

A molecular hybrid of the *H-2D^d* and *H-2L^d* genes expressed in the *dm1* mutant

(hybrid class I antigen/B10.D2-*H-2^{dm1}*/hemoglobin Lepore-like recombination)

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ABSTRACT Sequential immunoprecipitates show that *H-2^{dm1}* mutant cells express a hybrid “H-2D/L” antigen exhibiting determinants normally associated with two different gene products of the parental *d* haplotype—i.e., the *H-2D^d* and *H-2L^d* antigens. The hybrid *H-2D/L^{dm1}* antigen appears to consist of a portion of the NH₂-terminal extracellular half of the *H-2D^d* antigen “fused” to a portion of the COOH-terminal extracellular half of the *H-2L^d* antigen. This structure is inferred from the reactivity of *dm1* antigens with cytotoxic T lymphocytes specific for *H-2L^d* determinants and with monoclonal antibodies specific for determinants in the structural domains of *H-2L^d* or *H-2D^d*. The *H-2D/L^{dm1}* molecule apparently retains all of the third external domain (C2 or $\alpha 3$) and part of the second external domain (C1 or $\alpha 2$) of *H-2L^d*, but its first external domain (N or $\alpha 1$) derives from *H-2D^d*. From these findings and from previous peptide mapping studies, we propose that the *H-2D/L^{dm1}* antigen is the product of a hybrid gene that has resulted from an unequal crossover between the parental *H-2D^d* and *H-2L^d* genes, leaving the N exon and part of the C1 exon of the *H-2D^d* gene joined to the *H-2L^d* gene beginning somewhere within its C1 exon.

One informative approach to defining structure–function relationships for major histocompatibility complex (MHC) class I antigens has been to characterize several naturally occurring mutants of these molecules (1, 2). The mutations in murine H-2 antigens are generally found to be of two basic types—those in which the primary amino acid sequence of an H-2 antigen has changed and those in which the expression of H-2 antigens has been altered or lost. For example, most of the “*bm*” mutants (e.g., *bm1*, etc.) of the *b* haplotype show various amino acid substitutions in the *H-2K^b* antigen (3) as a consequence of changes in small segments of the MHC *H-2K* gene DNA sequence, perhaps occurring through some type of gene conversion event (4, 5). However, several other mutations, particularly those affecting H-2 antigens derived from the *H-2D/L* genes of the MHC, appear to show complete or partial losses in the expression of at least one antigen—the *H-2L^d* antigen in the *dm2* mutation (6, 7); the *H-2D^d* antigen in the *dm6* mutation (8); and the *H-2D1^b* antigen in the *bm13* mutation (9).

At least one H-2 antigen mutant, the *dm1* mutant (10), clearly has a different phenotype than those described above. This mutant involves more than one H-2 antigen, and both basic types of alterations discussed above appear to be in effect. Specifically, histogenetic, serological and structural analyses of the *dm1* mutant indicate that major alterations in the structures and/or the expression of at least three *H-2D/L*-linked antigens are affected in this mutant (6, 11–16). Although the phenotype of this mutant is quite complex,

most of its properties can be accounted for by two relatively simple observations: separate molecular allomorphs of the parental *H-2D^d* and *H-2L^d* antigens do not appear to be expressed in *dm1* mutant cells (unpublished observation), whereas one molecule with a combination of structural properties of these two antigens does appear to be expressed (14). This “hybrid” molecule, here designated the *H-2D/L^{dm1}* antigen, is further characterized in the present study by using monoclonal antibodies (mAbs) and cytotoxic T lymphocytes (CTL), both directed against structurally defined determinants or regions of the parental *H-2D^d* and *H-2L^d* antigens.

MATERIALS AND METHODS

Mice. All mice came from breeding colonies described previously (14, 17) and maintained at the University of California (Santa Barbara). The strains used (with *H-2* haplotype designations in parentheses) are as follows: B10.D2(*H-2^{dm1}*); B10.D2/nSnJ(*H-2^d*); A/J(*H-2^a*); C57BL/6(*H-2^b*); B6.C(*H-2^{bm1}*); BALB/cBy(*H-2^d*); BALB/c(*H-2^{dm2}*); and C58(*H-2^k*).

Antisera. Hyperimmune allo-anti-H-2.4 (*k/b* anti-*a*) and rabbit anti-mouse IgG were prepared in the laboratory of D.W.S. (University of California, Santa Barbara), while mAb ascites was prepared in the laboratory of K.O. (National Institutes of Health). The hybridoma designations (18, 19) of four mAbs used in this study and the H-2 antigen domain specificities (20) of these mAbs are listed in Table 1. The anti-*H-2K^b* mAb, 5F1.2 (21), was used for control immunoprecipitates.

Preparation of Radiolabeled Antigen. All procedures for metabolic incorporation of amino acids into splenocytes, for Nonidet P-40 extraction of H-2 antigens and for glycoprotein purification by lentil lectin chromatography have been described (14).

Immunoprecipitation Reactions and NaDodSO₄/PAGE. With the following inclusions, the procedures for direct sequential antibody precipitates, using heat-killed *Staphylococcus aureus*, and for analysis of NaDodSO₄/PAGE (including counting procedures) have been described (22). In this study, rabbit anti-mouse IgG (together with *S. aureus*) was used to optimize mAb precipitation efficiencies. For the discontinuous, NaDodSO₄/PAGE of 2-mercaptoethanol-reduced samples, 10-cm cylindrical resolving gels were made with 13% (wt/vol) acrylamide. The radioactive profiles shown in Figs. 1 and 2 are only for the 1.0- to 8.5-cm portion of the respective gels, because no radioactivity above background levels was detected outside this region.

CTL and Unlabeled-Target Inhibition Analysis. These procedures have been detailed (17).

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Abbreviations: MHC, major histocompatibility complex; mAb, monoclonal antibody; CTL, cytotoxic T lymphocyte.
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Table 1. mAbs specific for the extracellular domains of H-2D^d and H-2L

Hybridoma*	Specificity	
	H-2 antigen	Domain(s) [†]
34.5.8	H-2D ^d	N-C1
34.2.12	H-2D ^d	C2
30.5.7	H-2L ^d	N-C1 [‡]
28.14.8	H-2L ^d	C2

*Hybridomas and H-2 antigen specificities are described in refs. 18 and 19.

[†]H-2 antigen domain specificities were determined in ref. 20.

[‡]From the present study, the domain specificity of this mAb appears to map to the C1 domain of H-2L^d.

RESULTS

Reactivity of Domain-Specific Monoclonal Antibodies with *dml* H-2 Antigens. H-2^{dml} antigen glycoproteins extracted by Nonidet P-40 detergent from concanavalin A-treated metabolically radiolabeled *dml* splenocytes were tested for cross-reaction with mAbs specific for determinants in the different structural domains of the H-2D^d and H-2L^d antigens. The four mAbs used in this study and their domain specificities are summarized in Table 1. Fig. 1 shows the NaDodSO₄/PAGE profiles [³H]arginine-labeled proteins immunoprecipitated from equivalent amounts of *dml* glycoprotein extract by each of these mAbs and by an anti-H-2.4 alloantiserum for comparison. The radioactive antigens observed in the peaks near the centers of these gels have electrophoretic mobilities corresponding to average molecular weights of ≈44,000. In addition to expressing H-2 antigens that cross-react with the H-2D^d-specific anti-H-2.4 alloantiserum (Fig. 1A), mutant cells also express antigens that cross-react with the domain-specific mAbs for determinants within N-C1 (Fig. 1C) and C2 (Fig. 1E) of H-2L^d and N-C1 of H-2D^d (Fig. 1D). However, *dml* cells do not express a glycoprotein antigen that cross-reacts with the mAb specific for a determinant in the C2 domain of H-2D^d (Fig. 1B). This pattern of cross-reactivities was confirmed by complement-mediated cytotoxicity assays, in which the ability of each of these mAbs to bind to *dml* cells was determined by measuring the lysis mediated by rabbit anti-mouse IgG added to mAb precoated target cells (data not shown; see ref. 23); again, only the H-2D^d(C2)-specific mAb did not cross-react with *dml* cells, indicating the complete absence of a cell-surface molecule bearing the corresponding determinant.

Sequential Immunoprecipitates Localize Both H-2D^d and H-2L^d Antigenic Determinants to the Same Molecule in the *dml* Haplotype. The three domain-specific mAbs and the anti-H-

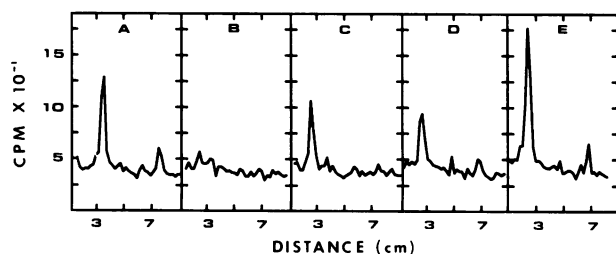


FIG. 1. NaDodSO₄/PAGE of immunoprecipitates of [³H]arginine-labeled *dml* glycoprotein. Sample radioactivity (cpm) is plotted versus the electrophoretic migration distance (cm) for immunoprecipitates with the following antibodies: (A) anti-H-2.4 alloantiserum; (B) 34.2.12, anti-H-2D^d(C2); (C) 30.5.7, anti-H-2L^d(N-C1); (D) 34.5.8, anti-H-2D^d(N-C1); and (E) 28.14.8, anti-H-2L^d(C2). Except for B, where no significant peaks are discernible, two main peaks were resolved—one migrating near 3.5 cm, of apparent $M_r \approx 44,000$, and another migrating near 7 cm, of apparent $M_r \approx 12,000$, which is the M_r of β_2 -microglobulin.

2.4 alloantiserum used in Fig. 1 were sequentially reacted with *dml* mutant or *d* parent glycoprotein extracts to ascertain which of the antibody-defined determinants co-exist on the same molecule. In such experiments, equal aliquots of *dml* or *d* glycoprotein extract reacted 3 times in sequence with different combinations of antibodies, and the resulting sequential immunoprecipitates were analyzed by NaDodSO₄/PAGE. Fig. 2 shows representative results of several triple sequential immunoprecipitate reactions carried out in this study. Here, the NaDodSO₄/PAGE profiles of the antigen immunoprecipitated in the third (or "test") step of a sequence are illustrated. Fig. 2 A–D shows the relative amounts of anti-H-2.4-reactive *dml* glycoprotein that remained after treatment (2 times) with 30.5.7 (Fig. 2A), 28.14.8 (Fig. 2B), a combination of these two mAbs (Fig. 2C), or the irrelevant anti-H-2K^b mAb, 5F1.2 (Fig. 2D). In samples treated with either H-2L^d-specific mAb, the level of anti-H-2.4-reactive antigen was significantly decreased (73% and 88% decrease in Fig. 2 A and B, respectively) as compared to the control sample (Fig. 2D). Although neither of the H-2L^d-specific mAbs alone was effective in completely precipitating the anti-H-2.4-reactive antigen in *dml* extracts, a combination of the two mAbs virtually eliminated all anti-H-2.4-reactive antigen (Fig. 2C). [It was generally difficult to find conditions in which individual mAbs could completely precipitate their target antigens, even from *d* extracts (data not shown) and even when a second anti-IgG antibody was used in conjunction with *S. aureus* and the mAb. However, in keeping with findings by others (24), combinations of different mAbs were usually more effective in precipitating antigen.] It is concluded that a single molecular species in *dml* mutant extracts bears H-2L^d as well as H-2D^d determinants.

Several other triple sequential immunoprecipitates of *dml* antigens using reciprocal combinations of the same antibodies gave similar results to those just described. Summarizing these data (not shown), the 30.5.7 and 34.5.8 mAbs and the anti-H-2.4 alloantiserum all reciprocally react with the same molecules in *dml* extracts, as determined by the finding that two successive reactions with any one of these antibodies significantly decreased the level of antigen (>80% decrease) detected by any other one of these antibodies. Also, the 28.14.8 mAb virtually eliminated all *dml* antigens reactive with any one of the other three antibodies above. Thus, it is concluded that *dml* cells express a molecule that bears all of

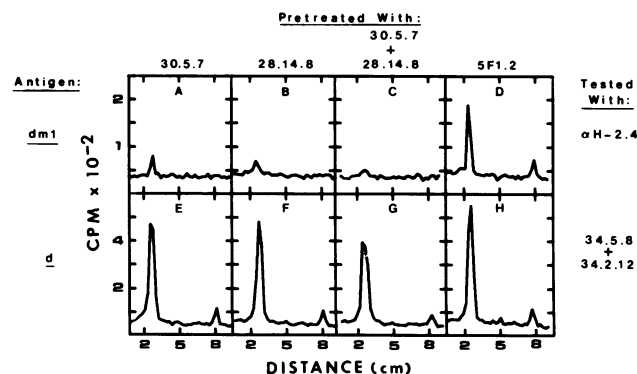


FIG. 2. NaDodSO₄/PAGE of representative third immunoprecipitates obtained in several triple sequential precipitation reaction sequences. (A–D) Gels of the third ("test") immunoprecipitates of *dml* glycoprotein extracts using the anti-H-2.4 alloantiserum; (E–H) The third precipitates of *d* extract glycoproteins using a combination of the two anti-H-2D^d-specific mAbs, 34.5.8 and 34.2.12. The following are the "pretreatment" antibodies used for the first two precipitates (gel analysis not shown): (A) 30.5.7; (B) 28.14.8; (C) 30.5.7 and 28.14.8; (D) 5F1.2; (E) 30.5.7; (F) 28.14.8; (G) 30.5.7 and 28.14.8; (H) 5F1.2.

the determinants defined by the 30.5.7, 34.5.8, and 28.14.8 mAbs and by the anti-H-2.4 alloantiserum.

These results with *dm1* mutant antigens contrast those obtained with radiolabeled glycoprotein antigens of the *d* parental haplotype. Two successive immunoprecipitates of *d* extract aliquots with 30.5.7 (Fig. 2E), 28.14.8 (Fig. 2F), or a combination of these two mAbs (Fig. 2G) had no significant effect on the normal level of the H-2D^d antigen, as determined after two successive reactions with 5F1.2 (Fig. 2H). (In these experiments, the H-2D^d antigen was immunoprecipitated using a combination of mAbs 34.5.8 and 34.2.12, which together reacted with all anti-H-2.4-reactive material in *d* extracts as found in other experiments; data not shown.) A quantitative difference between the amounts of anti-H-2.4-reactive glycoprotein in *d* as compared to *dm1* extracts (cf. Fig. 2D and H) has now been observed in several experiments.

Recognition of *dm1* Antigens by Anti-H-2L^d- and Anti-H-2D^d-Specific CTL. The H-2 antigens expressed by *dm1* cells were further characterized by their effectiveness in inhibiting the action of CTL specific for determinants of the parental H-2L^d and H-2D^d antigens. Fig. 3 shows representative results of several unlabeled-target inhibition studies, in which unlabeled concanavalin A blasts of splenocytes from mice of various haplotypes were titrated for their ability to inhibit the lysis of ⁵¹Cr-labeled targets by CTL of various specificities. Fig. 3A shows the inhibition patterns obtained when *b* haplotype CTL educated against *a* stimulator cells are cross-tested on *bml* targets. As previously shown and discussed (17), the strong cross-reaction by *b* anti-*a* CTL on *bml* targets is mediated primarily by the *b* anti-H-2L^d CTL subpopulation, which detects shared structural determinants created by the uniquely homologous stretch of amino acid sequence in H-2L^d and H-2K^{bm1}, including Ala¹⁵²-Tyr¹⁵⁵-Tyr¹⁵⁶. As Fig. 3A shows, *dm1* cells significantly inhibit this

cross-reactive lysis of *bml* targets, although the degree of inhibition is less than that found for the homologous combinations using cells of the *a* or *d* (B10.D2) haplotypes. As expected, *dm2* cells that completely lack expression of the H-2L^d antigen (8) inhibit only at the nonspecific background levels of *b* inhibitors, the effector cell haplotype. It is concluded from this experiment that *dm1* cells express H-2 antigens having at least partial amino acid sequence homology to the H-2L^d antigen in the region spanning residues 152-156.

In spite of the fact that *dm1* cells do appear to express certain H-2L^d-related determinants, other such determinants appear to be completely lacking in mutant cells, as shown in Fig. 3B. Here, cells of various haplotypes were tested for their abilities to inhibit *d* haplotype target cell lysis by *dm2* anti-*d* (i.e., *dm2* anti-H-2L^d) CTL. In contrast to parental type *d* cells, *dm1* cells are no better than effector haplotype *dm2* cells at inhibiting *dm2* target lysis. Thus, it is concluded that *dm1* mutant cells appear to have completely lost (or only weakly express) determinants associated with the H-2L^d antigen, as recognized by alloreactive anti-H-2L^d CTL.

In the final inhibition analysis (Fig. 3C), *dm1* cells were tested for their ability to inhibit the *k* anti-*a* CTL tested on *dm2* targets. Here, *dm1* cells inhibited lysis at an intermediate level compared to *d* and *dm2* cells (which behaved identically). This result indicates that *dm1* cells express antigens with a significant degree of determinant homology to the H-2D^d antigen, although this homology is only partial, as indicated by the lower plateau level for *dm1* cell inhibition.

DISCUSSION

A model for the structure of the H-2D/L^{dm1} antigen, which best fits the data of this and a previous study (14), is pro-

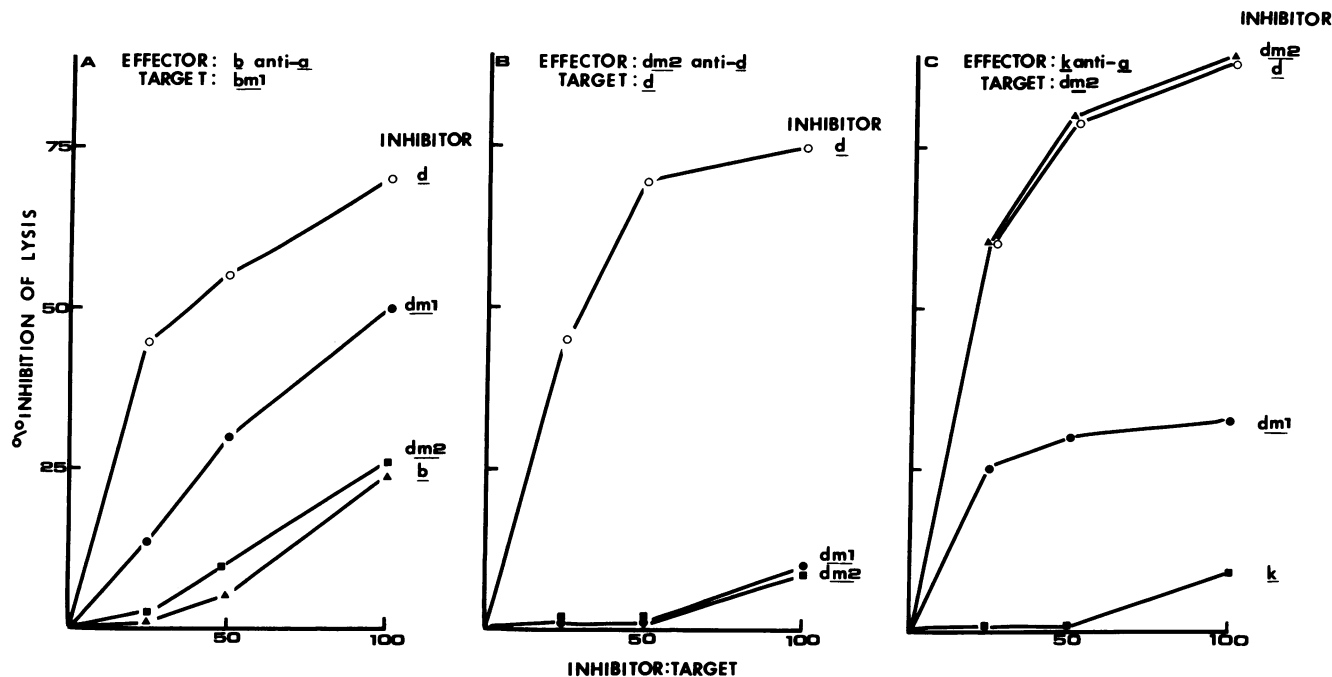


FIG. 3. Analysis by unlabeled-target inhibition of H-2 antigen cross-reactivities as tested by allo-specific CTL. The CTL effector/stimulator/target combinations and the various inhibitor cell haplotypes are indicated at the top of each panel. The percent specific inhibition of killing (ordinate) at a fixed effector/target cell ratio (E/T) is plotted versus the inhibitor/target cell ratio (abscissa). Percent specific inhibition was calculated by the following formula: % inhibition = $100 \times [1 - (OR - SRC)/(ER - SRC)]$ where OR is the observed release of ⁵¹Cr (in cpm) from the target cells caused by effectors in the presence of inhibitor cells; ER is the effector-mediated release obtained in the absence of any inhibitor cells; and SRC is the spontaneous release control for target cells in the absence of any effector cells. The following are the values for ER, SRC, FRC (the full-release control for detergent-extracted ⁵¹Cr from targets) and the E/T for all data points: (A) ER = 5254 cpm; SRC = 1097 cpm; FRC = 10,843 cpm; E/T = 50:1. (B) ER = 8429 cpm; SRC = 2451 cpm; FRC = 12,021 cpm; E/T = 50:1. (C) ER = 7589 cpm; SRC = 1423 cpm; FRC = 15,673; E/T = 35:1.

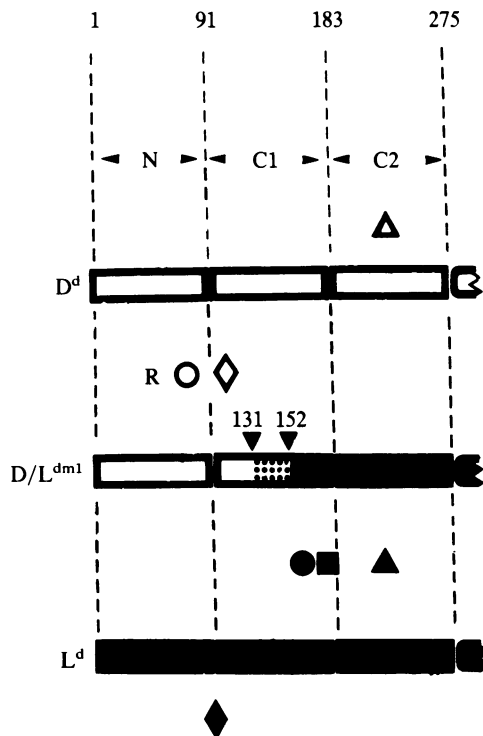


FIG. 4. Proposed hybrid model of the H-2D/L^{dm1} antigen aligned between the parental haplotype H-2D^d and H-2L^d antigens. The symbols located between adjacent structures indicate homologies. The open and closed symbols indicate H-2D^d and H-2L^d properties, respectively. R, arginine-containing tryptic peptides; ○, mutant antigen; ◇, anti-H-2D^d-specific CTL; ▲, anti-H-2L^d(C2)-specific mAb; △, anti-H-2D^d(C2)-specific mAb; ◆, *dm2* anti-*d* CTL; ●, anti-H-2L^d(N-C1)-specific mAb; ■, unique H-2L^d sequence, spanning residues 152–156.

posed in Fig. 4.[§] The mutant antigen is depicted between the H-2D^d and H-2L^d antigens. The boundaries of the three extracellular domains of H-2 antigens (25) are delineated by the amino acid sequence positions indicated at the top. The domains are encoded by the second through fourth exons—respectively designated N, C1, C2 (26) [or alternatively, $\alpha 1$, $\alpha 2$, $\alpha 3$ (27)]—of the genes for these H-2 antigens (26, 28, 29). (The first 5'-exon, L, encodes a leader sequence.) Because all three of the antigens have similar molecular weights (as determined by NaDodSO₄ gel electrophoresis), the mutant antigen is also likely to be organized into three extracellular domains of comparable size. As suggested by its white and black structural segments in Fig. 4, the mutant antigen appears to be a structural mosaic of the two parental antigen structures, because it is found to share a number of properties with each. The shared properties, as indicated by the symbols located between adjacent structures in this figure, are summarized in the following paragraph.

As proposed previously (14), the NH₂-terminal extracellular portion of the H-2D/L^{dm1} antigen (white structural segment of this molecule in Fig. 4) is likely to derive from the corresponding region of the H-2D^d antigen. Such appears to be the case because the arginine-containing tryptic peptides of these two molecules are almost identical (12, 14), in contrast to the lysine-containing tryptic peptides. The arginine-peptide homology suggests that the N and at least part of the C1 domains of the H-2D/L^{dm1} antigen are very similar, if not identical, to those of the H-2D^d antigen, which contains 15

arginine residues and only 1 lysine residue in its first 130 amino acid positions before the second lysine residue is encountered in the sequence (29, 30). The proposed NH₂-terminal structure homology is consistent with two observations made in the present study: (i) The anti-H-2D^d(N-C1)-specific mAb cross-reacts (Fig. 1D) with the mutant antigen; and (ii) mutant cells strongly (but not completely) cross-react (Fig. 3C) with anti-H-2D^d-specific CTL, which detect determinants only in the N-C1 regions of H-2D^d (31).

A substantial portion of the COOH-terminal extracellular region of H-2D/L^{dm1} (black structural segment in Fig. 4) appears to derive from the corresponding region of H-2L^d. In addition to the fact that there is significant structural homology between these two molecules, as indicated by their nearly identical lysine-containing tryptic peptides (14), the mutant antigen cross-reacts (Fig. 1E) with the anti-H-2L^d(C2)-specific mAb and not (Fig. 1B) the anti-H-2D^d(C2)-specific mAb. It is predicted that the mutant antigen lacks a significant portion of the NH₂-terminal extracellular segment of the H-2L^d structure, because *dm1* cells are not cross-reactive (Fig. 3B) with *dm2* anti-*d* CTL, which detect determinants only in the N-C1 (but not C2) domains of H-2L^d (31, 32). The proposed absence of the NH₂-terminal half of the H-2L^d antigen in *dm1* cells is consistent with the recent demonstration that *dm1* mice fail to make anti-vesicular stomatitis virus CTL and that vesicular stomatitis virus-infected *dm1* cells fail to serve as targets for *d* haplotype CTL, which are found to be exclusively restricted to the H-2L^d antigen (33).

Although the apparent junction or "fusion" point between the H-2D^d and H-2L^d structures within the H-2D/L^{dm1} antigen is not precisely located by the methods used in this study, two observations indicate that the mutant antigen retains at least part of the C1 domain of H-2L^d. First, the mutant antigen cross-reacts (Fig. 1C) with the anti-H-2L^d(N-C1)-specific mAb. (The tryptic peptide homologies discussed above suggest that this mAb is more likely to detect a determinant in the C1 domain.) Second, *dm1* mutant cells cross-react (Fig. 3A) with *b* anti-*a* CTL specific for determinants originating from some or all of the amino acid residues in positions 152–156 of the C1 domain of H-2L^d, as described (17). A structural interpretation of the latter observation depends on the number of different H-2D/L-related antigens that *dm1* cells express on their surfaces. Because only one H-2D/L-related antigen has been clearly identified for *dm1* mutant cells, as discussed in the following paragraph, it is proposed that the hybrid antigen sequence includes at least a portion of the unique H-2L^d sequence spanning residues 152–156.

As demonstrated by sequential precipitation analysis in the present study (Fig. 2 A–D), a single molecular species in the *dm1* mutant appears to bear all of the parentally defined antigenic determinants that are known to be associated with at least four molecules in the parent *d* haplotype. Recent structural and serological studies (unpublished observations) of *dm1* mutant H-2 antigens indicate that *dm1* cells express only one H-2D/L-related molecule having distinctive tryptic peptides, and this molecule has both H-2D^d- and H-2L^d-related allodeterminants. Specifically, no tryptic peptide differences were found in these studies among H-2D^{dm1} (isolated by anti-H-2.4 alloantiserum) and H-2L^{dm1} (isolated by anti-H-2.28 alloantiserum or by 28.14.8 precipitation of anti-H2.4 nonreactive *dm1* antigen; see ref. 34). In agreement with conclusions reached by others (15), these studies cast doubt on the supposition that separate allomorphs of the H-2D^d and H-2L^d antigens are expressed in *dm1* cells; they also indicate that no counterpart of the H-2M^d antigen (defined as anti-H-2.4-reactive, anti-H-2.28-nonreactive; see refs. 13 and 22) exists in *dm1* mutant cells, again in agreement with findings by others (13). Although the 28.14.8 mAb appeared

[§]This model and part of the supporting data were presented in the "Biochemistry of MHC Products" Workshop at the Fifth International Congress of Immunology, 1983.

to react with more than one antigen—for example, it consistently yielded larger immunoprecipitates as compared to 30.5.7 (cf. Fig. 1 *C* and *E*), and its target antigen in *dml* extracts could not be completely cleared by any of the other antibodies (data not shown)—the structural studies, as noted above, indicate that 28.14.8 may react with only one antigen with a distinct amino acid sequence. Thus, the immunoprecipitation differences between the various mAbs are probably explained by the inefficiency that mAbs generally show in immunoprecipitation reactions when used individually. In any case, only one conventionally defined H-2D/L-related glycoprotein has been clearly identified for *dml* mutant cells, and other such antigens, if they exist, are likely to be relatively minor species or highly homologous in structure to H-2D/L^{dml}.

Because an H-2D^d/H-2L^d structural discontinuity probably exists within the C1 domain of the hybrid antigen on the COOH-terminal side of residue 131 and on the NH₂-terminal side of residue 152 (dotted region of H-2D/L^{dml}), it is proposed that the mutant antigen originates from a hemoglobin Lepore-like hybrid gene (35, 36) rather than from an RNA splicing abnormality. The latter possibility would more likely affect structures in or near the domain boundaries, which are determined by exon/intron splicing patterns. In any event, the *dml* mutant is phenotypically like all other mutants involving H-2D/L-related gene products (as discussed in the Introduction), because it also exhibits a major deletion of genetic information normally expressed in the parent haplotype. Unequal recombination between the genes for MHC antigens may be an important mechanism for generating H-2 antigen polymorphism.

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