Structural comparisons of TL antigens derived from normal and leukemia cells of TL+ and TL− strains and relationship to genetically linked H-2 major histocompatibility complex products

(cell surface glycoprotein/tryptic peptide analysis/tumor antigen/Tla/MHC region)

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ABSTRACT Comparative tryptic peptide analysis was used to probe the structure of the TL products coded for by normally expressed TL alleles (Tla* and Tla haplotypes) and the structure of the TL product on ERLD, a TL− leukemia occurring in a strain that does not normally express TL antigens (Tla haplotype). In mice that have the Tla haplotype, the peptide maps of TL glycoproteins from normal thymocytes and from TL− leukemias were identical, a finding that is consistent with the indistinguishable serologically defined TL phenotype of these cells. Analysis of the TL product on ERLD leukemia cells (TL phenotype, TL.1,2,4) indicates a single TL glycoprotein species with the TL.4 determinant (restricted to leukemias of Tla* and Tla haplotypes) coexisting on the same glycoprotein as the TL.1 determinant (normally expressed by thymocytes of Tla* haplotype). Comparison of the peptides of the TL product of ERLD and the products of the normally expressed Tla* and Tla loci showed a high degree of structural similarity (i.e., 70–80%) of shared peptides. In contrast, the products of the Tla*, Tla−, and Tla loci shared relatively few peptides with the products of the K, D, and L loci of the closely linked major histocompatibility complex (15–35%). The TL family, therefore, consists of alleles that are highly homologous. This contrasts with the marked diversity and polymorphism among the alleles of the K, D, and L loci.

The TL system of cell surface antigens was discovered almost 20 years ago during studies of mouse leukemia (1). A number of unique features set TL apart from other surface antigenic systems (2). In certain strains of mice (designated TL− strains), TL has the characteristics of a differentiation antigen, expression being limited to thymocytes and leukemia cells of thymic origin. In other strains (TL− strains), TL is not normally expressed on thymocytes, but can be detected on leukemia cells. The usual explanation given for these findings is that all strains of mice have the genetic information for TL, but strains differ with regard to regulatory sequences controlling expression vs. nonexpression of TL on normal thymocytes. Leukemogenesis disrupts this regulatory control of TL in TL− strains, resulting in the expression of normally silent TL genes and the anomalous appearance of TL antigens on leukemia cells.

Mendelian analysis of TL expression in normal mice showed that the controlling locus (designated Tla) is closely linked to the H-2 complex on chromosome 17, 1 to 2 centimorgans to the right of the H-2D locus, and there are several reasons to believe that this is also the site of the TL structural gene (3). In addition to close genetic linkage between Tla and H-2, the products of these two loci show certain biochemical similarities (4, 5), and antibody blocking studies indicate a close spatial apposition of these two components on the cell surface (6). Both the major histocompatibility complex (MHC) H-2 and the TL antigens consist of similar molecular complexes, containing a Mr 45,000 glycoprotein and a noncovalently associated Mr 12,000 protein (4, 5, 7–9). Despite these similarities, H-2 and TL differ in a number of ways. H-2 antigens are expressed on virtually all cells of the body, whereas TL antigens are normally restricted to thymocytes in TL− strains. In contrast to the remarkable polymorphism of H-2 (10), only six TL specificities have been defined to date, one of them, TL.4, never having been detected on normal cells (2, 11). Furthermore, unlike H-2, TL antigens do not serve as transplantation antigens capable of causing graft rejection or eliciting cytotoxic T cell responses. The reason for this may have to do with the rapid conformational change in TL antigens induced by exposure to TL antibody, leading TL− cells to become resistant to the action of TL antibody and complement, a phenomenon known as antigenic modulation (12–15).

Past structural studies have focused on TL antigens on thymocytes and leukemia cells of TL− strains. To gain information about both normally and abnormally expressed TL antigens, the technique of tryptic peptide analysis has been used to probe (i) the structure of the TL products coded for by normally expressed TL alleles (Tla* and Tla); (ii) the structure of the TL product expressed by ERLD, a TL− leukemia arising in a TL− strain (Tla); and (iii) the relationship between the products of the Tla*, Tla−, and Tla loci and the K, D, and L MHC products.

MATERIALS AND METHODS

Mice. C57BL/6 (H-2b, Tlaa), B6-Tla* (H-2b, Tla), A (H-2a, Tla) and BALB/c (H-2d, Tlac) came from our colonies.

Leukemia Cells (2). A strain origin: spontaneous leukemias ASL1 and ASL6 and x-ray-induced leukemia RADA1; phenotype TL.1,2,3. B6 origin: x-ray-induced leukemia ERLD; phenotype TL.1,2,4. EL4 is a TL− leukemia of C57BL origin.

Antiserum. TL antisera (2); (B6 × A-Tla)*F1, anti-ASL1 (αTL.1,2,3), B6 anti-ASL1 (αTL.1,2,3), (B6-Tla × A)*F1, anti-ERLD (αTL.4). The hybridoma line (TL.m3) producing monoclonal TL.3 antibody (αTL.3) was originally provided by U. Hämmerling (16). Sera of nu/nu mice bearing TL.m3 were used, and the TL.3 specificity of this monoclonal antibody was confirmed in our laboratory. [Reactions against Qa-1-related antigens were excluded by the use of αTL.3 in tests with Tla thymocytes or leukemia cells, B6 anti-ASL1 with ERLD leukemia, and (B6 × A-Tla)*F1 and ASL1 with BALB/c (Tla) thymocytes.] H-2 antisera: (B6 × C3H)*F1, anti-B10.A (αH-2a, C3H.OL anti-C3H (αH-2a, 23), (B10.BR × LP.RIII)*F1, anti-

Abbreviations: CAMIG, goat anti-mouse immunoglobulin; MHC, major histocompatibility complex; MuLV, murine leukemia virus; NMS, normal mouse serum.
B10.A(2R) (αH-2.28), H2C anti-EL4 (αH-2.33), and A/anti-B10.H or B10.A(2R) (αH-2.2) (5). Normal mouse serum (NMS) was obtained from BALB/c mice. Goat anti-AKR murine leukemia virus (MuLV) envelope glycoprotein was prepared by E. Fleissner and rabbit anti-Rauscher MuLV was provided by W. D. Hardy, Jr. Goat anti-mouse immunoglobulin (GAMIG) was prepared by immunization with purified immunoglobulin from NMS.

Source of Radiolabeled TL and H-2 Antigens. TL and H-2 were isolated from leukemia cells cultured in Dulbecco’s modified Eagle’s medium/10% fetal bovine serum or from thymocytes cultured in RPMI-1640/10% fetal bovine serum in the presence of radiolabeled amino acid by a modification of published methods (17, 18). After treatment with NMS and GAMIG, the Nonidet P-40 extract containing 1 mM phenylmethanesulfonyl fluoride (Sigma) was partially purified by using a Lens culinaris hemagglutinin Sepharose 4B affinity column (19). The fraction that eluted with 0.1 M a-methyl-d-mannoside (glycoprotein pool) was cleared of MuLV-related proteins by affinity chromatography with a mixture of goat anti-MuLV envelope glycoprotein and rabbit anti-MuLV sera (20, 21). The unbound fraction was pooled and recycled twice. TL and H-2 antigens were recovered from this partially purified glycoprotein pool by indirect immunoprecipitation with aTL or aH-2 K, D, L and GAMIG or Staphylococcus aureus Cowan I (22). The preparation of H-2L\(_d\) antigen used the aD\(_d\) immunoadsorbant method (23), except that 50 mM diethylamine, pH 10.5/0.25% Nonidet P-40 was used for elution.

Reduction and Alkylation, Bio-Gel A Chromatography, Trypsin Digestion, and Ion Exchange Chromatography. These procedures were carried out as described (17), except that the elution buffer for the Bio-Gel A column contained 0.02 M dithiothreitol (A grade, Calbiochem) and the trypsin digestion buffer included 0.1 M ammonium bicarbonate, pH 8.55.

Discontinuous NaDodsSO\(_4\)/Polyacrylamide Gel Electrophoresis. Samples were fractionated by electrophoresis using the discontinuous buffer system (24).

RESULTS

General Biochemical Properties of TL Antigens. By using procedures similar to those reported (17, 18), TL antigens were prepared from the lentil lectin-positive glycoprotein fraction of Nonidet P-40 lysates of TL\(^+\) thymocytes or leukemia cells. TL antiserum, as well as NMS, precipitated several protein peaks from the glycoprotein pool, including a number of high molecular weight components presumably unrelated to TL. To remove these unrelated components, the glycoprotein pool was fractionated on an affinity column of Sepharose coupled to heterologous anti-MuLV sera. Conventional TL antiserum (aTL 1,2,3) and monoclonal aTL 3 precipitated a single major protein (\(M_0 = 45,000\)) as well as several smaller variably occurring peaks (\(M_0 = 28,000\) and \(≈ 21,000\)) from previously cleared extracts (Fig. 1A). In addition, a \(M_0 = 12,000\) peak was present (Fig. 1B). Monoclonal aTL 3 precipitated a smaller amount of the high molecular weight contaminants. However, even with monoclonal antibody, clearing of extracts was an essential step to eliminate these unrelated materials from the immunoprecipitates.

Comparative NaDodsSO\(_4\)/polyacrylamide gel electrophoresis analysis of the TL heavy chain and the MHC H-2K or D heavy chains showed that TL has a lower mobility than H-2, corresponding to a \(M_0 = 46,000\) for TL and 45,000 for H-2K\(^d\) and H-2D\(^d\) (Fig. 1C and D). The smaller protein associated with the TL heavy chain in immunoprecipitates (Fig. 1B) was isolated by Sephadex G-75 and G-50 chromatography and analyzed by gel electrophoresis. Peptide mapping and NH\(_2\)-terminal amino acid sequence analysis have shown that this M\(_0 = 12,000\) component is identical to the β-2 microglobulin found in association with H-2 antigens (unpublished results). All reactivity with aTL 1,2,3 was removed by clearing with aTL 3, suggesting that the three TL determinants are carried by a single molecular species (9).

The overall recovery of arginine and lysine radioactivity in the glycoprotein fraction of Nonidet P-40 extracts of TL\(^+\) or TL\(^-\) leukemia cells was 2–5%. The comparable figure for recovery of glycoproteins from extracts of normal thymocytes was ≈1%. After immunoprecipitation and gel electrophoresis, the recovery of TL from normal or leukemia cells was one order of magnitude lower than that for H-2 antigens (0.1–0.5% vs. 2–5%).

Comparison of TL Products from Normal Tla\(^+\) and Tla\(^-\) Mice. Normal thymocytes of B6-Tla\(^+\) mice (Tla\(^+\)) express TL 1,2,3 determinants, whereas thymocytes of BALB/c mice (Tla\(^-\)) express only the TL 2 determinant. Tryptic peptide analysis of TL glycoproteins from thymocytes of these two strains shows that the Tla\(^+\) and Tla\(^-\) products are similar (Fig. 2). Nearly 70% of the arginine and lysine peptides were found to be shared.

Comparison of TL Products from Normal Thymocytes and Leukemia Cells of Tla\(^+\) Mice. Past studies have not shown any
**FIG. 2.** Comparative tryptic peptide analysis of Tla* and Tla* products. [14C]Arginine-labeled Tla* from B6-Tla* thymocytes (---) and [14C]arginine-labeled Tla* from BALB/c thymocytes (----) immunoprecipitated with αTL.1,2,3.

The difference in the TL phenotypes of thymocytes and TL leukemia cells of mice that have the Tla* haplotype; both normal and leukemic cells express TL.1,2, and 3 determinants. Tryptic peptide analysis showed that TL glycoproteins from Tla* thymocytes and Tla* leukemia cells (RADA1, ASL1, and ASL6) were identical (Fig. 3).

**Studies on the TL Product Expressed by Leukemia Cells Arising in Tla** Mouse. Normal thymocytes of mice with the Tla* haplotype have a TL* phenotype. Leukemias of thymic origin arising in Tla* strains, such as B6, frequently have a TL* phenotype. The anomalous product (designated Tla*) on B6 leukemia cells carries three serologically defined TL determinants, TL.1, TL.2, and TL.4. TL.1 and TL.2 are serologically identical to TL determinants normally expressed on thymocytes of Tla* and Tla* mice, whereas TL.4 has never been detected in normal mice of any strain.

To explore the nature of Tla antigens expressed by leukemias of Tla* mice, we have analyzed the Tla* product of ERLD, a B6 x-ray-induced leukemia. As shown in Fig. 4, two separately prepared αTL.1,2 precipitated a M, 46,000 peak from glycoprotein extracts of ERLD. A similar peak was precipitated by αTL.4. Sequential precipitation analysis indicated that TL.4 and TL.1,2 determinants were carried on the same molecule: αTL.1,2 cleared all reactivity with αTL.4 and αTL.4 cleared all reactivity with αTL.1,2. H-2D<sup>b</sup> and H-2K<sup>b</sup> products were also identified in ERLD extracts by precipitation with αH-2.2 and αH-2.33. Clearing with these H-2 antisera did not alter the reactivity of either the αTL.1,2 or the αTL.4, nor did clearing with αTL alter reactions with αH-2. αTL.3 did not precipitate any components from ERLD and clearing with αTL.3 did not alter the reactions with αTL.1,2 or 4; these results are in accord with the TL.3* phenotype of ERLD cells.

Direct comparative peptide analysis was performed on TL antigens precipitated by αTL.4 or αTL.1,2 from ERLD extracts. As shown in Fig. 5, identical patterns of both arginine- and lysine-labeled peptides were found. Thus, the results of sequential precipitation and tryptic peptide analysis indicate that TL.4 and TL.1,2 determinants are carried on the same molecule.

**Comparison of the Tla** Product and the Products of the Tla* and Tla* Alleles. Fig. 6 shows a comparative tryptic peptide analysis of the Tla* and Tla* products. Approximately 80% of the peptides were identical, when both lysine as well as arginine peptides were compared. A similar comparison of Tla* and the Tla* products showed that these molecules share ~70% of their arginine peptides. Table 1 summarizes the results of comparative peptide analysis of Tla*, Tla*, and Tla* products. The findings indicate that the products of these three Tla* alleles show a high degree of structural similarity.

**Comparison of Tla and H-2K, D, and L Products.** Comparative tryptic peptide analysis was carried out on (i) the Tla product and the H-2K<sup>b</sup>, D<sup>b</sup>, and L<sup>b</sup> products of RADA1 leukemia cells and (ii) the Tla* product and the H-2K<sup>b</sup> and D<sup>b</sup> products of ERLD leukemia cells (Fig. 7). The results are summarized in Table 2. It is clear that the Tla and the H-2 products share considerably fewer peptides than are shared by the members of the TL family. Thus, the Tla* product shares 13–24% arginine peptides and 28–31% lysine peptides with K<sup>b</sup>, D<sup>b</sup>, or L<sup>b</sup>. The comparison of Tla* with K<sup>b</sup> and D<sup>b</sup> products indicates a somewhat higher degree of sharing: 27–31% arginine and 28–34% lysine peptides. Tla* and D<sup>b</sup> products showed the...
highest degree of sharing: 31% of arginine and 34% lysine peptides.

**DISCUSSION**

Comparison of the TL products of the three Tla haplotypes (Tla*, Tla+, and Tla0) analyzed by tryptic peptide mapping showed a high degree of structural homology. As summarized in Table 1, the products of the three alleles shared 70–80% of their peptides. This degree of sharing is comparable with that found with MHC mutant products of the K* haplotype, which share 85–95% of the peptides and for which only one or two amino acid differences have been noted between mutant and parental products (25). This is in sharp contrast to the limited degree of peptide homology shown by allelic products of the MHC K and D regions. For example, K* and K9 products share only 35–40% of the arginine- and lysine-labeled peptides (17).

At the primary sequence level, this degree of peptide homology means that 90% of the amino acid sequences are identical (26). We estimate that the percentage homology at the amino acid sequence level for the TL family would be close to 95%. Comparison of the TL products with H-2K, D, or L products shows that they share only a limited number of similar peptides. Although the scatter of values is large for different combinations of TL and H-2 products, the range is 15–35%. Tryptic peptide comparisons of this magnitude are difficult to interpret in terms of primary structural homology because peptides scored as similar by one dimensional chromatographic analysis may not be identical when analyzed with different criteria. In fact, preliminary primary sequence analysis reveals that some putative similar or shared peptides between K* and Tla0 are not identical (unpublished observations). Thus, it appears that the homology between K, D, and L products and TL products may be lower than the peptide profiles indicate. These findings suggest that the TL and H-2 family of molecules should be considered as distinct families structurally with different biological properties. They do, however, share a number of overall biochemical properties. Thus, both TL and H-2 products are glycoproteins with an approximately 45,000 Mr subunit heavily chain noncovalently associated with a 12,000 Mr subunit, which, in the case of H-2, has been shown to be β-2 microglobulin. Peptide profile analysis and amino acid sequence analysis demonstrate conclusively that the 12,000 Mr subunit of TL is also β-2 microglobulin (unpublished data). Further structural studies, presently in progress, are directed toward elucidating more specifically the structural relationship of the TL and H-2 molecules.

**Table 1.** Tryptic peptide comparisons of Tla*, Tla**, and Tla0 products

<table>
<thead>
<tr>
<th>Allelic products compared</th>
<th>Labeled residue</th>
<th>% total peptides shared</th>
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<tbody>
<tr>
<td>A: Tla* vs. Tla**</td>
<td>Arginine</td>
<td>81 ± 5</td>
</tr>
<tr>
<td></td>
<td>Lysine</td>
<td>81 ± 3</td>
</tr>
<tr>
<td>B: Tla* vs. Tla0</td>
<td>Arginine</td>
<td>68</td>
</tr>
<tr>
<td>C: Tla** vs. Tla0</td>
<td>Arginine</td>
<td>70 ± 2</td>
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</table>

Results represent the ratio of the average number of coincident peaks to the total number of peptides after double-label comparative tryptic peptide analysis (for calculation, see ref. 17). A: Tla* product from RADA1, ASL1, or ASL2 (A strain leukemia) or B6-Tla0 product from ERLD (B6 leukemia); B: Tla* product from RADA1 vs. Tla* product from BALB/c thymocytes; C: Tla** product from ERLD vs. Tla0 product from BALB/c thymocytes.
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TL* leukemias express TL.1, 2, and 4. Tla* normal thymocytes express only the TL.2 determinant, whereas TL* leukemias in these mice express TL.1 and TL.4 in addition to TL.2. In the present study of ERLD, a prototype TL* leukemia of C57BL/6 (Tla*) mice, sequential precipitation studies indicate that TL.1 and TL.4 determinants were on the same molecule. In addition, the peptide maps of the TL product precipitated by either aTL.1 or aTL.4 were identical. Analysis of the anomalous Tla* product of ERLD cells showed that its peptide structure is as closely related to normally expressed Tla* and Tla* products as these products are to each other. It appears, therefore, that there are considerable constraints to mutational or other types of genetic change in the TL region of the genome, even though in certain strains these genes remain unexpressed throughout normal life.

With regard to TL antigens expressed on leukemia cells, a comparison of Tla* products expressed on normal thymocytes and on leukemia cells showed that they were identical (within the limits of our analysis). In addition, the fact that no TL molecules reactive with aTL.1 remained after preclearing with aTL.3 suggests only a single species of TL molecules on Tla* leukemia cells and excludes the possibility that silent TL genes coding for TL.1 are activated as a consequence of leukemogenesis in mice of the Tla* haplotype. Activation of normally silent TL genetic information is a key feature of the TL system, as shown by the anomalous appearance of TL antigens in the leukemias of mice having the Tla* or Tla* haplotypes (2). In Tla* mice, normal thymocytes express no TL specificities, whereas

Table 2. Tryptic peptide comparisons of TL products and H-2K, D, and L products

<table>
<thead>
<tr>
<th>Products</th>
<th>Arginine</th>
<th>Lysine</th>
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<tbody>
<tr>
<td>A: Tla* vs. K*</td>
<td>13 ± 2</td>
<td>28 ± 4</td>
</tr>
<tr>
<td>Tla* vs. D*</td>
<td>22 ± 3</td>
<td>31 ± 5</td>
</tr>
<tr>
<td>Tla* vs. L*</td>
<td>24 ± 6</td>
<td>29 ± 7</td>
</tr>
<tr>
<td>B: Tla* vs. K*</td>
<td>27 ± 1</td>
<td>28 ± 8</td>
</tr>
<tr>
<td>Tla* vs. D*</td>
<td>31 ± 6</td>
<td>34 ± 8</td>
</tr>
</tbody>
</table>

Results are expressed as described in the legend to Table 1. A: Tla* product from RADA1 or ASL1 vs. K* (H-2.23), D* (H-2.4), or L* (H-2.28) products from RADA1 or ASL1; B: Tla* product from ERLD vs. K* (H-2.33) or D* (H-2.2) from ERLD.

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