The Thymus Leukemia Antigen Binds Human and Mouse CD8

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Summary

The thymus leukemia antigen (TLA) is a class Ib, or ‘nonclassical’ class I molecule, one of several encoded within the T/a locus of the mouse major histocompatibility complex (MHC). It structurally resembles the H-2K, D, and L class I transplantation antigens, which present processed peptides to cytotoxic T lymphocytes (CTLs). Although their function(s) are unknown, there has been recent speculation concerning the possibility that class Ib molecules may present antigens to T cells that express γδ T cell antigen receptors (TCRs). In this report, using both a cell-cell adhesion assay and adhesion of T lymphocyte clones to purified plate-bound TLA, we provide evidence that TLA can bind to both human and mouse CD8. We also show that a chimeric class I molecule containing the peptide antigen binding site of Ld and the α3 domain, transmembrane, and cytoplasmic segments of TLA, can support a CD8-dependent immune response by CTLs. These results demonstrate for the first time binding of a class Ib molecule to CD8 with a functional outcome, as is observed for the class I transplantation antigens. The capacity to interact with CD8 has been conserved despite the extensive sequence divergence of TLA in the peptide antigen binding site, suggesting this interaction is highly significant. TLA is expressed by epithelial cells in the mouse small intestine. As these epithelial cells are in close contact with intestinal intraepithelial lymphocytes that are nearly all CD8+, and many of which express the γδ TCR, the data are consistent with the hypothesis that TLA is involved in antigen presentation, perhaps to γδ-positive lymphocytes in this site.
is not yet clear whether these results, obtained from very few T-cell clones and hybridomas, are physiologically relevant for immune responses by populations of γδ T cells. A class I b molecule named the thymus leukemia antigen (TLA) is encoded by the T18\(^b\) gene located within the Tla subregion (nomenclature according to reference 14). Historically, TLA was first identified as a leukemia-specific cell surface antigen in X-irradiated A strain mice (15), although its expression was soon detected in immature cortical thymocytes as well as leukemias of particular mouse strains. By inspection of its gene organization and amino acid sequence, TLA appears similar to the transplantation antigens and contains separate exons encoding for external α1, α2, and α3, as well as transmembrane, and cytoplasmic domains (16). Comparison of the TLA amino acid sequence with an H-2 consensu sequence shows a 60% identity in the α1 domain, a 66% identity in the α2 domain, and an 86% identity in the α3 domain (6). TLA contains three potential sites for N-linked glycosylation at positions 86, 90, and 283 compared with H-2 consensus glycosylation sites at positions 86, 176, and 256 (6). Four conserved cysteines are present in TLA at positions 101, 164, 203, and 259, as is also seen in all the transplantation antigens (17, 18). These cysteine residues are required for disulphide bond formation and proper folding in all class I molecules. TLA also associates noncovalently with β2-microglobulin, as do K, D, and L class I antigens (6). Despite all these similarities to the transplantation antigens, a cell-mediated immune response to TLA has never been reported, possibly due to its low levels of expression outside of the thymus and intestine (19, 20). If TLA were to function similarly to a transplantation antigen, perhaps it would also be bound simultaneously by both the TCR and CD8 as in the co-receptor model for antigen recognition. To determine if this is the case, the ability of TLA to interact with CD8 was assessed.

Materials and Methods

Gene Cloning and Transfections. A T18\(^b\) cDNA clone was obtained from Dr. F.-W. Shen, Tampa Bay Research Institute (St. Petersburg, FL). Constructs pDT18\(^b\)GH and pHBAprTL18\(^b\) were generated by standard molecular biology methods using pBluescript KS+ (Stratagene, La Jolla, CA) or pBR322-based cloning vectors. The H-2D\(^b\) promoter, human β-actin promoter-intervening sequence 1 (IVS 1), and human growth hormone coding sequence 1 (IVS 1), and human growth hormone coding sequence were described elsewhere (21-23). The plasmid pLdneo, containing the H-2L\(^d\) promoter and genomic coding sequence 1 (IVS 1), and human growth hormone coding sequence 1 (IVS 1), and human growth hormone coding sequence was a gift of Dr. Martha Zuniga, UC Santa Cruz, Santa Cruz, CA. A chimeric L\(^d\)/TLA MHC class I gene construct, designated pHBAprLT18\(^d\), was generated from a 1.8-kb XbaI genomic fragment encoding the \(L^d\) leader, α1, α2 domains, and 4.5-kb XbaI genomic fragment of pTLA1 encoding the T18\(^b\) α3, transmembrane, cytoplasmic, and 3’ UTR (16, 24). The human β-actin promoter was inserted 5’ of this chimeric gene in the proper orientation for expression in the cloning vector pBluescript KS+.

20 μg of the appropriate construct was coelectroporated (Gene Transactor 300; BTX Corp., San Diego, CA) with 10 μg of pSVneo (25) into either 10\(^5\) CIR B cells or L cell fibroblasts. G418 (Gibco BRL, Gaithersburg, MD) selection at 600 μg/ml active drug for CIR transfectants and 400 μg/ml active drug for L cell transfectants began 48 h postelectroporation in 96 well flat-bottom microtitre plates. Stable transfectants were chosen at 3 to 4 wk, grown in drug-free RPMI 1640 supplemented with 10% FCS, and stained for cell surface expression of TLA or \(L^d\). Staining was done as previously described (20) with TL-m4 primary mAb (a kind gift of Drs. S. Kimura and E. Boyse, Sloan-Kettering Memorial Cancer Institute, NY) followed by a FITC-conjugated goat anti-mouse Ig second reagent (CalTag, South San Francisco, CA) for detection of TLA. L\(^d\) expression was assessed using mAbs 30-5-7 (specific for the α2 domain of \(L^d\)), which stains both \(L^d\) and L\(^d\)/TLA positive transfectants, and 28-14-8 (specific for the α3 domain of \(L^d\)), which detects only L\(^d\) transfectants (26, 27). Cells were fixed in 1% paraformaldehyde and analyzed on a Coulter EPICS V flow cytometer (Coulter Electronics, Inc., Hialeah, FL) with FITC. A high expressing CIR TLA (+) transfectant, using the pHBAprTL18\(^b\) construct, was used in both the cell-cell adhesion assays and to purify TLA glycoprotein. L\(^d\) and L\(^d\)/TLA cell transfectants that express approximately equal levels of surface protein, as measured by staining and flow cytometry with mAb 30-5-7, were used as targets for primary CTLs.

Cell-Cell Adhesion Assay. This assay has been described in detail previously (3, 28, 29). Briefly, 3 to 4 d before assay between 5 \times 10\(^4\) and 10\(^5\) human CD8\(^{\alpha+}\) (+) or CD8\(^{\alpha-}\) (−) adherent Chinese hamster ovary (CHO) cells per well were placed in 96 well flat-bottom microtitre plates. Optimal confluent monolayers, as judged by visual inspection, were used on the day of assay. Parent or transfectant CIR B cells were labeled for 2 h with \(^{35}S\)methionine \(0.5-1.25 \muCi/10^5\) cells, >600 Ci mmol\(^{-1}\); Amersham, Arlington Heights, IL) in 1x MEM without l-methionine (Flow Laboratories, McLean, VA). The CIR cells were added to the plate wells and centrifuged for 3 min at 160 g to initiate contact with the CHO monolayers. The plates were then incubated at 37°C for 1 h while shaking gently. Following incubation, the wells were washed 10-12 times with assay buffer and the remaining bound cells were solubilized in 1% Triton X-100 for scintillation counting. For the antibody blocking experiments, 10\(^5\) labeled CIR cells were preincubated with 10 μg of the appropriate mAb, or adherent CHO cells in plate wells were preincubated with 2.5 μg of the appropriate mAb. Incubations were carried out for 30 min at 4°C in 0.01% NaN\(_3\), 1x PBS (pH 7.4) and 10% FCS (" assay buffer"). The mAbs used in the blocking studies were the anti-TLA mAbs TL-m4 and TL-m9 and the anti-CD8 mAbs 28-14-8 (specific for the 3’ domain of La), which detects only L\(^d\) transfectants (26, 27).

Purification of Class I and Class Ib Molecules. D\(^b\) was purified as described previously (31). TLA was purified from the CIR TL-J transfectant according to a slightly modified version of this protocol (31). Briefly, cells grown in vitro were lysed by nitrogen cavitation and the membrane fraction isolated by centrifugation. Membranes were extracted with 0.5% Triton X-100 in 10 mM Tris (pH 7.2) with 0.15 M NaCl and the solubilized protein applied to a column prepared from the rat anti-TLA mAb HD168 (a kind gift of Drs. L. Old and E. Stockert, Sloan-Kettering Memorial Cancer Institute, NY) followed by a FITC-conjugated goat anti-mouse Ig second reagent (CalTag, South San Francisco, CA). The CIR cells were added to the plate wells and centrifuged for 3 min at 160 g to initiate contact with the CHO monolayers. The plates were then incubated at 37°C for 1 h while shaking gently. Following incubation, the wells were washed 10-12 times with assay buffer and the remaining bound cells were solubilized in 1% Triton X-100 for scintillation counting. For the antibody blocking experiments, 10\(^5\) labeled CIR cells were preincubated with 10 μg of the appropriate mAb, or adherent CHO cells in plate wells were preincubated with 2.5 μg of the appropriate mAb. Incubations were carried out for 30 min at 4°C in 0.01% NaN\(_3\), 1x PBS (pH 7.4) and 10% FCS (" assay buffer"). The mAbs used in the blocking studies were the anti-TLA mAbs TL-m4 and TL-m9 and the anti-CD8 mAbs 66.2 and 51.1 (28, 30). All mAbs were protein A-Sepharose affinity-column purified.

1 Abbreviations used in this paper: CHO, Chinese hamster ovary; IEL, intraepithelial lymphocyte, TLA, thymus leukemia antigen.
sayed for total protein content using the BCA Protein Assay (Pierce Chemical Co., Rockford, IL), and for TLA glycoprotein by direct ELISA using mAb HD168 and HRP-goat anti-rat Ig second antibody (CalTag; reference 31).

Cytotoxic T Lymphocyte (CTL) Adhesion to Plate-Bound Class I Major Histocompatibility Complex (MHC) Glycoproteins. Purified TLA and D\(^d\) antigens were immobilized in microtiter plate wells by incubation for 1.5 h at 25°C, followed by four washes with 0.15 ml of 1× PBS as previously described (31). The amount of input protein/well is indicated in Fig. 3 B. The wells containing bound class I and class Ib molecules were then blocked by incubation with 2% FCS; control wells received only 2% FCS. V\(\beta^8\) CTL clones C35 and C11, specific for the K\(^d\) class I alloantigen (33, 34), were \(^{51}\)Cr-labeled (sodium chromate, 100 μCi/10⁶ cells, 250–500 mCi mg⁻¹ Cr; Amersham) by incubation for 1 h at 37°C. Labeled cells were then added to wells bearing the indicated class I or class Ib protein in the absence or presence of soluble anti-V\(\beta^8\) mAb. The anti-V\(\beta^8\) mAb F23.1 (35) was incubated with the clones at a concentration of 0.45 μg/ml for C35 and 0.9 μg/ml for C11 in 100 μl total volume. Plates were then centrifuged at 1200 rpm for 1 min to initiate contact and incubated for 2 h at 37°C, 5% CO\(_2\). Cell binding was determined as previously described by adding 0.1 ml ice-cold 1× PBS to each well and placing the plates in an ice water bath for 10 min (33). Unbound cells were removed by pipeting 10 times and discarding the supernatant. Well bottoms were then cut off and the remaining radioactivity was measured.

Cytotoxicity Assay. Primary anti-H-2\(^d\) effector CTLs were created by stimulating ~10⁶ C57BL/6 splenocytes (H-2\(^b\)) with ~10⁶ 2,000 r irradiated BALB/c splenocytes (H-2\(^b\)). Cells were incubated for 5 d in RPMI 1640 supplemented with 10% FCS at a concentration of 2 × 10⁶ total splenocytes per T25 tissue culture flask. Both C57BL/6 and BALB/c mice were bred and housed in the UCLA Vivarium, UCLA School of Medicine. \(^{51}\)Cr-loading (100 μCi/4 × 10⁶ cells; Amersham) of lightly trypsinized target cells was done for 1 h at 37°C, 5% CO\(_2\) with residual \(^{51}\)Cr removed by extensive washing. The targets included L cells transfected with the L\(^d\) gene, the L\(^d\)/TLA chimeric gene, and the pSv2neo gene, along with DBA/2 strain P815 (H-2\(^d\)) mastocytoma cells. Varying amounts of effector cells were incubated with 10⁴ labeled targets in 200 μl of medium containing RPMI 1640 supplemented with 10% FCS. Target cell lysis proceeded for 4 h in 96-well U-bottom microtiter plates at 37°C and 5% CO\(_2\). Measurement of specific \(^{51}\)Cr release was performed as described previously by removing 100 μl per well and assaying for \(^{51}\)Cr in the removed supernatant (36). For blocking studies, 2 ml of effectors were preincubated with 200 μl of the anti-Lyt2.2 mAb 41-3.48 (3.2 mg/ml, ammonium sulfate precipitation of ascites fluid) for 20 min at 37°C, 5% CO\(_2\) and then added to the microtiter plate assay wells (37).

Results

Cell-Cell Adhesion Assay. A full-length T18\(^d\) cDNA, encoding TLA serological determinants (38), was inserted into two different mammalian expression cassettes (Fig. 1 A). Transfectants were made using CIR cells that lack HLA-A and HLA-B genes and express only low levels of HLA-C. These nonadherent human B lymphoma cells were used in order to minimize background binding to CD8 due to endogenous human class I gene expression (28, 39, 40). Gene transfer and subsequent antibody staining of numerous clones generated from each type of construct showed that the \(\beta\)-actin-promoter construct yields a higher level of surface TLA expression than does the H-2\(^d\)-promoter construct (Fig. 1 B). Very high levels of gene expression for several other class Ib genes have been achieved using this \(\beta\)-actin-promoter expression cassette in CIR cells (data not shown).

The ability of TLA to bind to CD8 was measured through the adhesion of T18\(^d\) transfectants to monolayers of CHO cells that overexpress or that are negative for human CD8\(\alpha\) (28). Due to the previously reported weak avidity of HLA-A2.1-mediated CD8 binding (28), data presented here is from a transfectant expressing high levels of TLA surface protein generated using the pH\(\beta\)ApT18\(^d\) (\(\beta\)-actin promoter) construct. However, transfectants that express lower levels of surface TLA under the control of the H-2\(^d\) promoter also bind CD8 specifically, though not as tightly, in this assay.
but not to CD80(-) CHO monolayers (Fig. 2). Blocking A2.1 (HLA-A2.1-positive) transfectants bind well to CD80(+) with the mouse anti-TLA mAb TL.m4 reduces the binding is presented. Cell lines CIR A2.1, CHO.1 (CD80-negative), and CHO.4 (CD80-overexpressing) have been previously described (28, 40). Blocking mAbs TL.m4 and TL.m9 are both TLA-specific, but only TL.m4 recognizes the allele of TLA encoded by T16(1) (30). The anti-CD8 mAb 66.2 is characterized elsewhere (49). Error bars represent the standard deviations of triplicate samples.

Figure 2. Cell-cell adhesion assay. Binding of radiolabeled CIR cells to CD80(-) and to human CD80(+) adherent CHO monolayers is measured; the percentage of input CIR cells retained after extensive washing is presented. Cell lines CIR A2.1, CHO.1 (CD80-negative), and CHO.4 (CD80-overexpressing) have been previously described (28, 40). Blocking mAbs TL.m4 and TL.m9 are both TLA-specific, but only TL.m4 recognizes the allele of TLA encoded by T16(1) (30). The anti-CD8 mAb 66.2 is characterized elsewhere (49). Error bars represent the standard deviations of triplicate samples.

(data not shown). Both CIR TL.J (TLA-positive) and CIR A2.1 (HLA-A2.1-positive) transfectants bind well to CD80(+) but not to CD80(-) CHO monolayers (Fig. 2). Blocking with the mouse anti-TLA mAb TL.m4 reduces the binding of CIR TL.J to a level equivalent to the mock-transfected CIR parent. TL.m9, an isotype-matched mouse mAb that does not recognize the allelic variant of TLA encoded by T16(1) (30), does not block binding of CIR TL.J to the CD80(+) monolayer. Interestingly, the rat anti-TLA mAb HD168 also does not block the binding, although cell surface staining of transfectants that express L(A)/TLA chimeric molecules indicates that both HD168 and TL.m4 recognize an epitope encoded at least partially by the T16(1) α1 and α2 domains (data not shown). All three TLA-specific mAbs have no effect on binding of either CIR A2.1 or CIR parent cells to the monolayers. Anti-CD8 mAbs 66.2 and 51.1, which were previously shown to block HLA-A2.1-mediated binding to CD80(+) monolayers (28, 29), also block TLA-mediated binding (Fig. 2 and data not shown). This CD8 mAb blocking data argues that either TLA binds epitopes on human CD80 equivalent to those bound by human class I, as was also previously suggested for mouse transplantation antigens K(0) and D(0) (29), or that these antibodies sterically hinder more than one binding domain.

The blocking of TLA-CD80 adhesion by TL.m4 mAb is specific but could be due to the downregulation of TLA cell-surface expression upon antibody binding. Previously, downregulation of surface TLA was shown to be dependent on a number of factors including temperature, with low temperature inhibiting modulation; cellular metabolism, with modulation inhibited by NaN(3); and the cell-type expressing TLA, with downregulation most readily detected in T cell leukemia lines (41, 42). However, flow cytometric analysis of CIR TL.J transfectants at 4°C and at 37°C in the buffer used for cell-cell binding assays showed no difference in peak or mean channel fluorescence intensity as compared with TL.m4 staining used to determine high-expressing transfectants (data not shown). When observed in the fluorescence microscope, there was no evidence for either 'capping' or decreased antibody staining under the conditions used in the binding assays compared to conditions normally used to determine cell-surface expression by flow cytometry (data not shown). The lack of TLA modulation could be due to the use of the β-actin promoter in expressing TLA, the presence of NaN(3) in the assay buffer, the inability of exogenously introduced TLA genes in CIR B cells to be inhibited, or perhaps a combination of these and other factors.

Cytotoxic T Lymphocyte (CTL) Adhesion to Plate-Bound Thymus Leukemia Antigen (TLA) Glycoprotein. Recently it was demonstrated that CD8-mediated binding of CTLs to purified mouse class I transplantation antigens is activated via a TCR-mediated signal (33). In the absence of a TCR stimulus, which can be generated by soluble anti-TCR mAbs, CTL binding to purified, immobilized class I protein cannot be detected. In the presence of soluble anti-TCR mAb, a significant fraction of the CTLs bind to any immobilized K- or D-locus protein. Binding is independent of both the CTLs TCR specificity and polymorphic class I determinants, and is blocked by anti-Lyt-2 antibodies (33). Purified TLA was used to determine if it could function as a CD8 ligand in this TCR-activated adhesion system. Unlike the cell-cell adhesion assay, this assay measures interactions with mouse CD8α/β heterodimers expressed at physiologic levels on T lymphocytes, and allows a semiquantitative comparison of

![Graph](image-url)

Figure 3. Cloned CTLs are activated by anti-TCR mAb to bind to affinity-purified TLA glycoprotein. (A) Purification of TLA by elution from an anti-TLA HD168 mAb affinity column. Buffer 1, used to remove nonspecifically adherent proteins, and buffer 2, used to specifically elute affinity-purified TLA glycoprotein. (4) Purification of TLA by elution from an anti-TLA HD168 mAb affinity column. Buffer 1, used to remove nonspecifically adherent proteins, and buffer 2, used to specifically elute mAb bound TLA from the column, are described in Materials and Methods section. Eluted fractions were assayed for total protein content (· · ·) and for TLA (——). Fractions 30 to 40 were pooled yielding an enriched preparation of approximately 25 μg/ml TLA. (B) CTL binding assay. The amount of input class I-enriched total protein per well, in μg, is expressed in parentheses on the abscissa. Control wells were coated with 2% FCS. Results are expressed as % of cells bound as a result of activation. Activated binding % = % cells bound in the presence of anti-TCR mAb - % cells bound in the absence of anti-TCR mAb. Background binding in the absence of soluble F23.1 (35) mAb was 14% for C35 and 4% for C11. Error bars depict the standard deviations of triplicate samples.
TLA binding with binding results obtained for other class I molecules. TLA glycoprotein was purified from the CIR TLA transfectant using an HD168 mAb affinity column (Fig. 3 A). Contamination with HLA class I molecules is unlikely from CIR cells and, furthermore even if present, previous results suggest that human class I molecules probably would interact poorly with CD8 expressed on mouse CTLs (43). The purified TLA was directly immobilized on plastic microtiter wells. As previously demonstrated for K and D class I proteins (31), the immobilized TLA retained serologic determinants (Fig. 3 A) and remained associated with human β2-microglobulin based on ELISA using the anti-β2-microglobulin mAb BBM.1 (data not shown). Two different Vβ8+, Kβ alloantigen-specific CTL clones were examined for binding. In both cases, purified TLA was observed to function as a ligand for TCR-activated adhesion following stimulation with soluble anti-Vβ8 mAb F23.1 (Fig. 3 B). The percent of T cells bound was in the same range as that obtained using D- (Fig. 3 B) and K-locus encoded class I proteins as ligands (reference 33 and M. Mescher, unpublished results) at approximately equivalent protein concentrations used to coat the wells. These data suggest that the affinity of the TLA-CD8 interaction is similar to the affinity of K or D for CD8.

CD8-Dependent Cytotoxicity. To determine if the TLA-CD8 interaction can facilitate a CD8-dependent immune response, a chimeric class I gene was constructed with the peptide antigen-binding α1 and α2 domains encoded by Lα, and the α3, transmembrane, and cytoplasmic domains encoded by TLA (Fig. 4 A). Lα was chosen because primary, allereactive Lα-specific CTLs are CD8 dependent and blockade with anti-Lα or anti-Lyt2 mAbs (44). As class I-CD8 binding is mediated by the α3 domain by (3, 29), CD8 interactions with the chimeric molecule should depend on TLA sequences.

Before testing the Lα/TLA molecule as an alloantigen for primary CTLs, its ability to bind CD8 was assessed. CIR transfectants that express the Lα/TLA chimeric class I molecule bind human CD8a(+) CHO monolayers as efficiently as HLA-A2.1-positive and TLA-positive transfectants in the cell-cell adhesion assay (data not shown).

Table 1. Blocking of Primary Cytotoxic T Lymphocyte (CTL) Lysis with Anti-Lyt-2 mAb

| Cell type       | Percent lysis |
|-----------------|---------------|
|                 | 50:1          | 50:1*         | 25:1          | 25:1*         |
| Mock (pSV2neo)  | 15.7 ± 2.5    | 8.8 ± 0.8     | (43.9)        | 3.9 ± 1.1     | (68.5)        |
| Lα              | 38.5 ± 2.6    | 12.4 ± 1.8    | (67.8)        | 23.9 ± 1.1    | (60.2)        |
| Lα/TLA          | 39.9 ± 2.5    | 16.0 ± 3.8    | (59.9)        | 27.4 ± 0.7    | (58.4)        |
| P815 (H-2b)     | 69.3 ± 3.9    | 31.8 ± 1.9    | (54.1)        | 62.7 ± 6.4    | (70.1)        |

* Effector/target ratio.
† % lysis with mAb 41-3-48 incubation.
Parentheses indicate % inhibition by mAb blocking.
mAb 41-3-48 blocking of anti-H-2b primary CTL lysis of four L cell target lines. The % inhibition is calculated as [(% lysis unblocked - % lysis blocked)/ % lysis unblocked] x 100.

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brane, and cytoplasmic domains do not impede the normal function of target lysis by incubation with the anti-CD8 mAb that can also recognize H-2 k targets. Ld-specific cytotoxicity was measured using L cell (H-2 k) transfectant targets that express either the Ld or the Ld/TLA molecule or the L d alloantigen (Table 1). In sum, these results suggest that the TLA or3 domain is capable of delivering the appropriate signal(s) through CD8 binding for a CTL response, and that the TISd-encoded or3 domain retains an external-facing loop connecting two antiparallel B strands. Interestingly, many class Ib molecules possess this or3 domain sequence alone. The Q7d-encoded or3 domain retains a consensus sequence of Table 2.

Discussion

If the hypothesis that class lb molecules present antigens were correct, it should be possible to demonstrate that these proteins carry out functions similar to those of the class I transplantation antigens, including appropriate trafficking through the cell, loading of peptide antigen, and interaction with the TCR and CD8. It is particularly necessary to establish this for the TLA molecule, which has not been observed to participate in cell-mediated immune responses. In this report, it is demonstrated that TLA binds to human and mouse CD8, and that a chimeric molecule containing the COOH-terminal portion of TLA can support a CD8-dependent recognition of the L d alloantigen.

Previously reported results from our laboratory showed that TLA is highly expressed by both epithelial cells and intraepithelial lymphocytes (IELs) in the mouse small intestine. IELs are greater than 80% CD8 single-positive and approximately 50% of them express the γδ TCR. The ability of TLA to bind effectively to CD8 supports the hypothesis that it may be involved in antigen presentation to either TCR γδ(+) and/or αβ(+) IELs, although a critical test of this hypothesis will require the detection of antigen-specific TLA-restricted IELs. TLA is highly expressed by immature cortical thymocytes, most of which also express CD8. It is therefore possible that TLA-CD8 interactions occur in the thymus, and these interactions may have important consequences for T cell differentiation.

Using site-directed mutagenesis, a minimal consensus sequence encompassing at least 11 amino acids in the human MHC class I or3 domain has been defined that is required for binding to transfectants that express high levels of human CD8α (reference 3 and Table 2). The or3 domain has a conformation similar to an immunoglobulin constant region homology unit, and most of the critical amino acids for CD8 binding, including positions 223-229, 233, 235, 245, and 247, are on an external-facing loop connecting two antiparallel β strands. Interestingly, many class Ib molecules possess this or3 domain minimal sequence, or sequences that deviate from it only slightly. For example, compared to HLA-A2.1, TLA contains conservative amino acid substitutions at positions 223-225 and complete identity at positions 226-228, 229, 233, 235, 245, and 247. TLA differs from H-2Kb only at position 228 in this minimal sequence. In fact, a compilation of 23 mouse and human class I or3 domain sequences suggests that these 11 amino acids are extremely well conserved (reference 18 and see the consensus sequence of Table 2).

The relatively high degree of sequence conservation between different MHC class I or3 binding domains suggests that other class lb proteins in addition to TLA may also bind CD8. However, it is probably not possible to infer CD8 binding from the presence of the conserved, minimal or3 domain sequence alone. The Q7d-encoded or3 domain retains the minimal consensus sequence for CD8 binding (Table 2).
Yet, this α3 domain is incapable of supporting a primary CTL response directed at a Ld/Qa3/α3/Ld chimeric molecule, in which sequences other than α3 are encoded by Ld(48). Secondary CTL responses directed at this chimeric molecule, which are less CD8 dependent, can be obtained. These data suggest that the Qa3 domain is incapable of binding CD8 in a manner that allows a primary alloreactive response. Therefore, in the absence of experimental evidence, it cannot be assumed that any class Iib molecule other than TLA binds to CD8.

The hypothesis that class Iib molecules present antigens to TCR γδ-positive lymphocytes does not require that all of these molecules interact with CD8. Most γδ-positive T cells, including the few known to recognize class Iib molecules (5, 12, 13), are CD4/CD8 double-negative. These double-negative γδ T cells do not require CD8 as a co-receptor, despite the apparent evolutionary conservation of the minimal CD8 binding site in class Iib molecules. In contrast, nearly all of the TCR γδ-positive IELs of the mouse small intestine express CD8, which could act as a co-receptor if the TCRs expressed by these cells recognize an antigen presented by the TLA molecule.

We thank Paul Champoux for excellent technical assistance, Ms. Adrene Niederlehner for help with flow cytometry, and Drs. Grace Ku, Philip Mixter, and Jeffrey Safrit for help in preparing the figures.

This work was supported by National Institutes of Health grant CA-52511 and by American Cancer Society Institutional grants IRG-131 and IM-415D. M. Teitell is a predoctoral trainee supported by the Medical Scientist Training Program grant GM-08042 and C. Olson was supported by a postdoctoral training grant CA-09120.

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Received for publication 25 June 1991.

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