Cutaneous T Cell Lymphoma: Characterization by Monoclonal Antibodies

By Patrick C. Kung, Carole L. Berger, Gideon Goldstein, Paul LoGerfo, and Richard L. Edelson

Monoclonal antibodies to human T cells permit the characterization of the surface phenotype of cutaneous T cell lymphoma (CTCL). The majority of CTCL cells are reactive with OKT1 and OKT3 monoclonals, which identify peripheral T cells and mature thymocytes. The neoplastic cells also react with OKT4, which recognizes the inducer T cell subset; they are, however, unreactive with OKT5 monoclonal, which identifies cytotoxic/suppressor T cell subsets. These data are in agreement with previous functional studies demonstrating that CTCL is a neoplasm of inducer (helper) T cells.

Cutaneous T cell lymphoma (CTCL) is a neoplasm of T lymphocytes that preferentially infiltrate the skin and have an affinity for the T cell zone of lymph nodes and spleen. An evolution of the disease frequently occurs from chronic slowly progressive epidermotrophic forms (mycosis fungoides, Sezary syndrome) to a more aggressive nonepidermotrophic form. CTCL is probably more common than generally believed: a recent prospective study of leukemic blood and from involved lymph nodes ranged from 43 to 61 yr of age.

Utilizing techniques such as sheep red blood cell rosetting and immunofluorescent staining with rabbit antiserum to human T cells, it was established that CTCL is a disease of T lymphocytes. We now extend this analysis by using monospecific antibodies directed against surface differentiation antigens on T lineage cells. These antibodies permit definition of functional and developmental stages of normal T cells, and thus allow the comparison of the phenotype of CTCL cells with these stages.

In this communication, CTCL lymphocytes derived from leukemic blood and from involved lymph nodes were studied using previously described monospecific antibodies to T lineage cells. Most CTCL cells were reactive with OKT1, OKT3 and OKT4, but not with OKT5. This is the phenotype of inducer (helper) T cells and is consistent with previous functional studies suggesting that CTCL represents a neoplasm of inducer T cells.

MATERIALS AND METHODS

Source of Cells

 Portions of lymph nodes obtained for diagnostic purposes from CTCL patients, as well as from various benign conditions (appendicitis, gall bladder removal), were studied. Peripheral blood specimens were drawn from CTCL patients and normal donors who ranged from 43 to 61 yr of age.

Informed consent was obtained prior to specimen donation in accordance with the Columbia University guidelines on human investigation.

Leukocyte Isolation

 Portions of lymph nodes were finely minced with a scalpel in Hank's balanced salt solution (HBSS, Gibco, Grand Island, N.Y.) and passed through 40 μ sieves to obtain cell suspensions. Peripheral blood specimens were layered on Ficoll-Hypaque (Gallard-Schlesinger, Carle Place, N.Y.), and the interface containing the mononuclear leukocyte fraction was collected and washed twice in HBSS. Aliquots of the neoplastic cell suspensions were preserved for future testing by freezing at -70°C in 15% dimethyl sulfoxide/RPMI 1640-10% fetal calf serum (FCS, Gibco).

Rosette Procedures

In some cases, T cells were enumerated by neuraminidase treated sheep red blood cells (E) according to the method of Weiner et al. The presence of receptors (EAC + ) for the split product of the third component of complement (C3) on the surface of leukocytes was determined using the technique of Bianco et al.

Assay of T Helper Capacity

The lymphocyte culture method used to assay for pokeweed-induced polyclonal helper activity was based on the technique of Siegal and Siegal. Unseparated mononuclear leukocyte suspensions and T and B enriched fractions were cultured for 7 days with an equal volume of RPMI 1640/20% FCS or with pokeweed mitogen (10 μg/cc in the same medium). Co-cultures consisting of combinations of normal, T and B enriched fractions, or of T enriched fractions from the patients' neoplasms added to normal B cells were incubated under the same conditions. The cells were cultured at a 1:1 T to B cell ratio. All cultures were maintained in an incubator under a 5% CO2 atmosphere at 37°C.

After incubation, the cells were centrifuged (300 g for 10 min) and washed twice in phosphate-buffered saline (PBS, Gibco). The cells were returned to their initial volume in 3% bovine serum albumin/PBS, and cytocentrifuge (Shadon Southern Instruments, Sewickle, Penn.) slides were prepared for quantification of results by immunofluorescence.

The slides were fixed in 5% acetic acid/methanol at -20°C for 10 min, rinsed twice in PBS, and then incubated in a moist chamber with a 1:4 dilution of polyclonal fluorescein-conjugated goat antihuman immunoglobulin (anti-IgG, -IgM, and -IgA, Hyland Laborator-
ries, Costa Mesa, Calif.) for 30 min. After incubation, the slides were washed twice in PBS and examined by light and fluorescence microscopy with a Leitz Ortholux II microscope equipped with an epifluorescent illuminator (Leitz, Rockleigh, N.Y.). The number of brightly stained cells containing diffuse intracytoplasmic fluorescence present in 500 randomly encountered cells was determined.

Non-specific staining of nonviable cells by the fluorescein-conjugated reagent was ruled out by studies in which suspensions of nonviable frozen and thawed cells were incubated with fluorescein-conjugated goat antimouse immunoglobulin. In all instances, nonviable intact lymphocytes failed to stain with this labeled antihuman immunoglobulin.

Production and Reactivities of Antihuman T Cell and Other Monoclonal Antibodies

The production and reactivities of the monoclonal antibodies to human T cells were described previously; OKT1 and OKT3 are reactive with all peripheral T cells and mature thymocytes, whereas OKT4 reacts with the inducer (helper) T cells in peripheral blood.6,14-16 OKT5 reacts with peripheral T cells involved in cell-mediated cytotoxicity and suppression;17 OKT6 reacts with la-like molecules on peripheral B cells, monocytes, and a proportion of activated T cells;18 OKT8 reacts with a majority of immature thymocytes.19 OKT9 recognizes an antigen present on the surface of replicating T and B lymphocytes. The antigen is not expressed on most peripheral blood lymphocytes (~3%), but is present on 10% of the thymocytes.6 OKT10 is present on 96% of the thymocyte population, 5% of peripheral blood T-cells, 13% of the non-T cells,6,19 and on peripheral T cells stimulated by concanavalin A phytoshemagglutinin and allogeneic lymphocytes (unpublished data).

Cytofluorografic Analysis

Cytofluorograf analysis

Cytofluorograf analysis of mononuclear antibodies with all cell populations was performed by indirect immunofluorescence with fluorescein-conjugated goat antimouse IgG (G/M FITC, Meloy Laboratories, Springfield, Va.) utilizing a Cytocfluorograf FC200/4800A (Ortho Instruments, Raritan, N.J.). In brief, 1 x 10⁶ cells were treated with 0.1 ml of mononuclear antibody at 1:500 dilution, of ascites incubated at 4°C for 30 min, and washed twice. The cells were then reacted with 0.1 ml of 1:40 dilution G/M FITC at 4°C for 30 min, centrifuged, and washed three times. Cells were then analyzed on the Cytocfluorograf, and the intensity of fluorescence per cell and percentage of positively stained cells were recorded on a pulse height analyzer. Background staining was obtained by substituting a 0.1 ml aliquot of 1:500 ascites from a BALB/cj mouse injected intraperitoneally with a nonproducing hybridoma clone.

RESULTS

Phenotypic and Functional Characterization of CTCL Leukemic Cells

Peripheral blood mononuclear cells from three leukemic CTCL patients were studied with monoclonal antibodies. The percentage of T lymphocytes (OKT1, OKT3) was elevated in the mononuclear cell preparations in these patients. However, the number of T lymphocytes reactive with OKT3 was lower than the number of lymphocytes reactive with OKT1 in the 3 leukemic patients (Table 1). The value of OKT1 was used to determine total T lymphocyte counts. The calculations presented in Table 2 represent the absolute number of peripheral blood T lymphocytes reactive with OKT1 and OKT4 monoclonal antibodies. The total number of T lymphocytes and inducer T cells in the three leukemic patients was increased 6- to 200-fold (Table 2).

Peripheral T lymphocytes in two leukemic patients (M.B. and E.S., Table 1) were virtually all inducer T cells (OKT4⁺ Fig. 1). The third leukemic patient (E.M.) showed a normal percent of OKT4⁺ cells; however, when the percent was adjusted to reflect the absolute number of circulating inducer T cells present in the peripheral blood, the massive expansion of the inducer population could be appreciated (Table 2). The absence of OKT5⁺ cells in the peripheral blood of the leukemic patients (Fig. 1, Table 1) is a significant observation. Dissociation between phenotype and function was noted in one leukemic patient (E.M.) who had an expanded population of OKT4⁺ cells that were unable to mediate in vitro helper activity (Table 3).

The results of the helper activity study are presented in Table 3. Neoplastic T-lymphocytes isolated from 4 of the 5 patients were able to mediate
enhanced helper activity when added to control B-lymphocytes.

Monoclonal Antibody Reactivities of Lymph Node Cells

The cell surface phenotypes of mononuclear cells obtained from histopathologically diagnosed lymph nodes were determined (Table 4). In benign reactive nodes, the inducer/suppressor (IS) T cell ratio (OKT4⁺/OKT5⁺) ranged from 2.2 to 2.8. In histologically involved nodes, the OKT5⁺ cells were absent, a condition similar to the peripheral blood of leukemic CTCL patients (Table 1). OKT4⁺ cells in the malignant nodes were shown to exhibit varying degrees of helper activity, a trait usually associated with CTCL tumor cells (Table 3).

Studies performed on an aleukemic CTCL patient (C.O.) over a 20-mo period (Table 5) revealed a distinctive reproducible abnormality. Prior to treatment, her involved lymph node contained no OKT1 reactive cells. After therapy, fewer T cells were present in the lymph node and the majority still did not express OKT1 nor OKT5. The peripheral blood specimen from this aleukemic patient obtained at the same time as the lymph node biopsy, however, contained an OKT1 reactive population and OKT5⁺ cells.

One patient (E.S.) with longstanding leukemic CTCL demonstrated a characteristic CTCL phenotype when her peripheral blood leukocytes were studied (Fig. 1, Table 6). Unexpectedly, a totally different antigenic presentation was expressed on the mononuclear cells recovered from her new, rapidly enlarging skin tumors. Most of these cells were reactive with OKT9, OKT10, and EAC, but unreactive with OKT1, OKT3, OKT4, and OKT5 monoclonals.

Table 3. Helper Activity in Patients with Cutaneous T-cell Lymphoma

<table>
<thead>
<tr>
<th>Patient</th>
<th>Number Plasma Cells/500 Cells Counted</th>
</tr>
</thead>
<tbody>
<tr>
<td>M.B.</td>
<td>90 ± 1</td>
</tr>
<tr>
<td>E.M.</td>
<td>21.3 ± 11.4</td>
</tr>
<tr>
<td>J.W.</td>
<td>124.5 ± 10.5</td>
</tr>
<tr>
<td>E.O.</td>
<td>129.0 ± 18.6</td>
</tr>
<tr>
<td>C.O.</td>
<td>140.0</td>
</tr>
<tr>
<td>Controls (7 Subjects)</td>
<td>62.7 ± 22.7</td>
</tr>
</tbody>
</table>

*Represents the mean of 2 determinations with the exception of C.O. where only one determination was performed.
SD = standard deviation.

DISCUSSION

Monoclonal antibodies are valuable probes for defining the developmental and functional states of
Table 4. Monoclonal Antibody Reactivities of Lymph Node Cells

<table>
<thead>
<tr>
<th>Patient</th>
<th>Age, Disease</th>
<th>Histologic Diagnosis</th>
<th>In Vitro Helper Activity</th>
<th>Percentage of Mononuclear Cells Reactive With Monoclonals</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>OKT1</td>
</tr>
<tr>
<td>R.B.</td>
<td>43, Appendicitis</td>
<td>Benign reactive</td>
<td>ND</td>
<td>39</td>
</tr>
<tr>
<td>C.D.</td>
<td>18, Toxoplasmosis</td>
<td>Benign reactive</td>
<td>ND</td>
<td>56</td>
</tr>
<tr>
<td>L.V.</td>
<td>26, CTCL limited to skin</td>
<td>Benign reactive</td>
<td>ND</td>
<td>68</td>
</tr>
<tr>
<td>K.L.</td>
<td>20, CTCL limited to skin</td>
<td>Benign reactive</td>
<td>ND</td>
<td>64</td>
</tr>
<tr>
<td>J.W.</td>
<td>53, CTCL</td>
<td>Malignant involvement*</td>
<td>+</td>
<td>42</td>
</tr>
<tr>
<td>J.M.</td>
<td>70, CTCL</td>
<td>Malignant involvement</td>
<td>ND</td>
<td>86</td>
</tr>
<tr>
<td>E.O.</td>
<td>49, CTCL</td>
<td>Malignant involvement</td>
<td>+</td>
<td>99</td>
</tr>
<tr>
<td>C.O.</td>
<td>75, CTCL</td>
<td>Malignant involvement</td>
<td>+</td>
<td>0</td>
</tr>
</tbody>
</table>

*Malignant involvement: complete effacement of the lymph node by infiltrating neoplastic cells. This part of the study was limited to those individuals whose lymph nodes contained no histologic evidence of residual normal elements.
†Weak fluorescence.

Table 5. Sequential Phenotypic Studies on the Cells from CTCL Patient C.O.

<table>
<thead>
<tr>
<th>Age</th>
<th>Date</th>
<th>Tissue</th>
<th>OKT1</th>
<th>OKT3</th>
<th>OKT4</th>
<th>OKT5</th>
<th>OKT1</th>
<th>OKT5</th>
</tr>
</thead>
<tbody>
<tr>
<td>75</td>
<td>4/28/78*</td>
<td>Effaced lymph node</td>
<td>0</td>
<td>72</td>
<td>72</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>76</td>
<td>1/17/80†</td>
<td>Effaced lymph node</td>
<td>10</td>
<td>38</td>
<td>33</td>
<td>2</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>76</td>
<td>1/17/80</td>
<td>Aleukemic blood</td>
<td>83</td>
<td>89</td>
<td>45</td>
<td>50</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

*Specimen taken prior to therapy.
†Patient last received modified Price Hill chemotherapeutic regimen 2 mo prior to testing.

normal T lineage cells. Recent studies suggested that OKT10+ prothymocytes in bone marrow become OKT4+, OKT5+, OKT6+ and OKT8+ as they multiply and differentiate in the thymus. These immature thymocytes lose OKT6 reactivity, segregate into OKT4+, and OKT5+/OKT8+ subsets, and acquire OKT1 and OKT3 reactivities. The OKT1/OKT3 cells correspond to the mature thymocytes, and eventually give rise to the more functionally mature T cell subsets in the periphery.

Correlation of the cell surface phenotypes of various leukemias and lymphomas with differentiative stages of normal lymphocytes remains an area of much research interest. Surface and intracellular markers have been important tools for these investigations. Recently, surface phenotypes of T acute lymphoblastic leukemia (T-ALL) were determined by using anti-T cell monoclonals. The majority of T-ALL are reactive with OKT10, some being reactive with OKT4, OKT6, and OKT8. Most of the (T-ALL) leukemic cells are, however, unreactive with OKT3. These findings indicated that T-ALL is a disease of T lineage cells at early and functionally immature stages roughly corresponding to prothymocytes through early thymocytes.

The observation that CTCL cells from most of our patients react with OKT1 and OKT3 strongly suggests that these neoplasms represent more mature T lineage cells. Most of these OKT1+ and/or OKT3+ tumor cells also react with OKT4, and are unreactive with OKT5. This finding further indicates that CTCL cells are related to inducer (helper) T cells. Previous functional studies in CTCL supported in part in our present studies, have shown that: (1) CTCL cells often act as inducers of B cell differentiation in vitro and (2) the serum immunoglobulin level in CTCL patients is frequently elevated, suggesting in vivo effect of the tumor cells in inducing immunoglobulin secretion.

Table 6. Characterization of Peripheral Blood and Cutaneous Lesions of CTCL Patient E.S.

<table>
<thead>
<tr>
<th>Patient</th>
<th>Specimen</th>
<th>OKT1</th>
<th>OKT3</th>
<th>OKT4</th>
<th>OKT5</th>
<th>OKT6</th>
<th>OKT9</th>
<th>OKT10</th>
<th>OKT11</th>
<th>E+</th>
<th>EAC+</th>
</tr>
</thead>
<tbody>
<tr>
<td>E.S.</td>
<td>PB</td>
<td>94</td>
<td>86</td>
<td>99</td>
<td>0</td>
<td>0</td>
<td>3</td>
<td>0</td>
<td>0</td>
<td>80</td>
<td>14</td>
</tr>
<tr>
<td>E.S.</td>
<td>Skin</td>
<td>15</td>
<td>15</td>
<td>15</td>
<td>0</td>
<td>0</td>
<td>71</td>
<td>43</td>
<td>83</td>
<td>15</td>
<td>94</td>
</tr>
<tr>
<td>Controls</td>
<td>PB</td>
<td>70±7</td>
<td>74±7</td>
<td>45±7</td>
<td>28±7</td>
<td>3±1</td>
<td>4±2</td>
<td>17±5</td>
<td>12±4</td>
<td>63±8</td>
<td>15±5</td>
</tr>
<tr>
<td>n = 14</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
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<td></td>
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</tr>
</tbody>
</table>
Our phenotypic data supports the notion that CTCL is a neoplasm of inducer T cells.

It is noteworthy that one patient (E.M., Tables 1, 2, and 3), whose neoplastic cells were OKT3+ and OKT4+, was incapable of expressing inducer T cell activity in vitro, a finding that suggests that the helper phenotype can be dissociated from functional capacity in malignant T cells. In no case did neoplastic T cells from CTCL express an antigen reactive with OKT5, an antibody that specifically binds to cytotoxic/suppressor T cells. This correlates with our failure to demonstrate suppressor T cell activity in any of our patients with CTCL (unpublished data).

The presence of Ia-like antigens (Tables 1 and 4) on the surface of some of the neoplastic T-lymphocytes isolated from CTCL patients, probably reflects their derivation from normal T-lymphocytes which have been shown to express Ia-like antigen after stimulation. The possibility exists that the specific event of neoplastic transformation may have resulted in the expression of Ia-like antigens. It should be noted that Ia-like antigens have also been found on the leukemic cells from patients with non-T acute lymphoblastic leukemia.2,23

The absence of antigen reactive with OKT1 on the surface of OKT3+ malignant cells from one patient (C.O., Table 5) confirms an earlier report that OKT1 and OKT3 are different antigens. Since OKT1 and OKT3 are expressed on all peripheral blood T cells from normal donors, the isolated absence of OKT1 antigen on CTCL cells is apparently related to the evolution of the disease process. In addition, study of a CTCL patient (E.S., Table 6) in whom it was possible to simultaneously study leukemic T cells and newly developing cutaneous tumors was particularly instructive. The leukemic T cells were OKT1+, OKT3+, and OKT4+, whereas the cells infiltrating the cutaneous lesions were unreactive with these antibodies. Instead, the skin cells reacted with OKT1, OKT9, and OKT10 antibodies directed against antigens manifested on the surface of actively proliferating cells or immature T lineage cells. Furthermore, the cells infiltrating the skin in this CTCL patient differed from those in the peripheral blood in their failure to bind sheep erythrocytes but they did show C3 binding. The presence of a C3 receptor on thymocytes24 and some CTCL cells has been previously noted.25 Although we cannot exclude the possibility that the cutaneous tumors represented evolution of a second, independent lymphoma of T or B-cell origin, we favor the notion that the tumor cells are dedifferentiated malignant subclone(s) of the original tumor.

It should be noted that no tumor-specific antibody has yet been produced against CTCL cells. However, the data suggests that the absence of OKT5 reactivity and the multiple reactivities with OKT1, OKT3, and OKT4 of a population of T cells in the blood or nodes may be useful in the diagnosis of CTCL, although other causes of shifts in lymphocyte populations must be considered. Furthermore, those monoclonal antibodies would prove useful in monitoring the response of CTCL patients to treatment and may eventually be efficacious in the therapy of CTCL.

REFERENCES

Cutaneous T cell lymphoma: characterization by monoclonal antibodies

PC Kung, CL Berger, G Goldstein, P LoGerfo and RL Edelson