A new human T cell surface antigen, Leu-5, has been defined using a set of monoclonal antibodies that block rosette formation between T lymphocytes and sheep erythrocytes (SRBC). Four antibodies obtained from 2 different fusions using 2 immunized mouse strains all reacted with the same antigen. All these antibodies gave identical quantitative immunofluorescence (FACS) profiles, all gave the same staining profiles and intensities when used singly or in combinations, and all precipitated the same molecule. The antigen is a single polypeptide chain, 40,000 to 50,000 M₀, and is found on all thymocytes, peripheral T cells, and some null cells, but not on B cells. Leu-5 is a differentiation antigen that decreases in density as thymocytes mature to peripheral T lymphocytes. Thus, the Leu-5⁺ subpopulations ranked in order of decreasing Leu-5 density are: a subpopulation of subcapsular thymocytes > cortical and medullary thymocytes > peripheral T cells (cytotoxic/suppressor subset) > peripheral T cells (helper/inducer subset). The density distribution pattern of Leu-5 parallels the relative affinity of thymocytes and peripheral T lymphocytes for SRBC. We suggest that Leu-5 is either identical to or closely associated with the human T lymphocyte receptor for SRBC.

Human T lymphocytes display a surface marker that serves as a specific receptor for sheep erythrocytes (E),⁴ causing the formation of rosettes in the absence of antibody or complement. The initial observation of E-rosettes (1-3) and their subsequent identification as specific for T cells and thymocytes (4, 5) was the first discovery of a unique human T lymphocyte surface marker. This property has since emerged as the standard method for identifying and enumerating peripheral T cells (6) and has found wide use in T cell purification (6), fractionation (7), monitoring production of anti-thymocyte serum (8), and purification of T cell-specific surface molecules (8-11).

The recent advent of somatic cell fusion techniques (12) has allowed the production of a variety of homogeneous antibodies that react specifically with various populations of peripheral T cells and thymocytes (13-22). One group of well-characterized T cell-specific monoclonal antibodies, Evans et al. (20), Englemann et al. (21), and Ledbetter et al. (22), reacts with a series of cell surface molecules designated Leu-1, Leu-2, Leu-3, and Leu-4. Studies with these monoclonal antibodies have defined the subpopulation distribution, biochemical properties, and functional association of previously uncharacterized human T cell surface molecules. Thus, Leu-1 and Leu-4 are present on all peripheral T cells, whereas Leu-2 and Leu-3 define functionally distinct T cell subpopulations. Some of the antigens, notably Leu-1, Leu-2a, and Leu-2b, have been shown to be analogous to murine T cell differentiation antigens, whereas others (e.g., Leu-3) have no known murine counterpart (22). In addition, human T cell differentiation antigens have been described by Reinherz and Schlossman (13) using the OKT series monoclonal antibodies.

In order to produce a homogeneous antibody specific for human T lymphocytes to be used in cardiac transplant recipient immuno-suppression, we employed an E-rosette inhibition assay (23) to screen supernatants of hybridomas from the fusion of NS-1 myeloma cells with immune splenocytes. The immune splenocytes were obtained from mice immunized with human thymocytes. This assay detected a number of anti-thymocyte monoclonal antibodies, ATM-1, ATM-2, ATM-3. We show here that all these antibodies react with the same antigen, which is present on all thymocytes, all normal peripheral T cells, and some null cells. The target antigen, a single polypeptide with relative m.w. (M₀) = 40,000 to 50,000, is present at higher density on thymocytes than on peripheral T cells. In the peripheral population, greater amounts are expressed on the cytotoxic/suppressor subpopulation than on the helper/inducer subpopulation. We designate the antigen Leu-5, the 5th in a series of well-characterized human leucocyte cell surface antigens. Leu-5 is either an integral part of or is closely associated with the T cell receptor for SRBC.

MATERIALS AND METHODS

Cells. Fresh thymus tissue was obtained from young patients (under 12 mo old) undergoing cardiac surgery. Peripheral blood was collected from normal adult volunteers. Thymocyte and lymphocyte suspensions were prepared in either newborn calf serum (NBS) containing RPMI 1640 (for analysis) or Hanks' BSS (for radiiodination) using conventional methods (23, 25).

Antibody reagents. The production and initial characterization of the antithymocyte monoclonal (ATM) series antibodies has been reported by Bieber et al. (24). All these antibodies share the property of inhibiting rosette formation between human peripheral T lymphocytes and thymocytes and SRBC. Table I summarizes the properties of the ATM monoclonal antibodies used in this study. ATM 1.1, ATM 1.2, and ATM 3.2 were all obtained from fusion of NS-1 with AKR/J immune splenocytes, whereas ATM 3.1 was from fusion of BALB/C splenocytes with NS-1 myeloma cells.

The Leu series antibodies, produced by Evans et al. (20) and Englemann et al. (21), were obtained from Becton-Dickinson FACS Systems Monoclonal Antibody Center, Sunnyvale, CA. Table II lists the cellular distribution and m.w. of the target antigens for the Leu series antibodies. The antibodies used and their corresponding clones were Leu-1, 17F12; Leu-2, SK1; Leu-3, SK3; Leu-4, SK7. Fluorescein isothiocyanate- (FITC) rabbit-α-human IgG was purchased from Tago, Inc., Burlingame, CA. FITC-α-goat-μ-μ-μ-μ-μ-μ and FITC-α-goat-μ-μ-MFab were prepared from affinity-purified immune serum (26). All fluoresceinated reagents were centrifuged at 100,000 × G for 10 min just before use.

Immunofluorescent staining. Target cell suspensions were prepared in RPMI 1640 containing 10% NBS and 0.1% sodium azide (staining medium). All incubations were carried out on ice. Fifty microliters of the appropriate 1st-step antibody were added to 10⁶ cells in 50 μl of staining medium. After a 1-hr incubation, cells were washed 3 times in 300 μl staining medium and resuspended in 50 μl staining medium. Fifty microliters of an appropriate 2nd-step antibody were added, and the mixture was incubated for an...
**TABLE I**

Properties of E-rosette inhibiting monoclonal antibodies

<table>
<thead>
<tr>
<th>Antibody</th>
<th>Polypeptide Composition*</th>
<th>Lactose†</th>
<th>E-Rosette Titer</th>
<th>Protein A Binding†</th>
</tr>
</thead>
<tbody>
<tr>
<td>ATM-1.1</td>
<td>HL</td>
<td>IgG-1</td>
<td>1.40 µg</td>
<td>No</td>
</tr>
<tr>
<td>ATM-1.2</td>
<td>NIL‡</td>
<td>IgG-1</td>
<td>0.60 µg</td>
<td>No</td>
</tr>
<tr>
<td>ATM-3.1</td>
<td>HL</td>
<td>IgG-3</td>
<td>0.25 µg</td>
<td>Yes</td>
</tr>
<tr>
<td>ATM-3.2</td>
<td>HL</td>
<td>IgG-3</td>
<td>0.10 µg</td>
<td>Yes</td>
</tr>
</tbody>
</table>

* H, heavy chain; L, light chain from splenocyte.
† Determined by Ouchterlony immunodiffusion.
‡ Values are the minimum antibody necessary for complete inhibition of E-rosette formation between 5 × 10⁷ thymocytes and 5 × 10⁶ SRBC.

**TABLE II**

Human T cell antigens defined by monoclonal antibodies

<table>
<thead>
<tr>
<th>Name</th>
<th>Thymocytes</th>
<th>Peripheral blood T cells</th>
<th>M. W. (Kilodaltons)</th>
<th>E-Rosette Blocking</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Leu-1</td>
<td>&gt;95 100</td>
<td>67</td>
<td>No</td>
<td>17, 20-22</td>
<td></td>
</tr>
<tr>
<td>Leu-2a, 2b</td>
<td>70-90 20-40</td>
<td>30-40</td>
<td>No</td>
<td>17, 20-22</td>
<td></td>
</tr>
<tr>
<td>Leu-3a, 3b</td>
<td>&gt;90 50-60</td>
<td>50-60</td>
<td>No</td>
<td>17, 20-22</td>
<td></td>
</tr>
<tr>
<td>Leu-4</td>
<td>&gt;90 100</td>
<td>40-50</td>
<td>Yes</td>
<td>24</td>
<td></td>
</tr>
<tr>
<td>T-1</td>
<td>10 100</td>
<td>n.r.*</td>
<td>n.r.*</td>
<td>13, 18</td>
<td></td>
</tr>
<tr>
<td>T-3</td>
<td>10 100</td>
<td>n.r.*</td>
<td>n.r.*</td>
<td>13, 18</td>
<td></td>
</tr>
<tr>
<td>T-4</td>
<td>75 60</td>
<td>62</td>
<td>n.r.*</td>
<td>13</td>
<td></td>
</tr>
<tr>
<td>T-5</td>
<td>80 20</td>
<td>30, 32*</td>
<td>n.r.*</td>
<td>13</td>
<td></td>
</tr>
<tr>
<td>T-6</td>
<td>80 30</td>
<td>n.r.*</td>
<td>n.r.*</td>
<td>13</td>
<td></td>
</tr>
<tr>
<td>T-8</td>
<td>10 0</td>
<td>n.r.*</td>
<td>n.r.*</td>
<td>13</td>
<td></td>
</tr>
<tr>
<td>T-9</td>
<td>10 0</td>
<td>n.r.*</td>
<td>n.r.*</td>
<td>13</td>
<td></td>
</tr>
<tr>
<td>T-10</td>
<td>90 20</td>
<td>n.r.*</td>
<td>n.r.*</td>
<td>13</td>
<td></td>
</tr>
<tr>
<td>HuA-1</td>
<td>20-50</td>
<td>40-60</td>
<td>40, 54</td>
<td>16</td>
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<tr>
<td>HTA-1</td>
<td>85 0</td>
<td>49, 12*</td>
<td>12*</td>
<td>15</td>
<td></td>
</tr>
<tr>
<td>12E7</td>
<td>10 0</td>
<td>n.r.*</td>
<td>n.r.*</td>
<td>19</td>
<td></td>
</tr>
<tr>
<td>3A1</td>
<td>n.r.*</td>
<td>85 40</td>
<td>No</td>
<td>14</td>
<td></td>
</tr>
</tbody>
</table>

* Molecular weight by SDS-PAGE under reducing conditions.
† Cytotoxic/suppressor T subpopulation marker.
‡ These antigens had a multichain structure.
§ Helper/inducer T subpopulation marker.

additional 45 min. The cells were washed twice, resuspended in 50 µl staining medium, and immediately analyzed on a FACS-I.

Fluorescence-activated cell sorter (FACS) analysis. FACS analysis was carried out using the methods of Loken and Herzenberg (27). Details of the scatter gating and fluorescence analysis methods used here have been described by Herzenberg and Herzenberg (28). The lymphoid cells were analyzed on a FACS-II (Becton-Dickinson, Mountain View, CA) fitted with a logarithmic amplifier spanning a 4-decade range. The distinctive feature of logarithmic amplification is that low intensity signals are amplified much more than high intensity signals. Thus, for example, a shift in intensity of 0.1 U at the high end of the log scale represents a much greater difference in absolute fluorescence intensity (i.e., number of fluorescein bounds) than a shift of 0.1 U at the low end of the log scale. The logarithmic amplifier enables simultaneous analysis of a much larger range of fluorescence intensities than is possible with standard linear amplification. A log scale is used.

Radioiodination and immunoprecipitation of cell surface proteins. Human thymocytes were labeled with ¹²⁵I by a modification of the lactoperoxidase technique described by Ledbetter et al. (22). Immunoprecipitation were with 5 µg antibody per extract from 2 × 10⁶ radioactive labeled cells. Antigen-antibody complexes were collected by the addition of 10 µl of 10% fixed staph A-Cowan I strain (The Enzyme Center, Boston, MA). For the IgG1 antibodies that do not bind to protein A, the staph A was precoated with affinity purified rabbit a-mouse IgG1. The staph A-antibody-antigen complexes were washed 1 and extracted with sodium dodecyl sulfate (SDS) sample buffer for gel electrophoresis on 10% SDS polyacrylamide 1-dimensional gels (29). Autoradiography was with intensified screens (Cronex lighting plus screens, Du Pont, Wilmington, DE) using Kodak X-Omat R film at -70°C.

**RESULTS**

Distribution on thymocytes. The cell populations found in the normal human thymus can be distinguished on the basis of their forward angle light scatter intensity, which varies with both cell size and morphology (27). Figure 1a is the forward angle light scatter profile of the cells obtained from the thymocyte preparation. Erythrocytes and dead cells have a scatter intensity of 60 to 110. total live thymocytes of 110 to 230, large live thymocytes 160 to 230, and clumped cells >230. The distribution of Leu-5, on normal human thymocytes was determined by indirect immunofluorescence staining with ATM-1.2 and the other 3 ATM antibodies. The FACS profiles for ATM-1.2 are shown in Figure 1. The profiles for the other ATM antibodies used alone or in combinations are identical to those in Figure 1 (data not shown). The lack of any additive staining indicated that all ATM antibodies reacted with the same determinant or with several closely spaced determinants on the same antigen. In all subsequent staining experiments, identical results were found with any of the ATM antibodies, although only results for ATM 1.2 are shown. Cells staining with a log fluorescence intensity >1.5 were considered Leu-5 positive. Numerical integration of the curve in this region revealed that 96% of the total live thymocytes in this preparation expressed Leu-5. Other thymocytes gave 98 to 99% Leu-5-positive cells.

The antigen density distribution of Leu-5 on total live thymocytes was bimodal, with most of the cells (>90%) staining at a mean log fluorescence intensity of 2.16 and the rest at mean log intensity 2.55. When the Leu-5 antigen density of only the larger thymocytes (scatter intensity 160 to 230) was analyzed, the proportion of bright-staining cells was substantially increased (Fig. 1c). The cells in the bright-staining subpopulation had a factor of 2.45 more fluorescence than the duller cells. Thus, Leu-5 was expressed on greater than 95% of total live thymocytes and at approximately a 2-fold higher density on a subpopulation of large thymocytes compared with the rest of the Leu-5-positive thymocytes. The size of the cells in the bright subpopulation suggests that they are likely to be immature cells derived from the thymic subcapsular region, by analogy with the mouse thymus (30).

Distribution of cell/surface blood lymphocytes. The cell populations found in normal human peripheral blood can be distinguished on the basis of their forward angle light scatter intensity, which varies with both cell size and morphology (27). Figure 2a is the forward angle light scatter profile of Ficol-Hypaque purified blood mononuclear cells. The lymphoid cells had a scatter intensity of 100 to 160 and the monocytes of 160 to 230. The distribution of Leu-5 on normal human peripheral blood cells was determined by FACS analysis as shown in Figure 2b. Approximately 75% of peripheral blood lymphocytes (PBL) expressed Leu-5 (i.e., log fluorescence intensity >1.4). Complete resolution of Leu-5+ and Leu-5- populations was not possible due to the dull a-Leu-5 staining (i.e., low Leu-5 antigen density) on peripheral blood cells. The log mean fluorescence intensity of α-Leu-5 was approximately 0.45 log units (i.e., 65%) less on PBL than on the majority of thymic lymphocytes.

Immunofluorescent staining of peripheral blood lymphocytes (Fig. 2c). The distribution of Leu-5 was not found on these cells, for no significant staining above background was observed (Fig. 2c). The slight increase in staining of monocytes by α-Leu-5 was probably due to Fc receptor-mediated binding of the antibody. The small tail of brightly staining cells is attributable to a few contaminating lymphocytes in the monocyte scatter window (160 to 230).

**Distribution on T and B lymphocytes.** Since Leu-5 was not present on all PBL, it was of interest to determine the relationship between Leu-5 expression and the 2 major classes of lymphocytes, T and B lymphocytes. To that end, cells were stained with a-Leu-5 in combination with either α-lg (specific for B cells) or α-Leu-1 (specific for T cells). Comparison of the fluorescence intensity profile of cells stained simultaneously with α-Leu-5 and the α-lg reagent (or α-Leu-1) with profiles obtained from each reagent staining alone defines the relationship between these populations (i.e., Leu-5+, T cells, and B cells).

The fluorescence intensity profile of α-Leu-5 alone on PBL indicated that approximately 25% of the cells were Leu-5+ (Fig. 3a, log fluorescence intensity <1.4). The α-lg reagent alone stained approximately 25% of peripheral blood cells (log fluorescence intensity >2.2, Fig. 3b). In Figure 3c, cells were stained with both the α-lg and α-lg. All cells were stained. The size and shape of the intensity profile in the Leu-5+ region (log fluorescence intensity 1.4 to 2.5, Fig. 3c) was not significantly altered by the presence of the α-lg reagent. Furthermore, all the Leu-5+ cells (log fluorescence intensity >1.4) expressed Leu-5.

* Cell identity was determined using nonspecific esterase staining and Wright stain morphology on sorted PBL (Dr. A. Frankel, personal communication).
intensity <1.5, Fig. 3a) were stained by the α-lg reagent at an intensity approximating the cells stained by α-lg alone.⁶ These results demonstrate that all PBL are either Ig⁺ or Leu-5⁺, that the α-lg reagent did not bind to cells in the Leu-5⁺ population, and that α-Leu-5 does not bind to the Ig⁺ population. Therefore, Leu-5 is not expressed on B lymphocytes. Evidence that Leu-5 is expressed on T lymphocytes was obtained by simultaneous staining using α-Leu-5 and another monoclonal antibody specific for all peripheral T lymphocytes, α-Leu-1 (Table III). When cells were stained with both α-Leu-1 and α-Leu-5, the number of positive cells (71%) was very nearly the same as when α-Leu-1 or α-Leu-5 was used alone (71% or 74%, Table III). In addition, the mean linear fluorescence intensity of the positively stained cells when α-Leu-1 and α-Leu-5 were used together was

<table>
<thead>
<tr>
<th>Antibody</th>
<th>%PBL Positive</th>
<th>Mean Fluorescence Intensity</th>
</tr>
</thead>
<tbody>
<tr>
<td>α-Leu-1</td>
<td>71</td>
<td>1.96 ± 0.195</td>
</tr>
<tr>
<td>α-Leu-5</td>
<td>74</td>
<td>2.00 ± 0.185</td>
</tr>
<tr>
<td>α-Leu-1 + α-Leu-5</td>
<td>71</td>
<td>2.15 ± 0.150</td>
</tr>
<tr>
<td>None</td>
<td>0</td>
<td>0.67 ± 0.254</td>
</tr>
</tbody>
</table>

⁶ The significantly decreased staining of the Ig⁺ cells in Figure 3c (log fluorescence intensity >2.4) compared with Figure 3e is due to cross-reactivity of the rabbit α-human Ig with unbound goat α-mouse Ig used to detect the α-leu-5. The duller staining of the Ig⁺ cells does not affect the interpretation of the results.
the sum of the mean linear fluorescence intensities obtained with separate staining (Table III, column 3). These results demonstrate that Leu-5 is expressed on all cells carrying Leu-1 and that the Leu-1 and Leu-5 determinants are spaced distantly enough to permit simultaneous binding of both monoclonal antibodies to the same cell. Thus, Leu-5 is expressed on all peripheral T lymphocytes (Leu-1+ cells).

**Distribution on T lymphocyte subpopulations.** The fluorescence intensity profile of α-Leu-5 binding to PBL exhibited a characteristic "double-humped" appearance (Figs. 2b, 3a, 5a). The level of Leu-5 expression partially resolves peripheral T lymphocytes into 2 subpopulations, 1 of which expresses significantly more Leu-5 than the other. The relative size of these 2 subpopulations varied from donor to donor.

The relationship between the Leu-5 dull and Leu-5 bright subpopulations and the 2 well-characterized T lymphocyte subpopulations, cytotoxic/suppressor (TH,+) and helper/inducer (TH,-) was determined using monoclonal antibodies specific for each subpopulation in additive staining experiments with α-Leu-5. α-Leu-3 has been shown to specifically stain the helper/inducer (TH,-) subpopulation (20). Its fluorescence intensity profile on PBL (Fig. 3c (solid line)) was obtained. The profile for α-Leu-5 staining alone is shown as a dashed line in Fig. 4c. Leu-3" cells stain brightly with a mean log fluorescence intensity of 2.4. When PBL were stained with both α-Leu-3 and α-Leu-5, the fluorescence intensity profile in Figure 4c (solid line) was obtained. The profile for α-Leu-5 staining alone is included for reference (dotted line, Fig. 4c). It can be clearly seen that α-Leu-3 preferentially stained the duller subpopulation of the Leu-5+ cells (i.e., those at a log fluorescence intensity of 1.4 to 1.8). The relative number of cells staining as dull Leu-5+ is decreased when cells are simultaneously stained with α-Leu-3, whereas the number of brighter Leu-5+ cells (log intensity = 1.8 to 2.4) is relatively unaffected. Similarly, α-Leu-2, specific for the cytotoxic/suppressor (TH,-) subset, preferentially stains the brighter Leu-5+ cells (Fig. 4b,d). The fluorescence intensity profile for PBL stained with both α-Leu-2 and α-Leu-5 (Fig. 4d) shows that the relative number of cells staining at the brighter Leu-5+ intensity is decreased with respect to the duller Leu-5- cells. Thus, high Leu-5 antigen density correlates with the cytotoxic/suppressor (TH,-) subset, and low Leu-5 density correlates with the helper/inducer (TH,+) subset.

**Relation to other Leu series antigens.** Simultaneous staining with α-Leu-5 and α-Leu-1 (Table III), α-Leu-2 (Fig. 4d), α-Leu-3 (Fig. 4c), and α-Leu-4 (Fig. 5) shows that Leu-5 differs from the other Leu series antigens. In all cases, the staining pattern in the brightest region is sharpened and shifted towards brighter intensity when α-Leu-5 is used in combination with each of the other α-Leu series antibodies. Since α-Leu-5 does not block the binding of any of the other antibodies, α-Leu-5 and each of the α-Leu monoclonals react with distinctly spaced determinants and, as shown below, react with different molecules.

It is of interest that α-Leu-5 stains a small number of cells not stained by α-Leu-4, an antibody reactive with all peripheral T cells (Fig. 5). α-Leu-4 stains at a log fluorescence intensity of >2.3, whereas α-Leu-5 stains at a log fluorescence intensity of 1.4 to 2.4. α-Leu-4 intensity is decreased when cells are simultaneously stained with α-Leu-5 (log intensity = 1.5 to 2.4, Fig. 5b,a). Any cells stained by both antibodies will be found at a log fluorescence intensity >2.0. Cells stained by α-Leu-5 but not α-Leu-4 will reside in the range log intensity = 1.5 to 2.0. Figure 5c clearly shows that 4 to 5% of the cells are found in this region (indicated by the bar in Fig. 5c). Since Leu-5 is not present on Ig+ cells (see above), this suggests that Leu-5 is expressed on a population of Leu-4+ cells. These cells are presumably null cells, i.e., those cells not classified by α-Leu-5 and α-Leu-4 determinants.

**SDS-PAGE analysis.** SDS-PAGE analysis of the Leu-5 antigen immunoprecipitated by various ATM antibodies is shown in Figure 6. Surface iodination, solubilization, and immunoprecipitation of human thymocytes revealed that Leu-5 is a broadly running band of approximately 40,000 to 60,000 daltons (Fig. 6, lanes 5–7) or nonreducing conditions (data not shown). All ATM series antibodies immunoprecipitated the same band. No evidence for a disulfide-bonded subunit was obtained. Thus, Leu-5 appears to be composed of a single polypeptide chain, which exhibits considerable size heterogeneity, perhaps due to post-translational modifications involving carbohydrate or protease processing. The Leu-5 determinant clearly resides on a molecule that is distinct from the Leu-1 through Leu-4 molecules7 (Fig. 6, lanes 1–4).

**DISCUSSION**

Human T lymphocytes possess a variety of surface molecules that have been defined with heterologous antibodies, both polyclonal and monoclonal. Polyclonal heterologous antibodies do not block Epstein-Barr virus infected human T cell sera reacting with a relatively broad range of these antigens and usually have rosette-inhibition activity. None of the monoclonal antibodies described previously, however, have been reported to block E-rosette formation. The ATM series monoclonals produced by Bieber et al. (24) are unique in that they do block E-rosette formation. These studies characterize Leu-5, the antigen detected by the ATM antibodies, and show it to be a T lymphocyte surface differentiation marker.

Previously defined T lymphocyte-specific surface markers in man have been shown to distinguish functional populations of T lymphocytes. These markers have been defined by 2 series of monoclonal antibodies, the Leu series (17, 20–22) and the T series (13) and several other individual monoclonal antibodies (14–16, 19, Leu-2a, Leu-2b, T-8, and T-5 mark the cytotoxic/suppressor T cell subpopulation, whereas Leu-5a, Leu-3b, and T-4 mark the helper/inducer subpopulation). Leu-1 is found on all T cells, but in greater amounts on the helper/inducer subpopulation than on the cytotoxic/suppressor subpopulation. Leu-4 is present on all T cells, as are T-1 and T-3. The rest of the T series antigens are found on various subsets of thymocytes (see Table II).

Leu-5 is distinguished from the other Leu and T series antigens by its quantitative distribution on PBL and thymocytes, its m.w., and its polypeptide chain composition. Thus, the distribution of Leu-5 on thymocytes and peripheral T cells distinguishes it from Leu-2, Leu-3, T-5, and T-4, and its relative m.w. distinguishes it from Leu-1, which is the most abundant of the Leu series antigens in the thymus. The broad band in Figure 6, lane 1 is due to the overloading of Leu-1 with respect to the other Leu series antigens. The additional band in Figure 6, lane 2, is due to a slight spillover of the Leu-1 antigen into the Leu-2 lane. SDS-PAGE analysis for Leu-1-Leu-4 has been published previously (17, 20–22).

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7 Leu-1 is the most abundant of the Leu series antigens in the thymus. The broad band in Figure 6, lane 1 is due to the overloading of Leu-1 with respect to the other Leu series antigens. The additional band in Figure 6, lane 2, is due to a slight spillover of the Leu-1 antigen into the Leu-2 lane.
from Leu-1, Leu-4, T-1, and T-3. An extension of the comparison between α-Leu-5 and each of the other reported monoclonal antibodies shows Leu-5 to differ from all markers previously defined by monoclonal antibodies (Table II).

The α-Leu-5 antibodies were produced by selecting for E-rosette-inhibiting activity in hybridoma supernatants. All the antibodies reacted with the same molecule even though they were derived from different mouse strains and different fusions. The target antigen of these monoclonals is therefore a candidate for the E-rosette receptor. Leu-5 possesses several properties expected for the E-rosette receptor. Peripheral T cells show two relative densities for SRBC (7), whereas Leu-5 shows two relative densities on peripheral T cells. Thymocytes form high affinity rosettes (7), whereas Leu-5 is expressed in greater amounts on thymocytes than on peripheral T cells. In addition, null cells (i.e., NK and ADCC cells) apparently have low affinity receptors for SRBC, since they have been found by some (31) but not by others (32) among the E-rosetting population of PBL. A comparative staining experiment suggested that Leu-5 is present on a null cell (Leu-5-) population. Thus, a strong correlation exists between the quantitative expression of Leu-5 and the affinity of E-rosette formation. These observations, together with the fact that all monoclonal antibodies that inhibit E-rosette formation bind to Leu-5, implies that Leu-5 is either identical to the E-rosette receptor or very closely associated with it on both thymocytes and peripheral T cells.

Heterologous antisera that inhibit rosette formation contain reactivity to multiple antigens on the T cell surface (9-11). Certain of these antigens have been designated as the E-rosette receptor (9, 11). Biochemical studies on the antigens recognized by some E-rosette-inhibiting heteroantisera have revealed glycoproteins of M, different from Leu-5 (9, 10). Ades et al. (10) produced an E-rosette-inhibiting antiserum that contained major reactivity to proteins of M, = 15,000 and 25,000. The major determinants recognized by this antiserum clearly differed from the receptor, however, since SRBC binding did not co-cap with the target antigens (8). Thus, the major target antigens identified by an E-rosette-inhibiting serum are not necessarily identical with the E-rosette receptor. The molecules recognized by the antiserum produced by Owen and Fanger (9) (M, = 30,000 and 65,000) were capable of blocking the rosetting-inhibiting activity of the antiserum. It was also possible to co-cap the target antigen and the rosette receptor. Direct inhibition of rosette formation by purified antigen was not demonstrable, however. It is possible that the major determinants recognized by their antiserum differed from the E-rosette receptor. The target antigen could be in close proximity to or noncovalently associated with the E-rosette receptor and thereby cause antibody binding to block E-rosette formation or co-cap the E-rosette receptor with the target antigen. In any case, these results do not contradict the possibility that Leu-5 is the E-rosette receptor.

The heteroantisera recently produced by Gross and Brown (11) possess potent E-rosette-inhibiting activity and immunoprecipitates a protein of M, equal to that of Leu-5. Based on its expression on normal and leukemic lymphocytes and its trypsin sensitivity, they tentatively identified the 45,000 M, glycoprotein as the E-rosette receptor. These results are in excellent agreement with our tentative assignment of Leu-5 as the E-rosette receptor.

The identity of the E-rosette receptor could be established by purification of the appropriate molecule(s) and demonstration of specific binding to SRBC and/or direct inhibition of E-rosette formation. Until direct binding or direct inhibition studies with purified antigen are successful, the identity of the rosette receptor remains an open question. Further experiments along these lines are underway and should clarify the relation of Leu-5 to the E-rosette receptor.

The lower density of Leu-5 on peripheral T cells compared with thymocytes defines Leu-5 as a marker of human T lymphocyte differentiation that decreases during T cell maturation. The large thymocytes, which have the greatest amount of Leu-5, presumably correspond to the subcapsular cells, the least mature thymic T cell precursors. In the periphery, functionally distinct subpopulations of T cells express different levels of Leu-5. The cytotoxic/suppressor subset expresses more Leu-5 than does the helper/inducer subset. This is in contrast to Leu-1 and Lyt-1 (in the mouse), which are found in greater amounts on T cells of the helper/inducer subset (22, 33). Thus, although the function of Leu-5 is not known, its differential expression during T cell development suggests a role for it in T lymphocyte maturation or function.

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Note Added in Proof. Since the time of writing, several more T lymphocyte specific antigens have been defined by monoclonal antibodies. One of these antibodies produced by Kamoun et al. (Kamoun, M., P. J. Martin, J. A. Hansen, M. A. Brown, A. W. Siadak, and R. C. Nowinski. 1981. Identification of a human T lymphocyte surface protein associated with the E-rosette receptor. J. Exp. Med. 153:207) recognizes a determinant that appears to
be identical to Leu-5. We found that antibody blocks binding of fluorescein-conjugated anti-Leu-5.

REFERENCES


