MOLECULAR BASIS OF THE dm1 MUTATION IN THE MAJOR HISTOCOMPATIBILITY COMPLEX OF THE MOUSE: A D/L HYBRID GENE

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The major histocompatibility complex (MHC)[1] of mammals encodes molecules that play important roles in the immune response. The H-2 region of the murine MHC contains at least three gene families. Cytotoxic T lymphocytes recognize foreign antigens in the context of the gene products from one of these families, the class I transplantation antigens (1). The transplantation antigens are integral membrane glycoproteins with a molecular weight of about 45 kilodaltons (kD). They are associated noncovalently with β_2-microglobulin and are present on the surfaces of most somatic cells.

The combination of alleles at all loci within the H-2 complex is termed its haplotype and is denoted by a superscript. The inbred BALB/c mouse has the H-2^d haplotype, and three H-2 class I transplantation antigens, K^d, D^d, and L^d, have been defined both serologically and biochemically. The genes encoding these antigens have been cloned and characterized (2). The K^d gene maps to the proximal (relative to the centromere) end of the H-2 complex, while the D^d and L^d genes map to the D region at the distal end of the H-2 complex. The presence of additional H-2^d class I antigens, e.g., M^d, R^d, has been suggested by serological analyses (3, 4). These antigens have not been well characterized.

The transplantation antigens are composed of three external regions (α1, α2, α3), each about 90 residues in length, a transmembrane region, and a cytoplasmic region. The genes are split into eight exons, corresponding to the structural components of the protein. The first exon encodes the leader or signal peptide, exons 2, 3, and 4 encode the three external regions, exon 5 encodes the transmembrane region, and exons 6, 7, and 8 encode the cytoplasmic region.

Mutations in the H-2 genes that cause the transplantation antigens to gain and/or lose antigenic determinant(s) can be selected by graft rejection. 30 mutant transplantation antigens have been identified (5–10). Studies of these mutants have yielded valuable information on the structure-function relationship of the class I antigens (6, 11). In most characterized mutants, the mutation can be localized to a small number of amino acid substitutions of the protein (7, 12).

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[1] *Abbreviations used in this paper:* CTL, cytotoxic T lymphocyte; mAb, monoclonal antibody; MHC, major histocompatibility complex; NC, nitrocellulose; SDS, sodium dodecyl sulfate.

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One mutant, dml, however, is unique in that it extensively affects both the H-2D^d and H-2L^d antigens.

The dml mutant was derived from the inbred strain B10.D2 of the H-2^d haplotype (13). Serological and transplantation studies showed the mutant is of the gain-and-loss type, i.e., it has gained and also lost antigenic determinant(s) (13–16). The mutation was mapped to the H-2D region (17, 18). The most interesting feature of this mutation is that both the H-2D^d and the H-2L^d antigens are affected (19–21). In the mutant, only a single H-2D- or -L-related class I antigen can be identified (22–25). This molecule was called the D^{dml} antigen because it shares an H-2D^d-specific serological determinant (22). The D^{dml} antigen shares only ~70% of its tryptic peptides with those of the D^d antigen, in contrast to the ≥90% peptide homology between most of the mutant K^b molecules and their wild-type counterparts, indicating a significant change that cannot be easily explained by point mutations (22). Tryptic peptide comparison and analyses with monoclonal antibodies (mAb) have suggested that the D^{dml} antigen is a hybrid protein between the D^d and L^d antigens (24).

This study investigates the basis of the dml mutation at the DNA level, and shows that the D^{dml} antigen is the product of a hybrid gene formed by fusion of the 5′ part of the D^d gene and the 3′ part of the L^d gene. The mutation probably resulted from a simple recombinational event with the region between the D^d and L^d genes deleted.

Materials and Methods

Mice. BALB/cJ and B10.D2 were obtained from The Jackson Laboratory, Bar Harbor, ME. B10.D2-H2^{dm1} was kindly provided by D. Sears (University of California, Santa Barbara).

Hybridization Probes. All probes were cloned in M13 vectors. Probe 59.2A was provided by A. Winoto (25a). Probe pH-2^{d}27.51 was a gift from M. Steinmetz (Basel Institute for Immunology, Basel, Switzerland) (26). Probe 18.1B is a 0.2 kilobase (kb) Alu I fragment from a 3.5 kb Bam HI/Nru I fragment of cosmid clone 18.1 subcloned into Sma I site of M13mp8 (B. Sher, Stanford University School of Medicine, unpublished observation). Probe 59.2B is a 0.7 kb Sau 3A fragment from a 3.2 kb Sma I/Hpa I fragment of cosmid clone 59.2 subcloned into Bam HI site of M13mp8.

Since M13mp vectors contain part of the *lac* region from *E. coli*, probes labeled by nick translation or primer extension will also hybridize to the *E. coli* chromosome. For screening cosmid libraries, probes were either gel-purified inserts or were prepared as follows. Single-stranded phage DNA was labeled by primer extension using the M13 universal sequencing primer (27). After the reactions, the sample was ethanol-precipitated and resuspended in 10 mM Tris, pH 8.0, 1 mM sodium EDTA. The insert was cut out by digestion with Eco RI and Pst I at the polylinker. The sample was adjusted to 1 M ammonium acetate and passed through nitrocellulose (NC) filter that was premoistened with 1 M ammonium acetate. The insert, now fully double stranded, will pass through, while the partially single-stranded vector fragment will be bound to the NC filter. The NC filtering step could be easily done by putting a small piece of NC filter in a 0.5 ml Eppendorf tube with a hole in the bottom. This small tube was placed in a 1.5 ml Eppendorf tube. The sample was spotted onto the NC filter. The eluate was collected in the 1.5 ml tube as the double tube was spun in an Eppendorf microfuge for 30 s. Probes had a specific activity of ~10^8 cpm/μg of insert DNA.

Cosmid Library Construction and Screening. The dm1 cosmid library was constructed using Mbo I partially digested and size-selected B10.D2-H-2^{dm1} liver DNA cloned into the Bgl II site of the vector pTL5 following the procedure of Steinmetz et al. (26). The library contains ~360,000 colonies distributed on 60 NC filters (137 mm). The cloning

efficiency was ~10^6 CFU (colony-forming units)/μg of size-selected insert DNA. The BALB/cJ cosmid library was similarly constructed using liver DNA from BALB/cJ mice, except that the nylon membrane, Biotrans (ICN Biomedicals, Inc., Irvine, CA), was used instead of the NC filters.

Hybridization is carried out in 5× SSPE (0.18 M NaCl, 10 mM NaH_2PO_4, pH 7.4, 1 mM EDTA), 5× Denhardt's solution, 100 μg/ml denatured, sheared salmon sperm DNA, 0.1% sodium dodecyl sulfate (SDS), 10^5 cpm/ml of each probe, at 68°C for 16–18 h. The filters were washed with 0.2× SSPE and 0.1% SDS, at 68°C. Exposures were for 8–10 h at −70°C with an intensifying screen.

Cosmid Clones. Cosmid clones 16.1, 59.2, and 49.2 were isolated from a BALB/cCum sperm DNA cosmid library and have been described (26–28). Cosmid clone 12.1 was isolated from a BALB/cJ sperm DNA cosmid library constructed by G. Siu (California Institute of Technology, unpublished observations). Cosmid clone 17.1 was isolated from a BALB/cJ liver DNA cosmid library constructed in this study. All dm1 cosmid clones were isolated from the B10.D2-H-2^{dm1} liver DNA cosmid library constructed in this study.

Restriction Mapping of Cosmid Clones. Restriction map analyses were performed using a combination of the standard double-digestion method and a new, rapid method. The new method is a modification of the method by Smith and Birnstiel (29) and by Rackwitz et al. (30). Briefly, two 40-mer oligonucleotides were synthesized corresponding to sequences flanking each side of the Sal I recognition site in pTL5. Sal I cleaves once in the vector but very rarely cuts within the insert. The cosmid DNA were linearized with Sal I, partially digested with the restriction enzymes used for mapping, electrophoresed on agarose gel, blotted onto NC, and hybridized to the radioactively-labeled oligonucleotides. The two end-specific oligonucleotides will each hybridize to a set of partial fragments, all with a common terminus but of different lengths, forming a ladder of bands that can be detected by autoradiography. The positions of all the restriction sites relative to the Sal I site can therefore be easily deduced. Detailed procedures will be published separately.

DNA Sequencing. The 2.4 kb Bam HI fragment containing the 5′ half of the D^{dm1} gene was subcloned into the Bam HI site of M13mp8. This subclone was mapped with the restriction enzymes Sma I, Pst I, and Xba I. From the cloned 2.4 kb fragment, a 300 base pair (bp) Sma I/Pst I fragment and a 500 bp Pst I/Xba I fragment were separately subcloned into the corresponding sites in both M13mp10 and mp11; a 300 bp and a 50 bp Sma I fragment were subcloned into M13mp8 Sma I site. DNA sequence analyses were carried out by the chain-terminating method of Sanger et al. (31).

Transfection and Radioimmunoassay. Transfection of mouse L cells with cosmid clone 40.2 and cell-surface radioimmunoassay were performed as described previously (27, 32).

Results

DNA Sequences 3′ to the D^d Gene and 5′ to the Ld Gene are Deleted in the dm1 Mouse. Because both D^d and L^d antigens are affected in the dm1 mutant, we first investigated whether the mutation affected the structural genes. Since the class I gene family contains 33 crosshybridizing members (26, 33), it is difficult to use coding sequences as probes to distinguish among the L^d, D^d, and other class I genes. Instead, several low copy-number sequences were subcloned from the more divergent regions flanking the L^d and D^d genes (Fig. 1). These were then used as hybridization probes in genomic Southern blot analyses to detect differences between the dm1 mutant and its parental strain B10.D2. BALB/c DNA was also included in the study because the D^d and L^d cosmid clones were generated from this strain (26). The BALB/c H-2 complex appears to be identical to the B10.D2 H-2 complex.

Probe 59.2A, located 2–4 kb 3′ of the L^d gene (Fig. 1), detects four Bam HI bands in BALB/c and B10.D2 liver DNA (Fig. 2). These four fragments have

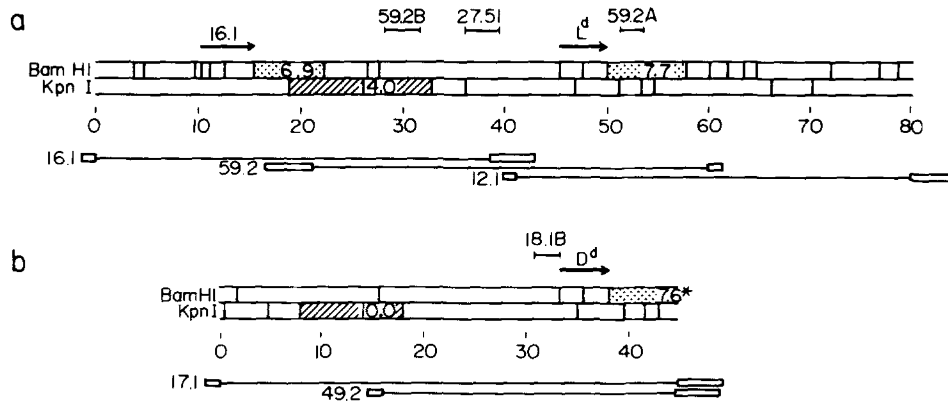


FIGURE 1. Restriction map of the L^d and D^d clusters and location of hybridization probes. Restriction map of the cosmid clusters containing the L^d gene (*a*) and the D^d gene (*b*). The regions contained in each cosmid clone are shown below the map with open boxes at the ends denoting the vector arms split at the single Sal I site. The class I genes are denoted by a heavy bar with arrow indicating the transcription orientation. Location of the hybridization probes are shown by a bar above the restriction map. The Bam HI fragments that hybridize to probe 59.2A are dotted, the Kpn I fragments that hybridize to probe 59.2B are crosshatched. The sizes of these fragments are shown in kbp. Bam HI fragment (*) is not complete in clone 17.1, but a cosmid clone, 2.20, overlapping with clone 17.1 (M. Steinmetz, Basel Institute for Immunology, personal communication), contains the complete fragment, which is 7.6 kb (data not shown).

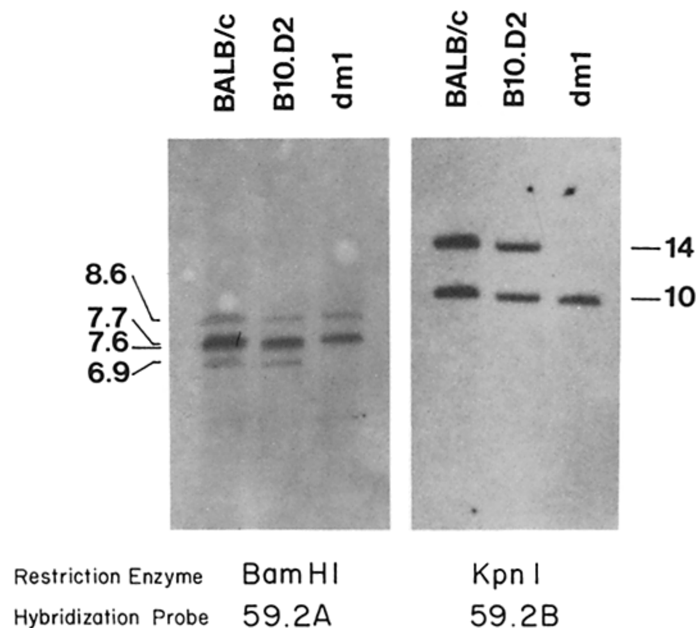


FIGURE 2. Southern blot analyses of genomic DNA showing deletion in the dm1 mutant. 10 μg of liver DNA from the mouse strains BALB/c, B10.D2, and B10.D2-H-2^{dm1} were digested with Bam HI or Kpn I, electrophoresed on a 0.6% agarose gel, blotted, and hybridized to radioactively-labeled probes 59.2A or 59.2B. All of the hybridizing restriction fragments have been identified in BALB/c cosmid clones. Their locations are shown in Fig. 1.

been identified in cosmid clones (Fig. 1). They all lie immediately 3′ to an H-2 class I gene. The 8.6 kb fragment is associated with gene 17.1, which is ~19 kb 3′ of the K^d gene (data not shown). The 7.7 kb fragment, from which the probe was generated, is associated with the L^d gene. The 7.6 kb fragment is associated with the D^d gene (see Fig. 1 legend). The 6.9 kb fragment is associated with gene 16.1, which is 5′ to the L^d gene. Southern blot analysis of liver DNA with probe 59.2A shows that the 7.6 kb and 6.9 kb Bam HI fragments are missing in the dm1 mouse, indicating that the DNA in the regions 5′ to the L^d gene and 3′ to the D^d gene have been deleted.

Probe 59.2B, located 13 kb 5′ of the L^d gene (Fig. 1), detects 14 and 10 kb Kpn I bands in BALB/c and B10.D2 liver DNA (Figs. 1 and 2). The 14 kb band corresponds to the fragment in clone 59.2 from which the probe was generated. This fragment is not present in dm1, indicating the region 5′ to the L^d gene has been deleted. Probe pH2^{d}27.51, located 5 kb 5′ of the L^d gene (Fig. 1), also fails to detect its corresponding fragment in dm1 (data not shown), indicating that the 5′ deletion extends within 5 kb of the L^d gene. Hybridization with probe 18.1B, located immediately 5′ to the D^d gene (Fig. 1), shows that the corresponding fragment is present in dm1 (data not shown).

These results indicate a deletion of the region 5′ to the L^d gene, and the region 3′ to the D^d gene, while the regions 3′ to the L^d gene and 5′ to the D^d gene are unaltered.

The dm1 Mutant Gene Is a D/L Hybrid Gene. To study the extent of the deletion, a cosmid library was constructed from dm1 liver DNA in order to clone the altered region. The library was screened using probes 59.2A and 59.2B, and 14 positive clones were found. By comparing single digests using several restriction enzymes, the clones were shown to be overlapping, and their order was determined. Three clones (38.1, 40.2, 7.2) that cover the whole region were then mapped in detail (Fig. 3, *A* and *B*) using a rapid restriction mapping method in combination with the conventional double-digest method. The cosmid cluster spans 90 kb and contains one class I gene. When the restriction map is compared with those of the L^d and D^d cosmid clusters, the region upstream of this gene is identical to that of D^d, while the region downstream of this gene is identical to that of the L^d gene (Fig. 3*C*). A 15 kb region including the *dm1* class I gene is indistinguishable by restriction map (Fig. 3 *C*) with the corresponding region of the L^d and the D^d gene clusters. These data suggest that the mutation resulted from a deletion removing the DNA between the D^d and L^d genes, possibly forming a hybrid gene.

To further define the region where the deletion occurred, restriction site polymorphisms around the L^d and D^d genes were identified in order to distinguish the two loci. The Sma I site in the second intron of the D^d gene was resolved into a doublet spaced 52 bp apart (28), while only one Sma I site is found in the corresponding region of the L^d gene. The additional Sma I site is present in the dm1 gene (data not shown), indicating that the D^d locus contributed the 5′ region of the dm1 gene extending at least 3′ to the position of the Sma I site. Studies with cytotoxic T lymphocytes (CTL) specific to amino acid residues 152–156 of the L^d antigen suggested that the 3′ end of the gene, extending 5′ to at least the codon for amino acid residue 152 is donated by the L^d gene (24).

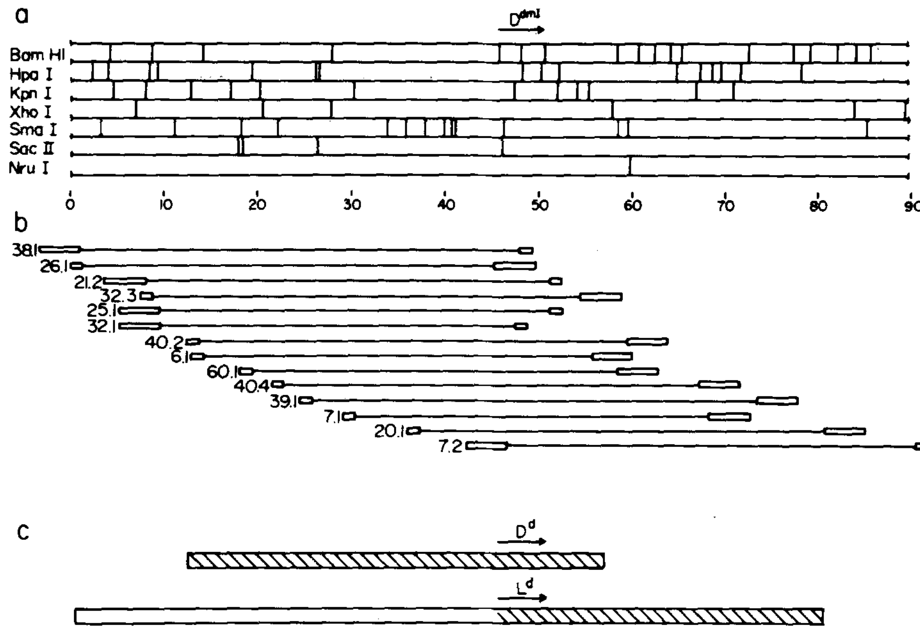


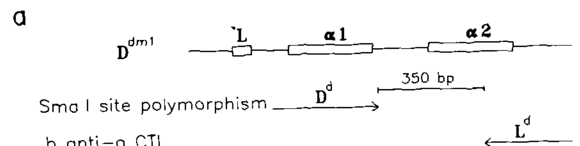
FIGURE 3. Restriction map for the 14 overlapping dm1 cosmid clones. (*a*) The composite restriction map is shown. Sal I and Cla I sites were not found. The mapping was done by a combination of double digestion and a rapid method as described in Materials and Methods. Lengths given in kb. (*b*) The regions contained in each cosmid clone are shown in lines with open boxes at the ends denoting the vector arms split at the single Sal I site. (*c*) Comparison of restriction map of the D^d and L^d with the dm1 cosmid clusters. Hatched region denotes indistinguishable restriction map to the dm1 clones.

Combination of the data from the Sma I site polymorphism and the CTL studies suggests that the *dm1* gene is a hybrid gene formed by a fusion between the 5′ portion of the D^d gene and the 3′ portion of the L^d gene (Fig. 4*A*). The recombination point apparently lies within a region of ~350 bp. This D/L hybrid gene is called the D^{dm1} gene because it is presumably transcribed from the D^d promoter.

DNA sequence analysis was carried out in this region of the D^{dm1} gene and compared against D^d and L^d sequences. The recombination point occurs in exon 3, which encodes the $\alpha 2$ region. As shown in Fig. 4*B*, the sequence from the beginning of exon 3 to position 827 (following the numbering in the D^d sequence) is identical to the D^d sequence (28), while the 3′ portion of the exon beginning at nucleotide 927 is identical to the L^d sequence (34). The 99 bp of sequence between these points is identical in the L^d and D^d genes. The sequence analysis clearly shows that the recombination event occurred within this 99 bp stretch. The resulting D/L hybrid antigen has the $\alpha 1$ and two-thirds of the $\alpha 2$ region identical to the D^d antigen, and two-thirds of the $\alpha 2$ region and the carboxyl half of the molecule identical to the L^d antigen (Fig. 4*C*).

The D^{dm1} Gene Is Expressed in Transfected Fibroblasts and Correlates with the Mutant Phenotype. The D^{dm1} gene was transfected into mouse L cells. The transformant line K31-25 was tested with a panel of mAb by cell-surface radioimmunoassay (Fig. 5). These mAb recognize specific regions of different H-2 class

a



b

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                                                      lySerHisThrLeuGlnTrpMetAlaGlyCysAspValGluSerAspGlyArgLeuLeuArgGlyTyrTrpGlnPheAla 117
D^dm1 91 GGGCCGGGGCGGGTGAGCGGGGCTGACCGGGGTCCCGCAGGCTCTCACACACTCCAGTGGATGGCTGGCTGTGACGTGGAGTCGGACGGGCGCCTCCTCCGCGGGTACTGGCAGTTCGCC
D^d      ------------------------------------------------------------------------------------------------------------------------
                                                        Thr                     Tyr              Gly                            Glu
L^d      -----------------------------------------------A-----------------------TAC--------------G------------------------------GA---------

D^dm1 118 TyrAspGlyCysAspTyrIleAlaLeuAsnGluAspLeuLysThrTrpThrAlaAlaAspMetAlaAlaGlnIleThrArgArgLysTrpGluGlnAlaGlyAlaAlaGluTyrTyrArg 157
          TACGACGGCTGCGATTACATCGCCCTGAACGAAGACCTGAAAACGTGGACGGCGGCGGACATGGCGGCGCAGATCACCCGACGCAAGTGGGAGCAGGCTGGTGCTGCAGAGTATTACAGG
                                                                                                                              ArgAsp
D^d       ----------------------------------------------------------------------------------------------------------------AGAG--C--
                  Arg
L^d       --------C-T-----------------------------------------------------------------------------------------------------------------

D^dm1 158 AlaTyrLeuGluGlyGluCysValGluTrpLeuHisArgTyrLeuLysAsnGlyAsnAlaThrLeuLeuArgThrG                                            182
          GCCTACCTGGAGGGCGAGTGCGTGGAGTGGCTCCACAGATACCTGAAGAACGGGAACGCGACGCTGCTGCGCACAGGTGCAGGGCCGCGGGCAG
                                          Arg
D^d       --------------------------------G--------------------------T------------------------------------
L^d       -----------------------------------------------------------------------------------------------
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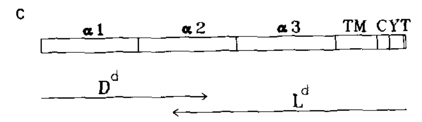
c



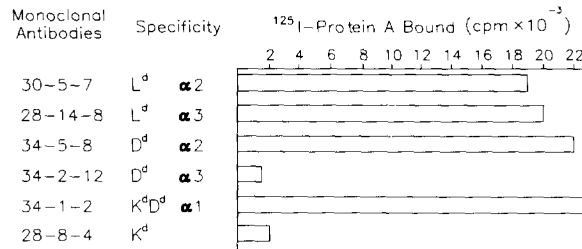


FIGURE 5. Cell-surface radioimmunoassay of the transformant K31-25. Cosmid clone 40.2 was used to transfect mouse L cells. The transformant line K31-25 was tested with a panel of mAb. The regions that the mAb recognize are indicated (35, 36).

I antigens (35, 36). The transformant line reacts with mAb that recognize either the D^d $\alpha 1$ region or the L^d $\alpha 3$ region. These results confirm that the cloned gene encodes a hybrid class I antigen with its $\alpha 1$ region derived from the D^d gene and the $\alpha 3$ region derived from the L^d gene. mAb 30-5-7 and 34-5-8, specific for the $\alpha 2$ region of L^d and D^d antigens, respectively, both react with the transformant. Taken together with the DNA analyses, these results further assign the specificity of mAb 30-5-7 to the carboxyl-terminal third of the L^d $\alpha 2$ region, and the specificity of mAb 34-5-8 to the amino-terminal third of the D^d $\alpha 2$ region.

The pattern of reactivity of the transformant with the panel of mAb is identical to that of dm1 spleen cells (24, 46). Preliminary results from two-dimensional gel analysis of the immunoprecipitated D^{dm1} antigen from the transformant also indicate it is identical to that from the dm1 spleen cells (M. McMillan, University of Southern California, Los Angeles, CA, personal communication). These data unequivocally show that the D^{dm1} gene codes, for the mutant class I antigen.

Discussion

Possible Mechanisms for Generating the Fusion Gene. We demonstrate here that the cloned D^{dm1} gene is a hybrid of the 5′ end of the D^d gene and the 3′ end of the L^d gene, with the region in between deleted. This gene fusion can presumably occur by either intrachromosomal deletion or interchromosomal unequal crossover (Fig. 6). In either case, it is most likely the result of homologous recombination, since the region where the recombination occurred is a 99 bp stretch of sequences identical between the D^d and the L^d genes. The reciprocal product (the L/D hybrid gene) in the recombination event presumably segregated from

FIGURE 4. The *dm1* gene is a D/L hybrid gene. (*a*) The left boundary of the 350 bp region is defined by the polymorphic Sma I site at 12 bp 3′ of exon II (encoding the $\alpha 1$ region). The right boundary is at amino acid residue 151, as defined by the b anti-a CTL. Recombination occurred within this 350 bp region. (*b*) The 2.4 kb Bam HI fragment containing the 5′ half of the dm1 gene was subcloned and mapped. ~1.2 kb has been sequenced from M13 subclones from the 2.4 kb Bam HI fragment. DNA sequence is only shown for the region immediately containing the third exon, where the recombination occurred. The dm1 sequence is aligned with the DNA sequence of L^d (34) and of D^d (28). Identity is indicated by a dash. Translated protein sequence of the D^{dm1} gene is shown above its DNA sequence. Differences in L^d and D^d are indicated. (*c*) The predicted D^{dm1} protein structure is shown. The $\alpha 1$ region and two-thirds of the $\alpha 2$ region are identical with the D^d antigen. Two-thirds of the $\alpha 2$ region, the $\alpha 3$ region, the transmembrane (*TM*) region, and the cytoplasmic regions (*CYT*) are identical to the L^d antigen.

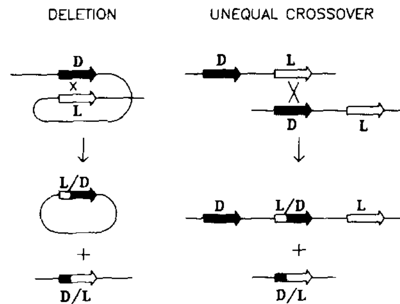


FIGURE 6. Two models for the occurrence of the dm1 hybrid gene.

the D/L hybrid gene in meiosis and was not passed on to progeny. Mice bearing such a reciprocal mutation have not been described.

In chromosomal walking experiments (Y. Sun, unpublished observations), the L^d gene has been linked to the Qa-2,3 cosmid cluster, with the transcription direction of L^d towards the *Qa-2,3* gene. This establishes that the D^d gene is located upstream of the L^d gene. This is consistent with the gene order reported in a recent study (47) using a recombinant between the H-2D and H-2L genes. In the above models, it is assumed that the D^d and the L^d genes have the same transcriptional orientation. In this configuration, only a single recombination event is required to create the mutation. Since the D^d gene has not been physically linked to the distal L^d gene nor to the proximal S region, which contains the class III genes, it remains to be shown whether the D^d or L^d genes do have the same transcriptional orientation.

Implications for Evolution of Multigene Families. Other examples of gene fusion have been found. Two hemoglobin variants, Lepore-Boston and Kenya, have been characterized by restriction site analyses (48, 49) of genomic DNA, and were shown to be due to gene fusion. Based on restriction map comparison, the BALB/c *Q7* gene has been suggested (50) to be a fusion between the equivalents of genes *Q8* and *Q9* of the B10 mouse. The *Q3* gene of the BALB/c mouse has also been suggested to have resulted from a gene fusion (S. Hunt, California Institute of Technology, personal communication).

Homologous but unequal recombination, as suggested by these examples of gene fusion, can serve to expand or contract the number of genes in a multigene family. Contraction and expansion of the class I gene repertoire is supported by many studies (2, 50–52) comparing different mouse inbred strains and different *Mus* species. Our study of the dm1 mutation is the first direct evidence of the contraction of the size of the H-2 class I gene family. Preliminary results show that the dm2 mutation (L^d loss mutant) also involves a DNA deletion, possibly including the L^d gene (Y. Sun, unpublished data), providing another example of this contraction process. Recombination at regions within the structural genes not only changes the size of the gene family but also can immediately create new (hybrid) members in the gene family, hence it serves as a mechanism for directly generating polymorphism. The dm1 mutant also provides an example of this process.

One of the most interesting and perplexing aspects of the H-2 transplantation

antigens is their extremely high polymorphism (37). Gene conversion and unequal crossover have been suggested as mechanisms for generating the polymorphism (2). Both mechanisms probably begin with the same molecular configuration of pairing between homologous sequences. However, a notable difference of their operation is noted in the mutants of two H-2 haplotypes. Of the 21 H-2^b mutants, 14 have been analyzed biochemically, and the alterations have been localized to 1–4 amino acids (7, 38). Gene conversion of short stretches of DNA sequence has been suggested as the mechanism generating these mutations, based on the following observations: the altered amino acids are clustered in each mutant; some of the clustered changes occurred in several independently derived mutants; and in several cases, a potential donor class I gene can be identified (38–41 and J. Geliebter, Albert Einstein College of Medicine, New York, NY, personal communication). On the other hand, mutants in the H-2^d have more extensive alterations. The dm1 and dm2 mutations involve DNA deletion. The dm5, an H-$2K^d$ mutant, can be shown by Southern blot analysis to have a significantly altered pattern of class I genes (R. Goodenow, unpublished data). The dm6 mutant fails to express the D^d antigen (19), possibly also as a consequence of DNA deletion. The basis for this predominance of gene conversions in the H-2^b haplotype and recombination in the H-2^d haplotype is not known.

Functional Dissection of the Transplantation Antigens. Using hybrid class I genes constructed by exon-shuffling methods, it has been possible to determine which regions of the H-2 class I antigen are important for the interactions with antibody and CTL (35, 36, 42–45). The dm1 mutation provides a natural hybrid gene. Whereas the hybrid genes artifically constructed to date have exchanged complete axons, the D^{dm1} gene has a hybrid $\alpha 2$ exon, allowing a finer functional dissection within a single external region. For example, as shown in the previous section, the position responsible for the specificity by mAb 30-5-7 and 34-5-8 can be narrowed down to only one-third of the $\alpha 2$ region.

Studies with constructed hybrid class I genes have shown that the $\alpha 1$ and $\alpha 2$, but not $\alpha 3$, regions are important in determining the specificity of recognition by alloreactive or antiviral CTL (43, 44). It has also been shown (45, 53) that the $\alpha 1$ and $\alpha 2$ regions cannot be independently recognized by CTL, suggesting that CTL recognition requires both regions. CTL from lymphocytic choriomeningitis virus–infected dm1 mouse are restricted by the D^{dm1} antigen, but not by the K^d, D^d, or L^d antigens (54). Since the D^{dm1} and the D^d antigen share the $\alpha 1$ region and two-thirds of the $\alpha 2$ region, it can be concluded that this shared region is not sufficient, and the last one-third of the $\alpha 2$ region is important in determining the specificity of CTL recognition. The last one-third of the α_2 region, however, is not sufficient because the L^d antigen shares the same sequence, but does not restrict dm1 CTL. Anti-L^d alloreactive CTL do not lyse dm2 cells (D^d-positive, L^d-negative) as expected, but show strong, although incomplete, crossreactivity for dm1 target cells (55), again showing that the last one-third of $\alpha 2$ region is important in CTL recognition. These data are consistent with conclusions cited above that the CTL recognize determinants that are formed by or are sensitive to interactions between the α_1 and α_2 regions, which have been suggested by x-ray crystallography studies (56) to fold and jointly form a domain.

None of the Additional H-2^d Class I Antigens Is Found in the dm1 Mouse. Several additional H-2^d class I antigens, including M^d, R^d, L2^d, identified either by sequential immunoprecipitation or cocapping experiments, have been mapped (3) to the *H-2D^d* region. The molecular basis of these additional class I antigens is not clear. They may be encoded by genes other than L^d and D^d, or be derived from the L^d or D^d gene via an alternative RNA splicing pathway or with different posttranslational modification (27). None of these additional antigens can be detected on the dm1 spleen cell nor in the cytoplasm (24, 25, 57). This suggests that the genes encoding these antigens have been deleted, or that the regulatory mechanisms for their expression are not functional in the dm1 mutant.

From our study of the dm1 mutation, two points can be made regarding these proposed mechanisms. First, putative alternative splice donor and acceptor sites have been identified in the second exon (encoding the α1 region) of the D^d gene (28). These sites are also present in the D^{dm1} gene. Since neither M^d nor R^d antigens were found in the dm1 mouse, they are probably not derived from the D^d gene by alternative RNA splicing. A study with the L^d transformants (58) also suggested that the R^d antigen is not derived from the L^d gene. Second, gene 16.1 (see Fig. 1) could encode the R^d antigen. The R^d antigen is probably a secreted or cytoplasmic molecule because it can be detected in cell lysate (46), but not on the cell surface (59). It also has a lower molecular mass (58), consistent with lack of a transmembrane region. Mouse L cells transfected with gene 16.1 do not express any new cell surface molecules recognized by the available H-2^d antisera and mAb. It may be a pseudogene, or it may encode a secreted or cytoplasmic antigen, possibly the R^d antigen. Consistent with the latter hypothesis is the absence of the R^d antigen in dm1 mouse, in which gene 16.1 has been deleted. Its location upstream of the H-2L^d gene is also consistent with the gene order of D-R-L (C. David, Mayo Medical School, Rochester, MN, personal communication). Analysis of cell lysate from L cells transformed with this gene will readily provide a test for this hypothesis.

Summary

The H-2^{dm1} mutation is unique among all described H-2 mutations in that two transplantation antigens, the H-2D^d and the H-2L^d, are affected. Here, we show that the mutant gene, D^{dm1}, is formed by fusion of the 5′ part of the D^d gene and the 3′ part of the L^d gene, with the region in between deleted. The recombination junction is located in the third exon, which encodes the α2 region of the protein. When the hybrid gene is transfected into mouse L cells, serological and biochemical analyses indicate the D^{dm1} antigen expressed in the transformant line is identical to the mutant molecule in dm1 spleen cells. These results demonstrate that the D/L hybrid gene is most likely responsible for the dm1 mutant phenotype.

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