MOUSE ISOANTIGENS: SEPARATION OF SOLUBLE TL (THYMUS-LEUKEMIA) ANTIGEN FROM SOLUBLE H-2 HISTOCOMPATIBILITY ANTIGEN BY COLUMN CHROMATOGRAPHY*

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Serological study of cell membrane antigens in the mouse has revealed several isoantigenic systems demonstrable by cytotoxic isoantisera. Four of these systems of antigens are exclusively or predominantly represented on thymocytes. One of these, TL (thymus-leukemia) (1), is exclusively represented on thymocytes: two (Ly-A and Ly-B) have predominant representation on thymocytes and are present also on lymphocytes, but not on any other cell type (reference 2 and unpublished data); another, designated θ (3), also is predominantly represented on thymocytes and in lower concentration on lymphocytes, but is found in at least one other tissue—adult brain. The major histocompatibility isoantigen of the mouse, H-2, also is demonstrable on thymocytes by the cytotoxic test, but in contrast to the first four systems (TL, Ly-A, Ly-B, and θ), H-2 is represented poorly on thymocytes in comparison with lymphocytes and some other tissues.

Of these five systems, TL has particular importance in tumor immunology because of its appearance in leukemias of strains that do not have the antigen on their normal thymocytes. In normal mice TL has the properties of an organ-specific isoantigen. Mice of TL+ strains cannot form TL antibody but mice of TL− strains can do so. TL+ leukemias, however, occur not only in TL+ strains but also in TL− strains. The conclusion is that all strains have a structural gene for TL synthesis, but that phenotypic expression on normal thymocytes is restricted to TL+ strains. In relation to TL there evidently are two consequences of leukemogenesis. First, in TL− strains the mechanism repressing TL synthesis is modified so that the TL character is now expressed. Secondly, in

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1 Ly-A and Ly-B are tentative designations for these two systems of isoantigens. (Ly, lymphocyte). Previously when less was known about them, the designation TSL (thymus-spleen-lymphocytes) was suggested (2) but this now appears unsuitable.
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TL+ leukemias of both TL+ and TL- strains, expression of the antigen is no longer dependent upon the thymic environment so that TL+ leukemia cells appear throughout the body.

The TL locus is complex and determines three antigenic specificities, TL. 1, 2, 3. Mice have one of three phenotypes: TL-; TL+ (TL. 2); and TL+ (TL. 1, 2, 3). Only the TL. 1 component is known to appear anomalously in strains that normally do not express it; thus TL+ leukemia cells have the phenotype TL. 1, TL. 1, 2 or TL. 1, 2, 3 according to the TL type of the strains in which they arise. In TL- strains and in TL+ (TL. 2) strains, TL. 1 must be regarded as a leukemia-specific antigen.

Genetic studies have shown that the locus determining the TL antigens is closely linked to H-2 and adjacent to the "D end" of this locus (4). Furthermore, the quantity of H-2 antigen in thymocytes is related to the presence or absence of TL antigen; TL+ thymocytes have less H-2 antigen than TL- thymocytes (5). This observation suggested the possibility of some relationship at the chemical level between H-2 and TL antigens.

Recent studies of H-2 isoantigens have revealed that these substances can be solubilized and purified to a point at least approaching homogeneity (6, 7). During the process of H-2 purification it has been shown that all of several non-H-2 specificities that were tested for are lost; indeed, many non-H-2 antigens appear to have properties distinguishing them as a group from H-2 antigens (8). Initial experiments showed that when H-2 antigens were extracted from TL+ leukemia cells TL specificity could be detected in the soluble material carrying H-2 activity. This encouraging finding led to the experiments described in this paper where it will be shown that TL antigens can be extracted and purified by the methods that have been used in chemical studies of H-2 antigens. Evidence has been given (7) that at least some of the several specificities determined by the H-2 allele of a particular mouse strain are carried on the same molecule. The possibility was entertained that the TL antigenic determinants also would be found on the H-2 molecule. But this proves not to be the case; as we report here, TL soluble antigen can be separated from H-2 soluble antigen by column chromatography.

Materials and Methods

Mice and Leukemias.—The following mouse stocks were used: A (H-2a, TL. 1, 2, 3), C57BL/6 (H-2b, TL-), 129 (H-2b, TL. 2) BALB/c (H-2d, TL. 2), H-2H (H-2h), H-2I (H-2i), C3H/An (H-2k), (C57BL X A) Fa, (BALB/c X C3H/An) F1, and C57BL/TL+ (H-2b, TL. 1, 2, 3). The C57BL/TL+ stock originated from a female of the cross-over phenotype H-2a: TL+ from a backcross of strain A (H-2a: TL+) to C57BL/6 (H-2b: TL-) (2). A serial backcross to C57BL/6 was initiated with this female. TL+ homozygotes were obtained by crossing heterozygous TL+ progeny of the eighth backcross. These homozygotes were inbred to produce the C57BL/6 stock which is congenic with C57BL/6, differing only at the TL locus (except for the effects of unrecognized residual heterozygosity).

The origins of the TL+ leukemias ASL1 (A strain spontaneous leukemia 1) and ERLD...
(C57BL/6 radiation-induced leukemia D) are described elsewhere (1). Ascites leukemia EL4 (TL—) was carried in C57BL/6 mice.

Cytotoxic Test Systems.—The activity of H-2 and TL preparations was measured by their capacity to inhibit the lysis of target cells exposed to isoantibody and guinea pig complement. The various antisera used and the specificities which they detect are indicated in Table I. The criterion of lysis employed in the studies reported here was mainly that of failure to exclude trypan blue, but release of radioactive label (chromium-51 chromate) was used in cytotoxic tests to scan column eluates for specific inhibitory activity, as previously described (6-8).

<table>
<thead>
<tr>
<th>Mouse strain immunized</th>
<th>Cells injected</th>
<th>Antibodies present</th>
<th>Absorption</th>
<th>Target cell</th>
<th>Specificities detected</th>
</tr>
</thead>
<tbody>
<tr>
<td>C57BL/6</td>
<td>Strain A leukaemia ASL1</td>
<td>TL: 1, 2, 3</td>
<td>—</td>
<td>C57BL/TL+ thymocytes</td>
<td>TL: 1, 2, 3</td>
</tr>
<tr>
<td></td>
<td>(IE*: TL: 1, 2, 3)</td>
<td>H-2: 1, 3, 4, 8, 10, 11, 13</td>
<td>—</td>
<td>Leukemia ERLD (C57BL/6)</td>
<td>TL: 1</td>
</tr>
<tr>
<td></td>
<td>129 thymocytes</td>
<td>—</td>
<td>—</td>
<td>H-2H lymph node cells</td>
<td>H-2 (“K region”) (H-2.1, 3, 8, 11)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>—</td>
<td>—</td>
<td>H-2I lymph node cells</td>
<td>H-2 (“D region”) (H-2.3, 4, 13)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>129 thymocytes</td>
<td>—</td>
<td>129 thymocytes</td>
<td>TL: 2</td>
</tr>
<tr>
<td>(BALB/c X C3H/A)F1</td>
<td>Leukemia ASL1</td>
<td>TL: 1, 3</td>
<td>In vivo in C57BL X AJF1 mice carrying leukemia ERLD</td>
<td>A thymocytes</td>
<td>TL: 3</td>
</tr>
<tr>
<td>BALB/c</td>
<td>A (spleen)</td>
<td>H-2: 4, 10, 13</td>
<td>—</td>
<td>BALB/c lymph node cells</td>
<td>H-2: 4, 10, 13</td>
</tr>
<tr>
<td>A</td>
<td>A (spleen)</td>
<td>H-2: 1, 5, 11</td>
<td>—</td>
<td>C3H lymph node cells</td>
<td>H-2: 1, 5, 11</td>
</tr>
<tr>
<td>A</td>
<td>Leukemia EL4/C57BL</td>
<td>H-2: 2, 22, 33</td>
<td>—</td>
<td>EL4 leukemia cells (C57BL)</td>
<td>H-2: 2, 22, 33</td>
</tr>
<tr>
<td>AKR</td>
<td>C3H thymocytes</td>
<td>3C3H</td>
<td>—</td>
<td>C3H thymocytes</td>
<td>#C3H</td>
</tr>
<tr>
<td>(C3H/An X I)F1</td>
<td>Leukemia EL4/C57BL</td>
<td>Ly-B.2 (and H-2)</td>
<td>—</td>
<td>C57BR thymocytes</td>
<td>Ly-B.2</td>
</tr>
</tbody>
</table>

* Congenic with C57BL/6, differing at TL loci (see text).
included titration of antigen in a "reciprocal" serum, (e.g. antigen from an H-2a source tested with serum H-2 anti-H-2); in no instance was inhibition observed, excluding the possibility of nonspecific inhibition from such effects as anticomplementary activity.

**Preparation of Antigen.**—This was performed as previously described in detail (8) except that whereas 0.7% NaCl had been used to extract normal lymphoid cells, the NaCl concentration was lowered to 0.3% to extract ASL1 leukemic cells because they are less fragile.

Crude lipoprotein "eluate" from 150 (C57BL × A)F1 hybrid spleens of mice that had been inoculated 7 days previously with ASL1 cells was prepared by repeated hypotonic salt extraction but not to the point of cell rupture. These cell-free extracts were pooled, centrifuged at 55,000 g, and the sediment suspended in water at 10 mg/ml and kept at +2°C with thymol as preservative. The 150 spleens weighed 108 g (approximately 20 g dry weight) and the yield of lipoprotein was 1.35 g (dry weight). This material reacted strongly not only for H-2 (H-2a) specificity but also in tests for TL specific activity.

Solubilization was carried out by the procedure described for H-2 antigen (6), in the knowledge derived from preliminary experiments indicating that TL had become solubilized under conditions of H-2 release from lipoprotein. Procedures were monitored by following H-2 activity using the systems H-2.4, 10, 13 and H-2.1, 5, 11. Lipoprotein (1.2 g) at 10 mg/ml was made 0.05 M with respect to tris buffer at pH 7.4 and incubated at 37°C for 2.5 hr. The suspension was cooled in ice and centrifuged in the cold at 105,000 g for 2 hr; the sediment was stored for further use and the supernatant dialyzed and freeze dried, giving 144 mg of crude soluble material.

**EXPERIMENTAL RESULTS**

**Use of P300 Bio-Gel.**—Crude soluble antigen (120 mg) was divided into three parts (40 mg each) and each part run through a P300 Bio-Gel column (Bio-Rad
Laboratories, Richmond, Calif.) measuring 1 × 20 cm. The pattern in each case was essentially that previously recorded for H-2 fractionations (7) and is shown in Fig. 1. Tests for H-2 antigen showed activity in the region of 30–50 ml of the column eluate and three pools were made: the higher molecular size fraction ("HMW", 10–29 ml), the H-2 active fraction (30–50 ml), and the lower molecular size fraction ("LMW", 51–80 ml). These were dialyzed and freeze dried to give HMW, 30 mg; H-2 active, 62 mg; and LMW, 15 mg. When tested for ability to give specific inhibition in the TL 1, 2, 3 combined system the higher and lower molecular size fractions that had no H-2 activity gave totally nega-

![Graph](https://via.placeholder.com/150)

**Fig. 2.** Cytotoxic inhibition tests on DEAE Sephadex fractions A, B, and C in the systems representing specificities determined by opposite ends (D and K) of the H-2 locus. ○——○, A (K); •——•, A (D); △——△, B (K); ▲——▲, B (D); □——□, C (K); ■——■, C (D). In both D and K systems fraction B gives 50% inhibition at approximately 80 µg/ml. Fractions A and C give no measurable inhibition at the highest level tested (1 mg/ml final concentration in test mixture).

ative results; the H-2 active fraction, however, was a potent inhibitor in the TL system.

**Use of DEAE Sephadex.**—The active fraction recovered from the P300 Bio-Gel columns, which served to remove inactive materials of higher and lower molecular weight, was dissolved (60 mg) in 5 ml of 0.1 M tris buffer and dialyzed overnight against 1 liter of the same buffer for equilibration. The solution was placed on a column of DEAE Sephadex A50 (Pharmacia Fine Chemicals Inc., Uppsala, Sweden) and eluted with a salt gradient (NaCl) from 0.1 to 0.4 M, the volume being 750 ml. The 280 µm absorbance pattern showed three major components, as had previously been described in H-2 antigen fractionations (7). The column eluate tubes were scanned for H-2 specific inhibitory activity in the cytotoxic test with the system H-2 1, 5, 11 and activity was found in the area of the second protein absorbance peak. Accordingly, three pools were made,
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one for each of the three major protein peaks, giving fraction A (22 mg), fraction B (H-2 active, 20 mg), and fraction C (16 mg).

The relative potency of the three fractions, A (eluted at low molarity), B (eluted at intermediate molarity), and C (eluted at higher molarity) was tested in the systems H-2. 1, 5, 11 and H-2.4, 10, 13 and negligible activity was recorded for fractions A and C. Similar tests were made for the “D end” and the “K end” of the H-2 locus (systems H-2.1, 3, 8, 11 and H-2.3, 4, 13 respectively) with similar results, as shown in Fig. 2. The correct H-2 specificity of the product (ASL1 cells are of A strain origin) indicated by these reactions for H-2.1, 3, 4, 5, 8, 10, 11, 13 was confirmed by failure to obtain any inhibition in the system H-2.2, 22, 33 (see Table I) with the active B fraction.

Tests on fractions A, B, and C for specific inhibitory activity in the TL systems showed a consistently different pattern. A TL.1, 2, 3 test illustrated in Fig. 3 shows that fraction C carried most of the activity, with some overlap into the H-2-active B fractions; fraction A gave a slight effect barely measurable at maximum concentration at the 50% inhibition level. Individual tests on the three fractions for TL. 1, TL. 2, and TL. 3 gave a consistent pattern as shown in the tests illustrated in Figs. 4 a–4 c; ignoring the effect of fraction A, not measurable in any instance at the 50% level, the following figures were obtained for the relative activities expressed as B/C: TL. 1, 2, 3—1/4; TL. 1—1/5; TL. 2—1/2.3; TL. 3—1/5.

Tests for other mouse isoantigens in fractions A, B, and C showed no reactivity for θ-C3H (3), in accordance with previous evidence that this antigen is destroyed during solubilization procedure (7), and none for Ly-B2.
Figs. 4 a–4 c. Cytotoxic inhibition tests on DEAE Sephadex fractions A (O——O), B (Δ——Δ), and C (□——□) in TL systems.

Fig. 4 a. System TL.1 (see Table I for definition), 50% inhibition for fraction A, not measurable; fraction B, 500 μg/ml; fraction C, 100 μg/ml.

Fig. 4 b. System TL.2, 50% inhibition for fraction A, 1 mg/ml; fraction B, 70 μg/ml; fraction C, 30 μg/ml.

Fig. 4 c. System TL.3, 50% inhibition for fraction A, not measurable; fraction B, 300 μg/ml; fraction C, 60 μg/ml.
DISCUSSION

The hypothesis tested in these experiments was that because of the close linkage of the TL (thymus-leukemia) and H-2 (histocompatibility-2) genetic determinants (4) there might be some relationship at the chemical level between the products of these genes. A functional relationship was suggested also by the finding that the quantity of H-2 antigen in thymocytes is reduced by the presence of TL antigens (5), as if there were competition for a defined number of sites at the cell surface. It seems clear from the data reported here that there is indeed such a chemical relationship, the precise nature of which awaits identification of the chemical structure of the soluble H-2 and TL products.

The lipoprotein starting material used in H-2 studies is essentially insoluble and contains not only H-2 antigen but all of a variety of non-H-2 antigens for which tests have been performed (8). As anticipated, TL specificity also was present in lipoprotein prepared from a TL+ source. On solubilization of H-2 antigen from lipoprotein by the autolytic procedure non-H-2 antigens sought thus far are not solubilized (7) although this may not be true of all. In the present study θ-C3H was shown not to be solubilized. Thus we may be justified in attaching significance to the finding that TL is solubilized with H-2. The efficiency of H-2 solubilization is low and that of TL has not yet been investigated. However, although the test systems are not strictly comparable because of their different sensitivities there are indications that the absolute values for concentration of material giving 50% inhibition are of the same order for H-2 and TL.

The fraction of intermediate molecular size that was separated on P300 Bio-Gel columns contained all the H-2 activity and also all the TL activity, the fractions of higher and lower molecular size being inactive in tests for both types of antigens. It has previously been shown that several specificities determined by a particular mouse H-2 allele are present on the same molecule (7), but the possibility that TL determinants also may be situated on the same molecule as H-2 is now ruled out in the light of the results from DEAE fractionation of the active fraction recovered from P300 Bio-Gel. Three main 280 mµ absorbing fractions were separated from DEAE columns. The middle fraction (B) contained essentially all the H-2 activity; tests for most of the specificities determined by the H-2* allele indicated no obvious deficiencies.

Tests for TL specificities all showed activity located predominantly in fraction C and overlapping fraction B to some extent. It must be concluded that the TL determinants are situated on molecules different from H-2. Evidently the TL product is not itself the C fraction 280 mµ absorbance peak but is closer to this than to the H-2 fraction. Thus the TL product cannot be homogeneous and it may be significant that the corresponding fraction from TL- sources has shown two bands on polyacrylamide gel electrophoresis (7); it is not yet known whether a TL analogue is present in TL- cells. The B/C fraction relative...
activities for TL specificities may be within the margins of error of the test systems and should not be taken to indicate that TL 1, TL 2, and TL 3 are separable.

It is too soon to speculate on the chemical relationship between H-2 and TL antigens; although they are clearly separable they are presumably somewhat similar. The possibility that one is derived by modification of the other is not ruled out but would require that all H-2 specificities are eliminated in the derivation of TL, or vice versa, and on the whole this is unlikely.

From the viewpoint of tumor immunology, TL is a model of considerable interest as an example of a tumor-specific antigen arising by activation of an ordinarily silent gene. Moreover TL antigen proves to be a molecular entity that can be extracted and purified for further study. Present studies on TL 1 antigen from leukemia cells of TL mice will show in what respects this molecule differs from the TL 1, 2, 3 complex of TL strains.

SUMMARY

Mouse H-2 histocompatibility antigen has been extracted, solubilized, and partly purified from the cells of an A strain spontaneous leukemia carrying TL (thymus-leukemia) antigens. H-2 and TL 1, 2, 3 activities were measured by inhibition of the cytotoxic effect of the corresponding isoantibodies.

TL activity was associated with the H-2 active fraction obtained by solubilization and fractionation by gel filtration. TL specificity was largely separated from H-2 antigen by subsequent chromatography on DEAE Sephadex as an adjacent component in a series of fractions.

The soluble H-2 antigen prepared from the leukemia cells was tested for most of the specificities determined by H-2* with no exceptional results.

TL 1, 2, 3 activities, measured as each component separately, were located in approximately the same position; there is no clear indication yet whether the three TL specificities are separable from one another.

It appears that in addition to the close genetic linkage between the H-2 and TL loci, and their reciprocal interaction in producing H-2 and TL antigens, these antigens exhibit some similarity at the chemical level.

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BIBLIOGRAPHY


