Recognition by Human and Rabbit Sera of Common Antigens to Leukemia Blast Cells, Peripheral Blood B-Lymphocytes, and Monocytes

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SUMMARY

A human serum (obtained from a multiparous and multiple-transfused patient with chronic myelogenous leukemia) and a rabbit antiserum (obtained by immunization with papain extracts from a B-lymphoblastoid cell line) showed reactivity against antigenic specificities (different from HLA) expressed on peripheral blood B-lymphocytes, unmarked lymphocytes, and monocytes. These antigenic determinants were expressed on myeloblasts and lymphoblasts from patients with acute leukemia (during the active phase of their disease) and on B-lymphoblastoid cell lines and lymphocytes from patients with chronic lymphocytic leukemia. Purified peripheral blood T-lymphocytes, mitogen (phytohemagglutinin)-activated T-lymphocytes, and lymphoblasts (with T-cell characteristics) obtained from patients with acute lymphoblastic leukemia or established lymphoblastoid cell lines lacked these antigenic specificities. Absorption experiments indicate that the antigen(s) detected on normal mononuclear cell populations, leukemia cells, and B-lymphoblastoid cell lines were either identical or highly cross-reactive.

MATERIALS AND METHODS

Medium

The work reported here was carried out using minimal essential medium (Grand Island Biological Co., Grand Island, N. Y.) with 10% FCS (K. C. Biological Co., Lenexa, Kans.) and HBSS.

Human Serum (H656)

Human serum (H656) was obtained from a multiparous woman (A. F.) 64 years old) with CML in August 1973 when the patient was admitted for pancytopenia suspected to be secondary to chemotherapy with Myleran. The drug, however, had been stopped 4 months earlier when her WBC was 5600. In the interim the patient had been transfused with 6 units of packed ABC 6 weeks prior to her admission, which was followed by a delayed transfusion reaction. The medications taken at the time were ferrous sulfate and prednisone, 60 mg p.o. every day. For control purposes we used 10 sera from young, healthy, nontransfused and nulliparous individuals. All sera were heat inactivated at 56° for 30 min before use.

Rabbit Antiserum

The method for obtaining this serum has been amply described (7). Normal rabbit sera obtained from 5 different
rabbits were used for control purposes. These sera have been absorbed with human RBC, peripheral blood mononuclear cells, lyophilized liver, and granulocytes. This serum, labeled R2-1 (T), is devoid of reactivity to HLA antigens (tested by Dr. A. Ting against 110 peripheral blood mononuclear cells with a complement-dependent microcytotoxicity assay).

**Complement**

Normal rabbit serum absorbed with a pool of 3 LCL’s and diluted 1:5 was used as a source of complement. This serum was cytotoxic against 10 to 20% of the target cells.

**Target Cells**

LCL’s. Eight established LCL’s (UCLA-109, -102, -81, -91, -173, and -176; RPMI-4265; and MOLT-4) and 10 newly established cell lines from patients with IM were used as target cells in a complement-dependent microcytotoxicity assay. The cultured cells were harvested daily and washed 3 times; viability was determined by trypan blue dye exclusion, and the cells were used only when viability was ≥90%.

Leukemia cells were obtained from the peripheral blood of patients with acute leukemia during periods of disease activity and were separated by Ficoll-Hypaque density gradient centrifugation (3). The cells so obtained contained between 50 and 90% blast cells (with most cell populations tested having ≥70%).

Peripheral blood mononuclear cells from normal donors, leukemia patients in remission (5 with AML and 5 with ALL), and patients with IM (during the active phase of their disease) were obtained by similar cell separation procedure. Those cells contained 70 to 80% lymphocytes, 20 to 30% monocytes, and 0 to 10% granulocytes, as determined morphologically with the use of May-Grünwald-Giemsa stain. On some occasions mononuclear cells separated by Ficoll-Hypaque density gradient centrifugation (in a concentration of 2 x 10^7 cells/ml) were mixed with 2 ml of lymphocyte-separating reagent to remove phagocytic cells, i.e., granulocytes and monocytes (16). The recovered cells consisted of ≥95% lymphocytes by morphological and histochemical criteria.

Peripheral blood leukocytes from patients with CML were obtained in a fashion similar to that used for bone marrow cells. The population obtained was composed mainly of cellular elements of the granulocyte series (70 to 80%), the rest being lymphocytes and monocytes.

**Bone Marrow Cells.** Bone marrow cells were obtained by aspiration from the posterior iliac crest of patients and controls. The cells were sedimented on plasmagel for 45 min at room temperature, and RBC contamination was removed by treatment with Boyle’s solution (Tris-ammonium sulfate buffer, pH 7.2) for 5 min. The cells were then washed 3 times in HBSS.

**Depletion of T-Lymphocytes.** The procedure used has been described by Wybrow et al. (22). Briefly, 1.5 x 10^7 mononuclear cells (obtained after Ficoll-Hypaque density gradient centrifugation) or lymphocytes (after removal of phagocytic cells with lymphocyte-separating reagent) were mixed with 1.5 x 10^8 sheep RBC in the presence of 50 μl of absorbed FCS in a total volume of 1 ml. The cells were centrifuged at 600 rpm for 20 min, 0.5 ml of the supernatant was removed, and the rest was resuspended gently so that the rosettes formed did not disrupt. The resuspended pellets were pooled and incubated at 37°C for 10 min, layered over 1.5 ml of Ficoll-Hypaque, and centrifuged at 1250 rpm for 20 min at room temperature. The interface cells contained a mixture of various cell populations depending on the type of cells used for fractionation. The interface obtained after depletion of lymphocyte rosetting with sheep RBC (E-RFC’s) consisted primarily of monocytes (50 to 70% of the cells stained positively with lipase), and we have designated them type 1. In 2 experiments monocytes were obtained after adherence to plastic Petri dishes (65 to 75% monocytes). The interface obtained after E-RFC depletion of lymphocytes consisted of a mixture of B-lymphocytes (40 to 60%) and unmarked lymphocytes (30 to 40%). The cells were labeled type 2. The rosetted cells recovered from the pellet consisted of ≥90% T-lymphocytes and were labeled type 3.

**Cytotoxicity Assay**

A modification of the microdroplet cytotoxicity assay was used (6). Wells of a microdroplet testing tray (Falcon Plastic Co., Oxnard, Calif.) were filled with 2 μl of oil to prevent evaporation. To each well 1 μl of antiserum was added, followed by 1 μl of target cell suspension (10^6 cells/ml, 10^5 cells/well). The plates were incubated for 30 min at 24°C; 5 μl of rabbit complement were added, and the plates were incubated for an additional 30 min. Then 3 μl of 5% aqueous eosin was added, followed after 3 min by 6 μl of 40% formaldehyde. A microscope slide (5 x 7.6 cm) was carefully placed over the plate, and the edges were fixed with hot Vaseline petroleum jelly. Reactions were read with an inverted phase contrast microscope with a x10 objective. Control wells containing normal serum of complement were screened first to determine base line cytotoxicity. Scoring of cytotoxicity was as follows: 1, 10 to 20% dead cells; 2, 21 to 30%; 3, 31 to 50%; and 4, 51 to 80%. Sera with cytotoxicity titers of ≥4 were considered reactive, and those with titers of ≤3 were considered nonreactive.

---

**Table 1**

<table>
<thead>
<tr>
<th>Disease</th>
<th>Clinical status</th>
<th>No. positive/no. tested</th>
<th>Titer</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal</td>
<td>Active^d</td>
<td>0/130 (0)</td>
<td>1:32</td>
</tr>
<tr>
<td>AML</td>
<td>Remission</td>
<td>13/16 (81)</td>
<td></td>
</tr>
<tr>
<td>ALL</td>
<td>Active^d</td>
<td>11/13 (85)</td>
<td>1:64</td>
</tr>
<tr>
<td></td>
<td>Relapse</td>
<td>0/4 (0)</td>
<td></td>
</tr>
</tbody>
</table>

^a R2-1 (T) rabbit antimembrane fraction of RPMI-4265 absorbed.
^b Dilution giving a cytotoxicity score ≥4.
^c Numbers in parentheses, percentage.
^d On peripheral blood differential count, ≥50% leukemic blasts.
Serum Absorption

Serum was incubated with packed cells (2 to 3:1, v/v) at 4°C for 2 hr with intermittent agitation. The cell suspension was centrifuged at 500 × g at 4°C for 15 min, and the supernatant was collected. Cells used for absorption included peripheral blood leukocytes (derived from healthy donors); LCL’s (RPMI-4265, MOLT-4, IM-1); and leukemia cells from patients with AML, ALL, and CML. All of the cells were washed 3 times in minimal essential medium before being used for absorption. When possible, microabsorption of 30 μl of sera was performed with 10⁶ cells.

Analysis of Cell Surface Characteristics

Surface immunoglobulin. One drop of a 1:4 dilution of fluorescein isothiocyanate-conjugated goat F(ab)’, anti-human IgG F(ab)’, or of goat anti-human immunoglobulin was incubated with 1 to 2 × 10⁶ lymphocytes for 45 min at 4°C. The excess of antiserum was removed by washing the cells 3 times in HBSS. After the final wash, 1 drop of a 50% solution of glycerol in phosphate-buffered saline was added to the cells; after resuspension, a small portion of the suspension was mixed in equal volumes with 5 × 10⁶ lymphocytes/ml trypsinized sheep ABC and incubated at 37°C for 30 mm, centrifuged at 500 rpm for 5 mm, and incubated at 4°C for 24 hr. One hundred μl of a crystal violet solution (15 mg of crystals per 10 ml of HBSS, kept at 56°C for 10 min and then centrifuged at 2000 rpm for 5 min to remove the undissolved crystals) were added, the pellet was gently resuspended, and 200 cells were counted each time.

E-Rosettes. A modification of the method described by Jondal et al. (10) was used. Briefly, 100 μl of a 0.5% sheep RBC suspension were mixed with 100 μl of a 5 × 10⁶ cells/ml lymphocyte suspension and 20 μl of FCS absorbed 3 times with sheep RBC. The tubes containing the cell mixture were incubated at 37°C for 5 min, centrifuged at 500 rpm for 5 min, and incubated at 37°C for 5 min, centrifuged at 500 rpm for 5 min, and incubated at 4°C for 24 hr. One hundred μl of a crystal violet solution (15 mg of crystals per 10 ml of HBSS, kept at 56°C for 10 min and then centrifuged at 2000 rpm for 5 min to remove the undissolved crystals) were added, the pellet was gently resuspended, and 200 cells were counted each time.

EA Rosettes. Sheep RBC were trypsinized prior to attachment of the antibodies to suppress their spontaneous binding to T-lymphocytes. A subagglutinating titer of rabbit anti-sheep RBC serum was mixed in equal volumes with 5 × 10⁶ cells/ml trypsinized sheep RBC and incubated at 37°C for 30 min (EA). Thereafter, the EA preparation was washed 3 times in HBSS and adjusted to a concentration of 10⁶/ml. To make rosettes, 100 μl of the 10⁶ cells/ml EA preparation were mixed with 100 μl of 5 × 10⁶ cells/ml lymphocyte suspension. The cells were mixed well, centrifuged at 500 rpm for 5 min, and counted immediately after resuspending them after the addition of 100 μl of crystal violet.

EAC Rosettes. The procedure was similar to that used for EA rosettes, except that IgM rabbit anti-sheep RBC were used. After the EA preparation (IgM) was prepared, 125 μl of zymosan-activated human complement were added to each ml of 10⁶ cells/ml EA (IgM) preparation. The cells were mixed well and incubated at 37°C for 30 min, after which they were washed 3 times in HBSS and resuspended at a concentration of 5 × 10⁶ cells/ml. To make EAC rosettes a procedure identical with that described for EA rosette formation was followed.

RESULTS

Reactivity of H656 against Histocompatibility Antigens (HLA) on Peripheral Blood Mononuclear Cells

The nonabsorbed antiserum (H656) was heat inactivated at 56°C for 30 min and kindly tested by Dr. A. Ting (at Dr. Terasaki’s laboratories, UCLA Medical Center, Los Angeles, Calif.) against a panel of 107 peripheral blood mononuclear cells obtained from healthy donors. Reactivity against 28 of 107 cells was demonstrated with the use of undiluted serum by the complement-dependent microcytotoxicity assay. The reactivity was directed mainly against HLA-A10 and HLA-Aw32 specificities. At a dilution of 1:10, a marked decrease in the cytotoxic reactivity was noted, with only 4 of 110 cells (all carrying HLA-A10) being reactive with the serum. To remove this reactivity, we absorbed the serum with pooled peripheral blood leukocytes from donors carrying HLA-A10 (AD HLA-A10, 29; B12) and HLA-Aw32 (JZ HLA-A1; B8; Aw32; Bw15). The antiserum so obtained (labeled H656(T)) had no complement-dependent cytotoxicity against a panel of 120 peripheral blood mononuclear cells tested. This serum was also not reactive against purified lymphocytes (≥95% lymphocytes) obtained from 10 healthy donors.

Reactivity of H656(T) and R2-1 (T) against Antigenic Determinant(s) Present on Mononuclear Cell Populations Depleted of T-Lymphocytes

Reactivity against Antigen(s) on Peripheral Blood Monocytes (Type 1 cells). Enriched populations of monocytes were obtained by depleting T-lymphocytes from the mononuclear cell fraction obtained after Ficoll-Hypaque density gradient separation of peripheral blood (Table 2). As shown, more than 50% of the cells obtained had morphological and histochemical features of monocytes. H656(T) and R2-2(T) had complement-mediated cytotoxicity against 10 of 10 type 1 cells obtained with a titer ranging between 1:20 and 1:200. In most of the experiments, the cytotoxic score was ≥4 (more than 80% of the cells were killed), suggesting that the other cells present, i.e., B-lymphocytes and unmarked lymphocytes, shared some or all of the antigenic specificities present on the monocytes. Identical results were obtained in 2 experiments performed on monocytes obtained by adherence to plastic surfaces (the cell populations consisted of 65 to 75% monocytes, as determined by morphological and histochemical techniques).

Reactivity against Antigen(s) on B-Lymphocytes and Unmarked Lymphocytes (Type 2 Cells). Enriched populations of B-lymphocytes and unmarked lymphocytes were obtained by depleting T-lymphocytes from purified lymphocyte populations obtained as described in "Materials and Methods." These cells were labeled type 2, and their surface characteristics and differential counts are summarized in Table 2. As shown in the table, this population consisted of a mixture of B-lymphocytes (40 to 60%) and unmarked lymphocytes (30 to 40%). H656(T) and R2-1(T) reacted against 6 of 6 type 2 cells with a titer ranging between 1:10...
The majority of the lymphocytes in these cell populations, even when the serum was used undiluted. Similar negative results were obtained when the serum was used alone or in combination with complement. The reactivity against type 1 and 2 cells was specific. Serum or complement alone were not cytotoxic. Furthermore, normal human or rabbit sera showed no cytotoxicity against any of these cell types.

Surface immunoglobulin on the various cell populations described was performed using a direct immunofluorescence assay with 2 types of fluorescein-labeled antibodies: (a) fluorescein isothiocyanate-labeled goat anti-human immunoglobulin and (b) fluorescein isothiocyanate-labeled goat F(ab')2 anti-human IgG F(ab')2. As shown in Table 3, the use of goat anti-human immunoglobulin almost doubled the percentages of SmIg-bearing cells in the fractionated and unfractionated cell populations when compared to the values obtained with the F(ab')2 fluoresceinated antisera. These results indicate that the fluorescein-labeled antibody molecules, when intact, will attach to Fc receptor-bearing cells, which are in reality SmIg(-), resulting in higher values that do not represent the true SmIg-bearing cells (B-cells) present in the cell mixture.

**Reactivity against T-Lymphocytes (Type 3).** Enriched populations of circulating T-lymphocytes were obtained from 8 healthy donors. These cells formed E-rosettes (≥90%) and were depleted of Fc receptor and SmIg-bearing cells. No cytotoxic reactivity was detectable against any of these cell populations, even when the serum was used undiluted. Similar negative results were obtained when the serum was reacted against mitogen (phytohemagglutinin)-activated T-lymphocytes. By contrast, 4 of 8 peripheral blood lymphocytes obtained from patients with IM during the active phase of their disease showed reactivity with both sera. The majority of the lymphocytes in these cell populations were atypical (>50%) and had surface characteristics of T-lymphocytes (E-RFC). No distinctive clinical or morphological features were identified between the reactive and non-reactive lymphocytes. Furthermore, no significant difference in the percentage of T-cells was discerned between the 2 groups.
complement-dependent cytotoxicity assay. H656(T) showed reactivity against 7 of 8 of the cell lines tested, with titers ranging between 1:164 and 1:320, without significant differences between cells derived from normal or leukemic donors. Four of 8 LCL's had B-cell characteristics, manifested by their ability to synthesize immunoglobulins in vitro. One of the cell lines, UCLA-109, has characteristics of a stem cell, and UCLA-102 and -176 have characteristics of monocytes, i.e., they synthesize lysozyme and are capable of migrating on a Boyden chamber in response to chemotactic factors (M. Jobin and B. Bonavida, personal communication). One cell line, MOLT-4, showed no reactivity with H656(T). To evaluate this further, we absorbed H656(T) with MOLT-4 and RPMI-4265 and then checked the reactivity of the absorbed serum against a panel of 4 different established cell lines (Table 5). As shown in the table, absorption with RPMI-4265 resulted in complete abrogation of complement-dependent cytotoxicity reactivity against all the cell lines, whereas absorption with MOLT-4 resulted in only a minimal decrease in the complement-dependent cytotoxicity titer, which is probably nonspecific in nature. H656(T) was reacted against 10 lymphoblastoid cell lines (recently established in culture) derived from peripheral blood mononuclear cells obtained from patients with IM during the active phase of their disease. The serum had reactivity against each of these cell lines, with a titer ranging between 1:40 and 1:320.

Specificity

Absorption experiments indicate that the antigenic specificities recognized on monocytes (type 1), B-lymphocytes, and unmarked lymphocytes (type 2) are either identical or highly cross-reactive with the antigens identified on leukemia blast cells and LCL's. As indicated in Table 6, leukemia myeloblasts (AML-E) and LCL's (RPMI-4265) removed completely the reactivity against the normal cells, as well as against the leukemia cells and LCL's. Similarly, absorption with type 1 and 2 cells gave identical results. When quantitative absorptions were performed, it was clear that cell types 1 and 2 had absorption capacities equivalent to those of the LCL's and leukemia blast cells.

**DISCUSSION**

Heterologous (rabbit) and human serum demonstrated high titer reactivity against antigens present on normal cells (B-lymphocytes, unmarked lymphocytes, and monocytes), B-LCL's, and leukemia blast cells. The reactivity was directed against identical or highly cross-reactive antigens, as evidenced by absorption experiments (Table 6). The data obtained with the heterologous serum confirm the observations of Billings et al. (1) and indicate that this serum is not detecting antigens specifically associated with acute leukemia cells as was originally inferred (6). These observations raise questions regarding the ability of rabbit antisera (2, 6, 12, 15) to identify leukemia-associated antigens. The data reported with nonhuman primate antisera (14) are different. These sera distinguish antigens on cells derived from myeloid leukemia from those on cells derived from lymphocytic leukemia. This suggests that nonhuman primate antisera might be capable of identifying antigenic specificities not recognized by rabbit antisera. This is further substantiated by the evidence presented by Metzgar et al. (13) that the antigens recognized by the nonhuman primate antisera are solubilized by trypsin but not by papain. By contrast, papain

![Table 4](image)

**Table 4**

Complement-dependent cytotoxicity reactivity of H656(T) against leukemia cells obtained during disease activity

<table>
<thead>
<tr>
<th>Cell type</th>
<th>No. positive/ no. tested</th>
<th>%</th>
<th>Titer</th>
</tr>
</thead>
<tbody>
<tr>
<td>ALL</td>
<td>6/6</td>
<td>100</td>
<td>1:80-1:320</td>
</tr>
<tr>
<td>ALL (T-variety)</td>
<td>0/2</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>ALL (Rem PBM)</td>
<td>0/5</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>ALL (Rem BM)</td>
<td>0/4</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>AML</td>
<td>19/21</td>
<td>90</td>
<td>1:80-1:640</td>
</tr>
<tr>
<td>AML (BM)</td>
<td>5/5</td>
<td>100</td>
<td>1:40-1:160</td>
</tr>
<tr>
<td>AML (Rem PBM)</td>
<td>0/5</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>AML (Rem BM)</td>
<td>0/8</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>CML</td>
<td>0/4</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>CLL*</td>
<td>5/5</td>
<td>100</td>
<td>1:80-1:160</td>
</tr>
</tbody>
</table>

* Final dilution giving >50% target cell lysis.

**Table 5**

Reactivity of H656(T) against LCL's after specific and nonspecific absorptions

<table>
<thead>
<tr>
<th>Serum</th>
<th>Complement-dependent cytotoxicity titer*</th>
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<tbody>
<tr>
<td>UCLA-81</td>
<td></td>
</tr>
<tr>
<td>RPMI-4265</td>
<td></td>
</tr>
<tr>
<td>UCLA-102</td>
<td></td>
</tr>
<tr>
<td>UCLA-173</td>
<td></td>
</tr>
<tr>
<td>H656(T)</td>
<td>1:64</td>
</tr>
<tr>
<td>H656(T1)</td>
<td>0</td>
</tr>
<tr>
<td>H656(T2)</td>
<td>1:48</td>
</tr>
<tr>
<td>H656(T3)</td>
<td>1:48</td>
</tr>
</tbody>
</table>

* Final dilution giving >50% target cell lysis.

**Table 6**

Cytotoxicity of H656(T) after absorption with type 1 and 2 cells, LCL's, and leukemia blast cells

<table>
<thead>
<tr>
<th>Absorbing cell</th>
<th>Type 1</th>
<th>Type 2</th>
<th>LCL</th>
<th>AML blast</th>
</tr>
</thead>
<tbody>
<tr>
<td>Unabsorbed</td>
<td>10/10</td>
<td>10/10</td>
<td>7/8</td>
<td>19/21</td>
</tr>
<tr>
<td>10* RPMI-4265</td>
<td>4/4</td>
<td>3/3</td>
<td>3/3</td>
<td>5/5</td>
</tr>
<tr>
<td>10* RPMI-4265</td>
<td>0/4</td>
<td>0/3</td>
<td>0/3</td>
<td>0/5</td>
</tr>
<tr>
<td>10* AML-E</td>
<td>3/3</td>
<td>3/3</td>
<td>2/2</td>
<td>4/4</td>
</tr>
<tr>
<td>10* AML-E</td>
<td>1/3</td>
<td>0/3</td>
<td>0/3</td>
<td>1/4</td>
</tr>
<tr>
<td>10* type 1</td>
<td>4/4</td>
<td>3/3</td>
<td>3/3</td>
<td>4/4</td>
</tr>
<tr>
<td>10* type 1</td>
<td>1/4</td>
<td>1/3</td>
<td>1/3</td>
<td>1/4</td>
</tr>
<tr>
<td>10* type 2</td>
<td>4/4</td>
<td>3/3</td>
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<td>4/4</td>
</tr>
<tr>
<td>10* type 2</td>
<td>0/4</td>
<td>1/3</td>
<td>0/3</td>
<td>0/4</td>
</tr>
</tbody>
</table>

* Positive test, >50% target cell killing.
digestion was the method used to solubilize and prepare antisera to B-antigens (4, 6).

The development of this antibody in our patient was probably the result of repeated transfusions with allogeneic blood products. The high titer encountered could result from a possible recall reaction triggered by the preceding transfusion reaction. The lack of reactivity with CML cells reinforces this notion. Both sera had reactivity against AML and ALL blast cells. The reactive lymphoblasts were of the "null cell" variety, i.e., they had no surface characteristics of either B- or T-lymphocytes. The origin of the null cell variety of ALL is unclear. In the circulating blood of normal individuals, a population of lymphocytes devoid of recognizable surface receptors but carrying the antigens identified by these sera was termed "unmarked lymphocytes." Null cell ALL could be originating from the malignant transformation of these cells.

The expression of these antigens on leukemia myeloblasts is intriguing. Normal granulocytes (end stage cells differentiated from normal myeloblasts) were devoid of these antigens, which agrees with the observations reported by Billings et al. (1). It remains unclear whether normal myeloblasts express these antigens. We have examined bone marrow cells from 2 patients with solid tumors who had developed pancytopenia (secondary to chemotherapy) and were in the recovery phase. The cells obtained (containing 30 to 40% myeloblasts, promyelocytes, and early myelocytes) were nonreactive (unpublished results), which indicates that normal immature elements of the myeloid series lack the antigens with which these sera react. It is quite possible that the appearance of this reactivity is associated with the neoplastic transformation. The significance of the appearance of B-like antigens on leukemia cells in unclear. Creswell and Geier (4) reported on the ability of a rabbit antiserum prepared against papain extracts from RPMI-4265 [this serum is probably similar to or identical with R2-1(T)] to inhibit activation of T-lymphocytes in a mixed lymphocyte reaction. Both sera described here induced strong inhibition of proliferation induced by allogeneic leukemia myeloblasts. This indicates that the antigens are important in triggering proliferation in a mixed cell culture. Their expression might enhance their ability to stimulate specific immune responses.

Both sera had reactivities similar to those described for sera identifying B-cell-specific alloantigens (11, 19-21). Their reactivity against B-lymphocytes, unmarked lymphocytes, and monocytes indicates that these antigens are not restricted to B-lymphocytes (1) and that they behave in a fashion similar to murine Ia antigens (8). We shall demonstrate, therefore, the antigenic specificities recognized by the sera as Ia-like antigens (8). We shall demonstrate that the sera described here recognize B-antigens in B-lymphocytes, null cells, macrophages, epidermal cells, and spermatozoa by using sera absorbed with platelets from multiparous patients. Platelets, erythrocytes, and fibroblasts lacked these antigens. The rabbit and human antisera thus far have showed positive reactivity against all cell populations tested. Similar data were reported by Billings et al. (1) using heterologous sera. It is possible that the system consists of common antigens present on all cells that are recognized by the sera discussed here and a group of allelic determinants detected by other human sera (20).

Normal, mitogen-activated T-lymphocytes and established LCL's with T-cell characteristics lacked Ia-like antigens. These results indicate that in humans these antigens are not readily detectable on T-lymphocytes. Mouse T-lymphocytes have Ia antigens (18), and it is feasible, therefore, that with a more sensitive methodology they might be detected in humans as well. Alternatively, these antigens are only present on a subset of T-lymphocytes, which could not be recognized without further fractionation procedures. Atypical lymphocytes from patients with IM carry Ia-like antigens. One possible explanation is that certain activation processes (viral infection) trigger the expression of these antigens on T-cells. Alternatively, the infection induced an expansion of cell clones that carry these antigens, enriching the circulating blood with these subsets of cells. Similar data have been obtained by examining activated T-lymphocytes obtained from a mixed lymphocyte culture. We are currently proceeding with studies to clarify further these intriguing observations.

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Complex and the I. Immune Response Region: Genetic Variation, Func-
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