Cell Surface Characterization of Malignant T Cells From Lymphoblastic Lymphoma Using Monoclonal Antibodies: Evidence for Phenotypic Differences Between Malignant T Cells From Patients With Acute Lymphoblastic Leukemia and Lymphoblastic Lymphoma

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A series of monoclonal antibodies was used for the characterization of malignant T cells from 21 patients with lymphoblastic lymphoma (LL). The tumor population from these patients showed a marked degree of phenotypic heterogeneity and a proportion (one-third) of patients had tumor cells that did not conform exactly with the cells normally detected in the thymus. However, these cell populations could be related to the early or common or late thymocyte population (about one-third of the patients in each category). This contrast, with the characterization of malignant T cells from 43 patients with acute lymphoblastic leukemia (ALL) that could be related to either early or common thymocytes, with an exception of two patients categorized as having a tumor population related to late thymocytes. Further phenotypic differences between cells from ALL and LL could be demonstrated by investigation with two additional monoclonal antibodies, A50 and U4. Among patients with malignant T cells related to common thymocyte, 0/12 patients with ALL had cells recognized by A50, whereas 5/8 patients with LL had A50+ cells. Among patients with early thymocytes, only patients with ALL had cells recognized by U4. In addition, 5 LL patients had cells reactive with J5, a monoclonal antibody recognizing the common ALL antigen (CALLA). Since CALLA was found on cells related to common and late thymocytes, CALLA is neither lineage specific, nor can it be viewed as being peculiar to malignant lymphoid cells arrested at very immature stages of differentiation.

Recent studies using heteroantisera and monoclonal antibodies have shown that profound changes in cell surface antigens mark the various stages of normal human T-cell differentiation. Cells accounting for approximately 10% of the total thymic population within the human thymus bear antigens shared by some bone marrow cells, T10, but lack mature T-cell antigens (stage I). With further maturation, thymocytes retained T10 and acquired a thymocyte-unique antigen T6, apparently analogous to TL in murine systems. Concurrently, these cortical cells express antigens defined by anti-T4, anti-T5, and anti-T8. The T4+, T5+, T6+, T8+, T10+ population accounts for approximately 70%-80% of the total thymic population and was found primarily within the cortical region of the thymus (stage II). With further maturation, thymocytes lost the T6 antigen and acquired the T3 antigen and segregated into T4+ and T5+/T8+ subsets. The latter cells were found primarily in the medullary region of the thymus (stage III). The peripheral compartment is composed of T3+, T4+ lymphocytes, which have been shown to define the human inducer cell, and a T3+, T5+/T8+ population defining the human cytotoxic/suppressor cell.

These studies support the view that it is possible in humans to detect T-cell lymphocyte populations with both a unique differentiation history and biologic functions on the basis of their cell surface antigenic components. The application of these studies to the diagnosis of malignancies of T-cell lineage and an understanding of their heterogeneity is now more appreciated. For example, the analysis of tumor cell populations in patients with T-cell chronic lymphocytic leukemia or Sézary's syndrome has indicated that these leukemias are derived from mature T cells. In contrast, studies of tumor cell populations in patients with T-cell acute lymphoblastic leukemia and T-cell lines derived from these patients have demonstrated that in those lymphoid malignancies, the tumor cells are thymocyte derived, and more importantly, restricted to discrete stages of differentiation. Virtually all patients with T-cell acute lymphoblastic leukemia were found to have tumor cells with the characteristics of either early thymocytes or the common thymocyte pool. Only 1 of 25 patients' tumor cells were from the most mature fraction of the human thymus (stage III).

In the present study, we have used a series of monoclonal antibodies that are selectively reactive with subpopulations of human T cells, B-cell antigens, and common acute lymphoblastic leukemia antigen (CALLA) to compare the phenotypes of patients with T-cell lymphoblastic lymphoma (LL)
with what was previously reported for T-cell acute lymphoblastic leukemia (ALL). By definition, patients with LL have no or minimal bone marrow involvement, whereas patients with ALL present with massive bone marrow and often blood invasion. However, the clinical similarities between T-ALL and T-LL have raised the question of their relationship. Are patients with T-ALL diagnosed at a more advanced stage than patients with T-LL, or are T-ALL and T-LL two different entities? In this respect it was noticed that patients with mediastinal LL seemed to have a better prognosis than patients with ALL involving the mediastinum under a similar chemotherapeutic regimen.

Here we show that the phenotype of the malignant T cells in LL can be related to discrete stages of differentiation as defined by monoclonal antibody and that the pattern of T-cell phenotypes encountered in LL is different from that seen in T-ALL.

MATERIALS AND METHODS

Patient Population and Tumor Specimens

The patients investigated in the present study were children or young adults below the age of 25 yr. They were cared for at the Institut Gustave Roussy at Villejuif, the Hôpital Saint-Louis in Paris, and the Sidney Farber Cancer Institute and Children's Hospital Medical Center in Boston. For our study, 21 patients with LL were available. The diagnosis of LL was established by several strict criteria. All patients presented with tumor masses and tumor cells from lymph nodes and mediastinal masses or pleural effusions were characterized using the standard morphological and histochemical criteria.

All patients underwent bone marrow aspiration from at least four different and distant sites. Whenever a patient had more than 25% lymphoblasts in one of the bone marrow smears, he was considered as ambiguous for categorization in either leukemic or lymphomaous diseases and therefore systematically removed from the study. Eighteen patients with leukemia retained in the study had massive bone marrow involvement — more than 95% — and in all but 2 cases, blood involvement with a leukocyte count above 25,000/cu mm.

In all cases, tumor cells were collected before initial chemotheraphy. Cells from pleural effusions (bone marrow or blood) were isolated by Ficoll-Hypaque density and centrifugation. Tumor masses in lymph nodes were gently teased, minced into single cell suspensions, and passed through stainless steel wire mesh. Tumor cells were readily distinguishable from normal lymphocytes by Wright-Giemsa morphology, and all neoplastic preparations analyzed had more than 90% abnormal cells.

Isolated tumor cells were stored at −196°C in liquid nitrogen, according to a previously described method, which usually allows collection of more than 90% living cells after thawing, as assessed by Trypan blue exclusion.

In three cases of LL (see Results) and four cases of ALL, tumor cells from different sources (cervical lymph nodes and pleural effusion for LL; bone marrow and blood for ALL) were investigated with exactly the same results (results not shown).

Surface Immunoglobulins and Rosette Formation With Sheep Erythrocytes

Cells carrying surface immunoglobulins were detected using a fluorescinated Fab', fragment of goat anti-human immunoglobulin (Cappel Lab. Inc. Dowington, Calif.), as described elsewhere. Rosette formation was investigated using sheep red cells pretreated with 2-aminoethylisothiouronium bromide hydrobromide (AET), and morphological identification of the E-rosetting cells was performed on smears.

Analysis of Tumor Cells With Rabbit Antisera

The preparation and specificity of rabbit antisera to human T cells has been described elsewhere. The malignant lymphoid cells were tested with the rabbit sera using a complement-dependent microcytotoxicity test, and the viability was assessed with Trypan blue exclusion.

Production and Characterization of Monoclonal Antibodies

Extensive characterizations of the cellular expressions of antigens defined by the monoclonal antibodies used has already been reported. Production and characterization of monoclonal antibodies anti-T3, anti-T4, anti-T8, anti-T6, and anti-T10 have been the subjects of previous reports. Monoclonal antibody la defined an HLA-D-related antigen, and monoclonal antibody B1 defined a unique B-cell antigen found on all peripheral B cells and specific to that lineage. Production and characterization of the J5 antibody to CALLA found on 70% of non-T-ALL cells has already been reported.

Flow microfluorometric analysis of these monoclonal antibodies with malignant cell populations was performed by indirect immunofluorescence with fluorescein-conjugated goat anti-mouse IgG (G-M FITC) (Meloy Laboratories, Springfield, Va.) using either a Cytoscan fluorograph FC200/4800A (Ortho Instruments, Westwood, Mass.) or a fluorescence activated cell sorter (Becton-Dickinson, Mountain View, Calif.) as previously described.

Monoclonal antibodies A50 and U4 were both obtained after immunization of Biozzi's high responder strain of mice with spleen cells from a patient with T-cell chronic lymphocytic leukemia. The analysis of malignant cells with A50 and U4 was performed using complement-dependent microcytotoxicity test. Briefly, A50 was shown to be restricted in reactivity among T cells to a subpopulation of thymocytes and all peripheral T cells. U4 was shown to react with all peripheral T cells and childhood thymocytes but was reactive with only a subpopulation of fetal thymocytes below the age of 28 wk.

Patterns of reactivity of the monoclonal antibodies used in the present study with human lymphoid cells are reported in Table 1.

RESULTS

Cell Surface Characteristics of T-Cell Lymphoblastic Lymphomas

Tumor populations from 21 patients with LL were shown to be of T-cell lineage as defined by their capacity to rosette with sheep erythrocytes and/or react with T-cell heteroantisera. As expected, these same populations lacked reactivity with antibodies to surface Ig, anti-Ia, and anti-B1 (results not shown). To define the nature of the malignant T-cell populations, further characterization was performed. As shown in Table 2, cells from patients 1–6 had a pattern of reactivity similar to stage I thymocytes, since they were almost exclusively reactive with anti-T10. Two patients, however, nos. 5 and 6, had a + / − reactivity with anti-T4 and anti-T8. In contrast, cells from
MALIGNANT T CELL SURFACE CHARACTERIZATION

Table 1. Reactivity of Monoclonal Antibodies With Human T and Non-T Lymphoid Cells

<table>
<thead>
<tr>
<th>Monoclonal Antibody</th>
<th>Percent of Cells Recognized</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Thymus</td>
<td>E PBL</td>
</tr>
<tr>
<td>Anti-T3*</td>
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<td>100</td>
</tr>
<tr>
<td>Anti-T4*</td>
<td>75</td>
<td>60</td>
</tr>
<tr>
<td>Anti-T8*</td>
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<td>0</td>
</tr>
<tr>
<td>Anti-T10*</td>
<td>95</td>
<td>5</td>
</tr>
<tr>
<td>J5*</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>A50†</td>
<td>30-60</td>
<td>70-90</td>
</tr>
<tr>
<td>U4†</td>
<td>90</td>
<td>85-95</td>
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</table>

*Percentage of positive cells using an indirect immunofluorescence method.  †Percentage of positive cells using a microcytotoxicity test.

patients 7–15 showed a general pattern of reactivity similar to stage II cortical thymocytes. Cells from these patients were strongly reactive with anti-T10, had almost all acquired the thymocyte-specific anti-T6, and/or coexpressed both T4 and T8 antigens. These patients, with one exception, had no reactivity with anti-T3, known to define a more mature thymic population as well as mature peripheral T cells. Thus, in general, these patients (nos. 7–15) appeared to coexpress T4/T8 and T6 and continued to express T10 antigen. Perhaps of greater interest was the unequivocal demonstration that cells from patients 13, 14, and 15 were reactive with J5 monoclonal antibody, which defines the non-T, non-B acute lymphoblastic leukemia antigen. Patients 16–21 showed an additional pattern not displayed by other patients, since virtually all of these tumors had acquired strong reactivity with anti-T3. Interestingly, cells from patients 16 and 17 expressed both T3 and T6 antigens, which are found in stage III and II thymocytes, respectively.

Although the above patterns of reactivity of the LL tumor cells appear to correlate generally with three major subpopulations of thymic lymphocytes, a proportion of patients had tumor cells with an unusual phenotype that did not conform with the cells

Table 2. Surface Antigens on Tumor Cells From Patients With Lymphoblastic Lymphoma

<table>
<thead>
<tr>
<th>Case No.</th>
<th>Source of Cells</th>
<th>Surface Antigens Defined by Monoclonal Antibodies</th>
<th>Anti-T1†</th>
<th>CALLA†</th>
<th>U4‡</th>
<th>A50‡</th>
<th>Rosettes (%)</th>
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<tr>
<td>1</td>
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<td>-</td>
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</tr>
<tr>
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</tr>
<tr>
<td>3</td>
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<td>NT</td>
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<tr>
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</tr>
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</tr>
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<td>13</td>
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<td>70</td>
</tr>
<tr>
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<td>17</td>
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<td>18</td>
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<tr>
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<td>-</td>
<td>-</td>
<td>NT</td>
<td>-</td>
<td>-</td>
<td>70</td>
</tr>
</tbody>
</table>

*LN, lymph nodes; PE, pleural effusion.
†Percentage of positive cells using an immunofluorescence method.  †Percentage of positive cells using a microcytotoxicity test.
†Percentage of positive cells using an indirect immunofluorescence method.  + = over 50% positive cells, ± = between 20% and 50% positive cells.  - = below 20% positive cells. NT = not tested.
normally detected in the thymus. Several examples of unusual antigen expression were seen in this patient population. For example, patients 16 and 17 displayed the mature T-cell antigen T3, but continued to coexpress T4, T6, and T8, as well as the common acute lymphatic leukemia antigen.

Further evidence for the heterogeneity of LL in terms of a cell surface phenotype is seen from the analysis of tumor cell populations with monoclonal antibodies U4 and A50. The antigen recognized by U4 is present on most mature T cells and thymus cells in children but is absent from fetal thymocytes until the age of 28 wk. In contrast, the antigen defined by A50 is present on all T cells in peripheral organs but lacking on 70% of thymocytes of both children and fetuses. As shown in Table 1, patients 7–21 reacted with either U4 or A50 or both, while in contrast patients in group 1–6 were reactive with neither of these antibodies. Tumor cells from two different sources, cervical nodes and pleural effusion, were investigated in these patients (nos. 7, 14, and 17) and displayed the very same phenotype.

Clinical features at the initial presentation of patients with LL are summarized in Table 3. As expected, a strong male predominance and the presence of a mediastinal mass were seen in all groups. A slight increase in the age of the patients was noted with the tumor populations that expressed a more mature phenotype, although these results were not statistically significant. Lymphadenopathy and any minimal bone marrow involvement was absent from patients 7–15 with common thymocyte phenotypes, in contrast with early or late thymic phenotypes. Continuation of this study will show whether differences in survival will appear in these groups. In contrast, hepatosplenomegaly was not a frequent finding in any age group. Finally, it is important to note that the convoluted morphological appearance of tumor cells themselves did not provide any insight into the phenotypic characterization of the tumor populations.

**Phenotypic Differences Between T Malignant Cells From Acute Leukemias and Lymphoblastic Lymphomas**

Previous investigation of 25 patients with T-cell ALL had shown that their tumor populations had the characteristics of either early or common thymocytes, except one patient who had cells with a late thymocyte phenotype. This is in sharp contrast with the findings from the present study with LL cells.

Further distinctions in immunologic phenotype between T-cell ALL and T-cell LL could be seen in our study. We were able to investigate 18 additional cases of T-cell ALL with both the anti-T series and monoclonal antibodies A50 and U4. Table 4 shows that among patients with tumor populations having the characteristics of common thymocytes, none of the 12 patients with ALL had cells recognized by A50, whereas 5 of 8 patients with LL had cells recognized by A50. Among patients with tumor populations of the early thymocyte type, only patients with ALL had cells recognized by U4. Lastly, in 4 patients with ALL, tumor cells from bone marrow and peripheral blood exhibited the very same phenotype (results not shown).

**DISCUSSION**

In this study, we have characterized the tumor cell populations from patients with LL using a panel of recently described monoclonal antibodies. The T-cell origin of these tumors was confirmed by their reactivity with sheep erythrocytes and/or specific T-cell heteroantisera as well as a lack of reactivity with anti-Ia antibodies and the anti-B1 monoclonal antibody. It was found that the phenotype of the LL could be related to the stages of intrathymic differentiation in which early thymocytes are recognized by anti-T10.
alone; common thymocytes are recognized by anti-T4, T6, T8, and T10, and late thymocytes by anti-T3, and T10, and either anti-T4 or T5/T8. In this report, 6 of 21 cases (29%) appeared the most analogous to the normal early thymocyte population, while 9 other cases (43%) were related to the common thymocyte populations, and 6 additional cases (29%) expressed late thymocyte antigens.

These findings should be contrasted with a previous study employing these same monoclonal antibodies to characterize tumor populations in 25 patients with T-cell acute lymphoblastic leukemia. In that study it was shown that T-ALL populations appeared to arise from early or common thymocytes, except in one case, which was related to late thymocytes. Utilization of two additional monoclonal antibodies, A50 and U4, has allowed us to observe further phenotypic differences between cells from ALL and LL by investigating another series of patients with T-cell ALL. A50 recognizes the majority of peripheral T cells and a subpopulation of thymocytes, as well as cells from B chronic lymphocytic leukemia. U4 recognizes an antigen present on virtually any T cell with the exception of fetal thymocytes below the age of 28 wk.

The distinctions in immunologic phenotype of T-cell acute lymphoblastic leukemia in comparison to LL, defined by monoclonal antibodies in the present study, are not surprising in the light of findings of earlier studies carried out with rabbit and horse antisera. Thus, use of antisera specific to thymic cells, and studies carried out with rabbit and horse antisera. Defined by monoclonal antibodies in the present study, has allowed us to observe further phenotypic differences between cells from ALL and LL by investigating another series of patients with T-cell ALL. A50 recognizes the majority of peripheral T cells and a subpopulation of thymocytes, as well as cells from B chronic lymphocytic leukemia. U4 recognizes an antigen present on virtually any T cell with the exception of fetal thymocytes below the age of 28 wk.

The above findings disclose a significant degree of phenotypic heterogeneity among the LL tumor populations themselves. A number of patients' tumor cells had a surface antigen distinct from the three major thymocyte populations. It is possible that the phenotype seen in these malignancies arises from normal counterparts present in low quantities within the thymus. In this regard, it is relevant to recall that the thymus is the site of intense destruction and that it has been postulated that less than 10% of thymocytes survive and are exported following intrathymic differentiation. Lastly, the malignant cells may be associated with alterations in the density or type of individual cell surface antigens. However, in many disorders of malignant T cells, including Sezary’s syndrome and chronic lymphocytic leukemia, the tumor populations maintain a cell surface expression similar to that of their normal counterparts.

It is clear from the present study that a considerable complexity of cell types exist even within the category of LL. Moreover, this heterogeneity is likely to be useful as we try to understand the nature of normal T-cell ontogeny and differentiation, and perhaps more important, the clinical variability seen within this disease entity. The development of additional immunologic techniques identifying further differentiation antigens found on normal T cells and expressed on certain tumors of T lineage will relate these malignancies to other normal T-cell counterparts. We believe that such an approach should result in a deeper understanding of the heterogeneity of T-cell lymphoblastic disease in humans.

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Cell surface characterization of malignant T cells from lymphoblastic lymphoma using monoclonal antibodies: evidence for phenotypic differences between malignant T cells from patients with acute lymphoblastic leukemia and lymphoblastic lymphoma

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