Brief Definitive Report

EXPRESSION OF THE THYMUS LEUKEMIA ANTIGEN BY ACTIVATED PERIPHERAL T LYMPHOCYTES*

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The thymus leukemia (TL) alloantigens are encoded by the *Tla* locus, located 1.5 centimorgans telomeric to *H-2D* on murine chromosome 17 (1). Six alleles have been defined (Tla^{a-f}) using alloantisera and monoclonal (mc) antibodies (1, 2). TL is a glycoprotein of ~45,000 mol wt (45 K) expressed at the cell surface in noncovalent association with β-2 microglobulin. Biochemical and DNA hybridization studies have demonstrated that TL is structurally homologous to other class I transplantation antigens (3–5).

TL has been defined as a differentiation antigen because it is expressed by thymocytes and some leukemias, but not by normal peripheral T lymphocytes (1). In this report we demonstrate that splenic T lymphocytes, activated by alloantigen or concanavalin A (Con A), express a TL antigen that is indistinguishable from that expressed by thymocytes, and a leukemia line as assessed by two-dimensional (2-D) gel analysis.

Materials and Methods

Mice. A/J and C57BL/6 (B6) mice were purchased from The Jackson Laboratory, Bar Harbor, ME. BALB/cCrgl mice were purchased from the Department of Cell Biology, Baylor College of Medicine. All other strains were from our breeding colony at Baylor College of Medicine.

Antisera. The anti-TL.m3 antibody[1] used in this study was prepared by ammonium sulfate fractionation of spent culture medium from the 18/20 hybridoma line (6). Normal mouse serum (NMS) and polyvalent goat anti-mouse Ig (GAMIg) were purchased from Pel-Freez Biologicals, Rogers, AR.

Cell Culture. Spleen cells (4×10^6/ml) were activated with Con A (2.5 μg/ml) in supplemented Mishell-Dutton medium (S-MDM) (7). After 24 h, viable cells were isolated by density gradient centrifugation and resuspended at 1×10^6/ml in S-MDM containing added interleukin 2 (IL-2). Exogenous IL-2 was from either a 24-h Con A-stimulated spleen cell supernatant (CAS) (final concentration 25%) or phorbol myristate acetate-stimulated EL4 cell supernatant that had been concentrated 20× by ammonium sulfate fractionation (final concentration 0.5%) (8). Con A-activated cells were passaged by a 1:2 to 1:3 dilution in the above medium every 24 h. For experiments examining the time course of appearance of TL, spleen cells were depleted of Ig-positive lymphocytes by panning on affinity-purified GAMIg-coated petri dishes (9) before activation with Con A.

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[1] The designation TL.m3 refers to the determinant recognized by the monoclonal antibody derived from the 18/20 cell line (6). This determinant is not necessarily the same as the TL.3 determinant defined by alloantisera.

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Radiolabeling and Immunoprecipitation. Cells were radioiodinated by the lactoperoxidase-catalyzed procedure, and detergent lysates prepared as described (10). Aliquots of the precleared lysates were then incubated with anti-TL.m3 or NMS and the complexes precipitated with GAMIg.

2-D Gel Electrophoresis. Immunoprecipitates were analyzed by 2-D gel electrophoresis under reducing conditions using a pH 5–7 isoelectric focusing gel in the first dimension and a 12.5% sodium dodecyl sulfate polyacrylamide gel in the second dimension (11). Dried gels were exposed at −80°C to Kodak XAR-5 film in the presence of intensifying screens. Proteins used as molecular weight markers were bovine serum albumin (68 K), ovalbumin (45 K), and carbonic anhydrase (30 K).

Flow Cytofluorometric Analysis. Viable cells were isolated by density gradient centrifugation, washed, and resuspended in phosphate-buffered saline containing 0.1% sodium azide (PBS-azide). 10^6 cells were incubated with a saturating amount of TL.m3 (or medium as control), washed, and then incubated with a saturating amount of fluorescein isothiocyanate (FITC)-conjugated polyvalent GAMIg. Stained cells were fixed in PBS containing 1% paraformaldehyde, and 10,000 cells were analyzed for fluorescence using an Epics V flow cytometer (Coulter Electronics Inc., Hialeah, FL).

Results

Time Course of Appearance of TL on Activated Peripheral T Cells. A/J splenocytes, depleted of Ig^+ cells by panning, were activated with Con A and maintained in culture for 6 d; exogenous IL-2 was added to the cultures after 24 h to help maintain proliferation and cell viability. At 24-h intervals, viable cells were isolated and examined for the expression of TL by flow cytometry (Fig. 1). TL was not detected on unactivated splenic T cells (0 h) and was minimally expressed by 24-h Con A blasts. By 48 h and at all subsequent times analyzed, TL was detected on a significant proportion (~40–60%) of the cells. We have also detected TL on 5-d mixed leukocyte cultures maintained in the absence of exogenous IL-2 and on cloned long term T cell lines (see below). Thus the expression of TL is a feature of both alloantigen- and Con A-activated T cells.

Strain Distribution Analysis of TL Expression by Con A-activated T Cells. To examine if the determinants detected by the mc anti-TL.m3 on activated peripheral T cells are encoded within the *Tla* region, we analyzed 6-d Con A blasts from several strains, including the *Tla* congenic pairs, A/J, A-*Tla*^{b}, B6, and B6-

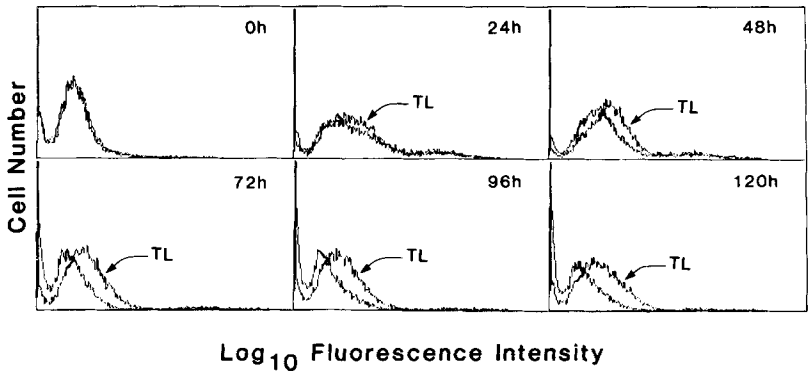

FIGURE 1. Time course of appearance of TL on activated T cells. Fluorescence histograms of normal (0 h) and Con A-activated (24–120 h) A/J T lymphocytes stained with mc anti-TL.m3. The profiles of staining with anti-TL.m3 plus FITC-GAMIg (denoted TL) are compared with staining with FITC-GAMIg alone.

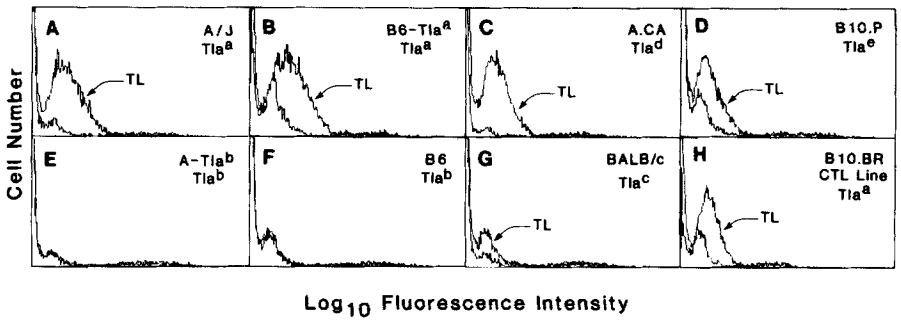

FIGURE 2. Strain distribution analysis of TL expression on activated T cells. Fluorescence histograms of 6-d Con A-activated splenocytes from various strains (*A–G*) and a long-term B10.BR CTL line (*H*) stained with mc anti-TL.m3. The profiles of staining with anti-TL.m3 plus FITC-GAMIg (denoted TL) are compared with staining with FITC-GAMIg alone.

Tla^a. As shown in Fig. 2, both A/J and B6-*Tla*^{a} Con A blasts stained for TL.m3; cells from the TL$^-$ (*Tla*^{b}) congenic partners, A-*Tla*^{b} and B6, were negative. Con A blasts from other TL$^+$ strains, BALB/c (*Tla*^{c}), A.CA (*Tla*^{d}), and B10.P (*Tla*^{e}) also expressed the TL.m3 determinant.[2] We have examined four B10.BR (*Tla*^{a})-derived long-term cytotoxic T lymphocyte (CTL) lines for the expression of TL and each was positive; a representative fluorescence profile of one line is shown in Fig. 2 *H*. These data demonstrate that the determinants detected with anti-TL.m3 on activated peripheral T cells are encoded by the *Tla* region.

2-D Gel Analysis of TL Immunoprecipitated From a Leukemia Cell Line, Thymocytes, and Con A-activated Peripheral T Cells. Fresh B6-*Tla*^{a} thymocytes, ASL-1 leukemia cells (1), and 6-d Con A blasts from B6-*Tla*^{a} and B6 were radioiodinated; aliquots of the cell lysates were immunoprecipitated with anti-TL.m3 or NMS and analyzed on 2-D gels (Fig. 3). Two sets of proteins of ~47 K and ~39 K mol wt were specifically precipitated with anti-TL.m3 from ASL-1, B6-*Tla*^{a} thymocytes, and Con A blasts, but not from the control B6 Con A blasts. Neither series of proteins represents the Qa-1 antigen as determined by 2-D gel analysis of anti-Qa-1 precipitates from normal splenocytes (unpublished observations). Although the relationship between the 47 K and 39 K mol wt forms of TL is not clear, both have been reproducibly isolated from *Tla*^{a} cells with the anti-TL.m3 antibody and with alloantisera. Based on both the size and charge, the TL molecules isolated from Con A blasts are identical to those isolated from thymocytes and the ASL-1 leukemia cell line.

Discussion

The *Qa-Tla* region encodes a series of cell surface antigens restricted in expression to cells of the hematopoietic lineage (1). While the Qa-1-6 antigens are expressed on subpopulations of peripheral T cells, the TL antigen has generally been regarded as a cell surface antigen restricted in expression to

[2] The BALB/c strain (*Tla*^{c}) was initially described as negative for the TL.m3 determinant as assessed by complement-mediated cytotoxicity (6). We have found that BALB/cCrgl thymocytes and Con A blasts are weakly positive for TL.m3, using more sensitive flow cytometric analysis. Alternatively, there may be differences among BALB/c sublines in the expression of TL specificities as has been reported for the expression of the Qa-2 and Qa-4 determinants (1).

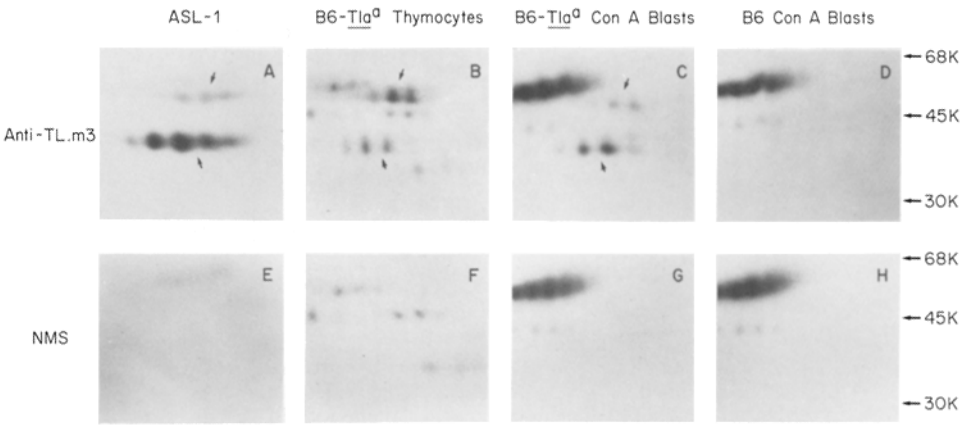

FIGURE 3. Two-dimensional gel analysis of the TL alloantigen. ASL-1 cells, B6-*Tla*^{a} thymocytes, 6-d B6-*Tla*^{a} Con A blasts, and 6-d B6 Con A blasts were radioiodinated and immunoprecipitated with mc anti-TL.m3 (*A–D*) or NMS (*E–H*). The basic end of the gel is at the left and the acidic end is at the right. Equivalent portions of each autoradiogram are presented. Downward and upward slanting arrows point to the ~47 K and ~39 K mol wt forms of TL, respectively. Radioactive CPM loaded on each gel and exposure times were as follows: *A* (350,000; 10 h), *B* (89,000; 84 h), *C* (99,000; 48 h), *D* (83,000; 48 h), *E* (112,000; 10 h), *F* (55,000; 84 h); *G* (96,000; 48 h), *H* (115,000; 48 h).

thymocytes (1). Using the sensitive techniques of flow cytometry and 2-D gel analysis, we have demonstrated that activated peripheral T lymphocytes from normal mice also express the TL alloantigen.

The TL antigen expressed by Con A-activated B6-*Tla*^{a} splenocytes appears identical to that expressed by B6-*Tla*^{a} thymocytes and the leukemia line ASL-1, based on migration in 2-D gel electrophoresis. Both the mc anti-TL.m3 and conventional anti-TL alloantisera (not shown) specifically precipitated two sets of cell surface iodinated proteins of ~47 K and ~39 K mol wt from all three cell sources. Previous studies by others (12, 13) have detected only one cell surface species (45–48 K range) precipitated by anti-TL mc antibodies and alloantisera. The ~39 K mol wt form probably does not represent cytoplasmic TL because the cells were cell surface-labeled by the lactoperoxidase-catalyzed procedure, and also because there is charge heterogeneity, which is not routinely observed for cytoplasmic forms of molecules. The ~39 K mol wt species may (*a*) possess the same polypeptide backbone as the ~47 K mol wt form but fewer carbohydrate side chains, (*b*) represent a proteolytic fragment of the ~47 K mol wt molecule, or (*c*) be the product of a second distinct *Tla* gene. Goodenow et al. (5) have demonstrated there are at least two *Tla* genes in the BALB/c genome. Experiments to address the structural relationship between the ~39 K and ~47 K mol wt forms of TL are underway.

The expression of TL on peripheral lymphocytes has been reported in two other instances. ~20–35% of spleen and lymph node cells from BALB/c mice exposed to total lymphoid irradiation express TL determinants (14). TL was not detected on peripheral cells if the animals were thymectomized before total lymphoid irradiation. In the other study, Ranges et al. (15) reported that up to 10 wk of age, ~100% of *nu/nu* splenic T cells were TL$^+$; by 16 wk of age the

percentage of TL^+ cells decreased to 8–10%. If there is a common mechanism that induces or allows the expression of TL on peripheral cells in these two types of abnormal mice, it is not clear. In the total lymphoid irradiation model, an intact thymus is required, but in *nu/nu* mice it is not. However, in both of these experimental systems the hormonal signals and microenvironments important to cellular differentiation and trafficking are undoubtably altered from the norm and thus may influence the expression of TL.

As described in this study, normal splenic T cells that have been activated in vitro with alloantigen or Con A express the TL antigen. TL was not detected on T cells isolated directly from the spleen but was detectable 48 h after activation with Con A. This TL^+ phenotype was retained for the duration of in vitro culture (8–10 d). We have also examined several long-term cloned CTL lines of the Tla^a genotype and each has been TL^+. These cell lines are Ly-1^-2^+; we are extending our analyses to cloned lines of other Ly phenotypes to determine if TL expression is a general feature of all or only specific subpopulations of activated T cells. Although our data and that of others (1) indicate that normal resting peripheral T cells are TL^-, these cells may express low levels of TL not detected in most analyses. Scollay et al. (16) have reported that the TL.m3 determinant is detectable by flow cytometry on 10–20% of normal peripheral lymphocytes; these cells are weakly TL^+ and may represent a normal subpopulation of resting T cells or T cells that have been activated in vivo.

Both spontaneous and radiation-induced leukemias can express TL (1). Included among these are leukemias derived from Tla^b strains that do not express detectable TL determinants on thymocytes. We have not as yet observed TL on activated cells from mice of the Tla^b genotype using anti-TL alloantisera, which potentially have reactivity for TL determinants known to be anomalously expressed on the Tla^b-derived leukemias. This implies that there is not a requisite association between cellular activation and TL expression, but a definitive answer awaits testing with other alloantisera and mc antibodies. Whether there is a central differentiative or functional state responsible for the expression of TL on thymocytes, activated peripheral T cells, certain leukemias, and peripheral T cells from total lymphoid irradiated mice and *nu/nu* mice, remains elusive. We emphasize, however, that although the expression of TL remains associated with differentiative events, it should no longer be considered a marker restricted to immature T cells and leukemias.

Summary

Peripheral T lymphocytes activated in vitro with concanavalin A (Con A) or alloantigens express the thymus leukemia (TL) alloantigen as assessed by staining with the monoclonal antibody TL.m3 and flow cytometric analysis. The determinants detected by TL.m3 on activated cells are encoded within the *Tla* region and are detected as early as 48 h after activation with Con A. Several long-term cloned cytotoxic T lymphocyte lines were also examined and each expressed TL. By two-dimensional analysis, the TL isolated from activated peripheral cells was indistinguishable from that found on thymocytes and the leukemia cell line ASL-1.

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