Leukemic Reticuloendotheliosis: "Hairy Cell Leukemia," Functional and Structural Features of the Abnormal Cell in a Patient With Profound Leukocytosis

By David H. Boldt, Stephen F. Speckart, Richard P. MacDermott, Geoffrey S. Nash, and Jan Edward Valeski

The development of profound leukocytosis in a patient with leukemic reticuloendotheliosis (LRE) enabled us to obtain purified LRE cells for the investigation of their structural and functional characteristics. The LRE cells of our patient bore surface immunoglobulin and had complement receptors but did not bear Fc receptors and did not form rosettes with sheep erythrocytes. By electron microscopy, the cells were observed to contain typical ribosome lamella structures and to phagocytize both 0.81 µm latex particles and complement-coated zymosan particles. They were adherent to both glass and nylon wool fibers. The mitogenic response to erythroagglutinating phytohemagglutinin was normal in magnitude but delayed chronologically. The binding of 125I-labeled plant lectins was used to characterize the surface topography of LRE cells. Results of these studies indicated that the LRE cell surface differed significantly from the surface of normal T and B lymphocytes and chronic lymphatic leukemia cells. The LRE cells were capable of both stimulating and responding in a one-way mixed lymphocyte culture. However, the LRE cells were not active as effector cells of either cell-mediated lympholysis, a T cell function, or antibody-dependent cellular cytotoxicity, a null cell function. In contrast, they were effector cells of lectin-induced cellular cytotoxicity showing that they did possess the capacity to function as cytotoxic effector cells. These data indicated that the LRE cells in our patient had surface and functional characteristics of both lymphocytes and monocytes.

LEUKEMIC RETICULOENDOTHELIOSIS (LRE) has engendered considerable interest both because of its unusual clinical behavior, characterized by a favorable response to splenectomy and generally poor response to chemotherapy, and because of the peculiar morphological features of the malignant LRE cell. Since the appearance of this cell is characterized by multiple microvillous projections, the name "hairy cell leukemia" has been applied to LRE. The LRE cell has been characterized as either a lympho-

Abbreviations used in this paper: LRE, leukemic reticuloendotheliosis; EAC, sheep erythrocyte sensitized with Cl, 4, 2, 3; EA, sheep erythrocyte sensitized with rabbit anti-sheep RBC IgG; E, sheep erythrocyte (unsensitized); E-PHA, erythroagglutinating phytohemagglutinin from Phaseolus vulgaris; L-PHA, leukoagglutinin from Ph. vulgaris; RCA-1, Ricinis communis agglutinin; Con A, concanavalin A; WGA, wheat germ agglutinin; CML, cell-mediated lympholysis; MLC, mixed leukocyte culture; ADCC, antibody-dependent cellular cytotoxicity; LICC, lectin-induced cellular cytotoxicity; CLL, chronic lymphatic leukemia.

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cyte\textsuperscript{13-17} or a histiocyte,\textsuperscript{1,7,18,19} but attempts to establish a definitive classification of LRE have been generally unsatisfactory.

Recently we have had an opportunity to study an LRE patient who developed profound leukocytosis consisting of greater than 95% LRE cells. We have utilized these cells in a variety of in vitro assays of both lymphocyte and histiocyte function as well as to examine a number of LRE cell surface characteristics. Our results indicate that the LRE cells from this patient possessed an unusual combination of lymphocytic and histiocytic functional capabilities and structural features.

**MATERIALS AND METHODS**

**Case Report**

M.F., a 47-yr-old man, became ill in November 1972 with headaches, cough, fever, and night sweats. He was found to have splenomegaly, a retroperitoneal mass, and inguinal adenopathy. Bone marrow examination revealed primitive lymphoreticular cells, and he was treated with cyclophosphamide, vincristine, and prednisone.

When first seen at Walter Reed Army Medical Center in January 1973, the patient had axillary and inguinal lymphadenopathy and splenomegaly. The hemoglobin was 12.8 g/dl, platelet count $67 \times 10^9$ liter, and leukocyte count $2.9 \times 10^9$ liter with 76% lymphoid cells, 10% LRE cells, and 14% polymorphonuclear leukocytes. Protein electrophoresis demonstrated no abnormalities. Bone marrow aspirate was not obtainable, but microscopic examination of a bone marrow biopsy specimen revealed a hypercellular marrow containing 75% atypical, reticular, histiocytic cells that were PAS positive. These cells contained tartrate-resistant acid phosphatase (determined by Dr. L. T. Yam, Boston). A diagnosis of LRE was made, and the patient underwent laparotomy and splenectomy.

Postsplenectomy the platelet count rose to normal levels and has remained stable, but the peripheral blood leukocyte count meanwhile has shown a progressive rise. Leukocyte counts (which ranged between 7.0 and $12.0 \times 10^9$ liter during 1973) rose to $20.0-25.0 \times 10^9$ liter in 1974, and during the past year have averaged $70.0-80.0 \times 10^9$ liter with greater than 95% LRE cells. Electron microscopic examination of these cells has shown the characteristic features of LRE, including cytoplasmic ribosome lamella structures and multiple filamentous cytoplasmic projections. Until recently, the patient has remained asymptomatic except for persistent fatigue.

Within the past year the patient was hospitalized for an episode of viral pneumonitis that resolved without complications. Subsequently he noted painful enlargement of inguinal lymph nodes, which on pathologic examination were infiltrated with characteristic LRE cells. The symptomatic inguinal node enlargement has responded to localized radiotherapy administered since these studies were done.

**Cell Preparation**

These studies were carried out using mononuclear cells isolated from defibrinated whole venous blood by dextran sedimentation of the erythrocytes followed by isopyknic centrifugation of the leukocyte-rich supernate on a Ficoll-Hypaque mixture.\textsuperscript{20} Resultant cell preparations consisted of greater than 99% LRE cells as judged morphologically.

**Electron microscopy.** Cell pellets were fixed in glutaraldehyde and embedded in epon. Thin sections were prepared with a Reichert OM43 ultramicrotome and stained with lead and uranyl acetate for examination on a Zeiss EM9S2 electron microscope.

**Surface markers.** The LRE cells were tested for a panel of lymphoid cell surface markers as previously described: surface immunoglobulin,\textsuperscript{21} complement receptors (EAC rosettes),\textsuperscript{22} Fc receptors (EA rosettes),\textsuperscript{23} and receptors for unsensitized sheep erythrocytes (E rosettes).\textsuperscript{22}

**Phagocytosis and adherence.** The phagocytic capabilities of the LRE cells were assessed using both 0.81-μm latex particles and zymosan. For latex particle ingestion, $2 \times 10^6$ cells and $40 \times 10^6$ latex particles were incubated in 1 ml Medium 199 (Microbiological Associates, Bethesda, Md.) containing 12% decomplemented fetal calf serum at 37°C for 1 hr with shaking.
Mitogenic response to plant lectins.

Zymosan particles were swollen and washed, preincubated in autologous serum with or without complement, then washed five times with Medium 199 prior to use. For zymosan ingestion, $2 \times 10^6$ cells and $40 \times 10^6$ zymosan particles were incubated in 1 ml Medium 199 containing $12\%$ decomponented fetal calf serum. The percentage of cells ingesting particles was determined by counting 1000 cells in 1-μm-thick sections fixed in glutaraldehyde, embedded in epon, and stained with toluidine blue.

Glass adherence was assessed by incubating $15 \times 10^6$ cells in 2 ml Medium 199 with $12\%$ decomponented fetal calf serum in 5 cm glass Petri dishes at $37^\circ C$ for 2 hr. The dishes were washed gently with Hank's balanced salt solution and the nonadherent cells counted. Nylon wool adherence was assessed as previously described.

Lectin-binding studies. The following plant lectins were prepared as previously reported: erythroagglutinating (E-PHA) and leukoagglutinating phytohemagglutinin (L-PHA), Ricinis communis agglutinin (RCA-I), concanavalin A (Con A), and wheat germ agglutinin (WGA). Purified lectins were radioiodinated by the chloramine-T method of Hunter, then used in binding experiments with the LRE cells as previously described. The amount of radioactivity bound to the cells was used to calculate the number of lectin-binding sites by the method of Steck and Wallich.

Mitogenic response to plant lectins. The response of LRE cells to stimulation by the plant

![Fig. 1. LRE cell showing characteristic ultrastructural features including numerous cytoplasmic projections and ribosome–lamella complexes (R-L). M, mitochondria; RER, rough endoplasmic reticulum; R, ribosomes. x 11,000.](image)
mitogen E-PHA was assessed and compared to the responses of normal and chronic lymphatic leukemia (CLL) lymphocytes using a microculture technique as previously described.21,25

In brief, \(1 \times 10^5\) cells of each cell type were placed in triplicate in five different microtiter plates and one plate pulsed daily after 3, 4, 5, 6, or 7 days with 0.05 ml of medium containing 0.2 \(\mu\)Ci methyl-\(^{3}H\)-thymidine (specific activity 1.9 Ci/mM; Schwarz-Mann, Orangeburg, N.Y.). After 4 hr in culture with the thymidine, the cells were harvested using a MASH II extractor (Microbiological).

**Mixed lymphocyte culture (MLC).** Standard one-way mixed lymphocyte cultures were performed as previously described.28,29 In brief, 2 \(\times\) \(10^5\) responding cells and 2 \(\times\) \(10^5\) mitomycin C-treated stimulating cells in 0.2 ml of culture medium were placed in triplicate in microtiter plates. After 6 days, the cultures were pulsed with 0.2 \(\mu\)Ci of \(^{3}H\)-thymidine for 16 hr and harvested with a MASH II extractor.

**Allogeneic cell-mediated lympholysis (CML).** This assay was carried out as previously described.28,29 In brief, killer (effector) cells were obtained by incubation of 7.5 \(\times\) \(10^6\) responding cells with 7.5 \(\times\) \(10^6\) allogeneic mitomycin C-treated lymphocytes in a total volume of 5 ml, in 30-ml flasks (Falcon Plastics, Oxnard, Calif.). The incubation was carried out at 37°C in a 5%, CO\(_2\)–95% air, humid atmosphere. After 6 days the killer cells were isolated and tested for cytotoxic capacity against appropriate \(^{51}Cr\) targets in a 4-hr \(^{51}Cr\) release assay. Utilizing this system, we have found that only T cells were effector cells of CML.28,29

**Antibody-dependent cellular cytotoxicity (ADCC).** Rabbit antichicken red blood cell antibody
was used to coat $^{51}$Cr-labeled chicken RBCs. Potential effector lymphocytes were then placed with the targets in a 4-hr $^{51}$Cr release assay. Utilizing this procedure, we, as well as others, have found that only null cells are effector cells of ADCC (R.P. MacDermott, unpublished results).

**RESULTS**

**Morphology.** Approximately 95% of M.F.’s peripheral blood leukocytes demonstrated the classical morphology of LRE cells, and mononuclear cell preparations obtained by density sedimentation on Ficoll–Hypaque consisted of greater than 99%, LRE cells. The characteristic electron microscopic appearance of these cells is seen in Figs. 1–4.

**Surface markers.** Table 1 presents the cell surface characteristics of the LRE cells. Approximately 95% of the cells bore surface immunoglobulin. Incubations with fluorescent antisera and subsequent washings were carried out at 4°C, thus minimizing nonspecific uptake of the label. Experiments using
monospecific antisera demonstrated that 80% of the LRE cells bore surface IgG with surface IgM on 27%, IgA on 21%, and IgD on 13% of the cells. These data indicated that IgG was the predominant surface immunoglobulin on the cells from our patient.

Following overnight incubation at 37°C, there was no change in the percentage of cells bearing surface immunoglobulin nor in the IgG predominance, indicating that this immunoglobulin was most likely a product of the LRE cells.

Table 1. Surface Markers of LRE Cells*

<table>
<thead>
<tr>
<th>Percentage of Positive Cells</th>
<th>Day 0</th>
<th>Day 1</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sig</td>
<td>97%</td>
<td>95%</td>
</tr>
<tr>
<td>EAC rosettes</td>
<td>54%</td>
<td>79%</td>
</tr>
<tr>
<td>EA rosettes</td>
<td>3%</td>
<td>10%</td>
</tr>
<tr>
<td>E rosettes</td>
<td>4%</td>
<td>3%</td>
</tr>
</tbody>
</table>

*Surface markers determined as described in Materials and Methods.
†Determinations performed both before (day 0) and after (day 1) overnight incubation at 37°C.
and not passively absorbed via Fc receptors. In addition to surface immunoglobulin a large percentage of the cells had complement receptors as detected by formation of EAC rosettes (Table 1). By contrast, very few LRE cells had Fc receptors and less than 5% of the LRE cells formed E rosettes.

**Phagocytosis and adherence.** Figures 2–4 demonstrate phagocytosis of both latex and zymosan particles by the LRE cells. The data for the phagocytosis and adherence studies are presented in Table 2; results from control mononuclear cell populations prepared by Ficoll–Hypaque centrifugation from normal individuals are presented for comparison. Fifty-one percent of LRE cells ingested 0.81-μm latex particles compared to 9% of control mononuclear populations (containing 10% macrophages as judged by staining for myeloperoxidase). Ingestion of the larger (~1.5 μm) zymosan particles by LRE cells was considerably less efficient, but was facilitated by the presence of complement (Table 2).

Compared to normal mononuclear cells, LRE cells demonstrated enhanced adherence to nylon fibers, but the percentage of LRE cells that was glass adherent was the same as normal (Table 2). When examined for myeloperoxidase by the method of Kaplow, only 0.2%, of purified LRE cell preparations stained positively.

**Lectin-binding studies.** Because plant lectins bind to specific carbohydrates on the cell membrane, they may be used as probes of cell surface structure. In Table 3 the results of lectin-binding studies on the LRE cells are compared to results obtained in our laboratory for lectin binding to normal human T, B, and null lymphocytes and to those of a group of patients with CLL.

### Table 2. Phagocytic and Adherent Properties of LRE Cells*

<table>
<thead>
<tr>
<th></th>
<th>LRE†</th>
<th>Normal†</th>
</tr>
</thead>
<tbody>
<tr>
<td>Percent cells ingesting latex</td>
<td>51.0</td>
<td>9.0</td>
</tr>
<tr>
<td>Percent cells ingesting zymosan:</td>
<td></td>
<td></td>
</tr>
<tr>
<td>+ Complement</td>
<td>10.0</td>
<td>8.5</td>
</tr>
<tr>
<td>- Complement</td>
<td>2.3</td>
<td>2.5</td>
</tr>
<tr>
<td>Percent glass adherent</td>
<td>47.0</td>
<td>46.0</td>
</tr>
<tr>
<td>Percent adherent to nylon fibers</td>
<td>74.0</td>
<td>50.0</td>
</tr>
</tbody>
</table>

*All assays performed as described in Materials and Methods.
†Normal mononuclear cell preparations contained 10% and LRE cell preparations contained 0.2% peroxidase-positive cells.

### Table 3. Lectin-binding Studies*

<table>
<thead>
<tr>
<th>Cell Type</th>
<th>Molecules Bound Per Cell x 10^-6 for the Lectins</th>
</tr>
</thead>
<tbody>
<tr>
<td>E-PHA</td>
<td>L-PHA</td>
</tr>
<tr>
<td>Normal T, B, or null cell†</td>
<td>2.43</td>
</tr>
<tr>
<td>CLL lymphocyte†</td>
<td>1.03§</td>
</tr>
<tr>
<td>LRE cell</td>
<td>2.80</td>
</tr>
</tbody>
</table>

*Lectin-binding studies performed as described in Materials and Methods.
†Data from Boldt et al.
§Data partially reported by Speckart et al. Numbers represent means of studies of 13 different patients.
ustain lecture-binding sites on CLL lymphocytes is significantly different from that on normal B lymphocytes (p < 0.001).
Fig. 5. Proliferative response of LRE cells stimulated by E-PHA. Cells from two normal volunteers and one patient with CLL used for comparison. Cells were placed in culture under conditions described in Materials and Methods, and proliferation was assessed by incorporation of $^3$H-thymidine at days 3, 4, 5, 6, and 7. Bars represent the mean ± SEM for the triplicate values of one experiment.

pared to normal T, B, and null lymphocytes, LRE cells had increased receptor sites for the lectins WGA, RCA-I, and Con A, whereas the number of receptors for E- and L-PHA was similar on LRE cells and normal lymphocytes. Identical results were obtained when the binding studies were carried out at 4°C, indicating that the increased number of receptors for WGA, RCA-I, and Con A on LRE cells was not due to uptake of the lectins.

The results for CLL cells presented in Table 3 represent studies on 13 individual patients. The complement of cell surface lectin receptors on the CLL lymphocytes was clearly different from that on both normal lymphocytes and the LRE cells. These data thus established clear differences of cell surface topography among normal peripheral blood lymphocytes, CLL cells, and the LRE cells from our patient.

Response to plant mitogens. The ability of the LRE cells to proliferate in response to nonspecific mitogenic stimulation was assessed using E-PHA. In Fig. 5, the response of the LRE cells is compared to the response of two normal

<table>
<thead>
<tr>
<th>Responding Cell</th>
<th>Stimulating Cell</th>
<th>$^3$H-Thymidine Incorporation (cpm)*</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>$A_m$†</td>
<td>6,147 ± 253</td>
</tr>
<tr>
<td></td>
<td>$LRE_m$</td>
<td>12,277 ± 439</td>
</tr>
<tr>
<td></td>
<td>$B_m$</td>
<td>16,784 ± 1107</td>
</tr>
<tr>
<td>LRE</td>
<td>$A_m$</td>
<td>2,814 ± 302</td>
</tr>
<tr>
<td></td>
<td>$LRE_m$</td>
<td>658 ± 22</td>
</tr>
<tr>
<td></td>
<td>$B_m$</td>
<td>4,911 ± 470</td>
</tr>
<tr>
<td>B</td>
<td>$A_m$</td>
<td>10,049 ± 848</td>
</tr>
<tr>
<td></td>
<td>$LRE_m$</td>
<td>10,753 ± 707</td>
</tr>
<tr>
<td></td>
<td>$B_m$</td>
<td>2,620 ± 168</td>
</tr>
</tbody>
</table>

*Mean ± SEM for one representative experiment done in triplicate.
†Stimulating cells $A_m$, $B_m$, and $LRE_m$ are cells from normal donors A and B and patient M.F. that have been treated with mitomycin C as described in Materials and Methods.
LEUKEMIC RETICULOENDOTHELIOSIS

Table 5. Cell-mediated Lympholysis

<table>
<thead>
<tr>
<th>Sensitized Effector WBC</th>
<th>Sensitizing WBC</th>
<th>Per Cent Cytotoxicity of $^{31}$Cr-labeled WBC From Normal Volunteer B</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>$A_m$†</td>
<td>1.3 ± 3.3†</td>
</tr>
<tr>
<td>A</td>
<td>$B_m$</td>
<td>9.5 ± 2.0</td>
</tr>
<tr>
<td>LRE</td>
<td>LRE$_m$</td>
<td>−1.3 ± 1.1</td>
</tr>
<tr>
<td>LRE</td>
<td>$B_m$</td>
<td>0.9 ± 0.6</td>
</tr>
</tbody>
</table>

*Effector cells sensitized by cells from donor A or B or patient M.F., then tested for cytotoxicity against cells from normal donor B.
†Sensitizing cells $A_m$, $B_m$, and LRE$_m$ are cells from normal donors A and B and patient M.F. that have been treated with mitomycin C as described in Materials and Methods.
‡Mean ± SEM for one representative experiment done in triplicate in a 4-hr $^{31}$Cr release assay at a killer-to-target cell ratio of 25:1.

volunteers and a patient with CLL. The normal cells demonstrated a peak response on day 4, while both the CLL and LRE cells exhibited a delayed time course with the peak response at day 6.

One-way MLC. LRE cells stimulated allogeneic cells from two normal volunteers to proliferate in vitro (Table 4). Normal WBC from individual A showed a twofold stimulation and WBC from individual B a fivefold stimulation in response to the LRE cells. The LRE cells in turn demonstrated a fivefold stimulation in response to A cells and an eightfold stimulation in response to cells from B. Thus in MLC, LRE cells functioned both as stimulator and responder cells.

Cell-mediated cytotoxicity (CML, ADCC, LICC). We examined the ability of LRE cells to mediate killing of target cells in a variety of cytotoxic systems. In the assay for CML, a T-cell function, LRE cells failed to kill allogeneic lymphocytes to which they had been sensitized in vitro (Table 5). The fact that in vitro conditions were adequate for sensitization was shown by the proliferative response of the LRE cells in the MLC (Table 4). Therefore the LRE cells, though capable of a proliferative MLC response, were incapable of differentiating into effector cells of CML.

When the LRE cells were tested for ADCC, a null-cell function, they did not kill antibody-coated chicken RBC, while in a parallel experiment cells from a normal individual exhibited 73.5% killing (Table 6). In order to rule out the possibility that passively adsorbed immunoglobulin might be blocking ADCC activity, the LRE cells were incubated overnight at 37°C, washed with warm medium, and retested. Again, no ADCC activity was produced by the LRE cells.

In the assay for LICC, normal T, B, and null lymphocytes as well as monocytes killed autologous or allogeneic RBC in the presence of WGA.

Table 6. Antibody-dependent Cellular Cytotoxicity

<table>
<thead>
<tr>
<th>Effector Cell</th>
<th>Per Cent Cytotoxicity of $^{31}$Cr Chicken RBC</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Media Alone</td>
</tr>
<tr>
<td>None</td>
<td>0</td>
</tr>
<tr>
<td>Normal WBC</td>
<td>−5.1 ± 0.8</td>
</tr>
<tr>
<td>LRE cells</td>
<td>−5.7 ± 2.0</td>
</tr>
</tbody>
</table>

*Mean ± SEM for one representative experiment performed in triplicate at a killer-to-target cell ratio of 50:1.
Table 7. Lectin-induced Cellular Cytotoxicity

<table>
<thead>
<tr>
<th>Effector Cell</th>
<th>Normal Target RBC</th>
<th>LRE Target RBC</th>
</tr>
</thead>
<tbody>
<tr>
<td>None</td>
<td>1.1 ± 0.9*</td>
<td>12.9 ± 10.0</td>
</tr>
<tr>
<td>Normal WBC</td>
<td>22.2 ± 4.0</td>
<td>45.3 ± 28.7</td>
</tr>
<tr>
<td>LRE Cells</td>
<td>29.2 ± 1.1</td>
<td>25.2 ± 5.1</td>
</tr>
</tbody>
</table>

*Mean ± SEM for one representative experiment performed in triplicate at a killer-to-target cell ratio of 25:1 and a WGA concentration of 8.3 μg/ml.

LRE cells killed both autologous (25.2%) and allogeneic (29.2%) RBC in this system (Table 7).

DISCUSSION

Because of his profound LRE cell leukocytosis, our patient presented an unusual opportunity to obtain large quantities of pure LRE cells by the routine procedure of density sedimentation. We have used these purified cells to examine a number of structural and functional features of the LRE cells.

Studies of the surface characteristics of the LRE cells indicate that these cells are surface immunoglobulin- and EAC rosette-positive but EA and E rosette-negative (Table 1). Preincubation studies at 37°C suggest that the surface immunoglobulin detected is a product of the LRE cells and not passively absorbed cytophilic antibody. This conclusion is strengthened by the finding of only a small percentage of Fc receptor-bearing LRE cells.

The surface markers on the LRE cells suggest a B-cell origin. Furthermore, the delayed proliferative response of the LRE cells to E-PHA (Fig. 5) is similar to that seen in lymphocytes from patients with CLL, a B-cell leukemia. We have previously shown that normal B cells will proliferate in response to mitogenic lectins provided that a small number of T cells are present to provide helper activity. We interpret the data in the present study to indicate that the LRE cells, which predominantly bear surface immunoglobulin, proliferate in response to E-PHA but may require a small percentage of T cells in order to respond.

The adherence of LRE cells to nylon fibers and to glass surfaces is consistent with either a B-cell or monocyte origin. A B-lymphocyte origin for the LRE cell has been inferred by other investigators. However, certain studies are inconsistent with a B-cell derivation for the LRE cells from our patient. Thus the cells have been found to phagocytize latex particles efficiently (Figs. 2 and 3; Table 2), a monocyte function. Phagocytosis of the larger zymosan particles is less efficient, but 10% of the LRE cells clearly ingest complement-coated zymosan particles (Fig. 4, Table 2).

The ability of LRE cells to stimulate an MLC reaction is consistent with observations that T, B, and null lymphocytes as well as monocytes can all stimulate an MLC response. Thus the LRE cell as either a B cell or a monocyte could carry out this function. The ability of the LRE cells to respond to allogeneic cell surface antigens is, however, unexpected since this is a T-cell function. We cannot exclude the possibility that the small number of normal T cells present are the cells proliferating in the MLC; but LRE cells overwhelmingly predominate in these cultures (>90%) and it seems more likely that
the LRE cells themselves are proliferating, thus exhibiting T-cell as well as B-cell and monocyte characteristics.

Despite the ability of the LRE cells to proliferate in an MLC setting they are unable to function as effector cells of CML (Table 5), another T-cell function.28,29 Recent work has demonstrated that MLC and CML activity may be dissociated among different lymphocyte subtypes. For example, Stout et al. have found that, in the mouse, T cells with Fc receptors both proliferate in MLC and carry out CML, but T cells lacking Fc receptors do not carry out CML although they proliferate in MLC.42 The cells from our patient which lack Fc receptors may be analogous to the latter group of T cells.

In addition to their inability to carry out CML, the LRE cells are incapable of performing ADCC (Table 6), a null-cell function dependent on membrane Fc receptors.30,31 This finding is consistent with the lack of Fc receptors on the cells from our patient. At variance with our results, recent reports have noted the presence of Fc receptors on LRE cells from some patients,19,38 suggesting that the clinical syndrome of LRE may be associated with cells bearing different surface features.

Because the cells from our patient did not participate in either CML or ADCC, the question arose as to whether they could carry out cytotoxic effector function under any circumstance. The results of the LICC assay in which the LRE cells killed appropriate targets in the presence of WGA (Table 7) indicate that the cells from our patient are able to carry out a cytotoxic effector function given an appropriate in vitro stimulus. Therefore their failure to carry out either CML or ADCC must represent specific rather than general functional deficiencies.

These structural and functional studies identify the LRE cells from our patient as cells with some features of both T and B lymphocytes and of monocytes. Similar conclusions have been drawn by other investigators.38,43 The unusual nature of the LRE cell is further supported by the results of our lectin-binding studies (Table 3). Using a panel of five different plant lectins, we have shown that the LRE cell carries a unique complement of cell surface lectin receptors, and that the surface topography of the LRE cell is quite different from that of normal lymphocytes on the one hand and CLL lymphocytes on the other. Whether the LRE cell arises from malignant transformation of a recognizable histiocytic or lymphoid cell line, if such a cell type is present in small and undetectable numbers in normal lymph nodes, spleen, or bone marrow, cannot be decided based on current information.

Our results, combined with a number of other recent studies,19,38,43 suggest that the clinical and morphological entity of LRE may actually represent a heterogeneous group of diseases at the molecular and functional level. Attempts to assign the LRE cell to either the lymphocyte or the histiocyte category do not give a true picture of the variable structural and functional features of this cell.

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