Erythrocyte Receptors and Thymus-associated Antigens on Human Thymocytes, Mitogen-induced Blasts, and Acute Leukemia Blasts

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SUMMARY

Human thymus cells and blasts from some patients with acute lymphoblastic leukemia (ALL) express similar cell surface properties. This suggests that this type of ALL originates in the thymus or alternatively that these properties of ALL blasts reflect changes occurring during blastogenesis. To test these possibilities, we determined whether mitogen-induced blasts would express erythrocyte (E) receptors and thymus antigens found on blasts from E⁺ ALL and on normal human thymocytes. E-rosettes of blood T-lymphocytes dissociated at 37°; in contrast, rosettes formed by E⁺ ALL blasts, human thymocytes, and pokeweed mitogen-stimulated blasts were stable at this temperature. Blasts that formed stable rosettes did not have cytoplasmic Ig, indicating that they were T-lymphoblasts. By immunofluorescence and a radiolabeled antibody assay we demonstrated a thymus antigen(s) that was present on the membrane of E⁺ ALL blasts and on normal thymocytes, but not on normal blood T-lymphocytes [TL-like antigen(s)]. This antigen was not expressed on blasts induced by mitogens. The finding that mitogen-induced blasts form temperature-stable rosettes, but lack TL-like antigen(s), indicates that this antigen is not required for the expression of E-receptors stable at 37°. The results support the concept that E⁺ ALL results from the malignant transformation of thymus cells.

INTRODUCTION

The presence of receptors to sheep erythrocytes is considered a cell surface marker of normal human T-cells (thymus derived) (2). This marker also has been demonstrated on neoplastic lymphoid cells, e.g., the lymphoblasts from 20 to 25% of children with ALL form E-rosettes (E⁺ ALL) (8, 9, 11, 21). There are, however, certain differences between E-rosettes formed by various normal or neoplastic lymphoid cells. E-rosettes formed by blood T-lymphocytes dissociated at 37° for 1 hr, while those formed by thymocytes and E⁺ ALL blasts do not (21). These findings suggest that E⁺ ALL is of thymic origin.

Moreover, recent studies suggested that E-rosette-forming blasts from children with ALL have at least 2 distinct T-cell surface antigens. One is T-cell specific and is present on normal peripheral blood T-lymphocytes and on thymus cells (θ-like antigen). The other is thymus specific and is present on thymus cells but not detectable on peripheral T-cells (TL-like antigen(s)) (16)). Therefore, thymocytes and E⁺ leukemic blasts appear to share a common surface antigen(s) [TL-like antigen(s)] that is absent on blood T-lymphocytes.

On the basis of these data, we have postulated that E⁺ ALL results from the malignant transformation of thymus cells or of a subpopulation of peripheral blood T-cells that is indistinguishable from thymus cells. Another possibility is that these surface properties of leukemic cells are related to blast transformation and might therefore be expressed on peripheral blood T-lymphocytes undergoing blastogenesis. We tested this possibility by comparing temperature stability of E-receptors and the presence of TL-like antigen(s) on the cell surface of E⁺ ALL blasts, normal human thymocytes, and mitogen-induced blasts. The data to be presented indicate that, although mitogen-induced blasts form temperature-stable E-rosettes, they lack the TL-like antigen(s) expressed by some leukemic blasts and normal thymocytes.

MATERIALS AND METHODS

Source and Preparation of Normal and Leukemic Cells. Normal peripheral blood was obtained from healthy volunteers, and the lymphocytes were separated by centrifugation on a Ficoll-Hypaque gradient (7). Blood was defibrinated, diluted 1:4 with McCoy's 5A tissue culture medium (Grand Island Biological Co., Grand Island, N. Y), layered on the Ficoll-Hypaque mixture, and centrifuged at 400 × g for 40 min at 20°. The lymphocytes were obtained from the interphase, and the layer of serum diluted with medium was recovered and used to culture these cells.

Bone marrow and peripheral blood cells were obtained from children with ALL at time of diagnosis and were processed as described previously (3). Samples of thymus tissue were obtained from children undergoing cardiac surgery, and a suspension of cells was prepared (16).

Preparation of Rabbit Antithymocyte Serum. The prepa-
ration of the antiserum was described in detail previously. Rabbits were given injections of thymus cells obtained from a 6-year-old child and were boostered 5 months later with fetal thymus cells and then bled 7 days later. Sera were absorbed with human AB erythrocytes. Raji cells, normal human bone marrow cells, and lymphoid cells from patients with chronic lymphocytic leukemia, as previously reported (16).

E-Rosette Formation and Detection of Cytoplasmic Ig. Assay for E-rosette formation was performed by mixing the 0.2 ml of medium containing 1.5 x 10⁶ lymphoid cells with an equal volume of 0.5% sheep erythrocytes in Hanks' balanced salt solution (Grand Island Biological Co.) containing 10% fetal calf serum previously absorbed with sheep erythrocytes. The suspension of cells was incubated at 37°C for 5 min, centrifuged at 200 x g for 5 min, and incubated at 4°C or at 37°C for 1 hr. The cell pellet was gently resuspended and counted in a hemocytometer chamber. To make smears, the supernatant was removed, 1 drop of fetal calf serum was added, the pellet was gently resuspended with a Pasteur pipet, and 1 drop of the suspension of cells was placed on the slide. The smears were made with a brush previously moistened with medium. The smears were air-dried and fixed in cold acetone:methanol (1:1) for 3 min. The slides were washed with PBS (pH 7.2) and incubated at room temperature for 40 min with fluorescein isothiocyanate-conjugated goat anti-human IgG diluted 1:5 (Meloy Laboratories, Springfield, Va.). They were washed with PBS and mounted with Elvanol.

Lymphocyte Cultures. Lymphocytes (5 x 10⁶) were suspended in 1 ml of McCoy's 5A medium with autologous serum previously removed from the Ficol-Hypaque gradient. This medium-serum mixture was diluted with McCoy's 5A medium to obtain a 10% concentration of autologus serum. The lymphocytes were cultured with and without PWM (Grand Island Biological Co.) or PHA (Difco Laboratories, Detroit, Mich.) in loosely capped glass tubes (7.5 x 1.25 cm) at 37°C in an incubator with a humid atmosphere of 95% air and 5% CO₂. PWM was resuspended in 5 ml of sterile 0.9% NaCl solution and diluted to 1:80. One-tenth ml of the solution was added to each culture tube containing 5 x 10⁶ lymphocytes. Cells were harvested after 1 to 7 days of culture. Three hr before the cultures were harvested, 1.5 μCi of [³H]thymidine (Schwarz/Mann, Orangeburg, N. Y.) (specific activity, 3.0 Ci/m mole) were added to each tube. Cultures were processed by acid precipitation for liquid scintillation counting as previously reported (5). Additionally, at the end of the culture period, cells from several tubes were pooled, spun down, and washed 2 times with TC 199 (Grand Island Biological Co.). Total and differential cell counts and viability by the trypan blue dye exclusion test were performed. Smears were made, fixed, and stained with Wright-Giemsa. In some experiments, the cells were incubated with sheep erythrocytes as described above. Smears were made and the slides were processed for radioautography with Kodak Nuclear Track Emulsion Type NTB2 (diluted 1:3 in distilled water). After 7 days exposure, the cells were stained with Wright-Giemsa.

Immunofluorescence with Antithymocyte Serum. The reactivity of the antiserum to lymphoid cells was tested by indirect immunofluorescence (16). One and one-half million cells were incubated for 30 min at 4°C with 50 μl of the antithymus serum at the appropriate dilutions or with the control serum. After incubation, the cells were washed with PBS and incubated for another 30 min at 4°C with 50 μl of fluorescein isothiocyanate-conjugated goat and anti-rabbit IgG (Meloy Laboratories) diluted 1:5. The cells were washed and mounted with PBS:glycerol under a coverslip. By immunofluorescence, the thymus antiserum reacted with >80% thymus cells, approximately one-half of the blood lymphocytes, and <10% of bone marrow lymphocytes (16).

Preparation of ¹²⁵I-labeled Goat IgG Anti-rabbit Immunoglobulin. Details of the preparation of the antiserum and iodination of goat Ig has been previously reported (16). Briefly, goat IgG was separated from other serum proteins by repeated precipitation with ammonium sulfate and was eluted from a DEAE-cellulose column. Goat Ig G was labeled with ¹²⁵I according to the method of McConahey and Dixon (14).

Measurement of Binding of Antithymocyte Serum to Lymphoid Cells by an Indirect Radiolabeled Antibody Test. One million test cells were incubated for 30 min at 4°C with 50 μl of rabbit antithymocyte serum or control serum, both in the appropriate dilutions. The cells were washed twice in PBS and incubated for another 30 min at 4°C with 50 μl of ¹²⁵I-labeled goat IgG anti-rabbit Ig. The cells were then washed 3 times and the final pellet was resuspended in 1 ml of PBS and counted in a Packard Auto-Gamma scintillation spectrometer. The results are reported as specific binding (antithymocyte serum cpm/10⁶ cells) — (control serum cpm/10⁶ cells). All values for specific binding were corrected to account for the radioactive decay of the ¹²⁵I-labeled goat antibody. Assay of various dilutions of anti-thymus serum with a constant number of either thymus cells or peripheral blood T-lymphocytes demonstrated a linear relationship between cpm bound to the cells and antibody concentration. The higher variation from the mean cpm between replicate samples was ±15% (16).

RESULTS

Temperature Stability of Rosettes Formed by E⁺ALL Blasts, Thymus Cells, and PWM-induced Blasts. Data from a small sample of patients suggested that E-rosettes formed by E⁺ALL blasts and by normal thymocytes are stable at 37°C (4). The results presented in Table 1 confirm and extend this observation in 13 patients with E⁺ALL. All patients with E⁺ALL had a significant number of blasts that formed E-rosettes after 1 hr of incubation of 37°C. Similarly, normal

Table 1

<table>
<thead>
<tr>
<th>Temperature dependence of rosette formation by lymphoid cells</th>
<th>% E-rosettes</th>
</tr>
</thead>
<tbody>
<tr>
<td>No.</td>
<td>4°C</td>
</tr>
<tr>
<td>E⁺ALL blasts</td>
<td>13</td>
</tr>
<tr>
<td>Thymocytes</td>
<td>8</td>
</tr>
<tr>
<td>Normal lymphocytes</td>
<td>6</td>
</tr>
</tbody>
</table>

* Numbers in parentheses, range.
human thymocytes formed stable rosettes, while rosettes of peripheral blood T-lymphocytes dissociate at 37°C. To determine whether in vitro blastogenesis would induce the formation of rosettes that are stable at 37°C, we studied this property in normal lymphocyte cultures with PHA and PWM. However, due to technical problems, the evaluation of rosette formation by PHA-induced blasts was not feasible. Despite extensive washing, PHA remained on the cell surface of nonstimulated lymphocytes and caused agglutination of sheep erythrocytes. These rosettes were stable at 37°C. In contrast PWM, a mitogen known to stimulate T- and B-lymphocytes, did not agglutinate red cells on the membrane of nonstimulated lymphocytes. The proportion of rosettes formed by lymphocytes incubated with PWM and cultured for 1 to 12 hr was not higher than that of control cultures without PWM, and these rosettes dissociated at 37°C (Table 3). Thus, the following studies were performed with PWM-induced blasts. The percentage of lymphocytes and blasts that form rosettes at 4°C and 37°C after 3 days in culture with PWM are shown in Table 2. Although lymphocytes formed rosettes at 4°C, very few remained at 37°C while more than 50% PWM-induced blasts formed rosettes at both temperatures. This finding was confirmed by radioautography. After 3 days of culture, labeled cells formed rosettes at both temperatures, while unlabeled lymphocytes formed rosettes only at 4°C. In 3 experiments, the range of [3H]thymidine labeled cells was 90 to 130/10^6 cells and the proportion of labeled cells that formed rosettes at 4°C (48 to 66%) and at 37°C (42 to 68%) was similar.

These results indicate that, like E⁺ leukemic blasts and thymocytes, PWM-stimulated blasts form rosettes that do not dissociate after 1-hr incubation at 37°C. There was a direct correlation between cell stimulation and the proportion of stable rosettes as a function of time in culture (Table 3). After 1 to 12 hr of incubation with PWM, the lymphocytes did not form 37°C-stable rosettes, indicating that under these experimental conditions PWM attached to the cell surface does not stabilize rosette formation at 37°C. After 168 hr (7 days), 22% of the cells formed rosettes that were stable at 37°C. This was an actual increase, since the number of recovered viable cells did not change significantly over the 168-hr culture. By size and cytomorphology, some of these cells were intermediate between small lymphocytes and large blasts. This may explain why, after 168 hr of culture, the percentage of E-rosettes at 37°C was higher than the proportion of large blasts.

PWM also stimulated B-cells and after 6 to 7 days of culture, we found a significant proportion of Ig-synthesizing blasts (B-blasts). The question arose as to whether E-receptors were an exclusive property of mitogen-stimulated T-blasts or whether they were also present on B-blasts (blasts with cytoplasmic Ig). To answer this question we used a combined method for the simultaneous assay of E-rosettes and cytoplasmic Ig. After 7 days of culture, the cells were incubated with sheep erythrocytes and slides were made, fixed, and stained with fluorescein-conjugated goat anti-human Ig. As shown in Table 4, E-receptors were not present on blasts with cytoplasmic Ig. This suggests that PWM induces at least 2 types of blasts (T and B) with different cell surface properties and supports the contention that the formation of stable rosettes at 37°C is a characteristic of T-blasts. Another approach to the study of cell membrane is to determine the kinetics of migration of membrane components that are linked to antigen or antibodies, i.e., cap formation. Since sheep erythrocytes attached to T-lymphocytes are also capable of inducing translational movements on the plane of the membrane, we compared the kinetics of rosette dissociation (Chart 1, upper panel) and cap formation (Chart 1, lower panel) at 37°C between blood lymphocytes, E⁺ALL blasts, and PWM blasts. There was rapid dissociation of rosettes formed by lymphocytes, but not by E⁺ALL blasts and PWM blasts. Chart 1 (lower panel) shows the kinetics of the appearance of cap-forming cells, that is cells with sheep erythrocytes localized to 1 pole of the cells or distributed over less than half of the cell surface. Within the 1st 10 to 20 min of incubation at 37°C, cap-forming E⁺ALL and PWM blasts increased and the percentage of caps formed by both cells did not change significantly over the next 2 hr. Thus, cap formation by these 2 types of cells appears to have similar kinetics.

**TL-like Antigen(s) on E⁺ALL Blasts, Thymus Cells, and Lymphoblasts Induced by PWM.** By immunofluorescence

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**Table 2**

Identification of PWM-stimulated cells that form E-rosettes at 37°C

<table>
<thead>
<tr>
<th>Blasts/10⁶ cells</th>
<th>% lymphocytes forming E-rosettes</th>
<th>% blasts forming E-rosettes</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>4°C</td>
<td>37°C</td>
</tr>
<tr>
<td>Mean</td>
<td>135%</td>
<td>36%</td>
</tr>
<tr>
<td>Range</td>
<td>73-210</td>
<td>21-48</td>
</tr>
</tbody>
</table>

*Mean of 5 experiments. Day 3 of culture with PWM.*

**Table 3**

Increase in the proportion of E-rosette-forming cells at 37°C as a function of culture time

<table>
<thead>
<tr>
<th>Time (hr)</th>
<th>Blasts/10⁶ viable cells x</th>
<th>E-rosettes (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>4°C (0-52) or 37°C</td>
</tr>
<tr>
<td>1</td>
<td>4.1</td>
<td>0</td>
</tr>
<tr>
<td>12</td>
<td>5.1</td>
<td>0</td>
</tr>
<tr>
<td>24</td>
<td>5.0</td>
<td>2.1 (1-3)</td>
</tr>
<tr>
<td>48</td>
<td>4.9</td>
<td>19 (17-21)</td>
</tr>
<tr>
<td>168</td>
<td>5.2</td>
<td>17.3 (15-19)</td>
</tr>
</tbody>
</table>

*Mean of 3 experiments.

Numbers in parentheses, range.
and by a radiolabeled antibody assay, we have found that heterologous antibody against normal human thymus binds to thymus cells and E\(^+\)ALL blasts. This reactivity is not abolished by absorption with peripheral lymphocytes (Table 5). Antithymus serum was tested before and after absorption with allogeneic lymphocytes against leukemic blasts and remission lymphocytes from the same patient with E\(^+\)ALL. The reactivity of antithymocyte serum with E\(^+\)ALL blasts was reduced when the antiserum was absorbed with 5 \(\times\) 10\(^8\) lymphocytes, but the remaining activity did not change after absorption with increasing concentrations (5, 10, and 25 \(\times\) 10\(^8\)) of blood lymphocytes. Conversely, 10 and 25 \(\times\) 10\(^8\) allogeneic lymphocytes absorbed all reactivity against remission lymphocytes from the same patient. These data confirm previous findings indicative of thymus-associated antigens on E\(^+\)ALL that are not present on peripheral T-lymphocytes [TL-like antigen(s)].

The question arose whether this TL-like antigen(s) would be expressed by blasts derived from normal T-lymphocytes after mitogen stimulation. The data presented in Table 6 compare the binding to thymus, E\(^+\)ALL, and mitogen-stimulated blasts by antithymocyte serum before and after absorption with peripheral blood leukocytes. The reactivity of the antithymus serum to E\(^+\)ALL blasts from 4 children with ALL and cells from 2 normal human thymuses was not abolished when the serum was absorbed with 25 \(\times\) 10\(^8\) allogeneic T-lymphocytes. The same results were obtained when the ALL blasts were derived from bone marrow (3 patients) or blood (1 patient). Conversely, the absorbed antiserum did not react with fresh peripheral blood lymphocytes (Day 0, Table 6) or lymphocytes cultured for 3 to 6 days in the absence of mitogen. Similarly, blasts induced by PWM reacted with antithymus serum prior to absorption, but not with the antiserum absorbed with peripheral blood T-lymphocytes. The data obtained with the radiolabeled assay were confirmed by immunofluorescence. Although a high proportion of blasts and lymphocytes incubated with unabsorbed antiserum had a bright fluorescent ring, all cells were negative when tested with the absorbed antiserum. Conversely, thymus cells and E\(^+\)ALL were positive. The same results were obtained regardless of whether the cells used for absorption were autologous or allogeneic lymphocytes. Absorption of the antiserum with 25 \(\times\) 10\(^8\) allogeneic or autologous lymphocytes also removed all reactivity to PHA-stimulated blasts. Thus, blasts induced by these mitogens do not express on their surface the TL-like antigen(s) found on E\(^+\)ALL blasts and normal human thymus cells.

**DISCUSSION**

This study demonstrates that, after PWM stimulation, a subpopulation of peripheral blood T-lymphocytes acquires the property of forming E-rosettes that are stable at 37\(^\circ\). By this characteristic they are similar to normal human thymus cells and E\(^+\)ALL blasts. In contrast, a cell surface antigen(s), TL-like antigen(s), that is detected on thymocytes and E\(^+\)ALL blasts is not expressed on PWM- and PHA-stimulated blasts. Thus, E-receptors that are stable at 37\(^\circ\) and TL-like antigen(s) appear to be independent characteristics of the cell membrane.

Several authors have investigated changes on cell surface properties of mitogen-stimulated lymphocytes. Using E-rosettes to identify T-cells and anti-Ig to recognize B-cells, they had shown that the same markers present on T- and B lymphocytes in their nonstimulated stage are expressed by mitogen-induced T- and B-blasts (10, 12, 13, 15, 19, 22). Although most agree that blood lymphocytes stimulated by mitogens are predominately T-blasts, and form spontaneous rosettes with sheep erythrocytes, little is known about the temperature stability of these rosettes. This study demonstrates that T-blasts induced by mitogens form E-rosettes that do not dissociate at 37\(^\circ\), while rosettes formed by blasts derived from normal T-lymphocytes [TL-like antigen(s)] dissociate at 37\(^\circ\).
These data suggest that there are several independent T-specific molecules, one of which is the E-receptor. Our finding sera, found no strict correlation between these 2 markers. Additionally, Brouet et al. (8) and Gaļ-Bezwalska et al. (11), who have studied cell surface properties of leukemic blasts by rosette formation and by cytotoxicity of antithymocyte sera, found no strict correlation between these 2 markers. These data suggest that there are several independent T-specific molecules, one of which is the E-receptor. Our finding by blood lymphocytes are unstable at this temperature. This finding could be used as a tool to identify and separate normal T-lymphocytes from stimulated T-lymphocytes.

It has been previously reported that PWM-stimulated blasts with surface Ig do not have E-receptors (19, 22). Our study demonstrates that this T-marker is also absent on B-blasts at a more differentiated stage, i.e., on cells with cytoplasmic Ig. Incubation of T-lymphocytes with sheep erythrocytes at 37° induces the migration of the red cells to 1 pole of the lymphocytes, i.e., cap formation (24). The kinetics of cap formation in early and late rosettes differs significantly, suggesting 2 different subsets of lymphocytes (25). Our data indicate that the distribution of E-receptors on the surface of both E-ALL blasts and PWM blasts also changes when the cells are incubated at 37° and the kinetics of this phenomenon were similar for both types of cells.

Previous studies on the blocking of rosette formation by antithymus sera suggested a close association between E-receptors and thymus-associated antigens (18, 20). Antiserum raised against peripheral blood T-cells and E-ALL also inhibited rosette formation, while antiserum to B-cells did not block E-receptors (20). Although these findings suggest a direct binding of the anti-T-serum to E-receptors, one cannot exclude the binding to membrane antigens closely associated with E-receptors or changes of the cell membrane as a consequence of the binding to other cell membrane components. Moreover, these studies did not determine whether the antiserum that blocks rosette formation reacts against only 1 or several thymus-associated antigens. Our data indicate no association between TL-like antigen(s) and E-receptors, since PWM-stimulated blasts form temperature-stable rosettes, but do not express this antigen(s) which is present on normal thymocytes and E-ALL blasts. Additionally, Brouet et al. (8) and Gaļ-Bezwalska et al. (11), who have studied cell surface properties of leukemic blasts by rosette formation and by cytotoxicity of antithymocyte sera, found no strict correlation between these 2 markers. These data suggest that there are several independent T-specific molecules, one of which is the E-receptor. Our finding of antigenic determinants present on thymus but not on blood lymphocytes confirm data of other investigators (18, 23). Additionally, we have shown that thymus antiserum absorbed with blood lymphocytes reacts against blasts from patients with E-ALL but not with mitogen-induced blasts. Mohanakumar and Metzgar (17) reported that, after absorption with normal blood lymphocytes, myelogenous leukemia blasts, and cells from a lymphoblastoid cell line, their thymus antiserum was still cytotoxic to lymphoid leukemia and normal thymus cells. However, whether the absorbed antiserum reacted with mitogen-induced blasts.

In contrast to our findings, Thomas and Phillips (23) found that their antithymocyte serum absorbed with blood lymphocytes binds to PHA-induced blasts. Differences in the preparation of reagents and in assays used may explain this discrepancy. Their antiserum was prepared with postnatal thymus and was tested only by immunofluorescence. Their antiserum reacted with less than 10% of thymus cells derived from older children, while our antiserum bound to >80% thymus cells, independent of donor's age. This emphasizes the need for standardization and comparison between thymus antisera produced in different laboratories using various immunization schedules and diverse cell sources. It also suggests that, similar to what has been found in the murine system (6), more than 2 differentiation antigens may be present in human thymus.

The finding that blast transformation of normal T-lymphocytes does not induce the expression of TL-like antigen(s) that is present on blasts from E-ALL provides further support for the concept that this type of leukemia results from the malignant transformation of thymus cells. Childhood ALL with T-markers is a disease that predominates in older boys and is characterized by thymic enlargement and/or high initial WBC (21). Although, in children with this type of ALL, chemotherapy may be effective at the beginning of treatment, remissions are usually short and the prognosis is poor. Further characterization of thymus differentiation an-

### Table 6

<table>
<thead>
<tr>
<th>Cells</th>
<th>No. of experiments</th>
<th>Culture time (days)</th>
<th>Specific binding*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Thymus cells</td>
<td>2</td>
<td>5026, 1398</td>
<td>1768, 716</td>
</tr>
<tr>
<td>E-ALL blasts</td>
<td>4</td>
<td>2700</td>
<td>(1572-4221)*</td>
</tr>
<tr>
<td>Blood lymphocytes</td>
<td>2</td>
<td>757, 1051</td>
<td>0, 0</td>
</tr>
<tr>
<td>3</td>
<td>953, 1059</td>
<td>0, 0</td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>509, 1130</td>
<td>0, 35^d</td>
<td></td>
</tr>
<tr>
<td>PWM-stimulated lymphocytes</td>
<td>2</td>
<td>969, 1142</td>
<td>0, 46^d</td>
</tr>
<tr>
<td>PHA-stimulated lymphocytes</td>
<td>2</td>
<td>1036, 1655</td>
<td>0, 0</td>
</tr>
<tr>
<td></td>
<td>1644, 1819</td>
<td>0, 0</td>
<td></td>
</tr>
</tbody>
</table>

* Specific binding (Antithymocyte serum cpm/10^6 cells) minus (control serum cpm/10^6 cells). The binding of normal rabbit serum ranged from 50 to 125 cpm/10^6 cells.
^ Numbers in parentheses, range.
^ This value is within the range of binding by control sera and is considered negative.
tigens on these leukemic cells might provide a better understanding of the pathogenesis and possibly new approaches to the treatment of T-cell leukemia.

ACKNOWLEDGMENTS

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REFERENCES

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