Surface Antigens on Malignant Sézary and T-CLL Cells Correspond to Those of Mature T Cells

By Laurence Boumsell, Alain Bernard, Ellis L. Reinherz, Lee M. Nadler, Jerôme Ritz, Hélène Coppin, Yolande Richard, Louis Dubertret, Françoise Valensi, Laurent Degos, Jean Lemerle, Georges Flandrin, Jean Dausset, and Stuart F. Schlossman

Tumor cells from eight adult patients with T-cell chronic malignancies were investigated with a series of monoclonal antibodies recognizing T-cell differentiation antigens. This series allowed definition of discrete subpopulations of mature T cells with functional specialization. All six patients with Sézary syndrome and one patient with T-chronic lymphocytic leukemia had cells with the same phenotype as normal helper/inducer T cells, whereas the other patient with T-chronic lymphocytic leukemia had cells with the same phenotype as normal cytotoxic/suppressor T cells. Some clinical manifestations observed in these patients may reflect retention of functional activities by their malignant cells.

CERTAIN STUDIES have shown that malignant cells in Sézary syndrome (SS) and rare cases of chronic lymphocytic leukemia (CLL) are T cells.1,1 In other studies it has been shown in a few cases that these cells have some functional properties of mature T cells.6-8

The expression of a particular cell phenotype by normal T cells is linked to their level of differentiation. This was shown by heteroantisera raised in rabbits and horses.9,12 The analysis of Sézary cells and T-CLL cells with these antisera have shown their antigenic phenotype to be indistinguishable from the phenotype of mature T cells.11,13,14 Recently, the development of monoclonal antibodies with specificity for T cells has permitted the definition of series of T-cell differentiation antigens15-17 and the recognition of mature T-cell subsets with a particular antigenic phenotype linked to a specialized function for which the cells are programmed.18-21

Here we report the analysis of the antigenic phenotype of the malignant cells in Sézary syndrome and T-CLL using these monoclonal antibodies. We show that the malignant cells from every patient have a phenotype identical to a given functionally specialized T-cell subset. Moreover, some of the clinical manifestations of disease in these patients may reflect retention by the malignant cells of a normal functional program.

MATERIALS AND METHODS

Isolation of Tumor Cells

Cells obtained from patients suffering from the following diseases were used in this study: T chronic lymphocytic leukemia (2 patients) and Sézary syndrome (6 patients). Malignant cells were obtained from blood specimens or from spleen specimen (in case 2 with T-CLL). Human mononuclear cells were isolated by Ficoll-Hypaque density centrifugation. Cell suspensions containing 80% or more malignant cells were analyzed. Tumor populations were cryopreserved in liquid nitrogen as described,22 until the time of surface characterization. With this method, cell viability was greater than 90% after thawing.

Cell Surface Markers

Surface immunoglobulins (SIg) and the E-rosette formation were assayed as described elsewhere.15,12

Analysis of Tumor Cells With Rabbit Antisera

Preparation and specificity of rabbit antisera to human T cells have been described elsewhere.15,12 The malignant lymphoid cells were tested with the rabbit sera using a complement-dependent microcytotoxicity test.

Production and Characterization of Monoclonal Antibodies

Production and characterization of monoclonal antibodies anti-T1, anti-T3, anti-T4, and anti-T8 were the subject of previous reports.15,20 In brief, these antibodies were shown to be restricted in their reactivity to normal cells, to cells of the T lineage. Anti-T1 and anti-T3 reacted with 100% of peripheral T cells and approximately 10% of thymocytes.15,18,19 The anti-T1 reactive (T,*) thymocyte population contained the functionally mature thymocyte population responsive to alloantigens in MLR. In contrast, anti-T4 and anti-T8 reacted with the majority of thymocytes and with 55% and 20% of the peripheral T cells. Functionally, anti-T4 defined the human inducer (helper) T-cell subset, while anti-T8 defined the human suppressor/cytotoxic population.15,20 Monoclonal antibody anti-T6 was shown to be restricted to common thymocytes, while monoclonal antibody anti-T9 was on early thymocytes.21 Monoclonal antibodies Ia and B1 were produced and characterized using previously described techniques following immunization of BALB/c mice with B-cell lymphoma.21 An extensive characterization of the cellular expression of antigens defined by these latter monoclonal antibodies will be the subject of a future report.21 Production and characterization of a monoclonal antibody recognizing an antigen...
on the cells from common acute lymphoblastic leukemia (CALLA) has already been reported. Cytofluorographic analysis of these aforementioned monoclonal antibodies with malignant cell populations was performed by indirect immunofluorescence with fluorescein-conjugated goat anti-mouse IgG (G/M FITC) (Meloy Laboratories) using either a cytofluorograph FC 200/4800 A (Ortho Instruments, Westwood, Mass.) or a fluorescence-activated cell sorter (FACS-I) (Becton-Dickinson, Mountain View, Calif.) as previously described. Production and characterization of monoclonal antibodies A50 and U4 have been the subject of previous reports. These monoclonal antibodies were both obtained after immunization of Biozzi's high responder strain of mice with the spleen cells from patient 2 with T-CLL. The analysis of malignant cells with A50 and U4 was performed using a complement-dependent microcytotoxicity test. Briefly, A50 was shown to be restricted in its reactivity toward all peripheral E-rosette-forming cells and a subpopulation of thymus cells, but it also reacted with the cells from almost one-half of the patients with T-CLL. U4 was shown to be restricted to all E cells, including child thymus cells, but was reactive only with one subpopulation of fetal thymus cells.

RESULTS

Clinical Features and Laboratory Findings in Patients With Chronic T-Cell Lymphoproliferative Disorders

Table 1 shows clinical data and Table 2 laboratory findings of the two patients with chronic T-cell lymphoproliferative disease.

Case 1 was a 47-yr-old woman with a past history of exposure to benzene. For the first 4 yr she had shown clinically asymptomatic CLL (case 1A in tables) diagnosed on a systematic survey of her blood that showed a lymphocytosis. A blastic transformation occurred in the fifth year with a rapid enlargement of lymphoid organs, while lymphosarcoma cells appeared in the bone marrow and blood. Subsequently, leukemic dissemination occurred in the skin and CNS (this second phase is referred to as case 1B in the tables). During the second phase of the disease, blood cells consisted of 95% lymphosarcoma cells. They did not respond to phytohemagglutinin (PHA) and pokeweed mitogen (PWM); yet they were shown to be T cells (E-rosette forming and β-glucuronidase positive cells). Response in mixed lymphocyte reaction (MLR) was deeply depressed, while stimulation was subnormal.

Case 2 was a 57-yr-old man. Splenomegaly was the main symptom of this T-cell CLL (Table 1). A monoclonal IgGλ was the main laboratory finding (Table 2).

Clinical Features and Laboratory Findings in Patients With Sézary Syndrome

Ages ranged from 58 to 73 yr in this group of patients. Two patients were female, and four were male. They all presented a typical clinical pattern: in each case, we observed an intense erythrodermia, with lymphadenopathy, but not hepato- or splenomegaly. Typical large Sézary cells and/or the small cell variant of Lutzner were observed in the blood, skin, and in lymph node biopsies whenever they were performed. Skin biopsies revealed a typical T-cell pattern. The levels of serum Ig were normal in four patients and elevated in two. However, no monoclonal Ig was detected in any of these six patients.

Table 1. Clinical Features of Two Adult Patients With T-Cell Lymphoproliferative Disease

<table>
<thead>
<tr>
<th>Patient</th>
<th>Age</th>
<th>Sex</th>
<th>Lymphnodes</th>
<th>Spleen</th>
<th>Liver</th>
<th>Skin</th>
<th>CNS</th>
<th>Infections</th>
<th>Treatment</th>
<th>Follow-up</th>
</tr>
</thead>
<tbody>
<tr>
<td>1A</td>
<td>47</td>
<td>F</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>Chlorambucil</td>
<td>4 yr</td>
</tr>
<tr>
<td>1B</td>
<td>51</td>
<td>F</td>
<td>Enlarged</td>
<td>Enlarged</td>
<td>—</td>
<td>—</td>
<td>+†</td>
<td>+* Viral, fungal</td>
<td>CHOP†</td>
<td>Died of infection</td>
</tr>
<tr>
<td>2</td>
<td>57</td>
<td>M</td>
<td>—</td>
<td>Enlarged</td>
<td>—§</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>Splenectomy</td>
<td>Alive in good status after 2 yr</td>
</tr>
</tbody>
</table>

*Meningeitis with leukemic cells.
†Leukemic infiltration of the dermis and hypodermis sparing the epidermis shown by skin biopsy.
‡Cyclophosphamide, Adriamycin, vincristin, prednisone.
§Leukemic infiltration in the liver biopsy.

Table 2. Laboratory Features of Two Adult Patients With T-Cell Lymphoproliferative Disease

<table>
<thead>
<tr>
<th>Patient</th>
<th>Hb (g/dl)</th>
<th>Platelets (x 10^5)</th>
<th>WBC (x 10^9)</th>
<th>Differential</th>
<th>Lymphocytoma</th>
<th>Lympho</th>
<th>MGG*</th>
<th>Bone Marrow Biopsy</th>
<th>Anti-</th>
<th>Ig</th>
<th>Monoclonal Ig</th>
</tr>
</thead>
<tbody>
<tr>
<td>1A</td>
<td>13.0</td>
<td>230</td>
<td>10</td>
<td>45</td>
<td>45</td>
<td>10</td>
<td>Small lymphocytes</td>
<td>Lymphosarcoma cells, cytoplasmic granules</td>
<td>NT</td>
<td>Nodular</td>
<td>—</td>
</tr>
<tr>
<td>1B</td>
<td>10.0</td>
<td>130</td>
<td>127</td>
<td>2</td>
<td>93</td>
<td>5</td>
<td>+</td>
<td>Massive invasion with myelobrosis</td>
<td>—</td>
<td>21.5</td>
<td>—</td>
</tr>
<tr>
<td>2</td>
<td>10.0</td>
<td>50</td>
<td>31</td>
<td>14</td>
<td>80</td>
<td>6</td>
<td>Massively invasion</td>
<td>—</td>
<td>9.9</td>
<td>IgG λ</td>
<td></td>
</tr>
</tbody>
</table>

*May-Grünwald-Giesma.
Phenotype of Tumor Cells

Table 3 shows that tumor cells from all 8 patients formed spontaneous rosettes with sheep erythrocytes (E) and lacked detectable surface immunoglobulins (SIg). These cells were initially analyzed with multiple rabbit antisera made specific for human cells of T lineage. All cells were reactive with an antithymocyte serum prepared with child thymocytes, but lacked reactivity to antithymocyte serum prepared with fetal thymocytes. In addition, the cells from 6 of the 8 patients were recognized by an antiserum raised against the Sézary cells from patient 4, in a C-dependent cytotoxicity test, while the tumor cells from the 2 remaining patients (6 and 7) could absorb the activity of this antiserum (results not shown).

We next investigated the tumor cells from these 8 patients with monoclonal antibodies. In each case, the tumor cells displayed the antigens defined by the monoclonal antibodies anti-Tl, anti-T3, A50, and U4, which are present on normal mature T cells. No patient had cells displaying the antigens defined by the monoclonal antibodies anti-T6 and anti-T9, which are restricted to immature cells within the T-cell lineage. Moreover, the cells from all patients were unreactive with the monoclonal antibodies CALLA and B1.

Of greatest interest was the finding that all patients with Sézary syndrome and patient 2 with T-CLL displayed T1, T3, and T4 antigens shared by the normal inducer T-cell population. In contrast, the lymphosarcoma cells from patient 1 displayed T1, T3, and T8 antigens, found on the normal cytotoxic-suppressor population. It should be noted that a small number of tumor cells from patient 2 expressed both Ia and T-cell antigens, while the percentage of Ia+ cells in the other patients could be accounted for by B lymphocytes or monocytes.

**DISCUSSION**

In this study, peripheral tumor cells in 8 adult patients with T-cell lymphoproliferative disorders were analyzed with monoclonal antibodies that defined multiple T-cell differentiation antigens. Previous studies indicated that these monoclonal antibodies were reactive with discrete subpopulations of thymic cells and functionally mature subsets of peripheral cells. It was shown that circulating peripheral cytotoxic/suppressor cells were T1+ T3+ T5+/T8-, whereas peripheral inducer cells were T1+ T3+ T4+.

The results we have obtained are clear-cut. Cells from all patients we tested had the phenotype of mature T cells: they lacked the antigens defined by the monoclonal antibodies anti-T6, anti-T9 that are characteristic of immature T cells, but they carried the antigens defined by the monoclonal antibodies anti-T1, anti-T3, and A50, which are restricted to mature T cells. Further, the malignant cells from all the patients with Sézary syndrome and one of the two patients with T-CLL had the phenotype of normal mature inducer T cells (i.e., T1+ T3+ T4+, T8-), while the malignant cells of the other patient with T-CLL had a phenotype analogous to the mature cytotoxic/suppressor T cells (i.e., T1+ T3+ T8-, T4+). It was of interest that one patient with T-CLL appeared to coexpress Ia and T-cell antigens in tumor population.

La antigens have been found on a limited subpopu-

---

**Table 3. Antigenic Phenotype of T Cells in Adult T-Cell Lymphoproliferative Disease**

<table>
<thead>
<tr>
<th>Patient</th>
<th>E</th>
<th>Slg</th>
<th>Thymus</th>
<th>Fetal Thymus</th>
<th>Sézary</th>
<th>Anti-T5</th>
<th>A50</th>
<th>U4</th>
<th>B1</th>
<th>CALLA</th>
<th>Ia**</th>
</tr>
</thead>
<tbody>
<tr>
<td>1B</td>
<td>62</td>
<td>&lt;1</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>89</td>
<td>97</td>
<td>1</td>
<td>0</td>
<td>86</td>
<td>0</td>
</tr>
<tr>
<td>2</td>
<td>80</td>
<td>&lt;5</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>72</td>
<td>67</td>
<td>66</td>
<td>4</td>
<td>4</td>
<td>5</td>
</tr>
<tr>
<td>3</td>
<td>67</td>
<td>&lt;5</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>61</td>
<td>60</td>
<td>56</td>
<td>3</td>
<td>11</td>
<td>6</td>
</tr>
<tr>
<td>4</td>
<td>56</td>
<td>&lt;5</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>NT</td>
<td>75</td>
<td>78</td>
<td>9</td>
<td>0</td>
<td>NT</td>
</tr>
<tr>
<td>5</td>
<td>85</td>
<td>&lt;5</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>81</td>
<td>84</td>
<td>65</td>
<td>0</td>
<td>4</td>
<td>0</td>
</tr>
<tr>
<td>6</td>
<td>94</td>
<td>&lt;1</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>82</td>
<td>59</td>
<td>78</td>
<td>3</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>7</td>
<td>67</td>
<td>&lt;1</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>79</td>
<td>86</td>
<td>72</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>8</td>
<td>75</td>
<td>&lt;1</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>84</td>
<td>72</td>
<td>91</td>
<td>7</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

*The range of the cytotoxic index of an E-PBL from normal individuals is 90-100 for the antithymus cell antiserum, 0 for the antifetal thymus cell antiserum, 70-90 for the anti-Sézary cell antiserum.
†The cells could absorb the activity of the antiserum raised against Sézary cells.
§Percentage of positive cells.
††Values represent the cytotoxic index. Ranges of values on E-PBL of normal individuals are, respectively, 70-90 for A50 and 85-95 for U4.
‖Means and standard error of the means of blood mononuclear cells of normal individuals are, respectively: T1:64% ± 5%; T3:67% ± 3%; T4:41% ± 2%; T6:0%; T8:22% ± 1%; T9 3% ± 2%; B1 9.1% ± 2%; CALLA 0%.
** As previously reported, no E-PBL cells are stained by anti-Ia in normal individuals.
tion of normal mature T cells after stimulation with mitogens or allogeneic cells in patients suffering from infectious mononucleosis, from graft-versus-host reaction, or rheumatoid arthritis.29-31 The presence of Ia antigens in detectable amounts on mature T cells could thus reflect a functional specialization32 and/or stage of activation in the process of immune response.

Our results are in agreement with previous observations that Sézary cells in most patients share features of helper T cells,29-32 whereas T-CLL tumor populations may be derived from either helper or cytotoxic/suppressor T cells.29 Moreover, clinical and biologic manifestations observed in our patients appeared to correlate with the functional specialization of their malignant cells, as indicated by their surface phenotypes. Although patient 1, whose cells were characteristic of the suppressor phenotype, had repeated infections and suppressed proliferative responses to mitogens and alloantigens, one cannot exclude the possibility that the failure to respond was a result of drugs and/or a large leukemic population. The phenotype of the cells from patient 2 were of greater interest, since this individual’s T-cell CLL was of the inducer phenotype and associated with monoclonal IgGA in the serum. It is possible that these tumor cells induced a restricted B-cell population to make immunoglobulins. Finally, it is of note that skin infiltration was observed not only in patients with the inducer phenotype in Sézary’s disease but also in patient 1 with T-cell CLL of the cytotoxic/suppressor phenotype. This finding would suggest that both subsets can migrate into the skin and that migration is not restricted singularly to the inducer phenotype.

We feel that an important conclusion from these observations is that surface antigens maintained on malignant populations generally reflect a normal state of differentiation defined by their cell surface phenotype. This observation is supported by earlier studies demonstrating phenotypic heterogeneity of malignant immature T cells observed in T-ALL17 and T-cell lymphoblastic lymphomas (in preparation). Moreover, recent studies have suggested that although normal antigens may be expressed, their density may be aberrant.33,34

It is thus possible to identify malignancies with discrete clinical and biologic features on their cell surface phenotype using monoclonal antibodies. This identification can be achieved quickly on a small number of cells and one might assume that this type of characterization will become accessible for large series of patients, permitting further dissection of clinical entities in conjunction with the availability of additional cell markers.

ACKNOWLEDGMENT

We thank Danièle Pham and Laurent Janniere for their contribution in preparing the monoclonal antibodies A50 and U4; and Vicky Hawken for typing the manuscript and helping us with our struggles with her native language.

REFERENCES

17. Reinherz EL, Kung PC, Goldstein G, Levey RH, Schlossman
BOUMSELL ET AL.


Surface antigens on malignant Sezary and T-CLL cells correspond to those of mature T cells

L Boumsell, A Bernard, EL Reinherz, LM Nadler, J Ritz, H Coppin, Y Richard, L Dubertret, F Valensi, L Degos, J Lemerle, G Flandrin, J Dausset and SF Schlossman