CO-EXPRESSIOX OF AN EPITOPE ON HUMAN FREE \( \kappa \)-LIGHT CHAINS AND ON A CYTOPLASMIC COMPONENT IN ACTIVATED T CELLS

KAREN Z. WALKER, GEORGINA E. HAYDEN, CHRISTOPHER C. GOODNOW, HEATHER A. BOUX, ELIZABETH ADAMS, ANTONY BASTEN, AND ROBERT L. RAISON

From the Clinical Immunology Research Centre, Sydney University, Sydney, NSW 2006, Australia

K-1-21 is a murine monoclonal antibody that reacts with human \( \kappa \)-light chains in free form but not when they are associated with immunoglobulin heavy chains. K-1-21 was unexpectedly shown to bind to a determinant, STA (Sezary T cell antigen), detected by immunofluorescence in the cytoplasm but not on the surface of Sezary T cells isolated from peripheral blood (4/4 cases) and in Sezary T cells from lymph node and bone marrow (one patient). STA was detected in F2/F7, CCFW-CEM, Molt-4, and CCRF-HSB (four human T ALL cell lines), in JURKAT (a human T cell leukemia line), and in MLA144 (a Gibbon T cell lymphoma line). It also occurred in Leu-3a+ antigen-specific T cell clones (6/6 tested). Moreover, although STA was absent from freshly isolated normal T cells, its expression could be evoked in \( E^+ \) cells from peripheral blood by in vitro culture with phytohemagglutinin. Thus, STA appears to be a cytoplasmic marker for activated T cells.

Cytoplasmic inhibition immunofluorescence studies indicated that K-1-21 binding to STA in Sezary cells or T cell lines was inhibited by preincubation of the K-1-21 antibody with purified \( \kappa \)-Bence Jones protein. STA from radiolabeled MLA144 cell lysates was immunoprecipitated by K-1-21 and was identified on polyacrylamide gel electrophoresis under reducing conditions as a protein of m.w. 57,000. Additional experiments are underway to define the molecular basis of the interesting cross-reactivity between a determinant in T cells and the K-1-21 reactive epitope on free \( \kappa \)-light chains.

K-1-21 is a murine IgG1 monoclonal antibody raised against human \( \kappa \)-Bence Jones proteins. It reacts with human \( \kappa \)-light chains in free form (as both monomers and dimers) but does not bind to \( \kappa \)-light chains associated with heavy chains (1). Immunofluorescence experiments revealed that K-1-21 recognizes a determinant, \( \kappa \)-myeloma antigen (KMA), which is present on the surface of \( \kappa \)-myeloma cells but is absent from normal B cells and malignant cells of non-\( \kappa \) myeloma origin (1). From clamping studies (2), KMA appears to be an epitope on free \( \kappa \)-light chains held in stable association on the myeloma cell membrane. Although KMA cannot be detected on the surface of normal B cells, reactivity with K-1-21 was seen in the cytoplasm of normal \( \kappa \)-synthesizing cells, presumably because of the free light chain pool (3, 4). By contrast, KMA was absent from the surface of all normal and malignant T cells examined. This paper reports the unexpected finding that K-1-21 reacts strongly with a determinant in the cytoplasm of T cells isolated from patients with Sezary syndrome. This malignancy forms part of the spectrum of cutaneous T cell lymphomas (5) and usually involves proliferation of cells of the helper/inducer phenotype OKT4+ (Leu-3+), OKT8+ (Leu-2+) (6). The K-1-21-reactive determinant in Sezary cells, unlike KMA on B cells, was not detected by cytoplasmic staining with AMD-\( \kappa \) (a monoclonal antibody that reacts with \( \kappa \)-chains) or with a goat anti-human-\( \kappa \) reagent, and has therefore been termed Sezary T cell antigen (STA). STA was also found in the cytoplasm of several T cell lines, antigen-specific T cell clones, and normal T cells stimulated to proliferate in vitro with phytohemagglutinin (PHA). Thus, the presence of STA would appear to be an indicator of T cell activation.

**MATERIALS AND METHODS**

*Preparation of cell suspensions.* Peripheral blood samples were obtained from four patients with Sezary syndrome. In addition, bone marrow aspirates and portions of lymph node were available from one of them. Normal peripheral blood came from healthy laboratory workers, whereas patients undergoing cardiac surgery provided a source of normal bone marrow. Single cell suspensions from bone marrow, lymph node, and peripheral blood were prepared in heparinized (10 IU/ml) RPMI 1640 medium (RPMI) (GIBCO, Grand Island, N.Y.), and mononuclear cell (MNC) fractions were then separated on Ficoll-Hypaque gradients (specific gravity 1.078). After washing three times in RPMI, a population of cells enriched for T lymphocytes was obtained by separation of E rosetting (E+) cells (7).

*Antigen-specific T cell clones.* Antigen-specific human T cell lines were made by following the method of Apte and co-workers (8). Human E+ cells from peripheral blood were incubated with antigen-pulsed presenting cells (APC), and the progeny were cloned at limiting dilution in 384-well plates (Sterllin, Teddington, U.K.). The six resulting T cell clones were of the Leu-3+ phenotype as determined by surface immunofluorescence, and had specificity for either influenza A (X31) or influenza B (HB) viruses. They were dependent for growth on the presence of interleukin 2 (IL-2), antigen and APC.

*Human cell lines.* LICR LON/HMY2 (HMY2), an IgG-secrating human lymphoblastoid cell line came from the Ludwig Institute for Cancer Research (LICR), London, whereas human T cell lines CCRF-HSB, F2/F7, MOLT-4, and CCRF-CEM were provided courtesy of LICR, Sydney. JURKAT, a T cell leukemia line, was obtained from Professor J. Watson, Medical School, Auckland University, New Zealand. MLA144, a T cell line established from a gibbon lymphosarcoma (9), was made available by Dr. C. G. Pathman, Department
of Immunology, Stanford Medical Center. All cell lines were grown in RPMI with added HEPES (4.76 g/liter), bicarbonate (8.5 g/liter), and antibiotics (penicillin 100 U/ml and streptomycin 100 μg/ml) and 10% fetal calf serum, whereas K-1-21 was preincubated with excess VOR ε-light chains (1) or F-1-1, an affinity-purified IgG1 monoclonal antibody of irrelevant antigenic specificity, served as negative control. The monoclonal antibody UCHT1 has a pan-T reactivity (11) (Ortho Diagnos- 

tics, Raritan, NJ). Fluorescein isothiocyanate (FITC)-conjugated reagents were used including FITC-goat F(ab')2 anti-human total Ig (GaHig) and FITC-goat F(ab')2 anti-human light chains (GaLHig) (both from Kallestad Laboratories, Austin, TX), and FITC-sheep F(ab')2 anti- 

mouse Ig (SaMlg) (New England Nuclear, Boston, MA).

Surface immunofluorescent staining. Cells for surface staining were suspended at 2 x 10⁶ cells/ml in cold RPMI (4°C) containing 0.02% NaN₃ (RPMI-NaN₃). Cell pellets in RPMI-NaN₃ and were exposed for 30 min at 4°C. after which the cell sediments were washed twice in cold RPMI-NaN₃. Stained cells were resuspended in 10% phosphate-buffered saline (PBS) in glycerol, pH 8.6, and were mounted for examination under a Zeiss photomicroscope II (Ober- 

kochen, West Germany). Cells stained with anti-Leu-3a were ana-lyzed for immunofluorescence by the UCHT1 method. In each case the trays were washed as before, and the supernatant was radiolabeled according to the method of Ober- 

kochen. Cells deposited onto filters (Titertek. Flow Laboratories, Irvine, U.K.) with 1 to 200 pg/ml PHA stimulates surface K-1-21 were pulsed for 12 hr with 53 U/ml glucose oxidase (Sigma) (20 pl). After iodination had been la-


dressed with a source of interleukin 2 (53 U/ml) in RPMI-NaN₃ and were washed three times in PBS-Tween, and once PES alone. Bound proteins were eluted by the addition of 100 µl 0.05 M triethylyamine (pH 11.6) to each well, and after incubation at room temperature for 30 min the eluates from control and test wells were separately pooled and lyophilized.

Polyacrylamide gel electrophoresis. Lyophilized samples were dissolved in 25 to 50 μl sample buffer (0.125 M Tris-Cl, pH 6.8, containing 10% glycerol, 3% sodium dodecyl sulfate (SDS), and 4 mg/ml dithiothreitol) and were boiled for three min. Electrophoresis was performed on a discontinuous, SDS-containing 5 to 15% poly- 

acrylamide gradient gel at 30 mA per gel slab at 15°C for 8 hr [14]. Gels were stained with Coomassie Brilliant Blue and were destained with 25 to 50 ml of destaining solution (40% methanol and 10% acetic acid) for 12 to 24 hr with Kodak X-ARS film (Kodak Wallac, Finland).

Cytoplasmic immunofluorescent staining. Cells deposited onto slides with a cytocentrifuge were fixed with absolute methanol at 4°C, and were stained with K-1-21 and FITC-SaMlg or with FITC-GaHig by using described methods (2). Slides were examined under the fluorescence microscope. Cells stained for cytoplasmic immu-

nfluorescence were also examined by flow cytometry. In this case, 10⁶ cells were washed three times in RPMI in conical 10 ml glass centrifuge tubes (Sibberbrand, West Germany). One milliliter of cold RPMI-NaN₃ was added to the sedimented cells with vigorous shaking over a period of one min, followed by a five min incubation at 4°C. The cells were then washed three times in RPMI-NaN₃ and were exposed for 30 min at 4°C to K-1-21 alone (40 μg in 200 μl RPMI-NaN₃) or to the same concentration of K-1-21 preincubated with 250 μg of purified VOR reactive with the epsilon heavy chain-associated human K-light chains, which has pan-T reactivity (1) (Ortho Diagnos- 

tics, Raritan, NJ). Fluorescein isothiocyanate (FITC)-conjugated reagents were used including FITC-goat F(ab')2 anti-human total Ig (GaHig) and FITC-goat F(ab')2 anti-human light chains (GaLHig) (both from Kallestad Laboratories, Austin, TX), and FITC-sheep F(ab')2 anti- 

mouse Ig (SaMlg) (New England Nuclear, Boston, MA).

RESULTS

Reactivity of K-1-21 with Sezary T cells. E+ cells from the peripheral blood of four patients with Sezary syndrome were shown to comprise 93 to 99% T cells (UCHT-1 positive), and many were of the helper/inducer phenotype (53 to 97% Leu-3a positive), whereas less than 2% displayed surface or cytoplasmic Ig (Table I). A more detailed analysis of E+ cells from patient CW with a panel of monoclonal antibodies and fluoresceinated reagents. K-1-21, and other monoclonal antibody reactions against human T-cell determinants and K-light chains, was derived from a fusion between NS-1 and spleen cells from mice immunized with human ε-Bence Jones proteins; its pro-

duction and purification have been described (1). K-1-21 was either used alone or after preincubation with MOS-ε-Bence Jones proteins (1) (Ortho Diagnos- 
tics, Raritan, NJ) with 10 to 100 pg/ml monoclonal antibody. After three washes in RPMI-NaN₃, the cells were stained with FITC-SaMlg and were washed again before analysis by flow cytometry as described above. Antigen-
specific inhibition of K-1-21 antibody with VOR (λ) or MOS (λ) Bence Jones proteins (1) was used to assess nonspecific binding of radio-
labeled proteins in this system. Control wells (usually 12 to 16 per experiment) coated with K-1-21 were incubated for 2 hr at 37°C with VOR (1 mg/ml), whereas MOS (1 mg/ml) was added to the test wells.

<table>
<thead>
<tr>
<th>Patients</th>
<th>CW</th>
<th>SH</th>
<th>EF</th>
<th>AE</th>
<th>Control</th>
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<tr>
<td>Cytoplasmic K-1-21</td>
<td>92</td>
<td>67</td>
<td>62</td>
<td>53</td>
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</tr>
<tr>
<td>F.1.1</td>
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<td>0</td>
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</tr>
<tr>
<td>GalHig</td>
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<td>2</td>
<td>0</td>
<td>12</td>
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<td>UCHT1</td>
<td>96</td>
<td>99</td>
<td>84</td>
<td>72</td>
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</tr>
<tr>
<td>Surface</td>
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<td></td>
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<td></td>
</tr>
<tr>
<td>F.1.1</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>GalHig</td>
<td>2</td>
<td>0</td>
<td>0</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>Leu-3a</td>
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<td></td>
<td></td>
<td></td>
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<td>UCHT1</td>
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<td>Leu-3a</td>
<td>96</td>
<td>97</td>
<td>53</td>
<td>62</td>
<td></td>
</tr>
</tbody>
</table>

*Cells were either stained directly with FITC-GaHig or -GalHig or indirectly with monoclonal antibodies, followed by FITC-SaMlg. They were immunoprecipitated for surface or cytoplasmic immunofluorescence by fluo-

rescence microscopy.

**Normal peripheral blood E+ cells.

| ND | not done |
of monoclonal antibodies revealed an absence of Leu-2 and OKM1 but low levels of HLA-DR, the presence of which is a feature of activated rather than resting T cells. Immunofluorescence studies of E+ cells from all four patients with K-1-21 indicated that between 53 and 92% demonstrated cytoplasmic staining, denoting the presence of STA, whereas surface staining was negative. Staining for STA was detected by microscopy as bright granular fluorescence distributed evenly throughout the cell cytoplasm. No equivalent reactivity was seen with a polyclonal FITC-GaHK. It is also of interest that the monoclonal antibody UCHT-1 produced cytoplasmic staining of comparable intensity with its surface staining. Cytoplasmic staining did not occur with Leu-3a.

Peripheral blood E+ cells obtained from one patient (CW) were examined on seven separate occasions over a 1-yr period and consistently yielded 89 to 92% STA-positive cells. By contrast, K-1-21 did not react in the cytoplasm or on the surface of E+ cells isolated from the peripheral blood of normal donors. Lymph node and bone marrow samples were also examined from patient CW: 27% of CW bone marrow MNC stained in the cytoplasm (but not on the surface) with K-1-21. Much of this reactivity was due to the expression of STA in T cells, because the bone marrow MNC were mainly of T cell origin (83% reacted with UCHT-1) and only 11 or 7% were surface Ig positive or B-reactive. In contrast, in a sample of normal bone marrow the proportion of cells reacting with K-1-21 in the cytoplasm (32%) corresponded closely to the proportion of cells expressing k-light chain (34%). CW lymph node also contained STA-positive cells, because 41% of separated E+ cells from this tissue showed cytoplasmic (but not surface) reactivity with K-1-21, although only 4% reacted with GaHK.

Reactivity of K-1-21 with PHA-stimulated but not resting normal T cells. The demonstration of STA in the cytoplasm of Sezary cells, which constitute an activated T cell population, but its absence in cells from peripheral blood or bone marrow of healthy donors raised the question whether the determinant was restricted to malignant T cells or whether it could be expressed in normal T cells after activation. To test this, two approaches were used. In the first, E+ cells isolated from peripheral blood of normal donors were stimulated in vitro with PHA and were examined by surface and cytoplasmic immunofluorescence for the expression of STA. Cells were fixed with 50% ethanol before staining, which enabled the analysis of cytoplasmic immunofluorescence by flow cytometry. Comparison with samples prepared for microscopy indicated that similar results were obtained by each method. In the E+ cells cultured with 100 μg/ml PHA, cytoplasmic staining with K-1-21 was detected between day 3 and day 7. The highest proportion of reactive cells (93%) was seen on day 5, which coincided with the peak of the proliferative response as measured by [3H]thymidine incorporation (Fig. 1). In contrast, no binding of K-1-21 to the surface of these cells was detected. The peak proliferative response as well as the peak reactivity to K-1-21 (93% of the cells being positive) occurred in cultures stimulated with 100 μg/ml of PHA. Nevertheless, a large proportion of STA-positive cells (85%) could still be detected in cultures containing as little as 1 μg/ml PHA. These results therefore confirmed that STA appears to be expressed in activated but not resting T cells from normal donors.

Reactivity of K-1-21 with antigen-specific T cell clones. The second approach to resolving whether STA was a marker for T cell activation involved the use of influenza virus-specific T cell Leu-3+ clones. Six lines were made, all of which were Leu-3+ and exhibited STA in T cell lines. The values refer to the percentages of cells reactive with K-1-21 minus control values for staining with anti-Leu-3a plus VOR. All cells failed to react with K-1-21 by surface immunofluorescence. All and B4 also failed to stain with a GaHK reagent.

Values refer to the percentages of cells showing surface staining with anti-Leu-3a and FITC-SaMlg.
several sources. The goal of these experiments was two-fold: first to test whether STA was restricted to a particular type of malignant cell and/or to T cells belonging to the helper/inducer (Leu-3+) phenotype, and secondly to obtain an adequate source of cells for the molecular characterization of STA. Seven cell lines (Table III) were selected for study and were examined by surface and cytoplasmic immunofluorescence for reactivity with K-1-21. Four human T ALL lines (F2/F7, CCRF-CEM, CCRF-HSB, and MOLT-4), JURKAT (a human T cell leukemia line), and MLA144 (a Gibbon T cell lymphoma line) exhibited cytoplasmic staining (21 to 70% of the cells were positive) but not surface staining with K-1-21. The λ-expressing B cell line U266 was STA negative. Only two of the six STA-positive lines (F2/F7 and CCRF-CEM) reacted with anti-Leu-3a antibody (Table III), indicating that STA is not inevitably associated with the surface expression of Leu-3. Fluorescence profiles obtained for E+, CW Sezary cells and two STA-positive T cell lines (MLA144 and CCRF-CEM) are illustrated in Figure 2A, B, and C, respectively. In each case, fluorescence intensity was greatly reduced when the cells were stained with K-1-21 preincubated with VOR-κ-Bence-Jones proteins compared with staining with K-1-21 alone. The intensity of fluorescence seen when K-1-21 bound to STA in these T cells was not as great as that observed when K-1-21 binds to KMA on κ-chains in the cytoplasm of the B lymphoblastoid cell line HMly2 (Fig. 2D).

To determine whether the presence of STA was the result of inappropriately expressed κ-chains or κ-chain fragments, cytoplasmic fluorescence in MOLT-4 was examined after staining with K-1-21 preincubated with VOR-κ- or MOS-λ-Bence-Jones proteins. As shown in Figure 3A, marked inhibition of fluorescence intensity occurred in the presence of κ- but not λ-Bence Jones proteins. By contrast, no staining was observed with AMD-kappa, a monoclonal antibody that reacts with both free and heavy chain-associated human κ-light chains (Fig. 3B). Each of the other five STA-positive T cell lines also failed to react with AMD-kappa monoclonal antibody. Taken together, these findings suggest that although STA bears an epitope cross-reactive with κ-light chain, it is not κ-light chain itself.

Identification of STA by immunoprecipitation. The relationship between the K-1-21-reactive determinant on

<table>
<thead>
<tr>
<th>Cell Source</th>
<th>Origin</th>
<th>Cytoplasmic staining K-1-21</th>
<th>Surface staining Anti-Leu-3a</th>
</tr>
</thead>
<tbody>
<tr>
<td>CCRF-HSB</td>
<td>T ALL</td>
<td>21%</td>
<td>1%</td>
</tr>
<tr>
<td>F2/F7</td>
<td>T ALL</td>
<td>55%</td>
<td>93%</td>
</tr>
<tr>
<td>MOLT-4</td>
<td>T ALL</td>
<td>44%</td>
<td>1%</td>
</tr>
<tr>
<td>JURKAT</td>
<td>T cell leukemia</td>
<td>26%</td>
<td>5%</td>
</tr>
<tr>
<td>CCRF-CEM</td>
<td>T pseudodiploid ALL</td>
<td>58%</td>
<td>87%</td>
</tr>
<tr>
<td>MLA144</td>
<td>Gibbon T cell lymphoma</td>
<td>76%</td>
<td>1%</td>
</tr>
<tr>
<td>U266</td>
<td>λ-B cell line</td>
<td>0%</td>
<td>ND</td>
</tr>
</tbody>
</table>

*Cytoplasmic and surface staining was assessed by FACS analysis.

*Cells were stained with K-1-21 or K-1-21 plus VOR as in Table II. The values refer to the percentages of cells reactive with K-1-21 minus control values staining with K-1-21 plus VOR. All cells failed to react with K-1-21 by surface immunofluorescence. All lines were examined for cytoplasmic staining with AMD-kappa but failed to show reactivity.

*Percentage of cells showing surface staining with anti-Leu-3a and FITC-SaMig.

*Not done.

Figure 2. K-1-21 binding to STA in the cytoplasm of CW E+ Sezary cells from peripheral blood (A) or in continuous T cell lines MLA144 (B) or CCRF-CEM (C). Cells were stained either with K-1-21 alone or with K-1-21 preincubated with excess κ-Bence Jones proteins, and then with FITC-SaMig, before examination for cytoplasmic immunofluorescence by flow cytometry. For each analysis, 10,000 cells were counted, and fluorescence was read on a linear scale. The open histograms depict the binding of K-1-21, whereas the shaded histograms show the inhibition of K-1-21 binding after preincubation with excess VOR-κ-light chain. K-1-21 binding to STA is less than K-1-21 binding to κ-chains in the cytoplasm of the IgGk-secreting lymphoblastoid cell line HMly2 (D).

Figure 3. STA in the cytoplasm of MOLT-4 cells. Cells were stained with K-1-21 preincubated with either MOS-κ-light chains (A, open histogram) or VOR-κ-light chains (A and B, shaded histograms). Staining is compared with that obtained with AMD-kappa monoclonal antibody (B, clear histogram). After incubation with a second antibody, FITC-SaMig, cells were assessed for cytoplasmic immunofluorescence by FACS analysis. For each analysis, 10,000 cells were counted, and fluorescence was read on a linear scale.
free \(\kappa\)-light chains and cytoplasmic STA was examined by analysis of precipitates from MLA144 cells and unstimulated peripheral blood \(E^+\) cells that are STA negative (Table I). Lysates of STA-positive or -negative cells were labeled by the lactoperoxidase technique with \(^{125}\)I, and immunoprecipitation was carried out by using K-1-21 bound to PVC microtiter wells in the presence of either \(\kappa\)-(control) or \(\lambda\)-(test) light chains. Electrophoresis of the immunoprecipitates (Fig. 4) showed that under reducing conditions the immunoprecipitate from MLA 144 formed in the presence of \(\lambda\)-light chains contained a major component of 57,000 and minor components of 49,000 and 45,000. The two lighter components were also present in the immunoprecipitate formed in the presence of \(\kappa\)-light chains, whereas the 57,000 component was absent, thus identifying it as a protein specifically precipitated by K-1-21 from MLA144 cytoplasm. Bands from nonreduced immunoprecipitates were of high m.w. (>200,000) and did not enter the gel. Biosynthetic labeling of MLA144 cells with \(^{14}\)Cleucine before immunoprecipitation and electrophoresis of reduced samples revealed a similar 57,000 band. This 57,000 band was not detected in immunoprecipitates from unstimulated peripheral blood \(E^+\) cells or the U266 \(\lambda\)-myeloma cell line, which are STA negative.

**DISCUSSION**

K-1-21 is a murine monoclonal antibody that reacts with human free \(\kappa\)-light chains and with an antigen, KMA, selectively expressed on the surface of \(\kappa\)-myeloma cells (1). The findings described in this paper demonstrate that K-1-21 also reacts with a determinant, STA, present in the cytoplasm but not on the surface of Sezary T cells (Table I).

Sezary syndrome forms part of the spectrum of cutaneous T cell lymphoma and is characterized by the presence of circulating malignant T cells with distinctive cerebriform nuclei (5). Phenotypic characterization of Sezary cells with monoclonal antibodies (6, 15) has shown that the cells from the great majority of patients are negative for human thymocyte antigen (OKT6), positive for pan T cell antigens (OKT1 and OKT3), positive for helper/inducer T cell subset (OKT4 and Leu-3) antigens, and negative for cytotoxic/suppressor T cell subset (OKT8 and Leu-2) antigens. Most Sezary cells also characteristically fail to react with 3A1, a monoclonal antibody that defines an antigen found on thymocytes and on OKT8+ T cells, but present on only a subpopulation of OKT4+ T cells (16). Their derivation from a well-differentiated helper/inducer T cell subset receives additional support from the observation that they can provide help for in vitro polyclonal B cell responses (17). The STA-positive Sezary cells from patients described here all expressed Leu-3 and belonged to the helper/inducer subset. The finding of some HLA-DR on their surface was indicative of their activated state.

The question therefore arose whether STA is restricted to malignant cells of this type or has a wide distribution in other T cell lymphomas or in normal T cells, although freshly isolated \(E^+\) cells from normal donors proved to be STA negative (Table I). Additional investigation showed that STA was expressed in six antigen-specific T cell clones (Table II), in six continuous tumour T cell lines (Table III and Figs. 2 and 3), and in normal \(E^+\) cells from peripheral blood after in vitro stimulation with PHA (Fig. 1). Thus, STA appears to be a marker for activated (proliferating) but not resting T cells, as well as being present in the cytoplasm of a range of malignant T cell lines. In view of these findings, STA is of great potential interest from the point of view of T cell function.

Only two of the six STA-positive T cell lines (F2/F7 and CCRF-CEM) were Leu-3a positive (Table III) (18), whereas only two (MLA144 and JURKAT) have been shown to be constitutive IL 2 producers (12, 19, 20). Thus, although STA is a marker for T cell activation, its presence does not appear to correlate with the expression of Leu-3 or with the capacity to secrete IL 2.

The capacity of K-1-21 to recognize a determinant on free \(\kappa\)-light chains pointed to the possible expression of an Ig fragment in the cytoplasm of activated T cells. Apparently consistent with this interpretation were the cytoplasmic inhibition studies showing a marked reduction in binding of K-1-21 to STA when the antibody was preincubated with excess free \(\kappa\)-Bence Jones proteins (Figs. 2 and 3). However, STA-positive cells did not bind a polyvalent GaHK reagent or another monoclonal anti-\(\alpha\) reagent (AMD-kappa) (Tables II and III and Fig. 3). In view of this, STA is unlikely to form part of a \(\kappa\)-chain. STA from radiolabeled MLA144 cell lysates was immunoprecipitated by K-1-21 and was identified on polyacrylamide gel electrophoresis under reducing conditions as a protein of m.w. 57,000 (Fig. 4). The interesting question is whether the observed cross-reactivity between this mol-
ecule and free $\kappa$-chains is merely a chance occurrence or whether it signals some degree of sequence homology and a possible evolutionary relationship between the molecules involved. In a discussion of other cases of unexpected serohomology observed with monoclonal antibodies, the latter explanation was favored (21).

Recent evidence from the cloning and sequencing of cDNA from mouse and human cells suggests that both chains of the T cell receptor exhibit significant homology with Ig domains, particularly with respect to the positioning of intra-chain disulfide bonds (22–25). These findings have prompted the suggestion that the T cell receptor should be included in the so-called Ig "super-family" along with Ig class I and class II major histocompatibility antigens, the poly-lg receptor, and Thy-1 (26, 27). If the cross-reactivity between STA and free $\kappa$-light chain is based on structural homology, then STA may prove to be an additional member of this "super-family". In this context, it is of interest to note that the K-1-21-defined epitope of $\kappa$-chains is dependent on the integrity of intra-chain disulfide bonds and resides in the constant region of the $\kappa$-chain (1) (R. L. Raison and H. A. Boux, manuscript in preparation).

Finally, the failure to identify STA on the surface of human T cells with K-1-21 deserves comment. One explanation is that STA is associated with another polyepitope chain on the cell membrane that makes the K-1-21-reactive epitope unavailable for antibody binding. Consistent with this possibility is the previously reported failure of K-1-21 to bind to $\kappa$-light chains when they form part of an intact Ig molecule (1). Alternatively, STA, like other receptor-associated molecules, may be present at such low density on the surface of isolated T cells as to be unetectable by conventional labeling techniques such as immunofluorescence. This possibility is currently being tested by blocking and capping studies with K-1-21 by using antigen-specific T cell clones.

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REFERENCES