DISSECTION OF SEROLOGICAL AND CYTOLYTIC T LYMPHOCYTE EPITOPES ON MURINE MAJOR HISTOCOMPATIBILITY ANTIGENS BY A RECOMBINANT H-2 GENE SEPARATING THE FIRST TWO EXTERNAL DOMAINS

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Major histocompatibility (H-2) antigens are cell surface glycoproteins that serve as targets for both allospecific and virus-restricted cytolytic T lymphocytes (CTLs)1 (for reviews see references 1, 2). However, the precise nature of the molecular interactions involved in the recognition of the H-2 molecules is poorly understood. The nature of these interactions was originally defined by analyzing the ability of T cells to recognize target cells bearing genetically altered H-2 molecules. Early studies demonstrated that the determinants on the H-2 molecules that are recognized by T cells are affected by variations that have arisen spontaneously in mice (3) and humans (4). All of the variants contained alterations in the 44,000-dalton subunit of the H-2 antigen. Subsequent studies have begun to map the functional regions of this peptide more accurately.

The H-2 antigen is comprised of two subunits—β2-microglobulin (12,000 daltons) and H-2 (44,000 daltons). The portion of the H-2 subunit that sits on the cell surface appears to fold into three external domains (N, C1, and C2) (5). Each of these domains is encoded by a separate coding block in the H-2 gene. Through the application of recombinant DNA technology, we and others (6-15) have begun to define the domains of the H-2 molecule that encode the determinants recognized by the T cells. That is, H-2 genes have been cloned, altered in vitro and reintroduced into mouse L cells. The ability of T cells to...
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recognize the L cells bearing altered H-2 antigens on their surfaces has been examined. The initial constructs exchanged the N and C1 exons between the H-2Ld and H-2Dd genes and suggested that allospecific and virus-restricted CTLs utilize determinants encoded within the N and/or C1 domains (14, 15).

To further define the epitopes that are involved in immune recognition of these antigens, we have constructed a new H-2 gene. This H-2 gene was made by replacing the N-terminal domain (N) of the H-2Ld gene with the N domain of the H-2Dd gene. The biological function of the cells expressing this hybrid H-2 gene on its cell surface were explored for serologic and CTL recognition.

Materials and Methods

Plasmid Construction. A hybrid H-2 gene was made from restriction enzyme fragments using standard DNA technology (16). In both plasmids pDd-1 and pLd-4 SmaI sites were located in the intervening sequences between the leader, the N, and C1 exons. In plasmid pLd-4, however, one additional SmaI site is present, located 5' of the leader sequence in the promoter region. In order to remove this 5' SmaI site, plasmid pLM was used in which the normal H-2 promoter was replaced by the metallothionein promoter (J. W., and J. G. S., unpublished results). This was done by cloning a BamHI partial digested fragment encoding the H-2Ld gene into the BglII site of plasmid pMK (17; J. W. and J. G. S., unpublished results). However, plasmid pLM contains several SmaI sites in the 3' end of the plasmid (17; J. W. and J. G. S., unpublished results). To remove these SmaI sites, a 2.2-Kb BamHI fragment of pLd-4, encoding the 3' end of the H-2Ld gene (6) was ligated to the 8-Kb BamHI fragment of pLM and used to transform E. coli strain 322 (18). The resulting plasmid pLL was extensively mapped by restriction endonuclease cleavage to confirm the reconstruction.

Plasmids pLL and pDd-1 were subsequently digested with SmaI. A 250-bp SmaI fragment of pDd-1 encoding the first external domain, was isolated and ligated to the 10-Kb SmaI fragment of pLL. The ligation mixture was reintroduced into E. coli LE392 by CaCl2-induced transfection. Colonies were screened for the insert by colony hybridization, using the 250-bp SmaI fragment as a probe. Isolated plasmids were checked for the orientation of the SmaI insert by M13 sequencing. Briefly, plasmids were digested with Sall and subcloned into M13 mp9. Plaques were screened for inserts using the 250-bp SmaI fragment as a probe. The inserts of these M13 recombinants were sequenced by the dideoxy termination method (19, 20).

Transfection of Plasmid pLD into L Cells and Determination of Binding of Monoclonal Antibodies. Plasmid pLD, encoding the recombinant H-2 gene, was introduced into thymidine kinase-deficient mouse L cells (DAP-3) by DNA-mediated gene transfer using the herpes simplex thymidine kinase gene as a selectable marker (21, 22). Serological characterization of H-2 transformants by monoclonal antibodies having specificities for H-2Ld or H-2Dd was carried out by radioimmunoassay as described previously (13). Briefly, cells dissociated by trypsin treatment were seeded in 96-well microtiter plates at 1 X 10^5 cells/ml in 150 μl culture medium and incubated overnight at 37°C in 7% or 10% CO2 in humidified air. After medium was removed by flicking the plate, 40 μl of monoclonal antibodies contained in culture supernatant were added, and cells were incubated for 45 min at 8°C. Cells were washed three times with culture medium and 40 μl of 125I-sheep anti-mouse IgG (heavy chain and light chain specific) (Amersham, Arlington Heights, IL) and diluted in medium at 1:100. Cells were incubated an additional 45 min at 8°C, followed by three rinses. Cell-bound 125I-antibodies were recovered by dissolving the cells in 100 μl a 1% solution of Triton X-100 and radioactivity was measured in a γ-spectrophotometer. The cloned cell lines T1.1.1 and T4.8.3, encoding an H-2Ld and H-2Dd gene, and monoclonal antibodies specific for H-2Dd and a H-2Ld determinant have been described elsewhere (6, 9, 23, 24).

Immunoprecipitation of H-2 Antigens. The cloned cell lines T1.1.1 and T9.10.3 were iodinated with 125I as previously described (25). The immunoprecipitation was performed...
as described (26). SDS-polyacrylamide gel electrophoresis was carried out on a discontinuous vertical slab gel using a modification of the Laemmli procedure (27). A gradient gel was made in 10–15% acrylamide. The gels were dried and exposed for 3 d.

Assays of Cell-mediated Cytotoxicity. BALB/c mice were immunized parenterally with either vesicular stomatitis virus (VSV; 10^7 pfu) or influenza A/PR/8/34 virus (PR8; 100 HAU). 1 wk later the mice were sacrificed by cervical dislocation and their spleens used as a source of virus-immune lymphocytes. 5-d secondary in vitro antiviral CTL cultures were established with 5 × 10^6 primed lymphocytes and 1 × 10^6 irradiated BALB/c splenocytes, which were infected with either 10 pfu tsG41 VSV at permissive temperature or 10 EID~0 PR8 virus/cell in 17-mm Costar clusters with RPMI 1640 medium supplemented with 10% fetal bovine serum (FBS), penicillin, streptomycin, L-glutamine, and 5 × 10^{-5} M 2-mercaptoethanol. Primary allospecific CTLs were generated in 5-d cultures of 5 × 10^6 naive lymphocytes derived from either C57B1/6j or C-H-2^dm^- mice cultured with 1 × 10^6 irradiated C-H-2^dm^- or C3H or BALB/c stimulator cells using the same medium. Virus-specific or allospecific effectors were harvested and dispensed in duplicate at 1 × 10^6, 3 × 10^5, and 1 × 10^5 cells/V-bottom microtiter well (Linbro) in 0.1 ml MEM supplemented with 10% FBS, nonessential amino acids, penicillin, streptomycin, and L-glutamine. L cell targets, prepared by versene treatment, were divided in three portions and infected with either 10 pfu wt VSV or 10 EID~0 PR8 virus, or left uninfected. L cells were pulsed with 0.1 mCi Na_2^{51}CrO_4 for 1 h at 37°C, incubated an additional 1 h, and then washed three times. L cell targets were dispensed at 1 × 10^4 in 0.05 ml volumes into wells containing effectors, normal spleen cells or 0.1 ml 5% Triton X-100 (to measure spontaneous release and total release, respectively). Plates were centrifuged at 100 g and then incubated at 37°C for 5 h. Supernatants (0.1 ml/well) were harvested and counted for ^{51}Cr release. Percent specific release was calculated by the formula = 100 × (experimental cpm - control cpm)/(total cpm - control cpm).

Cold Target Inhibition of CTL Activity. Unlabeled targets were prepared by removing transformed L cells from flasks with versene. For each target equal portions were either untreated or infected with influenza A/PR/8/34 virus (10 EID~0/cell) and incubated at 37°C for 1 h. The cells were washed and counted and dispensed in 50-μl volumes (four serial twofold dilutions 2 × 10^6/ml to 0.25 × 10^8/ml in duplicate) into V-bottom microtiter wells. Next, ^{51}Cr-labeled targets, prepared as described above, were added in 50-μl volumes from a 2 × 10^4/ml suspension. Lastly, BALB/c effectors specific for PR8 virus were added in a 50-μl volume from a 2 × 10^5/ml suspension. Plates were spun as described above and incubated for 5 h before taking aliquots of supernatant for counting.

Results

Construction of a Novel H-2D^d/H-2L^d Recombinant Gene. The availability of transformed cell lines expressing new H-2 recombinant antigens has allowed us to map serological and CTL determinants to discrete portions of the H-2 molecule (13-15). In order to define more precisely the location of these determinants, we constructed a hybrid H-2 gene by replacing the H-2L^d exon encoding the N domain with the H-2D^d exon. Several restrictions endonuclease cleavage sites, including Smal sites, are conserved between the H-2L^d and H-2D^d sequences. Specifically, in plasmids pD^d-1 and pLL, two Smal sites are present. One Smal site is located in the intervening sequence between the leader and N-exon. Another Smal site is between the N and C1 exon of the H-2D^d gene (Fig. 1; C. M. and J. G. S., unpublished results). These constructions were digested with Smal and a Smal fragment of the H-2D^d gene encoding the N domain was ligated to the 10-Kb Smal fragment of pLM (Fig. 1, panel A). If correctly expressed, the product of the recombinant plasmid (pDL) would contain the N domain of one transplantation antigen and the C1, C2, TM, and internal domains of the other (Fig. 1, panel B).
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Expression of the Novel Recombinant H-2 Gene in L Cells and Determination of the Location of Serological Specificities. To determine whether the novel recombinant gene is expressed when introduced into mouse L cells, DNA from this recombinant gene together with the herpes viral thymidine kinase gene, was used to co-transform thymidine kinase-negative mouse L cells as previously described (21, 22). Transformants were selected in hypoxanthine-aminopterin-thymidine (HAT) medium and screened with monoclonal antibodies specific for H-2L^d and H-2D^d specificities. Antibody-positive cells were further characterized with a series of antibodies whose specificities were previously mapped to either N/C1 or C2 domains of the two antigens. The results are shown in Table I. As expected, monoclonal antibody 28.14.8, previously shown to bind to the C2 domain of the H-2L^d molecule (13), recognizes the recombined gene product of the transformant T9.10.3. Monoclonal antibody 34.2.12, recognizing a determinant of the C2 domain of the H-2D^d monoclonal antibody did not bind significantly to the recombinant molecule, confirming that the 3' end of this gene is derived from the H-2L^d.
Table 1

Monoclonal Antibody Binding to the N-C1 Hybrid H-2 Antigen*

| Monoclonal antibody | Specificity$ | Antibody class | Transformed cells ($^{125}$I bound, cpm/well) |
|---------------------|-------------|----------------|-----------------------------------------------|
|                     |             |                | DAP-3 | T1.1.1- (LdLdLd)* | T485- (DdDdDdDd) | T9.10.3- (DdLdLd) |
| 16.1.2              | KdDd        | IgG2a          | 2,341 ± 23 | 1,128 ± 19 | 1,952 ± 11 | 2,290 ± 21 |
| 17.3.3              | Ia$^b$      | IgG2a          | <200 | <200 | <200 | <200 |
| 27.11.13            | Dd & Ld     | IgG2a          | <200 | 1,331 ± 18 | 1,650 ± 11 | 1,703 ± 28 |
| 34.4.20             | Dd & Ld     | IgG2a          | <200 | 1,985 ± 23 | 1,580 ± 28 | 2,253 ± 20 |
| 28.11.5             | Dd & Ld     | IgM            | <200 | 438 ± 15 | 380 ± 23 | 518 ± 9 |
| 28.14.8             | Ld, C2      | IgG2a          | <200 | 1,609 ± 29 | <200 | 2,018 ± 45 |
| 23.10.1             | Ld N/C1 IgM | <200 | 615 ± 18 | <200 | 654 ± 8 |
| 30.5.7              | Ld N/C1 IgG | <200 | 1,885 ± 33 | <200 | 2,200 ± 18 |
| 34.2.12             | Dd C2       | IgG2a          | <200 | <200 | 1,428 ± 21 | <200 |
| 23.5.1              | Dd N/C1 IgM | <200 | <200 | 453 ± 5 | 638 ± 31 |
| 28.8.6              | Dd N/C1 IgG | <200 | <200 | 508 ± 18 | 378 ± 9 |
| 34.4.21             | Dd N/C1 IgM | <200 | <200 | 620 ± 5 | 598 ± 9 |
| 34.5.8              | Dd N/C1 IgG | <200 | <200 | 1,241 ± 17 | 388 ± 30 |
| 34.1.2              | Dd N/C1 IgG | <200 | <200 | 1,532 ± 38 | 1,980 ± 12 |

* The transformed cells were placed in wells of 96-well microtiter plates at the concentration of 1 × 10⁵ cells/well and incubated overnight. Monoclonal antibodies in supernates were added to the cells, and antibody binding was monitored by subsequent incubation with $^{125}$I-sheep anti-mouse IgG. Each value represents mean of triplicates ± standard deviation.

$^b$ The origin of the gene for each domain shown in the order of the N, C1, and C2.

$v$ Specificity of the antibodies for gene products and domains have been assigned previously (13, 23, 24).

Monoclonal antibodies 34.4.20, 27.11.13, and 28.11.5, which show cross-reacting specificities, reacted in all cases with transformant T9.10.3, confirming that the exon shuffling has not greatly altered the conformational structure of the protein. Because of the nature of $^{125}$I-anti-mouse reagents, IgM antibodies gave consistently lower counts than IgG antibodies.

Two monoclonal antibodies, 23.10.1 and 30.5.7, specific for N and/or C1 domain of H-2Ld reacted with the transformed cell line T9.10.3. Thus, the specificities of both monoclonals are likely to be mapped to the C1 domain of the H-2Ld antigen. Four of the five monoclonal antibodies, with specificities for H-2Dd determinants, recognized H-2 molecules on transformed T9.10.3, suggesting that reactivities of these antibodies were mapped to the N domain of the H-2Dd antigen. The level of these determinants expressed on the hybrid molecule was comparable to those expressed on native H-2Ld or H-2Dd molecules, since no major difference in antibody binding was found between the hybrid and native molecules of antibodies. However, very weak binding of monoclonal antibody 34.5.8 (H-2Dd) to the transformed T9.10.3 was detected, which may indicate that this antibody reacts with the C1 domain of H-2Dd antigens, or to a conformational determinant dependent upon the integrity of the N and C1 domains of H-2Dd. Although most of the anti-H-2Dd and all of the anti-Ld antibodies presented in this paper reacted with the transformant, lack of reactivity was found with some additional monoclonals reactive with N and/or C1 domains of H-2Dd or H-2Ld antigens. These monoclonal antibodies have been obtained recently, and their detailed characterization will be presented elsewhere. Thus,
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FIGURE 2. SDS-PAGE analysis of ^125I-labeled surface antigens immunoprecipitated from clone T1.1.1, expressing the complete H-2L^d gene and clone T9.10.3 expressing the recombinant gene.

this antigen represents "gain and loss" alteration with respect to the serological specificities of the H-2D^d and H-2L^d antigen.

Immunoprecipitation. In order to determine the molecular weight of the recombinant gene product and its association with $\beta_2$-microglobulin, the hybrid antigen was isolated from the cell surface. Membrane proteins were labeled by lactoperoxidase-mediated iodination of the transformed cells. The proteins were immunoprecipitated by monoclonal antibody 28.14.8, which recognizes a determinant of the C2 domain of the H-2L^d protein (13). The immunoprecipitates were analyzed by SDS-polyacrylamide gel electrophoresis (Fig. 2). The gel shows clearly that the recombinant H-2 antigen has a molecular weight (45,000 daltons) similar to that of the wild-type H-2 molecule. Moreover, the protein is associated with $\beta_2$-microglobulin.

Localization of Determinants Recognized by CTLs Using the Transformant T9.10.3. The ability of the recombinant gene product to serve as a target for allogeneic and virus-specific CTLs was tested. Target cells expressing this hybrid H-2 molecule also enabled one to localize CTL determinants to either the N or C1 domains.

a. Allospecific CTL recognition. CTLs generated by stimulating B6 (H-2^b) spleen cells with C3H (H-2^a) cells lysed all L cells (Table II). C-H-2^dm2 mice express H-2K^d and H-2D^d and background antigens of BALB/c mice, but do not express H-2L^d antigens, therefore, CTLs from cultures of dm2 splenocytes
Table II
Allospecific and Virus-specific Recognition of N-C1 Recombinant Line T9.10.3

| Effector* | H-2 gene | Target cells | T9.10.3 |
|-----------|----------|--------------|----------|
|           |          | P815         | T1.11    | T4.8.3   |
| I         |          | L* L* L* /  | D*D*D    |          |
| BALB/c    |          | 50, 35, 21   | 30, 26, 13| 8, 4, 0  |
| B6        |          | 77, 82, 29   | 42, 37, 17| 40, 34, 23 |
| dm2 anti-BALB/c |    | 95, 84, 93   | 80, 57, 37| 23, 9, 6  |
| II        |          | C3H         | 89, 88, 27| 4, 0, 0   |
| B6        |          | 22, 5, 0     | 56, 33, 17| 72, 39, 20|
| dm2 anti-BALB/c |    | 84, 81, 60   | 59, 30, 16| 11, 6, 5  |
| III       |          | ND          | 64, 50, 25| 66, 64, 48|

* Virus-specific effectors were generated by in vivo priming with either 10⁷ pfu VSV or 100 HAU influenza A/PR/8/34 virus 1 wk before sacrifice and in vitro restimulation of splenocytes with virus-infected syngeneic cells as described in Materials and Methods. Allospecific effectors resulted from primary in vitro culture of splenocytes with irradiated stimulator cells.

Virus-specific effectors were limited to recognizing the H-2L₃ molecule. These effectors lysed all cells expressing H-2L₃ products (Table II). The transformant T9.10.3 was recognized less efficiently than T1.11 (H-2L₃ transformant) or cells expressing the hybrid gene possessing the N and C1 domains of H-2L₃ (data not shown), suggesting that some but not all determinants recognized by alloreactive CTLs are located on the C1 domain of H-2L₃. The loss of determinant(s) recognized by effectors specific for H-2L₃ can be due to a determinant(s) that maps in the N domain or to an epitope(s) that is formed by the interaction of both the N and C1 domains of H-2L₃. Allospecific CTLs for the H-2D₃ antigen were prepared by sensitization of C3H splenocytes with dm2 cells. These CTLs lysed all lines expressing the H-2D₃ antigen including the recombinant, T9.10.3. This maps some allospecific epitopes to both the N and C1 domains.

b. Influenza virus-specific CTLs. We have previously shown that BALB/c effectors specific for influenza virus are restricted to both the H-2D₃ and H-2L₃ molecules expressed on L cells following DNA-mediated gene transfer (15). In order to test whether influenza virus specific CTLs could recognize infected cells expressing the recombinant H-2 molecule, BALB/c mice were immunized with PR8 virus. Secondary in vitro cultures elicited CTLs that lysed all of the transformants shown in Table II. The recombinant T9.10.3 was recognized less efficiently than T1.11 or T4.8.3, suggesting that some virus-restricting determinants were lost after recombination.

Cold target inhibition assays were used to further map determinants detected by influenza virus-specific CTLs. Specifically, the inhibition of influenza virus-specific assays revealed the presence of a virus-restricting epitope located on the C1 domain of H-2L₃.
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specific CTL lysis of $^{51}$Cr labeled (hot) targets was examined in the presence of excess unlabeled (cold) target cells. As shown in Fig. 3, lysis of labeled T4.8.3 and T1.1.1 was partially blocked by infected, but not uninfected, unlabeled T9.10.3 cells. Virally infected cold target cells expressing the hybrid molecule were not as efficient as were infected cold target cells expressing the same transferred gene as the hot target cells. These results suggest that some determinants that restrict influenza virus-specific CTLs are present in both the N domain of H-2D$^d$ and the C1 domains of H-2L$^d$.

c. VSV-specific CTLs. CTLs from BALB/c mice recognize VSV in association with H-2L$^d$, but not with H-2D$^d$ (28). Recently, we have shown that restricting elements recognized by VSV-specific effector cells are located in the N and/or C1 domain of the H-2L$^d$ antigen (15). Transformant T9.10.3 was used in an attempt to further map these VSV-restricting elements. BALB/c mice were immunized with VSV virus and these VSV-specific CTLs lysed infected P815 and T1.1.1 targets efficiently. However, infected T4.8.3 and T9.10.3 were not lysed by VSV-specific effector cells. Thus, it appears that determinants for VSV-specific CTLs either map exclusively to the N domain or are dependent upon conformation that is lost when external domains are exchanged.

Discussion

Major histocompatibility antigens are the primary target of both humoral and cellular responses. The portions of these antigens that are involved in the specific interactions that permit the specific recognition of cells bearing these antigens remains uncertain. One approach to dissecting the H-2 antigen has been to examine the ability of antisera and T cells to recognize genetically altered H-2 antigens on the cell surface. These studies have been extended by the application of recombinant DNA technology to generate hybrid H-2 genes that encode novel H-2 molecules. We have described here one such hybrid H-2 antigen-formed by exchanging the N domains of the H-2D$^d$ and H-2L$^d$ antigens. Using
TABLE III
Tentative Assignment of Polymorphic Determinants in the External Domains of the H-2 Antigen

| Antigen | Domain |
|---------|--------|
|         | N      | C1     | C2     |
| H-2L<sup>d</sup> | 30.5.7 | 28.14.8 |
|         | 23.10.1|
| H-2D<sup>d</sup> | 23.5.1 | 34.5.8*| 34.2.12 |
|         | 28.8.6 |
|         | 34.1.2 |
|         | 34.4.21|

cells expressing this hybrid H-2 antigen, we have further mapped serological and CTL determinants on the H-2 molecule.

Previously we had located serological and CTL determinants to the N and/or C1 domains of H-2D<sup>a</sup> and H-2L<sup>a</sup> using hybrid H-2 antigens created by exchanging exons from the genes of these two antigens. The recombinant H-2 antigens expressed on L cells following DNA-mediated gene transfer were used as targets for CTLs that were either H-2D<sup>a</sup> or H-2L<sup>a</sup> restricted. CTLs specific for four different antigens were used. All four different types of CTLs would only recognize L cells expressing the N and C1 domains of the restricting allele (14, 15). By contrast, serological determinants were identified that mapped to the C2 domain as well as the N and C1 domains of these H-2 antigens (13).

The hybrid H-2 antigen described here has been used to further map determinants that were previously mapped to the N-terminal two domains (N and C1). Serological determinants map to both the N and C1 domains (Table III). Furthermore, there are allospecific (Table II) and influenza virus–specific determinants that can be mapped to either the N or C1 domains (Table II and Fig. 3). Since CTLs and antibodies could recognize this hybrid molecule, we suggest that the conformation of the hybrid molecule is not grossly altered from that of the H-2L<sup>a</sup> or H-2D<sup>d</sup> molecule. However, there has clearly been a loss of some determinants recognized by the population of CTLs; these determinants are either localized to the domain that is missing in the recombinant molecule or to epitopes comprised of the interaction of the N and C1 domains in the native molecule. We assume that different CTL clones, although specific for the same protein, are interacting with different portions of the H-2 antigen. This conclusion was also made from studies of the ability of CTLs to recognize mutant H-2 molecules (29–32).

Although influenza virus and allospecific CTLs could recognize the hybrid H-2 antigen, VSV-specific CTLs could not recognize VSV in association with the hybrid antigen. This finding suggests that either the conformation of the hybrid antigen is sufficiently altered to prevent the VSV-specific CTLs from recognizing the cells expressing the hybrid antigen or the VSV determinants are exclusively located on the N domain of the H-2L<sup>d</sup> antigen. It should be remembered that this analysis has been performed with uncloned CTL populations. It is possible that a minor population of VSV specific clones exists that can recognize VSV in association with the C1 domain. We are currently constructing the hybrid
antigen in which the N domain is derived from the H-2L<sup>d</sup> gene. L cells expressing this hybrid antigen should be useful in determining which of these models are correct.

We have used a new hybrid H-2 antigen to further map the portions of the H-2 antigen that act as restricting elements for CTLs. The important conclusion of this analysis is that different CTLs utilize different portions of the H-2 antigen and in particular that there are sites on the C1 domain of the H-2L<sup>d</sup> antigen that are recognized by influenza-specific CTLs but not VSV-specific CTLs. Eventually further studies of this type, in conjunction with protein chemical studies, may reveal the precise molecular nature of the interactions that are involved in H-2-restricted recognition of target cells by CTLs.

**Summary**

A novel H-2 gene in which the first external (N) domain of the H-2L<sup>d</sup> antigen was replaced with that of the H-2D<sup>d</sup> antigen was constructed and introduced into L cells. A transformant expressing the products of the hybrid gene was studied for binding to monoclonal antibodies specific for H-2L<sup>d</sup> and H-2D<sup>d</sup> antigens. It was found that serological determinants are distributed both in the N (D<sup>d</sup>) and C1 (L<sup>d</sup>) domains. Determinants recognized by allospecific cytotoxic T lymphocytes (CTLs) and virus-specific CTLs also mapped to the N and C1 domains. Determinants recognized by vesicular stomatitis virus (VSV)-specific effect cells, however, were not present on the recombinant molecule. These results show that a recombinant gene of two H-2 antigens in which the first external domain has been reshuffled can express a functional H-2 antigen that can then be used to map serological and CTL determinants to specific domains.

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