CONCISE REPORT

Surface Phenotype of Japanese Adult T-Cell Leukemia Cells Characterized by Monoclonal Antibodies

By Tosio Hattori, Takashi Uchiyama, Toshio Toibana, Kiyoshi Takatsuki, and Haruto Uchino

We studied the surface phenotype and the functional activities of leukemic cells from three patients with Japanese adult T-cell leukemia (ATL) using the panel of OK and anti-Tac monoclonal antibodies, which react with differentiation antigens and define functionally distinct T-cell subsets or activated and terminally differentiated T cells. The phenotype of ATL cells were determined to be OKT1 T3 T4 T10 T5 T8 OKIa1 - , although cells from two patients suppressed pokeweed mitogen (PWM) induced normal B-cell differentiation, and cells from all patients lacked helper activity in this system. In addition, after cultivation with PWM, ATL cells from all patients were reactive with anti-Tac monoclonal antibody, and cells from one patient were reactive with OKIa1. These findings suggest that ATL cells arise from peripheral mature T-cell subsets and also suggest that the transition of surface phenotype of ATL cells to functionally mature and activated T cells occurs in culture.

RECENT DEVELOPMENT in hybridoma technology has permitted the production of monoclonal antibodies,1 which can define functional and developmental states of normal T cells.2 3 Using these monoclonal antibodies, it has been reported that leukemic cells in Sézary syndrome, which are known to act as helper cells in pokeweed mitogen (PWM) induced normal B-cell differentiation system,4 arise from helper/inducer T-cell subsets.9 Japanese adult T-cell leukemia (ATL) is a unique T-cell leukemia and is apparently different from the typical Sézary syndrome. The clinical features of ATL consist of both visceral and skin infiltrations of leukemic cells, a subacute or chronic clinical course, and clustering of birth places of patients.10 ATL cells from some patients possess human B-lymphocyte antigens11 and are known to suppress PWM-induced normal B-cell differentiation11,12 and the proliferative response of normal T cells to alloantigens.13 In the present report, the surface phenotype of ATL cells characterized by the panel of OK2 3,14 and anti-Tac15,16 monoclonal antibodies and their functional activities were described.

MATERIALS AND METHODS

Patients

All patients were referred to our laboratory for surface marker analysis and functional studies.

Culture Conditions and Assay of T-Cell Function

Normal peripheral blood lymphocytes (PBL) and ATL cells were separated by Ficoll-sodium metrizoate density gradient centrifugation. The viability of cells were counted by the trypan blue dye exclusion test. PBL were then separated into T and B cells by a neuraminidase-treated sheep red blood cell rosette method.11 One million PBL, normal T cells, B cells, or ATL cells were cultured separately in the presence or absence of PWM (GIBCO, Grand Island, N.Y.) in RPMI 1640 medium (Nissui, Tokyo) supplemented with 100 U/ml of penicillin, 100 μg/ml of streptomycin, 2 mM L-glutamine, and 10% fetal calf serum (Flow Lab., Stanmore, N.S.W., lot 81203). The suppressor activity of ATL cells was examined by the addition of ATL cells (0.5, 1.2 x 10⁶) to 10⁵ PBL. The helper activity of ATL cells was examined by the addition of ATL cells (0.1, 0.5 x 10⁶) to 10⁶ purifed normal B cells. In all experiments, cell densities were adjusted to 10⁸/ml and the concentration of PWM was 10 μg/ml. Following 7 days of culture in a humid atmosphere at 37°C under 5% CO₂, cells were harvested and the viabilities and cell numbers were counted. The relative proportions of cytoplasmic-immunoglobulin (CIg) positive cells among the intact cells were counted by direct immunofluorescence methods, using fluorescein (FITC) conjugated rabbit F(ab')² anti-human Ig (Hoechst, Marburg) under a Zeiss ultraviolet microscope equipped with an HBO 50W super-pressure mercury lamp and FITC 450-490 nm interference primary filter. The presence of suppressor activity was determined as detailed elsewhere.12 The helper activity was evaluated by comparing the numbers of CIg-positive cells generated from the mixtures of normal T and B cells from the same donor and those from the mixtures of ATL and normal B cells at the same ratio. In addition, ATL cells were cultured for 4 or 7 days in the presence of PWM, and these cells were used for the analysis of surface phenotype.

Cell Surface Marker Analysis

The panel of OK monoclonal antibodies were obtained from Drs. Patrick Kung and Gideon Goldstein. The preparation and specificity of the panel of OK monoclonal antibodies have been amply documented.2 3 OKT1 and OKT3 react with mature thymocytes and all circulating T cells. OKT4 and OKT5/8 react with both thymocytes and with 60% and 30% of peripheral T cells, respectively. OKT6 reacts with a majority of immature thymocytes, whereas OKT10 reacts with all immature thymocytes, some peripheral T cells, and null cells. OKIa1 is known to be specific for human la-like antigens.14 Anti-Tac monoclonal antibody reacts with activated and functionally mature T cells, including concanavalin-A-induced suppressor T cells, PWM-activated radioresistant helper T cells.

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PWM-activated radiosensitive suppressor T cells, and cytotoxic killer cells against allogenic cells, but no reactivity was noted with normal resting T cells, B cells, and EB-virus-transformed B cells.\(^{15,16}\)

Cell surface antigens were detected by indirect immunofluorescence methods, using FITC-conjugated goat F(ab')\(^2\) anti-mouse IgG (Cappel Lab., Cochranville, Pa.) as a second antibody. As a control, normal mouse IgG separated by DEAE cellulose chromatography from pooled mouse sera was used. All reagents were diluted by phosphate-buffered saline (pH 7.6, 0.15 M), supplemented with 1 mg/ml of bovine albumin (BSA) (Armour Pharmaceutical Co., Phoenix, Ariz.) and 0.1% sodium azide. The optimal dose of each reagent was carefully assessed, using normal peripheral blood T cells, PWM-stimulated T cells, Molt 4F, EB-virus-transformed cell lines from a normal person. Half a million of ATL cells were treated with 20 \(\mu\)l of monoclonal antibodies (1:40 dilution for the panel of OKT\(^1\)T\(^3\)T\(^4\)), mouse IgG (25 g/ml, Elcm/l%) for 30 min on ice. Cells were then washed twice with Hanks' balanced salt solution (Nissui, Tokyo) containing 1 mg/ml of BSA and 0.1% sodium azide. After labeling with 20 \(\mu\)l of a second antibody (1:20 dilution) for 30 min on ice, the percentage of reactive cells in at least 200 lymphocytes was determined under phase and fluorescent illumination as described above.

### RESULTS AND DISCUSSION

Three patients showed typical clinical features of ATL and cells had deeply indented and lobulated nuclei (Table 1). The relative proportions of leukemic cells in cell suspensions were over 90% as determined by May-Giemsa smears of cytocentrifuge preparations. ATL cells from all patients reacted positively with OKT1,T3,T4,T10 monoclonal antibodies; no reactivity was noted with OKT5,T6,T8,OKI\(^a\)I\(^a\) monoclonal antibodies. Cells from two patients reacted only slightly with anti-Tac monoclonal antibody (Table 1).

### Table 1. Surface Phenotype and Functional Activities of ATL Cells

<table>
<thead>
<tr>
<th>Patient</th>
<th>Age (yr)</th>
<th>Sex</th>
<th>WBC (x 10(^3)/l)</th>
<th>Lobulated Cells (%)</th>
<th>OKT1 (%)</th>
<th>OKT3 (%)</th>
<th>OKT4 (%)</th>
<th>OKT5 (%)</th>
<th>OKT6 (%)</th>
<th>OKT8 (%)</th>
<th>OKT10 (%)</th>
<th>OKI(^a)I (%)</th>
<th>Tac (%)</th>
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<tbody>
<tr>
<td>K.M.</td>
<td>30</td>
<td>Female</td>
<td>31.9</td>
<td>45</td>
<td>86</td>
<td>88</td>
<td>78</td>
<td>1</td>
<td>6</td>
<td>14</td>
<td>63</td>
<td>3</td>
<td>15*</td>
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<tr>
<td></td>
<td>Before culture</td>
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<tr>
<td>S.T.</td>
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<td>Male</td>
<td>67.2</td>
<td>57</td>
<td>70</td>
<td>65</td>
<td>74</td>
<td>&lt;1</td>
<td>3</td>
<td>51</td>
<td>4</td>
<td>78</td>
<td>82</td>
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<tr>
<td>H.I.</td>
<td>32</td>
<td>Female</td>
<td>22.5</td>
<td>30</td>
<td>73</td>
<td>82</td>
<td>86</td>
<td>&lt;1</td>
<td>3</td>
<td>2</td>
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* indicates faint fluorescence.

In functional studies, ATL cells from three patients apparently lacked helper activity on allogeneic B cells in the presence of PWM. Cells from two patients had a profound suppressive effect on the generation of CIg positive cells from normal PBL. However, leukemic cells from patient S.T. did not have suppressive activity (Table 1). Experiments were repeated two or three times in each case, and the results were reproducible. The data described above strongly suggest that ATL cells arise from peripheral helper/inducer subsets (OKT\(^1\)T\(^3\)T\(^4\)), although cells from two patients acted as suppressor cells. Thomas et al. reported that interactions between OKT\(^{+}\) and OKT\(^{8+}\) cells were necessary to induce suppressor effector cells.\(^{11}\) We are now investigating if ATL cells interact with normal T or B cells to act as suppressor cells.

We also studied the surface phenotype of ATL cells cultured with PWM. In case S.T., cultured cells were analyzed on day 4, because the viabilities of cells were less than 50% following 7-day culture. Cultured cells stably reacted with OKT1,T3,T4,T10, no reactivity was noted with OKT6,T8. Therefore, no phenotypic changes to suppressor/cytotoxic subsets or dedifferentiation to immature subsets (OKT\(^{6+}\)) occurred. However, ATL cells from all patients were highly positive for Tac antigen after cultivation. Leukemic cells from two patients had Tac antigen before culture, however, a significant difference in fluorescent intensity prior to and after cultivation was seen. Ia-like antigens were expressed on the surface of ATL cells in only 1 case (H.I.) after cultivation (Table 1). These findings could not be ascribed to the proliferations of a small number of antigen positive cells, because \(^3\)H-thymidine uptake by cultured cells was low, and cell numbers did not increase after cultivation (data not shown). Thus, the suppressor activity of ATL cells in the PWM system and the acquisition of Tac antigens on ATL cells during culture could be simultaneously seen. These findings suggest that ATL cells have the capacity to differentiate in vitro both phenotypically and functionally.

### ACKNOWLEDGMENT

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