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Immunology

Thymus-dependent membrane antigens in man: Inhibition of cell-mediated lympholysis by monoclonal antibodies to T_{H_2} antigen

(T cell antigen/receptor/functional T cell subsets/cytotoxicity)

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ABSTRACT **In prior studies a heteroantiserum to a surface membrane component termed T_{H_2} was used to define two subsets of human T cells ($T_{H_2}^+$ and $T_{H_2}^-$), which were found to express distinct sets of activities *in vitro*. In the present studies we prepared monoclonal antibodies to surface determinants that are restricted to T cells belonging to each of these two subsets. Two antibodies, termed αLeu-2a and αLeu-2b, which seem to define the same surface antigen identified by the original T_{H_2} antiserum, reacted with 57–84% of thymocytes and 22–46% of the erythrocyte-rosette-forming cells (ERF-C) in peripheral blood. Two other monoclonal antibodies, termed αLeu-3a and αLeu-3b, reacted with the same subpopulation of thymocytes (78–89%) and peripheral blood ERF-C (47–78%) but, unlike αLeu-2a and αLeu-2b, did not exhibit cross-blocking; i.e., labeling cells with αLeu-3a did not inhibit the subsequent binding of αLeu-3b. T cells reactive with αLeu-2a were shown to be unreactive with αLeu-3a, indicating that two separate subpopulations of T cells, Leu-2 (formerly $T_{H_2}^+$) and Leu-3 ($T_{H_2}^-$) T cells, were thereby defined. These two T cell subsets make up the subpopulation of ERF-C (80–95%) previously defined by a monoclonal antibody to a T cell membrane antigen (Leu-1) that has a thymus-dependent distribution on normal lymphocytes but is expressed by some surface-immunoglobulin-positive (sIg^+) leukemic lymphocytes. None of the Leu antibodies reported here reacted with sIg^+, Leu-1^+ leukemic cells, nor did they react with normal hematopoietic cells or lymphoid cells that had surface markers characteristic of B cells. Studies of the blocking effects of Leu antibodies on killing in cell-mediated lympholysis by effector T cells were carried out in the absence of complement. These experiments established the following points: (*i*) αLeu-2a abolished the killing by cytotoxic T cells of allogeneic phytohemagglutinin-stimulated blasts, (*ii*) inhibition of killing by αLeu-2b was markedly less than inhibition by αLeu-2a, and (*iii*) other antibodies, including αLeu-1, αLeu-3a, and αLeu-3b, had little or no effect on killing in cell-mediated lympholysis. The relevance of these findings to prior studies done in the mouse and in man are discussed.**

There is now good evidence in man, as in the mouse, that the thymus gives rise to functionally distinct subpopulations of T cells that can be defined by their unique surface phenotypes of thymus-dependent membrane antigens (1–7). This was demonstrated by the use of heterologous anti-human T cell sera (6, 7) and has been explored more recently by using monoclonal antibodies generated by somatic cell hybridization techniques (8, 9). These studies have shown that a thymus-dependent antigen, termed T_{H_2}, is expressed on 30–40% of circulating T cells, and that $T_{H_2}^+$ T cells mediate suppressor effects *in vitro* and are also responsible for most of the killing in cell-mediated lympholysis (CML) (7, 9). In contrast, $T_{H_2}^-$ T cells were shown to effect 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 virus, tetanus toxoid) (7).

On the basis of certain parallels between this system and the subclasses of murine Lyt-1 and Lyt-2,3 cells (7), it seemed likely that these two sublineages of human T cell differentiation were further delineated by other thymus-dependent surface antigens. To examine this possibility we prepared monoclonal antibodies to human T cell membrane determinants by somatic cell hybridization techniques (10, 11) and studied their specificities by indirect immunofluorescence analysis on a cytofluorograph.

Two antibodies, termed αLeu-2a and αLeu-2b, which seem to define the same surface antigen identified by the original αT_{H_2} serum, reacted with 57–84% of thymocytes and 22–46% of erythrocyte-rosette-forming cells (ERF-C) in peripheral blood. Two other monoclonal antibodies, termed αLeu-3a and αLeu-3b, reacted with the same subpopulation of thymocytes (78–89%) and peripheral blood ERF-C (47–78%) but, unlike αLeu-2a and αLeu-2b, did not exhibit cross-blocking; i.e., labeling cells with αLeu-3a did not inhibit the subsequent binding of αLeu-3b. T cells reactive with αLeu-2a were shown to be unreactive with αLeu-3a, indicating that two separate subpopulations of T cells, Leu-2 (formerly $T_{H_2}^+$), and Leu-3 ($T_{H_2}^-$) T cells, were thereby defined. These two T cell subsets make up a subpopulation of ERF-C (80–95%) previously defined by a monoclonal antibody to a T cell membrane component (Leu-1) that has a thymus-dependent distribution on normal lymphocytes but is detectable on surface-immunoglobulin-positive (sIg^+) leukemic lymphocytes from some patients with chronic lymphocytic leukemia (CLL) (12). None of the Leu antibodies reported here reacted with these sIg^+, Leu-1^+ leukemic cells, nor did they react with normal hematopoietic cells or lymphocytes that had surface marker profiles characteristic of B cells.

The recent demonstration that αLyt-2 and αLyt-3 block the cytotoxicity of effector T cells (13) prompted us to examine the blocking effects of the Leu antibodies on cytotoxicity in CML.

MATERIALS AND METHODS

Production of Monoclonal Antibody. BALB/c mice were immunized subcutaneously with 1×10^7 sheep-erythrocyte-rosetting human peripheral blood lymphocytes and boosted three times at 2-week intervals. Three days after the last immunization, splenocytes were fused with 3×63Ag-8 myeloma (NS-1) cells and cultured in RPMI medium (GIBCO) containing 15% fetal calf serum and hypoxanthine/aminopterin/thymidine, following the procedure developed by Köhler and Milstein (10, 11).

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Abbreviations: CML, cell-mediated lympholysis; α, anti-; ERF-C, sheep erythrocyte-rosette-forming cells; sIg, surface immunoglobulin; G/M FITC, fluorescein-conjugated goat anti-mouse IgG; CLL, chronic lymphocytic leukemia.

Specificity Analysis of the Monoclonal Antibody. *Isolation of T lymphocyte population.* Mononuclear cells were separated from fresh heparinized blood of normal individuals by Ficoll/Hypaque density gradient centrifugation. T cells were isolated from other mononuclear cells by a two-step process involving passage over nylon wool columns followed by erythrocyte-rosette formation at 4°C, using neuraminidase-treated sheep erythrocytes (14) and density gradient centrifugation.

Isolation of monocytes. Monocytes were isolated by plating peripheral blood mononuclear cells at a density of 5×10^6 per ml in RPMI medium containing 10% fetal calf serum over glass petri dishes for 2 hr. The nonadherent cells were removed by vigorous lavage and the adherent cells were recovered after incubation for 1 hr at 37°C in medium containing 0.1% EDTA. Greater than 90% of these cells had the morphological characteristics of monocytes or macrophages, having a reniform nucleus and a clearly different size distribution from lymphocytes.

Isolation of B cells. Lymphocytes depleted of phagocytic cells by carbonyl iron treatment were brought to a concentration of $10–20 \times 10^6$ per ml and mixed at a 1:1 volume with a 5% suspension of neuraminidase-treated sheep erythrocytes (13). The non-ERF-C (B cells) were separated from the ERF-C on Ficoll/Hypaque gradients.

Cytofluorographic analysis. Cytofluorographic analysis of all cell populations was performed by indirect immunofluorescence with affinity-purified, fluorescein-conjugated, goat anti-mouse IgG (G/M FITC) on a cytofluorograph (Ortho Instruments, Westwood, MA; model FC200-4800 A50) as described (6). In brief, 5×10^5 cells were treated with 0.15 ml of serum from mice bearing the antibody-secreting tumors at a dilution of $1:10^4$, incubated at 4°C for 1 hr, and washed twice. The cells were then mixed with 0.15 ml of G/M FITC at a 1:20 dilution for 1 hr, washed three times, and analyzed on the cytofluorograph. Background staining was obtained by using a serum from a *nude* mouse that was tumor-free.

Selection and cloning of hybridized cells. Approximately 10–14 days later, supernatants were collected from wells with hybrid cell growth and tested for antibodies specific to T cells by indirect immunofluorescence analysis. Hybrids from wells containing antibodies specific to peripheral T lymphocytes were harvested and cloned by a limiting dilution method in the presence of feeder cells and plated into a Linbro 96-well flat-bottom tissue culture plate for subsequent screening and recloning.

Nude *mice sera containing monoclonal antibody.* After positive hybrids had been recloned 8–10 times, 1×10^7 hybrid cells were implanted subcutaneously into Swiss strain background *nu/nu* mice. Sera were obtained from these tumor-bearing mice approximately 3 weeks later.

Assay for CML. Cytotoxic T lymphocytes were generated by culturing 10×10^6 peripheral blood mononuclear leukocytes (responding cells) with 10×10^6 irradiated [2000 roentgens (0.5 coulomb/kg)] stimulating cells, at a final concentration of 2×10^6 cells per ml in RPMI 1640 supplemented with 10% heat-inactivated fetal calf serum, 0.025 M Hepes buffer, 2 mM glutamine, and antibiotics. The cells were incubated in tissue culture flasks (Falcon) in a humidified incubator with 5% CO_2 for 7 days. Allogenic as well as autologous target cells (1×10^6 cells per ml) were cultured in the previously described medium and stimulated with phytohemagglutin (GIBCO) at 10 μg/ml for the last 4 days of the incubation. Before the assay 1×10^6 target cells were centrifuged at $200 \times g$ for 5 min, resuspended in 0.3 ml of medium, and labeled with 250 μCi of sodium [^{51}Cr]chromate (specific activity 200–500 mCi/mg of Cr; New England Nuclear; 1 Ci = 3.7×10^{10} becquerels) for 2 hr at 37°C. The target cells were washed three times with medium and resuspended at a concentration of 5×10^4 cells per ml. Effector cells were washed twice and incubated with various dilutions of monoclonal antibodies, as described in the text, for 2 hr at room temperature. Samples (100 μl) were mixed with 100 μl of target cells in round-bottom microtiter plates (Nunclon, Copenhagen) and incubated for 5 hr at 37°C in a humidified incubator with 5% CO_2. From the supernatants 100-μl samples were carefully withdrawn and their radioactivities were determined in a gamma counter (Packard Instruments, Downers Grove, IL). Percent cytotoxicity was determined from ^{51}Cr release as follows:

$$\% \text{ cytotoxicity} = \frac{\text{Experimental release} - \text{spontaneous release}}{\text{maximal release} - \text{spontaneous release}} \times 100.$$

Maximal release was determined by treating 100 μl of cells with 100 μl of 5% Triton X-100 for 5 hr at 37°C. Spontaneous release was determined by incubating target cells in medium alone for 5 hr at 37°C.

RESULTS

Screening and Preparation of the Monoclonal Antisera. Mouse splenocyte–myeloma cell hybrids producing antibodies that reacted with normal T but not B cells derived from the peripheral blood were screened by indirect immunofluorescence analysis on a cytofluorograph. Four such antibodies (αLeu-2a, IgG_1; αLeu-2b, IgG2a; αLeu-3a, IgG1; and αLeu-3b, IgG1) that reacted with subpopulations of ERF-C were cloned 8–10 times *in vitro* and implanted subcutaneously into *nude* mice. The resulting antisera exhibited detectable reactivity by indirect immunofluorescence to normal T cells at titers as high as $1:10^6$.

Distribution of Leu-2a, Leu-2b, Leu-3a, and Leu-3b on ERF-C. The specificities of the antibodies were studied by indirect immunofluorescence on a cytofluorograph, using techniques that have been described (6). Background fluorescence was assessed by using a serum at the same dilution ($1:10^4$) from a tumor-free animal of the same strain. Fig. 1 *A–C* shows representative binding curves of each antiserum and a normal *nude* mouse serum that had reacted with ERF-C isolated from the peripheral blood of a normal individual. T cells that had reacted with an excess of αLeu-2a and αLeu-2b either alone or in combination were stained with an identical frequency and intensity (Fig. 1*A*), indicating that the same subpopulation of T cells (32%) was defined and that complete cross-blocking occurred between αLeu-2a and αLeu-2b. Furthermore, it was demonstrated that labeling cells with an excess of the original rabbit T_{H_2} antiserum completely inhibited their subsequent reactivity with both these monoclonal antibodies (data not shown). The epitope(s) defined by these three reagents were identically distributed on other cell types reported here and are probably part of the same macromolecule. In contrast, cross-blocking did not occur between αLeu-3a and αLeu-3b, although the same subpopulation of ERF-C was defined by both antibodies. Thus, an identical percentage of T cells (62%) showed fluorescence greater than background when labeled with αLeu-3a or αLeu-3b either alone or in combination (Fig. 1 *B* and *C*). However, cells incubated with both antibodies were markedly brighter than cells labeled with an excess of either alone (Fig. 1*C*), indicating that Leu-3a and Leu-3b were different determinants on the same subset of T cells.

Leu-2a and Leu-3a Are Expressed by Separate T Cell Subsets. To establish the relationship of the subsets defined by αLeu-2a and αLeu-3a, the fluorescence profiles on ERF-C that

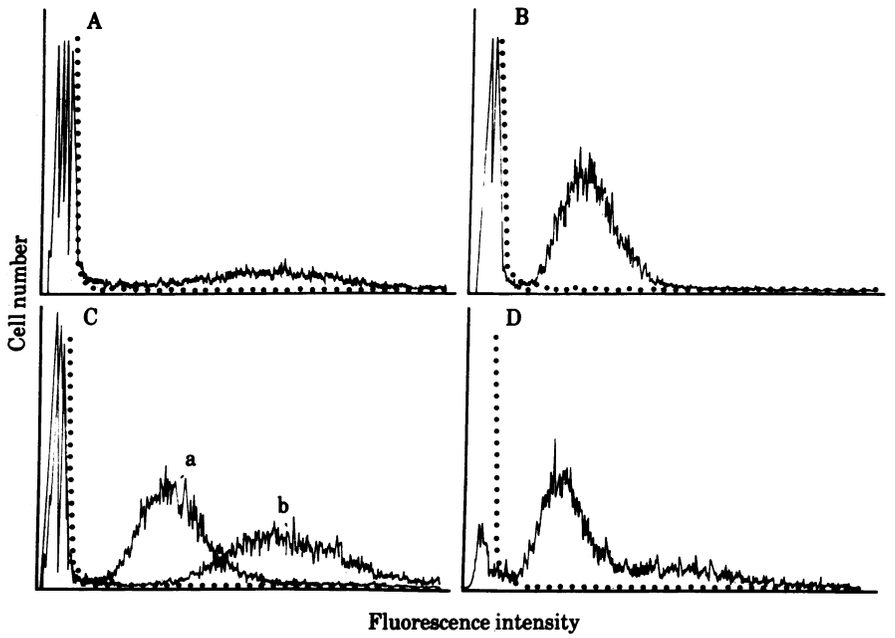

FIG. 1. Cytofluorographic analysis of indirect immunofluorescence staining of human peripheral blood ERF-C with sera from *nude* mice bearing hybrid tumors secreting monoclonal antibodies. Reactivity with specific *nude* mouse sera at a $1:10^4$ dilution (—) is assessed by using a control *nude* mouse serum (····) at the same dilution. For each experiment, 10,000 cells were counted. (*A*) αLeu-2a, αLeu-2b, or αLeu-2a and Leu-2b in combination produced identical profiles (32% +). (*B*) αLeu-3a (62% +). (*C*) αLeu-3b (curve a) or αLeu-3a and αLeu-3b in combination (curve b). The numbers of positive cells defined by curve a and curve b were nearly identical (6513/10,000 and 6529/10,000, respectively). (*D*) αLeu-2 and αLeu-3a in combination (92% +).

had reacted with these antibodies alone and in combination were determined as shown by a representative experiment (Fig. 1 *A*, *B*, and *D*). As can be seen, the number of T cells fluorescing above background when incubated with both antibodies (92%) closely approximated the sum of the numbers defined by αLeu-2a (32%) and αLeu-3a (62%). Similar results were obtained by examining the peripheral blood of 15 normal individuals, in which the numbers of ERF-C reacting with αLeu-2a and αLeu-3a ranged between 22% and 46% and between 47% and 78%, respectively (Table 1). While these results suggested that only two subsets of ERF-C were defined by αLeu-2a and by αLeu-3a (Leu-2a$^+$3a$^-$ T cells and Leu-2a$^-$3a$^+$ T cells), the presence of small numbers of a third subset having the Leu-2a$^+$3a$^+$ (Leu-23) phenotype remained to be excluded.

Table 1. Distribution of thymus-dependent membrane determinants on subpopulations of lymphoid and hematopoietic cells

Cells*	% reactive		
	αLeu-2a (IgG1)	αLeu-3a (IgG1)	αLeu-3b (IgG1)
Thymocytes (6)	57–84	78–89	78–89
Peripheral blood ERF-C†	22–46	47–78	47–78
Peripheral blood, ERF-C-depleted‡ (15)	<5	<5	<5
Tonsil ERF-C (10)	12–21	73–82	73–82
Tonsil, ERF-C-depleted§ (10)	<5	<5	<5
CLL Ig$^+$ (14)	0	0	0
Bone marrow, ERF-C-depleted (6)	<5	<5	<5
Peripheral blood monocytes (8)	<5	<5	<5

* Numbers in parentheses are the minimal number of individuals tested with each antibody.
† Rosetted at 4°C using neuraminidase-treated sheep erythrocytes.
‡ Depleted of phagocytic cells, 50–60% sIg.
§ Depleted of phagocytic cells, 80% sIg.

To this end, the number of ERF-C from five normal individuals that reacted with both αLeu-2a and αLeu-3a labeled with different fluorochromes was determined by fluorescence microscopy. Cells were first allowed to react with αLeu-3a and a G/M FITC as described and then stained with αLeu-2a that was directly conjugated to rhodamine. Examination of cells developed in this manner by double phase fluorescence microscopy failed to reveal detectable numbers of Leu-2a$^+$3a$^+$ cells.

Distribution of Leu-2a, Leu-3a, and Leu-3b on Thymocytes. As shown in Table 1, the majority of thymocytes obtained from six children undergoing cardiac surgery were reactive with αLeu-2a and αLeu-3a. As with peripheral T cells, the same population of thymocytes was stained by αLeu-3a and αLeu-3b (78–89%), whereas somewhat fewer were reactive with αLeu-2a (57–84%). These data, together with the demonstration that the great majority of Leu-2a$^+$ thymocytes were also Leu-3a$^+$, indicates that most of these cells express the Leu-2,3 phenotype.

Leu-2a, Leu-3a, and Leu-3b Are Restricted to Thymus-Dependent Cells. Table 1 shows the reactivity by indirect immunofluorescence analysis of αLeu-2a, αLeu-3a, and αLeu-3b on subpopulations of mononuclear cells derived from the blood, tonsils, or bone marrow. As indicated, these three antibodies did not react with ERF-C-depleted cells from any of these compartments, nor did they react with peripheral blood monocytes or sIg$^+$ leukemic cells from 14 patients with CLL. It would therefore seem that Leu-2a, Leu-3a, and Leu-3b are restricted to cells of the thymic lineage.

Data presented in Table 1 also indicate that the ratio of Leu-2 to Leu-3 cells was significantly less in the tonsils than in the peripheral blood.

Leu-2 and Leu-3 T Cells Are Contained Within the Leu-1$^+$ Population. We recently defined a T cell surface membrane

Table 2. Relative distributions of Leu-1, Leu-2a, and Leu-3a on ERF-C

Donor	% reactive			
	αLeu-1	αLeu-1 + αLeu-2	αLeu-1 + αLeu-3a	αLeu-2 + αLeu-3a
T.M.	93	95	94	95
S.R.	87	85	88	90
B.G.	83	84	87	85
A.F.	95	97	97	97
R.E.	79	79	83	81

component, Leu-1, by a monoclonal antibody that reacted with 80–95% of peripheral blood ERF-C and with sIg^+ leukemic cells from 11 of 14 patients with CLL but did not react with normal B cells (12). The number of Leu-1^+ ERF-C suggested that this population of cells might contain both the Leu-2 and Leu-3 T cell subsets. As shown in Table 2, staining ERF-C from five normal individuals with αLeu-1 alone closely approximated the number of cells stained with any of the following combinations: αLeu-1, αLeu-2a; αLeu-1, αLeu-3a; αLeu-2a, αLeu-3a. Leu-2 and Leu-3 T cells therefore make up and are contained within the population of Leu-1^+ cells.

Inhibition of CML by Leu Antibodies in the Absence of Complement. Cytotoxic T cells were recovered on day 6 from a standard one-way mixed lymphocyte culture, incubated for 2 hr at room temperature with a normal *nude* mouse serum or heat-inactivated Leu antisera at various dilutions, and allowed to react with the appropriate phytohemagglutinin-stimulated blasts in a 6-hr ^{51}Cr-release assay. The sera used in these experiments were brought to concentrations having titers between 1:4000 and 1:8000 as determined by indirect immunofluorescence. They were shown not to be cytotoxic to T cells when heat inactivated and not to induce killing of autologous lymphocytes by mixed lymphocyte culture-primed T cells, a killing that has been shown to occur when target cells are labeled with rabbit antisera (15, 16). Any effect resulting from the presence of the Leu antibodies in the cytotoxic cultures would therefore not be attributable to complement-dependent lysis or to antibody-dependent, cell-mediated cytotoxicity.

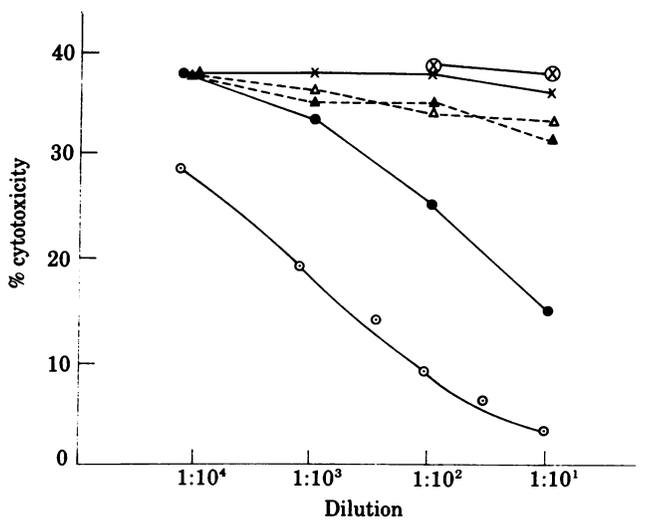

FIG. 2. Killer activity in cell-mediated lympholysis (CML) by allosensitized T cells incubated in a 5-hr ^{51}Cr-release assay with the appropriate allogeneic phytohemagglutinin-stimulated target cells in the presence of different concentrations of *nude* mouse monoclonal antisera. ⊗, Normal *nude* mouse serum; ▲, αLeu-1; ⊙, αLeu-2a; ●, αLeu-2b; △, αLeu-3a; X, αLeu-3b. Sera were brought to concentrations having titers between 1:4000 and 1:8000 before they were added to the cytotoxic cultures. The effector-to-target ratio was 40:1.

As shown by a representative experiment in Fig. 2, the results of five separate experiments established the following points: (*i*) killing in CML was abolished by αLeu-2a at low dilutions, (*ii*) blocking by αLeu-2b also occurred but was markedly less than blocking by αLeu-2a at various dilutions, and (*iii*) killing in the presence of other antibodies, including αLeu-1, αLeu-3a, and αLeu-3b, did not differ significantly from that in the presence of a normal mouse serum control. These results indicate that a membrane component that is critical to CML is selectively blocked by αLeu-2a and, to a lesser extent, by αLeu-2b.

DISCUSSION

We have defined cells that belong to two sublineages of thymus-dependent differentiation by monoclonal antibodies to discrete surface membrane determinants. Two antibodies, termed αLeu-2a and αLeu-2b, which defined the same surface antigen originally defined by the original T_{H_2} antiserum (7), reacted with 57–84% of thymocytes and 22–46% of peripheral blood ERF-C. Two other antibodies, termed αLeu-3a and αLeu-3b, defined another subpopulation of thymocytes (78–89%) and T cells (47–78%) and, like αLeu-2a, reacted only with thymus-dependent lymphocytes. T cells reactive with αLeu-2a were shown to be unreactive with αLeu-3a, indicating that these two T cell subsets do not overlap.

This demonstration, and the demonstration that the majority of thymocytes has the Leu-2,3 phenotype, suggest that these cells give rise to more differentiated Leu-2 or Leu-3 T cells, and that this differentiation step may normally occur in the thymic environment. However, this consideration should be conditioned by the following two points: (*i*) the presence of small numbers of Leu-2,3 T cells in other lymphoid compartments (tonsils, spleen) has not yet been formally excluded, and (*ii*) preliminary evidence indicates that T cells of this phenotype are detectable in the peripheral blood of certain patients with immune disorders. Nonetheless, it seems unlikely that significant numbers of Leu-2,3 T cells exist outside the thymus in most normal individuals.

These considerations are particularly interesting when this system is compared to the subclasses of murine T cells delineated by the Lyt alloantigens. A parallel has already been drawn between these two systems that was based on the functional profiles of $T_{H_2}^+$ (Leu-2) and $T_{H_2}^-$ (Leu-3) T cells defined by heterologous antisera (7). In brief, both Leu-2 and Lyt-2^+3^+ cells express suppressor and cytotoxic functions, whereas both Leu-3 and Lyt-1^+ lymphocytes amplify the functions of other cells. It is therefore interesting that a counterpart to the Lyt-$1^+2^+3^+$ subclass of "precursor" T cells is not prominent in the differentiation architecture defined by αLeu-2a and αLeu-3a, particularly in light of recent studies which indicate that T cells of this phenotype play an important role in maintaining immune homeostasis (17, 18). Elucidation of the structural homology between the Lyt alloantigens and antigens defined by these Leu antibodies will undoubtedly help resolve these issues.

It might also be emphasized that the structural variability of these antigens is considerably more restricted within a species than between two species as disparate as man and mouse. The heterologous determinants associated with a functional membrane component that can be defined by monoclonal antibodies are therefore potentially numerous and would undoubtedly serve as useful markers in the functional analyses of the antigens associated with Leu-1, Leu-2, etc. This is likely reflected in the demonstration that cytotoxicity by effector T cells in CML was

inhibited more effectively by αLeu-2a than by αLeu-2b, although their binding sites are sufficiently close to produce complete cross-blocking as determined by indirect immunofluorescence analysis on a cytofluorograph. Thus, killing in CML may have been blocked more effectively by αLeu-2a than by αLeu-2b due to the positions of their binding sites relative to a specific membrane structure. Alternatively, the different blocking efficiencies of these two antibodies may have been determined by their relative affinities, which might also have affected our results by producing different levels of agglutination or immobilization of the effector cells. While these points need further clarification, our data strongly suggest that cytotoxicity is inhibited by both αLeu-2a and αLeu-2b as the result of specific blocking of a membrane component critical to the lytic process.

In regard to the nature of this surface structure, it is relevant that, in the mouse, αLyt-2 and αLyt-3 block the cytotoxicity of effector cells in the absence of complement (13). This parallel, together with the similar functional profiles of Leu-$2a^+2b^+$ T cells and Lyt-2^+3^+ cells (7), suggests a homology between these murine antigens and the antigen(s) defined by αLeu-2a and αLeu-2b. An interesting implication of this possibility involves the observation that genes coding for immunoglobulin κ chains and genes coding for the Lyt-2 and Lyt-3 antigens are closely linked on chromosome 6 (19–21). Thus, inhibition of T cell cytotoxicity by a Lyt-2,3 and Leu-2a,2b antisera could involve blocking of homologous structures that are part of the T cell receptor in both species. Alternatively, hindrance of the receptor might result from antibody binding to an adjacent site on the Lyt-2,3 or Leu-2a,2b molecules. It should be emphasized, however, that these considerations would also apply to structures that take part in CML by mechanisms other than those involved in recognition and attachment. The resolution of these issues should be facilitated by study of the structural homology between the Leu-2a,2b and Lyt-2,3 antigens.

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