STRUCTURAL CHARACTERISTICS OF Tla PRODUCTS

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The Tla locus belongs to a family of some 36 class I genes (1, 2) that occupy the extended major histocompatibility complex (H-2:Qa:Tla) region of mouse chromosome 17. The relatedness of class I genes is indicated and defined by crosshybridization of H-2 DNA probes with members of this family, and by a characteristic product structure consisting of a heavy (H) chain of 40–50,000 mol wt, associated with a β-2 microglobulin subunit, coded elsewhere. The sharpest biological distinction between members of this family rests in differential expression, since H-2 products are probably expressed by all somatic cells, whereas the expression of Qa:Tla genes is restricted to only some cell lineages. Polymorphism ranges from extreme, in the case of H-2K (3), to little or none in the case of Qa-2 (4), otherwise there is little to categorically distinguish the products of different class I genes from one another.

The structure of the thymus leukemia antigen (TL) is of particular interest for several reasons (5), notably in the aberrant expression of TL by T cell leukemias of TL mouse strains that never normally express TL, and in the phenomenon of antigenic modulation (loss of the TL phenotype on exposure of TL⁺ cells to TL antibody), which completely deprives TL of any function whatever as a histocompatibility antigen, in striking contrast to its fellow class I H-2 antigens. Here we give evidence that TL, the Tla product, is distinguished by certain features of its H chain, and by the occurrence of additional products of higher molecular weight.

Materials and Methods

Mice. A/J, A.TH, BALB/cJ, B10.M, A.CA, B6D2F1, P/J, 129 and C57L/J were purchased from The Jackson Laboratory (Bar Harbor, ME). BALB/cBoy and B6-Tla⁺ congenic mice were from colonies kept at Memorial Sloan-Kettering Cancer Center.

Cells. Thymocytes for radiolabeling were obtained from male mice aged 8–12 wk. The B6 leukemia ERLD was passed in B6 females, and the cells were recovered from spleen. The source of ASL1 (A strain) leukemia cells was the ASL1.1 line adapted to culture by Dr. R. Hyman (Salk Institute, La Jolla, CA), kindly provided by Dr. E. Rothenberg (California Institute of Technology, Pasadena, CA).

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Abbreviations used in this paper: 2D IEF, two-dimensional isoelectric focusing; H, heavy chain; mAb, monoclonal antibody; NP-40, nonidet P-40; PAS, protein A-Sepharose; PBS, phosphate-buffered saline; SDS-PAGE, sodium dodecyl sulfate–polyacrylamide gel electrophoresis; TL, thymus leukemia antigen.
Antisera. The standard TL antiserum used was (B6 × A-Tla\(^+\))\(_F_1\) anti-A strain leukemia ASL1.

Radioiodination, Cell Lysis, and Immunoprecipitation. Essentially according to Vitetta et al. (6), 5 × 10\(^7\) thymocytes or leukemia cells were labeled with 2–20 mCi of Na\(^{125}\)I (Amersham Corp., Arlington Heights, IL) by lactoperoxidase catalysis. Cells to be labeled were suspended in 1 ml of phosphate-buffered saline (PBS) to which we added 200 \(\mu\)l of PBS containing 200 \(\mu\)g of lactoperoxidase (Sigma Chemical Co., St. Louis, MO). The reaction was initiated by addition of 10 \(\mu\)l of a 0.03% \(\text{H}_2\text{O}_2\) solution. After 5 and 10 min, an additional 10 \(\mu\)l of \(\text{H}_2\text{O}_2\) solution were added. 20 min after the first \(\text{H}_2\text{O}_2\) addition, labeling was terminated by washing the cells twice with 50 ml of ice-cold PBS. The cells were lysed in 2 ml of PBS containing 0.5% Nonidet P-40 (NP-40) and 1 mM phenylmethylsulfonyl fluoride. The lysates were cleared in an Eppendorf microcentrifuge and used immediately for immunoprecipitation.

Immunoprecipitation was effected by antibodies bound to Protein A–Sepharose 4 B (PAS) beads (Pharmacia Fine Chemicals, Piscataway, NJ). PAS beads (1.5 g) were first swollen with 15 ml of PBS containing 0.01% sodium azide and stored at 4°C. Generally, 10 \(\mu\)l of serum or ascites fluid was added to 100 \(\mu\)l of bead suspension, and the mixture was shaken for 15–30 min at 23°C. The antibody-bead complex was then washed three times with 15 ml of ice-cold PBS containing 0.5% NP-40. 200 \(\mu\)l of lysate (5 × 10\(^6\) cell-equivalents) was then added to the washed bead pellet, and the mixture was again shaken for 30 min at 23°C. Afterwards, the bound immune complexes were washed four times with PBS with 0.5% NP-40, and once with a low-salt detergent buffer (2 mM Tris, 0.5% NP-40, pH 7.4). After this last wash, the PAS beads were centrifuged through a cushion of low-salt buffer containing 20% sucrose.

Electrophoresis. Sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS-PAGE) was performed according to Laemmli (7) using 12% acrylamide. Molecular weight standards (Pharmacia Fine Chemicals) included phosphorylase b (94,000), bovine serum albumin (67,000), ovalbumin (43,000), actin (42,000), carbonic anhydrase (30,000), soybean trypsin inhibitor (20,100), \(\alpha\)-lactalbumin (14,400), and cytochrome c (13,400) (Sigma Chemical Co.). Two-dimensional isoelectric focusing (2D IEF)-SDS-PAGE was performed according to O'Farrell (8). All SDS-polyacrylamide gels were dried under vacuum and exposed to Kodak X-omat AR film at -70°C using Dupont Cronex intensifying screen.

Peptide Mapping. 2D peptide mapping of \(^{125}\)I-labeled TL (eluate of the specific SDS-PAGE band) was performed according to Elder et al. (9). Chymotrypsin was used at a concentration of 50 \(\mu\)g/ml in ammonium bicarbonate buffer, pH 8.3.

Results

SDS-PAGE Characteristics of TL

Thymocytes: doublet H chains. Figs. 1 and 2 show SDS-PAGE of TL precipitated from thymocytes with various anti-TL monoclonal antibodies (mAb) (10), and with conventional TL antiserum. TL appears as two closely migrating bands of similar intensity in the H chain region (45–50,000 mol wt). This doublet pattern, observed originally with B6-Tla\(^+\) thymocytes (11), was invariably seen also with thymocytes of all TL\(^-\) mouse strains tested (A, B6-Tla\(^+\), C58, SJL/J, A.TH [Tla\(^+\)]; BALB/cBoy, BALB/cj [Tla\(^-\)]; B10.M, A.CA [Tla\(^+\)]; BDP/J, P/J [Tla\(^-\)]; C57L/J, 129 [Tla\(^-\)]). H-2 precipitated with anti-H-2 sera from the same materials in no case showed a doublet H chain pattern. TL is less readily precipitated from Tla\(^+\) and Tla\(^-\) thymocytes than from thymocytes of other Tla types, but nevertheless, the characteristic H chain doublet was apparent. Fine differences in SDS-PAGE migration, which distinguish some but not all TL genotypes from one another, affect the two bands of the doublet equally.
FIGURE 1. SDS-PAGE of TL from Tla⁺ (BDP/J) thymocytes, showing the doublet H chain (47,000 and 49,000 mol wt) and TL products of higher molecular weight (52,000 and 53,000). The weak representation of the higher–molecular weight TL with mAb (TL.m4, 5, 6, and 8), as compared with antiserum (anti-TL), is due to the underexposure needed to distinguish the two bands of H chain doublet clearly. mAb TL.m9 is a negative control, because Tla⁺ thymocytes do not express TL.m9 antigen (10). The uppermost band with anti-TL is retroviral gp70 precipitated by anti-gp70 present in this antiserum.

FIGURE 2. SDS-PAGE of TL from Tla⁺ (B10.M) thymocytes, showing the doublet H chain and a TL product of higher molecular weight. The underexposure needed to show the H chain doublet distinctly with mAb is insufficient for showing the high–molecular weight band that is strongly seen in this figure only with TL antiserum (anti-TL). mAb TL.m9 and TL.m8 are negative controls (10). (See legend to Fig. 1.)

TL of higher molecular weight. A major feature of TL from Tla⁺ thymocytes (strains BDP/J [Fig. 1] and P/J [not shown]), which is not seen with thymocytes of the prototype TL⁺ strain A (Tla⁺), is the presence of additional Tla⁺ bands of
higher mol wt (~52,000 and 53,000). In the case of Tla \(d\) (strains B10.M [Fig. 2] and A.CA [not shown]), there was a single band of higher mol wt (~53,000). Neither the 53,000 mol wt nor any other specific band was obtained from controls in which B10.M spleen cells were substituted for B10.M thymocytes. No corresponding additional bands were seen with H-2 precipitates from the same material.

**Leukemia cells.** Although TL from thymocytes of all TL+ mouse strains, regardless of Tla genotype, yielded H chains showing the characteristic and equally intense doublet bands (see above), TL from leukemia ASL1 (a spontaneous A strain leukemia in culture), and from the B6 radiation leukemia ERLD, gave only a single H chain band. ERLD cells yielded an additional product of ~53,000 mol wt, evidently corresponding to the higher molecular weight products already noted. ASL1 cells, like A strain thymocytes, gave no such additional band.
FIGURE 4. 2D chymotryptic peptide maps of the two H chain bands from Tla~ thymocytes separated individually from an SDS-PAGE gel, showing no significant difference. The doublet bands from Tla~ thymocytes and from Tla~ thymocytes gave similar results.

2D IEF-SDS-PAGE Characteristics of TL

Thymocytes. Fig. 3 shows 2D IEF-SDS-PAGE patterns of TL from thymocytes, according to Tla genotype. In no instance were the two components of the H chain doublet distinguishable by charge. The patterns for H chains of Tla~ and Tla~ thymocytes, which differ serologically only for TL..m9 and TL..m7 antigens (10), were not distinguishable, but differed from the pattern typical of Tla~, Tla~, and Tla~ in being more basic and showing more microheterogeneity; thus the patterns of TL from normal thymocytes fall into two main groups.

The higher-molecular weight TL products that we find associated with Tla~ thymocytes, but not Tla~ are, in each case, distinguishable by charge from the respective H chain doublet, as is illustrated in Fig. 3 for Tla~ and Tla~.

Leukemia cells. In 2D IEF-SDS-PAGE, the single H chain band (45,000 mol wt) from ASL1 leukemia cells of the A strain (Tla~) differed from H chains of A strain thymocytes (although the peptide maps were the same, see below), but was somewhat similar to the 50,000 mol wt H chain of ERLD leukemia cells of the B6 strain (Tla~) (Fig. 3).

2D Chymotryptic Peptide Mapping of TL

Thymocytes. The two bands of the H chain doublet were separately removed from SDS-PAGE gels and examined by 2D-chymotryptic peptide mapping. Maps of the two H chain bands from Tla~ thymocytes (Fig. 4) were not distinguishable, nor could the maps of the two Tla~ H chain bands be distinguished from one another (not shown).

Comparison of H chain maps of TL from Tla~ and Tla~ thymocytes showed little difference, in keeping with the close similarity of serological phenotype and charge. H chain maps for Tla~ and Tla~ thymocytes were very similar to one another and clearly different from the Tla~ and Tla~ pattern, although there were shared features. These data, illustrated in Fig. 5, are in accord with the data of Yokoyama et al. (12), obtained with comparative tryptic peptide mapping.
FIGURE 5. 2D chymotryptic peptide maps of H chains of TL (eluted from SDS-PAGE bands) from thymocytes and leukemias of various Tla genotypes. The upper four panels represent thymocytes of mice of strains A (Tla<sup>a</sup>), BDP (Tla<sup>c</sup>), BALB/cJ (Tla<sup>d</sup>) and B10.M (Tla<sup>b</sup>); the lower two panels represent the A strain leukemia ASL1 (Tla<sup>a</sup>) and the B6 leukemia ERLD (Tla<sup>b</sup>). The Tla<sup>a</sup> and Tla<sup>c</sup> maps (top panels) are broadly similar. The maps of Tla<sup>a</sup> thymocytes (top left) and leukemia cells (bottom left) are not distinguishable.
**Leukemia cells.** Despite the marked differences in charge between TL H chains of A strain thymocytes and of A strain leukemia cells, noted above, the peptide maps did not differ (Fig. 5). The peptide map for B6 leukemia cells, though generally similar to the strain A pattern, is not the same, but in the case of B6, there is no thymocyte TL for comparison because B6 mice are TL<sup>-</sup> (*Tla<sup>b</sup>)*.

**Discussion**

The two characteristics reported here, which on present evidence distinguish TL from other class I products of the *H-2*:Qa:*Tla* complex are, first, the doublet H chain feature shown by TL of all TL<sup>+</sup> thymocytes regardless of *Tla* genotype but not by the two TL<sup>+</sup> leukemias studied, and second, the occurrence of putative TL products of higher molecular weight in TL<sup>+</sup> mice of all *Tla* types except *Tla<sup>a</sup>* (these higher–molecular weight products are being studied and are not discussed further here).

Since the two components of the H chain doublet could not be distinguished by charge or by peptide mapping, and since only a single internally labeled cytoplasmic TL precursor has so far been identified (11), the two H chain bands may differ only in glycosylation, without an effect on charge.

Only one H chain band could be found in the two TL<sup>+</sup> leukemias studied. One possible interpretation is that two forms of TL are expressed singly by different TL<sup>+</sup> thymocyte subpopulations, hence the single H chain band of clonal leukemias, but clearly there may be other explanations.

The differences in molecular weight and charge between TL of A strain thymocytes and A strain leukemia cells was not accompanied by any difference in the 2D peptide maps, as similarly observed by Yokoyama et al. (13) in comparative tryptic peptide mapping. A reason for the difference in charge may be found in differing degrees of glycosylation that are known to occur with other glycoproteins of normal as compared to transformed cells (14). In short, there is nothing here to indicate the action of more than one *Tla* gene in normal or transformed A strain T cells. The 2D peptide map of the single H chain band of B6 ERLD leukemia cells is distinctive, but there is no B6-constitutive TL with which to compare it, since B6 has the *Tla<sup>b</sup>* allele, which is null with respect to thymocyte phenotype.

2D peptide mapping and 2D IEF–SDS-PAGE categorically distinguish TL from other class I products, H-2 and Qa-2, which we have studied similarly, and the relatively small differences among TL of various *Tla* types reflects the lesser polymorphism of *Tla* as compared to H-2.

One of the aims of this study was to look for evidence of expression of more than one *Tla* gene in thymocytes or leukemia cells, since multiplicity of *Tla* genes has been inferred from the results of gene cloning and transfer (15). Our data are not decisive in that regard. BALB/c leukemias, which we are now studying, may be more favorable for answering this question, because BALB thymocytes (*Tla<sup>c</sup>*) express constitutive TL antigen, whereas BALB leukemias have additional TL antigens which, although normally expressed by thymocytes of other mouse strains (e.g. *Tla<sup>c</sup>*), are not expressed by BALB thymocytes (16).
Biochemical study of thymus leukemia antigen (TL) from thymocytes of various Tla genotypes and from leukemia cells revealed features that, given present evidence, are peculiar to TL among class I products of the H-2:Qa:Tla region of chromosome 17.

Sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS-PAGE) of TL from thymocytes of all TL+ mouse strains, precipitated by anti-TL antiserum or monoclonal antibodies, showed two closely migrating bands of equal intensity in the heavy (H) chain position (45–50,000 mol wt). Comparison of these two bands by two-dimensional isoelectric focusing (2D IEF)–SDS-PAGE and 2D chymotryptic peptide mapping showed no differences indicative of protein dissimilarity. Thus, the two components of the H chain doublet may differ only in a feature of glycosylation that does not affect charge. The two leukemias studied gave only a single band in the H chain position.

On 2D peptide mapping and 2D IEF–SDS-PAGE, the patterns for TL of Tla a and Tla e thymocytes, which are closely related serologically, were broadly similar, but clearly different from the pattern typical of Tla c and Tla d thymocytes. 2D peptide maps of TL from Tla a thymocytes and Tla a leukemia cells did not differ. Leukemia cells of Tla b origin (thymocytes TL−) gave 2D peptide and 2D IEF–SDS-PAGE patterns of a third type.

With the exception of Tla a, thymocytes of TL+ mice yielded additional TL products of higher molecular weight than the TL H chain.

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