H-2L-RESTRICTED RECOGNITION OF VIRAL ANTIGENS

In the H-2d Haplotype, Anti-Vesicular Stomatitis Virus Cytotoxic T Cells Are Restricted Solely by H-2L.

BY RICHARD CIAVARRA† AND JAMES FORMAN

From the Department of Microbiology, University of Texas Health Science Center at Dallas, Dallas, Texas 75235

A large body of evidence exists demonstrating that the recognition of viral antigens by cytotoxic thymus (T) -derived lymphocytes (CTL) is virus specific and restricted by gene products encoded in the major histocompatibility complex (1). In the mouse, these restricting genes have been mapped to the H-2K and H-2D regions (1). Indeed, a single mutation in one of these loci can result in failure of antiviral CTL to recognize virus-infected cells (2). Recent biochemical studies have demonstrated that the serologically detected H-2 antigens are the H-2K and H-2D region-encoded gene products that serve as restriction antigens for H-2-restricted recognition (3-5).

Viral specificity is a consequence of the insertion of specific viral proteins into the surface membrane; once integrated into the plasma membrane they presumably interact with H-2 antigens, creating immunogenic complexes that are recognized by antiviral effector cells. Two mechanisms that allow membrane insertion of viral proteins have been identified: (a) fusion of the viral envelope with the target cell membrane (6), and/or (b) insertion from the cytoplasmic side after viral protein synthesis (7, 8). In addition, studies with liposomes (9) and reconstituted membrane vesicles (8, 10, 11) have demonstrated that viral and H-2 antigens must be integrated into the same lipid bilayer to trigger antiviral CTL.

Once viral proteins have been inserted into the plasma membrane, it is not clear whether they remain independent of H-2 antigens or form transient, intermolecular associations. Co-capping studies have suggested a fairly stable association (reviewed in 12). In some studies, cell surface associations appear to be biologically important. For example, Friend virus effector cells recognize this virus in the context of only those H-2 antigens that are selectively associated into the mature particle (13). Antibodies to H-2Kb but not H-2Db co-cap vesicular stomatitis virus (VSV) antigens; recognition by anti-VSV CTL is restricted solely to the K-end in this haplotype (14). It should be noted, however, that these associations are induced and in many of these studies their significance has not been accurately assessed (12).

Recent studies have demonstrated that the H-2D region is more complicated than initially proposed. Immunochemical and serological techniques have identified at

* Supported by grants AI 13111 and AI 11851 from the National Institutes of Health.
† Present address: The University of Connecticut, Biological Sciences Group, Genetics/Cell Biology Section, Storrs, CT 06268.
1 Abbreviations used in this paper: Con A, concanavalin A; CTL, cytotoxic T lymphocytes; E/T, effector/target; PFU, plaque-forming unit; VSV, vesicular stomatitis virus.
least four H-2D-encoded gene products: D, L, M, and R (15–18). In view of this complexity, it is important to first identify which antigens function as restriction molecules before evaluating the biological significance of viral/H-2 antigen membrane associations. For this reason, we have performed experiments designed to identify which H-2 molecules function as restriction antigens for anti-VSV CTL. The results of our studies are the subject of this report.

Materials and Methods

Mice. Mice were obtained from our animal colony at the University of Texas Health Science Center. The H-2 haplotype of the strains used are listed in Table I.

Virus. VSV of the Indiana serotype (VSV IND) was prepared by passage of the virus stock in the rat cell line R(B77). The source and culture conditions of R(B77) have been previously described (19). VSV was titered by a plaque assay using R(B77) monolayers (19).

Cells. P815 tumor cells (a DBA/2 mastocytoma) were maintained by biweekly passage in vitro in RPMI 1640 medium supplemented with 10% fetal calf serum and antibiotics. Alloreactive cytotoxic cells were induced following previously published procedures (20). Spleen cells obtained from mice immunized 1–2 mo previously with 10^5 plaque-forming units (PFU) of VSV were restimulated in vitro with VSV-infected stimulator cells to generate secondary anti-VSV CTL. The procedure has been described in detail elsewhere (8). Secondary anti-VSV CTL were used in all experiments described in the report.

Assay for CTL Activity. CTL activity was measured using a standard chromium release assay (20). Concanavalin A (Con A) -stimulated lymphoblast targets were radiolabeled with 51Cr and left uninfected or infected with VSV as previously described (8). Various numbers of normal or immune lymphocytes were added to a constant number (10^4) of target cells in microtiter plates. After 4 h of incubation at 37°C, the cells were pelleted by centrifugation and the supernatant measured for radioactivity. Cold target competition analysis was performed following previously published procedures (21) using a constant number of effector cells (usually 10^6) and target cells (10^4) and various numbers of inhibitor cells.

When various antisera or hybridoma products were tested for their ability to inhibit CTL activity, labeled targets were incubated with the indicated quantities of antibodies for 45 min at 4°C. The effector cells were then added, and the mixtures were incubated and processed as described for the CTL assay.

<table>
<thead>
<tr>
<th>Strain</th>
<th>H-2 gene</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>K</td>
</tr>
<tr>
<td>BALB/c, B10.D2, DBA/2</td>
<td>d</td>
</tr>
<tr>
<td>H-2^d^</td>
<td>d</td>
</tr>
<tr>
<td>H-2^md</td>
<td>d</td>
</tr>
<tr>
<td>B10.D2(R103)</td>
<td>d</td>
</tr>
<tr>
<td>B10.D2(R107)</td>
<td>b</td>
</tr>
<tr>
<td>B10.A(5R)</td>
<td>b</td>
</tr>
<tr>
<td>B10 BR</td>
<td>k</td>
</tr>
</tbody>
</table>

* M and R molecules are assumed to be controlled by genes separate from H-2D or -L. However, these molecules could be precursors or modifications of H-2D or H-2L (17).
† Alleles of H-2 genes. Data from refs. 17, 18 and T. Hansen, personal communication. B10.D2 and DBA/2 are assumed to express H-2M§.
‡ Altered by mutation.
§ Alleloantigen not detected.
¶ Not typed.
** Typed by derivation; H-2M alloantigen is assumed.
Antisera and Monoclonal Antibodies. Mice were given multiple injections of $3 \times 10^7$ spleen cells approximately every 2 wk. Blood samples were obtained from the retro-orbital sinus and tested for cytotoxic activity. Only sera that contained high cytotoxic titers ($>1/2,000$) and could specifically immunoprecipitate H-2 antigens from cell-free lysates were used in these studies. Hybridomas specific for various H-2 antigens were kindly provided by Dr. Ted Hansen (Merck Institute for Therapeutic Research). The preparation and characterization of these reagents has been previously described (22). Two monoclonal antibodies have anti-H-2.65 specificity (an H-2L<sup>d</sup> determinant); 23-10-1 is an IgM antibody and 30-5-7 is an IgG<sub>2a</sub> antibody. A third monoclonal antibody, 34-2-12, has anti-H-2.4 specificity (this antibody reacts with H-2D<sup>d</sup> but not H-2L<sup>a</sup> or H2R<sup>d</sup>; K. Ozato, S. Epstein, J. A. Bluestone, S. O. Sharrow, T. Hanson, and D. H. Sachs, manuscript in preparation) and is an IgG<sub>2a</sub> antibody. Antisera and monoclones are listed in Table II.

Results

Failure of Anti-VSV CTL to Recognize VSV in the Context of H-2K<sup>d</sup>-encoded Gene Products. To determine if VSV is recognized in the context of H-2K<sup>d</sup>- and/or H-2D<sup>d</sup>-encoded gene products, BALB/c anti-VSV effector cells were tested against a panel of VSV-infected target cells. The results, presented in Fig. 1, demonstrate that these effector cells are H-2 restricted and virus specific, as uninfected or Sendai-infected B10.D2 (K<sup>d</sup> D<sup>d</sup>) targets are not lysed (panels A and B), whereas B10.D2-VSV targets are killed (panel C). The fact that maximum isotope release from B10.D2-VSV target cells (panel C) is only 55% of the total suggests that only a portion of VSV-infected Con A lymphoblasts are susceptible to lysis.

In some but not all experiments, weak lysis also occurred against both uninfected and VSV-infected C57BL/6 (H-K<sup>b</sup>D<sup>b</sup>) targets as well as several other H-2-unrelated target cells (data not shown). This result may reflect the presence of crossreactive CTL clones; or alternatively, the generation of anti-H-2 CTL during in vitro culture. Whatever the mechanism, it is clear that direct measurement of cytotoxicity is not the best approach to determine the restriction loci for VSV recognition. Therefore, to more accurately assess whether H-2K<sup>d</sup>- or H-2D<sup>d</sup>-encoded molecules associate with VSV to create target antigens for anti-VSV CTL, we performed cold target competition experiments using BALB/c (K<sup>d</sup> D<sup>d</sup>) anti-VSV CTL and B10.D2-VSV (H-2<sup>d</sup>) targets. These studies demonstrated that only B10.D2 (K<sup>d</sup> D<sup>d</sup>) and B10.A(5R) (K<sup>a</sup> D<sup>a</sup>)-infected inhibitors blocked the lysis of B10.D2-VSV targets (Fig. 2); B10.D2(R103)(K<sup>d</sup> D<sup>b</sup>) (R103)-VSV inhibitors did not block the cytotoxic activity of BALB/c anti-VSV CTL. Although the inhibitory activity of B10.A(5R)-VSV cells seen in this experiment was less than that of B10.D2-infected cells, this was not

![Table II](https://example.com/table2.png)

<table>
<thead>
<tr>
<th>Antiserum or monoclon e</th>
<th>Donor/recipient</th>
<th>Anti-H-2&lt;sup&gt;d&lt;/sup&gt; specificity</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>23-10-1</td>
<td>BALB/c/H-2&lt;sup&gt;b&lt;/sup&gt;</td>
<td>H-2L&lt;sup&gt;d&lt;/sup&gt; (2.65)</td>
<td>22</td>
</tr>
<tr>
<td>30-5-7</td>
<td>BALB/c/H-2&lt;sup&gt;b&lt;/sup&gt;</td>
<td>H-2L&lt;sup&gt;d&lt;/sup&gt; (2.65)</td>
<td>22</td>
</tr>
<tr>
<td>34-2-12</td>
<td>(BD)F&lt;sub&gt;1&lt;/sub&gt;/C3H1</td>
<td>H-2D&lt;sup&gt;b&lt;/sup&gt; (2.4)</td>
<td></td>
</tr>
</tbody>
</table>

The reactivity of the two antisera represents potential specificities. The monoclonal reagents are specific for the H-2<sup>d</sup>-encoded antigens indicated. Monoclon 34-2-12 does not react with H-2L<sup>b</sup> or -R<sup>d</sup> (T. Hansen, personal communication).
observed in a repeat experiment. The data from this experiment, however, may indicate that the presence of H-2K\textsuperscript{b}, an antigen that restricts in the H-2\textsuperscript{b} haplotype, affects antigen presentation by the D-end of H-2\textsuperscript{d}.

The data above suggest that BALB/c anti-VSV killer cells are restricted solely by
products encoded in the H-2D region of the H-2^d haplotype. Further observations support this view: (a) B10.A(5R) (K^d D^d) mice mount a strong secondary anti-VSV CTL response when tested against P815-VSV (K^d D^d) targets, whereas R103 (K^d D^h) mice are nonresponders (unpublished data); and (b) H-2^d^1 (K^d D^d^1) and H-2^d^2 (K^d D^d^2) mice do not mount a secondary anti-VSV CTL response (Table III).

TABLE III

<table>
<thead>
<tr>
<th>Strain</th>
<th>E/T ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>100</td>
</tr>
<tr>
<td>B10.D2</td>
<td>50‡</td>
</tr>
<tr>
<td>BALB/c</td>
<td>54</td>
</tr>
<tr>
<td>H-2^d^1</td>
<td>10</td>
</tr>
<tr>
<td>H-2^d^2</td>
<td>7</td>
</tr>
</tbody>
</table>

*Mice were primed in vivo with 10^3 PFU of VSV. Approximately 6 wk later, the animals were killed and their spleen cells restimulated in vitro for 5 d with VSV-infected syngeneic cells.

‡ Percent specific release from VSV-infected P815 targets. Percent specific release = percent release in restimulated cultures - percent release in unstimulated cultures.

Fig. 2. Failure of H-2^K^a region-compatible inhibitors to block the cytotoxic activity of BALB/c anti-VSV CTL. The competition assay was performed using various numbers of inhibitor cells together with a constant number of effector cells (10^6) and radiolabeled B10.D2-VSV targets (10^4). The percent release in the absence of inhibitors was 55%, whereas 20% of the isotope was released when target cells were incubated with primed cells that were not reincubated with VSV in vitro (control release). Percent inhibition = 1 - ([percent specific release in presence of inhibitors]/[percent specific release in absence of inhibitors]) X 100. B10.D2 (□); B10.D2-VSV (●); B10.A(5R)-VSV (+); R103-VSV (×); B10.D2-Sendai (×).
Evidence that BALB/c Anti-VSV Cytotoxic Cells Are Not Restricted by the H-2D<sup>d</sup> Molecule. The previous data suggest that in the H-2<sup>d</sup> haplotype, VSV is recognized in the context of H-2D<sup>d</sup> and not H-2K<sup>d</sup>. This hypothesis was tested by cold target competition experiments using the H-2 mutant mouse strains H-2<sup>dm1</sup> and H-2<sup>dm2</sup>. The H-2<sup>dm1</sup> strain has altered H-2D and H2-L molecules and lacks H-2M (18), whereas the H-2<sup>dm2</sup> strain possesses a normal H-2D molecule but does not express H-2L and H-2R (17). The results presented in Fig. 3 demonstrate that unlike BALB/c VSV inhibitors, H-2<sup>dm1</sup> and H-2<sup>dm2</sup> VSV-infected competitor cells do not block the lysis of
P815-VSV or BALB/c-VSV target cells in the presence of BALB/c anti-VSV killer cells. Because H-2^{dmd} mice possess a wild-type H-2D^{d} molecule, these data indicate that VSV recognition is not restricted by H-2D^{d}. The fact that H-2^{dmd} expresses H-2M^{d} suggests that this molecule is also not involved in VSV recognition, although it cannot be ruled out that H-2M was affected by mutation. H-2^{dm1} expresses a mutant form of H-2D and H-2L, expresses H-2R (T. Hansen, personal communication), although it is not known whether this molecule has undergone mutation, and lacks H-2M. Taken together, these data demonstrate that H-2D^{d} is not the H-2D region-restricting locus and suggests that H-2L^{d} may serve in this function.

Recognition of VSV is Restricted by the H-2L^{d} Molecule. Because the previous data suggest that H-2L^{d} rather than H-2D^{d} is involved in associative recognition of VSV (see Fig. 3), we directly evaluated the role of H-2L^{d} as a restricting antigen. Two possibilities can be envisioned: either the H-2L molecule influences the orientation or conformation of H-2D, which facilitates an immunogenic interaction with VSV, or the anti-VSV T cell receptor is restricted by H-2L. To discriminate between these possibilities, anti-H-2 sera and monoclonal anti-H-2 antibodies were tested for their ability to block the cytotoxic activity of anti-VSV CTL in the CTL assay.

Antisera prepared against the whole H-2^{d} complex (B10.BR anti-B10.D2) or antigens encoded by the D-end of H-2^{d} (R103 anti-B10.D2) blocked anti-VSV CTL activity.

![Inhibition of anti-VSV CTL activity with antibodies specific for the H-2L^{d} molecule.](image)

**Fig. 4.** Inhibition of anti-VSV CTL activity with antibodies specific for the H-2L^{d} molecule. P815-VSV targets were incubated with antisera or monoclones before the addition of BALB/c anti-VSV effector cells (see Material and Methods). Percent inhibition was calculated as follows: 1 - ([percent specific release in presence of antiserum]/[percent specific release in presence of normal mouse serum]) \times 100, where percent specific released equals percent isotope release in stimulated cultures (either in the presence of immune or normal mouse serum) minus percent isotope release in unstimulated cultures. In this experiment, spontaneous release was 7%, whereas 27% of the isotope was released in the presence of normal mouse serum. E/T ratio was 100:1. The antisera and monoclones were: B10.BR anti-B10.D2, anti-H-2^{d} (O); R103 anti-B10.D2, anti-H-2D^{d}-region (C); 25-10-1, anti-H-2L^{d} (*); 30-5-7, anti-H-2L^{d} (X); 34-2-12, anti-H-2D^{d} (+).
activity in a dose-dependent fashion (Fig. 4). The ability of the B10.D2(R103) anti-B10.D2 (anti-D region) serum to block anti-VSV activity against P815-VSV targets is consistent with the view that VSV is not \( H-2K \)-end restricted. That this inhibition was due to antibody binding to the H-2L molecule and not to H-2D can be seen by the ability of hybridomas specific for H-2L\(^d\) (H-2.65) to block CTL activity. This is particularly true for the 30-5-7 (IgG\(_{2a}\)) monoclonal, which was very inhibitory even at low concentrations. On the other hand, a hybridoma specific for H-2D\(^d\) (34-2-12), but not other H-2D-end-encoded molecules, had no inhibitory activity. Thus, these data clearly demonstrate that in the \( H-2^d \) haplotype, VSV is restricted solely by H-2L.

To verify the specificity of these reagents, alloreactive CTL specific for either H-2K\(^d\), -D\(^d\), or -L\(^d\) molecules were generated and various quantities of antibody were then tested for their ability to block CTL activity against particular H-2 molecules.
The data in Fig. 5 illustrate that R103 anti-B10.D2 (anti-D\textsuperscript{d-region}) serum is \textit{D-end} specific. For example, this serum blocked the lytic activity of H-2D-region-specific alloreactive CTL (R103 anti-BALB/c) (panel A), whereas it had no effect on the lytic activity of B10.D2(R107) (abbreviated R107) anti-BALB/c CTL (anti-H-2K-end) (panel B). As a control, we demonstrated that an antisem (B10.BR anti-B10.D2) directed against antigens from the entire anti-H-2\textsuperscript{d} haplotype blocks both anti-H-2K-end and anti-H-2D region CTL responses (panels A and B). A hybridoma specific for the H-2L\textsuperscript{d} molecule (30-5-7) inhibited the activity of H-2\textsuperscript{dm2} anti-BALB/c CTL (anti-H-2L\textsuperscript{d}) (panel C), but this antibody, as well as monoclonal 23-10-1 (anti-H-2L\textsuperscript{d}), was not inhibitory for R103 anti-H-2\textsuperscript{dm2} effector cells (panel D). It should be noted that H-2\textsuperscript{dm2} anti-BALB/c effector cells could potentially display lytic activity against H-2R, as H-2\textsuperscript{dm2} animals lack this molecule. However, all the CTL activity was blocked with the anti-H-2L\textsuperscript{d} monoclonal (anti-2.65) (panel C and data from two other experiments [not shown] where the net isotope release at effector/target (E/T) ratio = 100 was 64.9 and 20.9). Therefore, these data indicate that H-2R is not recognized by H-2\textsuperscript{dm2} anti-BALB/c alloreactive T cells. Finally, an H-2D\textsuperscript{d}-specific hybridoma (34-2-12) caused no inhibition of the anti-H-2L\textsuperscript{d} CTL reaction (panel C), whereas anti-H-2D\textsuperscript{d} CTL activity was inhibited in a dose-dependent fashion (panel D). Thus, these antisera and hybridomas are functionally specific for individual H-2 molecules.

In summary, because monoclonal 23-10-1 and 30-5-7 are specific for H-2L\textsuperscript{d} and do not react against H-2R\textsuperscript{d}, and H-2\textsuperscript{dm2} mice express H-2D\textsuperscript{d} and H-2M\textsuperscript{d} but their cells do not block CTL activity, these data indicate that H-2L\textsuperscript{d}, rather than H-2D, H-2M, or H-2R, restricts anti-VSV recognition in H-2\textsuperscript{d} mice.

Discussion

Anti-viral CTL are thought to play an important role in preventing the spread of virus infection. This is presumably achieved by the destruction of virus-infected cells by CTL during the eclipse period, a period before the assembly of mature infectious virus particles. This implies that viral antigens appear on the plasma membrane before the release of virus progeny, a view which is substantiated by kinetic studies in many systems (7, 12). With all viruses tested to date, H-2K- or H-2D-region compatibility between effectors and target cells is required for recognition of viral antigens by anti-viral CTL (1).

We have demonstrated that BALB/c (H-2\textsuperscript{d}) anti-VSV CTL are similarly restricted by these same loci; in this study, compatibility at H-2L\textsuperscript{d} was required, whereas H-2K\textsuperscript{d}-restricted recognition could not be demonstrated. In addition, H-2D\textsuperscript{d}-restricted recognition could not be detected by cold target competition analysis or blocking studies with an anti-H-2D\textsuperscript{d}-specific hybridoma. However, VSV recognition was completely inhibited by two H-2L\textsuperscript{d} specific monoclonal. Although two other H-2D-region-encoded gene products, designated H-2M and -R, have been recently identified in the H-2\textsuperscript{d} haplotype (17, 18), these molecules do not serve as restricting loci for VSV recognition in this mouse strain. Thus, VSV-infected cells obtained from H-2\textsuperscript{dm1} mice that express H-2M but not H-2L (18) do not block the lysis of BALB/c-VSV targets by BALB/c effector cells in a cytotoxic assay. Further, the 30-5-7 monoclonal (anti-H-2.65, anti-H-2L\textsuperscript{d}) does not react with the H-2R molecule (17), but completely inhibits recognition of VSV-cells by BALB/c CTL. Therefore, in H-2\textsuperscript{d} mice, VSV is recognized exclusively in the context of H-2L\textsuperscript{d}.
The failure to detect H-2K and D locus-restricted recognition of this virus may indicate that VSV-G protein, the envelope glycoprotein recognized by killer cells (25), may not associate in an antigenic fashion with H-2K^d and H-2D^d molecules on the cell membrane. However, other explanations are possible and further studies are required to determine the basis of this unresponsiveness.

Previous reports have indicated that T cells possess a more restricted repertoire of responses restricted by H-2L than with H-2K or H-2D. For example, H-2L-restricted cytotoxic responses have not been detected against trinitrophenol (26) and minor histocompatibility antigens (27). However, studies by Blanden et al. (28) have indicated a possible role for the H-2L molecule in T cell recognition of viral antigens. These investigators reported that a strong H-2D^d-restricted CTL response against ectromelia virus could be elicited in BALB/c but not H-2dm2 mice. Similarly, H-2dm2-infected macrophages could not elicit secondary, H-2D^d-restricted anti-ectromelia killer cells from in vivo primed cells. However, H-2dm2-infected targets were as susceptible to lytic attack by H-2D^d-restricted anti-ectromelia CTL as BALB/c infected targets. These authors suggested that the H-2L^d molecule was not necessary for H-2D^d-restricted recognition of this virus by activated cytotoxic effector cells, but rather that H-2L played some role, perhaps in the orientation or conformation of the H-2D^d molecule, which facilitated the putative associations between H-2D^d and viral antigens required for triggering CTL precursors.

Indirect evidence for H-2L-restricted recognition of viral antigens has been obtained using monoclonal antibodies specific for H-2D^k-encoded determinants (29). These antibodies blocked H-2D^k-restricted anti-influenza CTL activity but not anti-Bebaru virus killer cells. One interpretation of these data is that another D-end-encoded molecule, presumably H-2L^k, serves as the restriction antigen for Bebaru virus. However, an alternative interpretation is that influenza and Bebaru virus have different restriction determinants on the H-2D^k molecule. In the latter case, these determinants are sufficiently distant from the allodeterminants recognized by the monoclonal antibodies, so that no steric hinderance by anti-Bebaru T cells occurs. In support of this, Weyand et al. (30) have demonstrated with monoclonal antibodies that there are different target regions on H-2 molecules.

The first direct evidence for the involvement of H-2L gene products in virus recognition was reported by Biddison et al. (31), who showed, using anti-H-2L^d and H-2D^k-specific hybridomas, that influenza virus elicits two T cell subsets that are restricted by either H-2L or H-2D. However, because no recombinant strains have been detected that separate H-2L^d from H-2D^k, it cannot be determined if H-2L^d can restrict recognition without a similar activity by H-2D^d. For example, H-2L may contain only one exon that can encode a restricting region and that is duplicated in the H-2D gene. On the other hand, H-2L may contain an additional (nonduplicated) exon that can also restrict antigen recognition. This possibility would predict that whenever H-2L restriction is observed for a given antigen, H-2D restriction would also be found, but not vice-versa. However, the data in the present report demonstrate that mouse strains with the H-2d haplotype use H-2L exclusively as the restricting locus for anti-VSV CTL. Thus, H-2L uses a unique determinant involved in H-2-restricted recognition of virus, further substantiating the role of this molecule as a major class I antigen.

Finally, the fact that H-2dm2 effector cells sensitized against BALB/c (anti-H-2L^d,
H-2L-Restricted Antiviral Cytotoxic T Lymphocytes

H-2L-encoded gene products were analyzed as restriction antigens for anti-vesicular stomatitis virus (VSV) cytotoxic T lymphocytes (CTL). Cold target competition experiments revealed that VSV recognition was H-2D region-restricted; H-2K-end-restricted recognition of VSV could not be demonstrated. That VSV is not recognized in the context of K-region-encoded gene products is also supported by the observation that H-2^dm1 and H-2^dm2 mice, strains that contain H-2K^d but have an alteration in H-2L and/or H-2D/L, are nonresponders in the CTL assay.

Two different lines of evidence eliminated H-2D^d, H-2M^d, and H-2R^d as the restriction antigens: (a) H-2^dm1-VSV inhibitors that express H-2D^d and H-2M^d did not block the lysis of P815-VSV targets by BALB/c anti-VSV killer cells, and (b) a hybridoma specific for H-2D^d failed to inhibit killer cell activity in this same effector/target combination. However, two monoclonal antibodies specific for H-2L^a but not H-2R^a completely blocked anti-VSV cytotoxic activity. Taken together, in the H-2^a haplotype, anti-VSV CTL recognize VSV solely in the context of the H-2L^a molecule.

This is the first demonstration of the exclusive use by a mouse strain of the H-2L molecule only for H-2-restricted recognition, and thus supports the notion that H-2L plays a major role in restricting antigen specific recognition. Finally, the fact that an anti-H-2L^a monoclonal completely blocked an H-2^dm1 anti-BALB/c CTL response indicates that H-2R, a molecule absent in H-2^dm1 but not BALB/c, does not sensitize H-2 alloreactive CTL.

We wish to thank Julia Tsan and Phyllis Jones for their excellent technical assistance. We thank Ms. B. J. Washington for typing this manuscript.

Received for publication 19 April 1982 and in revised form 7 June 1982.

References


