2b and Lyt-3 easily destroyed by trypsin.

The molecules carrying the Leu and Lyt antigens have undergone evolutionary change since 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 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 through 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 (i.e., Lyt-2$^+$Leu-2a$^+$ to Lyt-2$^-$/Leu-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-2$^+$/Leu-2a$^+$ cells than on Lyt-2$^-$/Leu-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.

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