THE MURINE MHC CLASS I GENES, *H-2D^q* AND *H-2L^q*, ARE STRIKINGLY HOMOLOGOUS TO EACH OTHER, *H-2L^d*, AND TWO GENES REPORTED TO ENCODE TUMOR-SPECIFIC ANTIGENS

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The murine MHC, also known as the H-2 complex, encodes highly polymorphic cell surface glycoproteins that are involved in T lymphocyte recognition of foreign antigens (1). One class of these glycoproteins, the class I molecules, are encoded by genes within the K, D, Q, and TL regions of the MHC. The class I gene loci within the K and D regions display a high level of polymorphism (1), whereas few alleles of the *Q* and *TL* class I genes are demonstrable (2). Furthermore, K and D region class I genes demonstrate 10–20% nucleic acid sequence divergence in the coding segments of the genes (3, 4). Analyses of K and D region class I gene sequences from wild-type and mutant class I genes suggest that gene conversion-like events are a major force in the generation of their extensive polymorphism and diversity (5).

Although extensive polymorphism exists among the K and D region class I genes, the number of K region class I genes and products have been found to be constant in different H-2 haplotypes (6, 7). On the other hand, the number of D region class I genes expressed varies from one H-2 haplotype to another. Haplotype disparity in the number of expressed D region class I antigens has been demonstrated in both inbred laboratory and wild-derived mouse strains using serological and peptide mapping analyses (8–12). In three cases, molecular approaches have corroborated this disparity at the gene level. Only one class I gene has been found in the D regions of mice of two different H-2 haplotypes (7, 13, 14), H-2^b (C57BL/10) and H-2^k (AKR and C3H). However, the H-2^d haplotype (BALB/c) contains at least five D region class I genes (13), of which only two, the *L^d* and *D^d* genes, have been conclusively shown to be expressed as cell surface products. Thus, the haplotype disparity in the number of expressed D region class I antigens reflects real differences at the gene level. Furthermore, molecular studies of the H-2^d mutants dm1 and dm2 revealed deletions of class I genes in their D regions, most likely due to unequal crossover events (13, 15, 16). Thus, the D region provides a unique model system to study genetic expansion and contraction in the number of class I genes.

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[1] *Abbreviations used in this papar:* β_2m, β_2-microglobulin; FIGE, field inversion gel electrophoresis; PFU, plaque-forming units; TATA, tumor-associated transplantation antigen.

Peptide mapping comparisons of D region class I molecules from seven different H-2 haplotypes have revealed a second phenomenon distinguishing the class I molecules encoded in the D region from those in the K region. These comparisons demonstrate a cline or gradient of relatedness of D region class I molecules, with the L^d molecule being the prototypic structure (9, 11). The existence of this cline of related D region class I molecules suggests that these molecules are encoded by genes derived from a common ancestor. Certain features of their L^d-like structure may be selectively advantageous. Alternatively, this cline may indicate that the more related genes have diverged more recently from an L^d-like progenitor.

Since the B10.AKM mouse strain ($K^kI^kS^kD^q$) expresses multiple D region class I products, and since these products are more closely related to the L^d prototypic structure (8, 9), we have investigated the D region of B10.AKM using molecular approaches. Two genes isolated from B10.AKM were designated D^q and L^q based on comparison of the restriction endonuclease patterns of the flanking regions of these genes with the analogous patterns from the BALB/c D^d and L^d genes, respectively. These data combined with the linkage of another B10.AKM class I gene, $D4^q$, to the 5′ side of the L^q gene (analogous to the organization of the $D4^d$ and L^d genes in BALB/c) suggest a D region organization similar to that of BALB/c. As determined by DNA-mediated gene transfer to mouse L cells followed by serological analyses, the D^q and L^q genes encode products that correspond to previously characterized class I antigens. The nucleic acid sequence comparisons of the D^q and L^q genes with other D region class I genes revealed striking homologies to the L^d gene (99% for L^q and 97% for D^q). This sequence homology of D^q and L^q to L^d was also manifested at the functional level when assessed in CTL responses. The considerable homology (>97%) of L^q to D^q supports the notion that these genes resulted from a duplication of an L^d-like progenitor. The near identity of D^q and L^q with the previously reported tumor-specific genes, *A166* and *A149* (17), from the UV-induced $H\text{-}2^k$ fibrosarcoma 1591, was unexpected. This observation raises the distinct possibility that the *A166* and *A149* genes represent genetic contamination rather than novel class I gene products expressed by tumor cells.

Materials and Methods

DNA Isolation. DNA was isolated from B10.AKM mouse liver using a modified procedure of Chang et al. (18). Briefly, mouse livers were homogenized in 5% citric acid and then overlayed onto a 5% citric acid/30% sucrose cushion. The isolated nuclei were lysed with an SDS-proteinase K mixture and then were sequentially extracted with phenol/chloroform and ether. The DNA was concentrated with solid phase sucrose for a few hours and then dialyzed extensively against 10 mM Tris-HCl, pH 7.5, 1 mM EDTA.

Generation and Screening of Genomic Libraries. Genomic B10.AKM liver DNA was partially digested with Mbo I under conditions to optimize the quantity of fragments in the size range of 13–20 or 35–40 kb. The digested DNA was then size fractionated either by sucrose density centrifugation or by electrophoresis on a Bull's eye gel apparatus (Hoefer Scientific Instruments, San Francisco, CA).

EMBL3 arms were generated by inactivating the stuffer fragment according to the method of Frischauf et al. (19). Cosmid vector pTCF, which contains the selectable aminoglycosyl-3′-phosphotransferase gene, was linearized with Bam HI and then dephosphorylated with calf intestine alkaline phosphatase (Boehringer Mannheim Biochemicals, Indianapolis, IN). The 13–20-kb and 35–40-kb fragment inserts were then ligated into the EMBL3 arms and the linearized pTCF cosmid vector, respectively, using T4 ligase (International Biotechnologies

Inc., New Haven, CT). The ligated concatamers were then packaged into λ phage using Gigapack-plus packaging extracts (Stratagene, San Diego, CA). 2×10^6 recombinant EMBL3 plaque-forming units (PFU) were amplified on a P2 lysogen of LE392 yielding a library of $\sim 5 \times 10^{11}$ recombinant PFU. The bacteriophage library was screened by the standard Benton and Davis protocol (20) and the cosmid library by a modification of the protocol described by Hanahan and Meselson (21).

Primary, secondary, and tertiary screening of the bacteriophage and cosmid libraries were performed using a 3′ flanking probe isolated from the region flanking the L^d gene (probe 4, see reference 15) and a 3′ noncoding region probe from the K^q gene (pH8D, see reference 22). Additional probes isolated from the L^d gene used were a 0.4-kb Xba I–Bam HI 5′ flanking probe (5′ fl-400), a Sma I–Sau 3A α1 probe, a Bgl II–Pst I α3 probe, and a Pst I–Bgl II transmembrane probe. These probes were labeled by the random hexamer priming technique of Feinburg and Vogelstein (23) using α-[^{32}P]dATP and α-[^{32}P]dCTP (Amersham Corp., Arlington Heights, IL). In addition, four oligonucleotide probes were used to further screen the isolates: L^dα3A (5′-TTCACCTTTAGATCTGGGGTGATG-3′) complementary to the 5′ variable region in the α3 exon of the L^d gene, L^dM (5′-TCCAATGATGGCCATAGC-3′) complementary to the variable region of the transmembrane exon of the L^d gene, KM (5′-GCTCCAGTGACTATTGCAGCTCC-3′) complementary to the consensus K sequence in the transmembrane exon, and D4^{d}M complementary to the transmembrane exon variable region in the $D4^d$ gene (sequence not available; obtained from S. Hunt, University of North Carolina, Chapel Hill, NC). The oligonucleotide probes were labeled at the 5′ end with T4 kinase and γ-[^{32}P]ATP (New England Nuclear, Boston, MA).

Restriction Endonuclease Mapping and Alignment of Cosmid Clones. Recombinant cosmids purified by cesium chloride banding were used for mapping restriction endonuclease sites according to the method of Graham et al. (24). Briefly, recombinant cosmid DNA was linearized with restriction endonuclease Cla I, which cuts only in the vector in all of the cosmids mapped in this study. Then the linearized cosmids were partially digested with 0.1–0.2 U of restriction endonucleases per microgram of DNA, and the fragments generated by partial digestion were resolved by field inversion gel electrophoresis (FIGE) on a 1% agarose gel. Electrophoresis was performed for 20 h at 12°C in 0.5× TBE (89 mM Tris-Borate, 2 mM EDTA) at 350 V in the forward direction and 250 V in the reverse, with a switching time of 0.3 s. These conditions were optimal for resolution of fragments up to 50 kb. After blotting the gel onto nitrocellulose, the filter was hybridized at 65°C with random hexamer-labeled probes that correspond to the left (1.4-kb Hind III–Hpa I) or right ends (1-kb Cla I–Bgl II) of the linearized vector. The blot was washed twice at 65°C in 0.5× SSC for 30 min and then exposed to X-Omat AR film, for ~1 h with a single intensifying screen. The restriction endonuclease sites were calculated from the size of the partial fragments that hybridized to either the left or right end probes. The resulting restriction endonuclease maps were then compared with each other, and overlapping cosmids were grouped together. The alignment of these D^q region cosmids with the D^d region of BALB/c was performed by comparing the restriction endonuclease (Bam HI, Kpn I, and Hpa I) maps. The location of the three genes isolated were determined by DNA blotting and hybridization with class I gene probes.

DNA-mediated Transfer of Genes into Mouse L Cells. Recombinant bacteriophage and cosmid DNA was introduced into mouse DAP-3 (thymidine kinase–negative [tk$^-$]) L cells by calcium phosphate precipitation (25, 26). Briefly, DAP-3 cells were plated at 10^6 cells per 75-cm^2 flask in DME containing 10% FCS. 5 μg of the recombinant bacteriophage or cosmid DNA was added to 30 μg of carrier DAP-3 DNA; 250 ng of a plasmid containing the herpes simplex thymidine kinase gene were added to the recombinant bacteriophage DNA mixtures. These mixtures were sterilized by ethanol precipitation. The precipitates were resuspended in 250 μl of sterile 10 mM Tris-HCl, pH 7.5, 1 mM EDTA, and then 250 μl of sterile 0.5 M $CaCl_2$ was added to each sample. Each mixture was added dropwise to tissue culture plates containing 0.5 ml of 2× sterile Hepes-buffered saline (50 mM Hepes, 280 mM NaCl, 1.5 mM $Na_2HPO_4 \cdot H_2O$) and left undisturbed for 30 min at room temperature. These precipitates were added to the plated DAP-3 cells and incubated overnight in 5% CO_2 at 37°C. The next day the medium was replaced with selective medium containing either HAT or the neomycin analogue, G418. Clones that appeared in ~3 wk were picked and grown in mass culture.

Serological Analyses by Cytofluorometry. Cells (4×10^5) were placed in the wells of a round-bottomed microtiter plate and washed twice in cytofluorometric medium (HBSS lacking calcium, magnesium, and phenol red, but containing 0.2% BSA and 0.1% sodium azide). The cells were then incubated for 30 min at 4°C in the presence of a saturating concentration of mAb or alloantiserum. The cells were washed twice and resuspended in a saturating concentration of fluorescein-conjugated, affinity-purified $F(ab')_2$ fragment of goat anti-mouse IgG (Fc specific) or of goat anti-mouse IgM (CooperBiomedical, Malvern, PA). The cells were incubated for 30 min at 4°C, washed three times, and resuspended in cytofluorometric medium containing 10 μg/ml propidium iodide (used to exclude dead cells from the analysis).

Cells were analyzed on a FACS (FACS IV; Becton Dickinson & Co., Mountain View, CA) equipped with an argon laser tuned to 488 nm and operating at 300 mW of power. Cytofluorometric histograms were generated with logarithmic amplification of fluorescence emitted by single viable cells. Each sample analyzed comprised a minimum of 4×10^4 cells. Cells labeled with only fluorescein-conjugated antibody were always included as background controls.

DNA Sequencing. The Bam HI-Xba I fragments (1.4 kb) from bacteriophage A2 containing the D^q gene and from cosmid 33.3 containing the L^q gene were subcloned into the replicative forms of M13mp18 and M13mp19 digested with the same endonucleases. These 1.4-kb fragments were ascertained to contain the α1 and α2 exons by DNA blot analysis of the bacteriophage A2, cosmid 33.3, and the M13mp18 and M13mp19 subclones. Single-stranded DNA obtained from the M13 phages was subjected to dideoxy sequencing (27) using various primers, 5'-α-[^{32}P]dATP (Amersham Corp. or ICN Radiochemicals, Irvine, CA) and either the Klenow fragment of DNA polymerase I (Boehringer Mannheim Biochemicals) or Sequenase (using a Sequenase sequencing kit from U. S. Biochemical, Cleveland, OH). Universal primer (Boehringer Mannheim Biochemicals) and synthetic primers were used to sequence the 1.4-kb segment from both genes in both directions. The sequencing reactions were double-loaded on a 6%, 40-cm Poker face sequencing gel (Hoefer Scientific Instruments). The gels were then exposed to X-Omat AR film for 16–40 h.

CTL Assays. Allogeneic CTL were generated by priming C3H mice with B10.AKM tail skin grafts. After rejection of the skin grafts, splenocytes from the C3H mice were restimulated in vitro for 5 d in CTL sensitization medium with irradiated B10.AKM splenocytes, or L cell transfectants expressing the D^q, L^q, or L^d gene. This medium consisted of RPMI 1640 with 100 U/ml penicillin, 100 μg/ml streptomycin, 2 mM L-glutamine, 110 mg/l sodium pyruvate, 2×10^{-5} M 2-ME, nonessential amino acids, and 10% FCS (selected lot from Flow Laboratories, Inc., McLean, VA). The restimulation cultures was incubated in 24-well plates (Flow Laboratories, Inc.) with 5×10^6 responders and either 2.5×10^6 irradiated (2,000 rad) B10.AKM splenocytes or 10^5 irradiated (10,000 rad) L cell transfectant stimulators per well in 2 ml of medium. Another group of primed cells was incubated in the absence of irradiated stimulator cells as a control. Cytotoxicity was assayed in triplicates in a 4-h ^{51}Cr-release assay in RPMI 1640 medium plus 10% FCS using a range of E/T ratios. Full lysis and spontaneous lysis controls were performed on each target group. The specific lysis was calculated as: Percent specific lysis = 100 × [(the mean of the experimental lysis − the mean of the spontaneous lysis)/(full lysis − spontaneous lysis)]. The SEM was always <6% specific lysis.

Results

Isolation of the D^q and L^q Genes, and their Alignment with BALB/c D Region Genes. Strain B10.AKM is a natural intra-H-2 recombinant mouse strain whose K-S regions were typed as having a k haplotype origin derived from the AKR strain and whose D region was typed as having q haplotype origin derived from a noninbred mouse (28). The antigens encoded by the D^q region of B10.AKM mice, or its congenic partner, AKR.M mice, have no known serological or functional differences from the antigens encoded by the D^q region from DBA/1 or SWR/J mice (29). Previous serological and peptide mapping studies of the B10.AKM mouse suggested that it expresses multiple D region class I molecules (8, 9). Furthermore, those studies also

suggested that its D region class I molecules are closely related to the L^d molecule (9, 11). Thus, molecular approaches were undertaken to isolate the genes encoding those molecules. Since probes generated by cloning the coding regions of a class I gene tend to crosshybridize with many of the other members of this multigene family, low copy probes were developed from noncoding regions surrounding the gene(s) of interest. In fact, two probes, probe 4 (15) from the 3′ flanking region of the L^d gene and pH8D (22) from the 3′ noncoding region of the K^q gene, were demonstrated to be low copy probes that hybridized primarily to D, or K and D region class I genes, respectively. Thus, these two probes were used to screen the bacteriophage and cosmid libraries generated from B10.AKM genomic liver DNA. Two L^d-specific oligonucleotide probes, one complementary to the L^d sequence in the 5′ variable region in the α3 exon, L^dα3A (15), and the other complementary to the L^d sequence in the variable region of the transmembrane exon L^dM (30) were then used to further screen the isolates. The L^dα3A probe probably corresponds to the sequence encoding the epitope bound by the mAb 28-14-8 (unpublished data), whereas the L^dM probe has been demonstrated to hybridize to H-2^q cDNA clones containing the 3′ portions of genes with homology to that of the L^d gene (30). Since all of the previously described D^q region class I molecules react with the mAb 28-14-8, and since they were all found to be homologous by peptide mapping to the prototypic L^d molecule (8, 9), these L^d-derived oligonucleotide probes were used to identify the D^q region genes of interest.

As shown in Table I, all isolated clones (of both bacteriophage and cosmid library origin) that contained an entire class I gene could be classified into one of two major categories, those containing either K region or D region class I genes. The first group of clones hybridized only to pH8D but not to probe 4 nor the L^d-derived oligonucleotide probes (Table I). These clones hybridized to an oligonucleotide probe (KM) complementary to the consensus sequence in the variable region of the transmembrane exon of *K* class I genes (30). Thus, these data suggest that these clones contain the K^k gene from B10.AKM . The second group of clones hybridized to both of the screening probes as well as to both of the L^d-derived oligonucleotide probes, which suggests that they contain the D region genes of interest. In this second group, two subgroups were further distinguished by restriction endonuclease mapping (Fig. 1 and Table I). These maps were generated by FIGE of partial restriction endonuclease digestions of the cosmids which obviates the need to subclone smaller fragments. Also, the sizes of the restriction endonuclease fragments and the number of sites were confirmed by DNA blot analysis on complete digests of the cosmid clones. Based on alignment with the D^d gene from BALB/c, one subgroup of cosmids contained a gene that we are designating as D^q; representative cosmid and bacteriophage clones of this type (cosmids 5.1, 3.1, and 34.2, and bacteriophage A2 and A14) are shown in Fig. 1. The cosmid clone 33.3 (Fig. 1 and Table I) demonstrated a significantly distinct restriction endonuclease pattern from those cosmids containing the D^q gene (Fig. 1); based on its similarity to the 5′ flanking region of the L^d gene and on the hybridization of a linked gene to a $D4^d$ gene–derived oligonucleotide, the L^d-like gene on this cosmid was designated as the L^q gene. Furthermore, the other class I gene linked to the L^q gene on cosmid 33.3 was identified as the $D4^q$ gene (Fig. 1). Taken together, these data strongly suggest that B10.AKM has a D region organization similar to BALB/c.

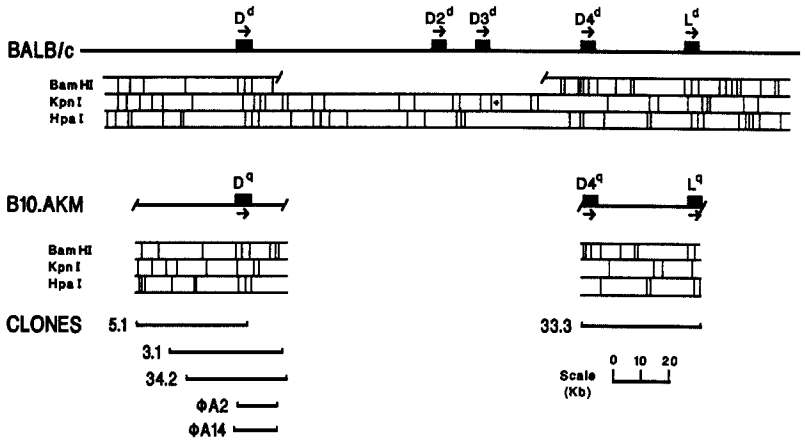

FIGURE 1. Tentative alignment of the B10.AKM D region class I genes with those in BALB/c (13, 16). The symbol (Ø) designates bacteriophage clones; all others are cosmid clones. The *D4*^{q} gene was designated as such due to its hybridization with a *D4*^{d} gene-derived oligonucleotide probe and to its position in relationship to the L^q gene.

TABLE I

Comparison of the Hybridization Patterns of B10.AKM Genomic Class I Clones with Class I Gene Probes

	Probes						
Clones	5'fl-400*	L^dα3A‡	L^dM‡	KM‡	$D4^d$M‡	pH8D*	Probe 4*
38.3 (K^k)§	+	–	–	+	ND	+	–
84.2 (K^k)	+	–	–	+	ND	+	–
ØA3 (K^k)	+	–	–	+	ND	+	–
ØA5 (K^k)	±	–	–	+	ND	+	–
ØA6 (K^k)	+	–	–	+	ND	+	–
3.1 (D^q)	+	+	+	–	–	+	+
5.1 (D^q)	+	+	+	–	–	+	–‖
34.2 (D^q)	+	+	+	–	–	+	+
60.5 (D^q)	+	+	+	–	–	+	+
80.1 (D^q)	+	+	+	–	–	+	+
ØA2 (D^q)	+	+	+	–	ND	+	+
ØA14 (D^q)	+	+	+	–	ND	+	+
33.3 ($D4^q$, L^q)	+	+	+	±¶	+	+‖	±‖¶
59.2 (L^d)	+	+	+	ND	–	ND	+
12.1 (L^d)	+	+	+	ND	–	ND	+
17.1 (D^d)	ND	–	ND	ND	–	ND	+

* Cloned probes; origin and location are described in Materials and Methods.

‡ Oligonucleotide probes; origin is described in Materials and Methods.

§ The clones in this group probably contain the K^k gene based on hybridization to the *K* gene-specific oligonucleotide probe, but sequence and transfection analysis are needed for confirmation.

‖ Truncated gene; this classification was chosen for these clones because they did not contain enough 3' prime flanking sequence to hybridize to probe 4 (Fig. 1).

¶ Weak hybridization, most likely due to the *D4*^{q} gene.

Despite the similarities in the restriction endonuclease patterns between the regions flanking the D^q and D^d genes, differences in the length of the fragments from the immediate 5′ flanking region of the D^q gene as compared with the analogous fragments flanking the D^d gene are apparent in Fig. 1. The fragments in BALB/c are ~5 kb longer than those in B10.AKM. This 5′ flanking region difference was confirmed in a side by side comparison of the restriction endonuclease maps of D^q gene cosmid clones and an analogous BALB/c cosmid clone (obtained from Dr. M. Steinmetz, F. Hoffman-La Roche and Co., Lt., Basel, Switzerland). The immediate 5′ flanking regions of the L^q, L^d, D^b, and D^k genes apparently lack this 5-kb insert; thus, it appears to be unique to the immediate 5′ flanking region of the D^d gene (3, 13).

Serological Analysis of L Cells Expressing Isolated D^q Region Class I Genes. The calcium phosphate precipitation method (25) was used to transfer recombinant bacteriophage clone DNA along with the HSV thymidine kinase gene or recombinant cosmid DNA containing the aminoglycosyl-3′-phosphotransferase gene into the mouse DAP-3 (tk^-) L cell line ($H\text{-}2^k$) followed by selection in medium containing HAT or the neomycin analogue, G418. The bulk transfectants and clones, or sorted populations of cells derived from those bulk populations, were then analyzed by cytofluorometric analyses using a battery of mAbs (Table II). Consistent with the restriction

TABLE II

Comparison of the Serological Reactivities of the D^q and L^q Molecules with the L^d, D^d, and D^b Molecules Using a Panel of mAbs

mAb*	Primary Reactivity	D^q	L^q	L^d	D^d	D^b	Domain‡
93	D^b	−	+	−	−	+	
143	D^v	−	+	−	−	−	
115	D^q	+	−	−	−	−	
Do4	K^d	+	−	−	+	−	α1/α2
Do7	K^d	+	−	−	wk§	−	
23-10-1	L^d	+	−	+	−	−	α1/α2
1634	L^d	+	−	+	−	−	
117	D^q	+	−	−	−	−	
113	D^q	+	−	−	−	−	
64-13-6	D^q	+	−	−	+	+	
66-3-5	D^q	+	−	+	−	−	
66-13-17	D^q	+	−	−	+	+	
30-5-7	L^d	+	wk	+	−	−	α1/α2
23-5-21	D^b	+	wk	−	+	+	α1/α2
57A1	D^q	+	wk	−	−	−	
66-2-4	D^q	+	wk	+	−	+	
28-14-8	D^b	+	+	+	−	+	α3
228	D^b	+	+	+	−	+	α3
248	D^d	+	+	−	+	−	α1/α2
66-4-8	D^q	+	+	+	+	+	
66-8-2	D^q	+	+	+	−	+	
66-13-5	D^q	+	+	+	+	+	

* The origin of these mAbs can be obtained in references 31–35.

‡ The domain specificities for some of these mAbs were reported in reference 36 or here.

§ Weak crossreaction detected.

endonuclease patterns of the isolated genes, the transfectants expressing those genes fell into two categories, as indicated in Table II: those expressing the D^q gene, and one expressing the L^q gene. All of the D^q gene transfectants showed nearly identical serological profiles indicating that the cosmid and bacteriophage clones contained identical D^q genes, as their restriction endonuclease patterns suggested. Preliminary data suggest that the L^q gene transfectant, constructed by DNA-mediated transfer of the entire cosmid 33.3 into mouse L cells, expresses only the L^q gene, as transcripts hybridizing to the $D4^d$ gene-derived oligonucleotide probe could not be detected; this apparent lack of expression of the $D4^q$ gene may reflect the absence of its promoter on this cosmid. In Fig. 2, *A* and *C*, both the D^q and L^q gene transfectants reacted with the mAb 28-14-8; this mAb reacts with the prototypic L^d molecule and another closely related molecule, D^b. Furthermore, D^q gene transfectants reacted strongly with the mAb 30-5-7 (Fig. 2 *A*), whereas the L^q gene transfectant reacted only weakly with this mAb (Fig. 2 *C*).

In Table II, only two mAbs, 93 and 143, were found to react exclusively with the L^q gene transfectant. The cytofluorometric profile of the D^q and L^q gene transfectants with the mAb 93 is shown in Fig. 2, *B* and *D*. 10 different mAbs were found to react exclusively with the D^q gene transfectant (Table II); the cytofluorometric analysis of two of those mAbs, 113 and 1634, on the D^q and L^q gene transfectants are also shown in Fig. 2. When tested on the two transfectants, the remaining mAbs fell into two serological patterns: 30-5-7-like or 28-14-8-like reactivity. However, it should be noted that all of these mAbs, with the exceptions of 28-14-8 and 228, and 66-4-8 and 66-13-5, detect different serological specificities. Moreover, in some cases, the mAbs can be distinguished by their domain specificity (Table II). The serological analysis of D^q and L^q gene transfectants included the use of the alloantiserum to the private specificity, H-2.30. This antiserum was used in the original

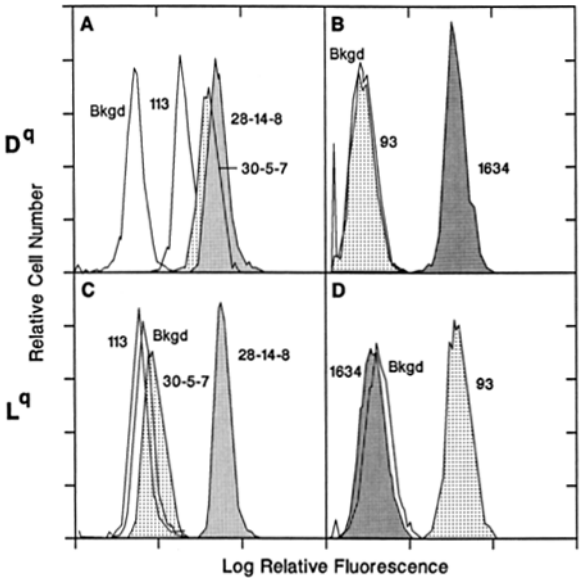

FIGURE 2. Cytofluorometric analyses of the D^q (*A* and *B*) and L^q (*C* and *D*) gene transfectants stained with mAbs and fluorescein-labeled goat anti-mouse IgG (*A* and *C*) or fluorescein-labeled goat anti-mouse IgM (*B* and *D*). Background (*bkgd*) indicates that no primary antibody was added.

serological (8), peptide mapping (9), and partial amino acid sequence (9) studies performed on B10.AKM splenocytes to distinguish and isolate the D^q molecule from other D^q region class I molecules. Using cytofluorometric analysis (data not shown), the H-2.30 antiserum was found to react exclusively with D^q gene transfectants and not with the L^q gene transfectant. The reason for this reactivity with the D^q and not the L^q molecule became clear after analysis of the sequence data presented below. The antiserum was produced in a mouse strain that expresses the L^d molecule and was produced against the D^q region molecules; since the L^d and L^q molecules are nearly identical, it is easy to understand why the antiserum is specific for the D^q molecule. This finding resolves a serological enigma of the past, namely why private specificities are difficult to detect on L^d-like molecules.

Sequence Analysis of the D^q and L^q Genes. The sequence comparisons of the 5′ regions of the D^q and L^q genes with the L^d gene are presented in Fig. 3; each region represents approximately half of each class I gene and contains most of the sites where polymorphisms among various other class I genes have been observed. The amino acid sequence derived from the nucleic acid sequence of the D^q and L^q genes correlates exactly with the partial amino acid sequence available for two D^q region class I molecules with one exception at amino acid position 83 of the D^q molecule (9). Furthermore, the sequence data presented in Fig. 3 corroborates several points suggested by the tryptic peptide map comparisons of D region class I molecules (9, 11). First, the D^q and L^q genes are quite homologous to the L^d gene, and second, the D^q and L^q genes are quite homologous to each other. These homologies are presented in quantitative form in Table III. In total, D^q is as homologous to L^q as it is to L^d, although these homologies are distributed differently in the various exons and introns. On the other hand, L^q is more homologous to L^d than it is to D^q, especially in the α1 exon and in introns 1 and 2. Indeed, the L^q gene sequence was found to differ from L^d at only 16 out of >1,400 positions compared. Interestingly, several of the nucleotides specific for the L^q gene and not the L^d gene were clustered in two sites and were identical to the D^q gene sequence: nucleotide positions 733–740 and 914–921 in the α2 exon (Fig. 3). These findings suggest that two distinct gene copy events occurred in a primordial L^d-like gene, and that the highly homologous D^q and L^q genes resulted from a relatively recent duplication event. In addition, Table III illustrates that the D^q and L^q genes are not only homologous in the 5′ region to the prototypic L^d gene but are also quite homologous to the D^b gene, a gene previously shown to be closely related to the L^d gene. Furthermore, it is noteworthy that the 3′ regions of class I genes are less polymorphic in general, and that the D^q, L^q, L^d, and D^b genes hybridize to and contain sequences identical to the aforementioned 3′ region L^dα3A and L^dM oligonucleotide probes (Table I). Thus, it is likely that the sequences in the 3′ region of the D^q, L^q, L^d, and D^b genes are identical or nearly identical. Therefore, taken together, these data provide further evidence for a group of genes closely related to the apparently prototypic L^d gene.

An additional unexpected observation is illustrated by Tabel III. The D^q and L^q genes were found to be extremely homologous to the *A166* and *A149* genes, respectively. In fact, within the coding sequences, the D^q and L^q genes are identical to the *A166* and *A149* genes, respectively. These latter two genes (17) were isolated from a C3H (H-2^k) UV-induced fibrosarcoma, 1591, and were reported to encode tumor-

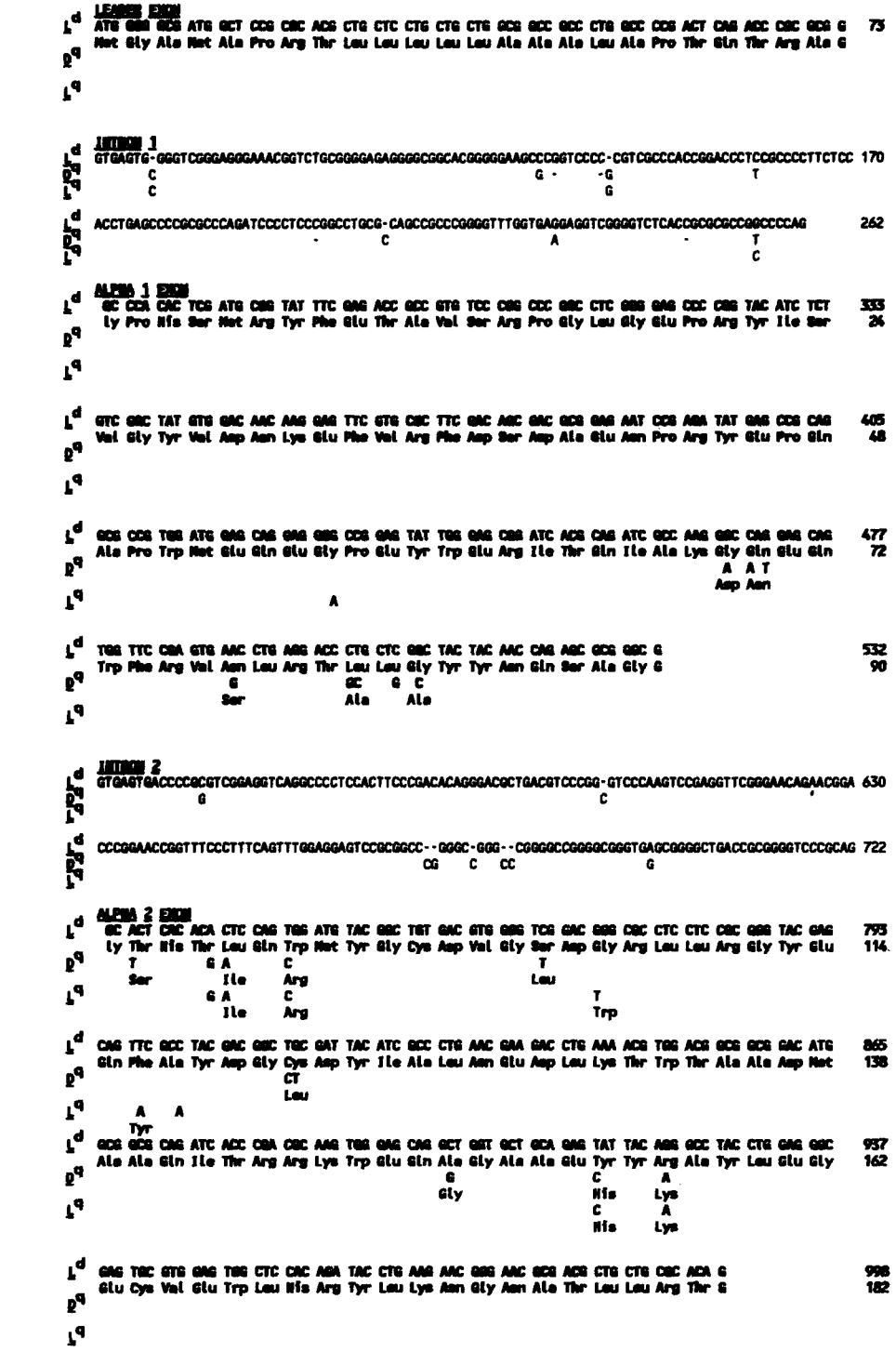

FIGURE 3. Continued on facing page.

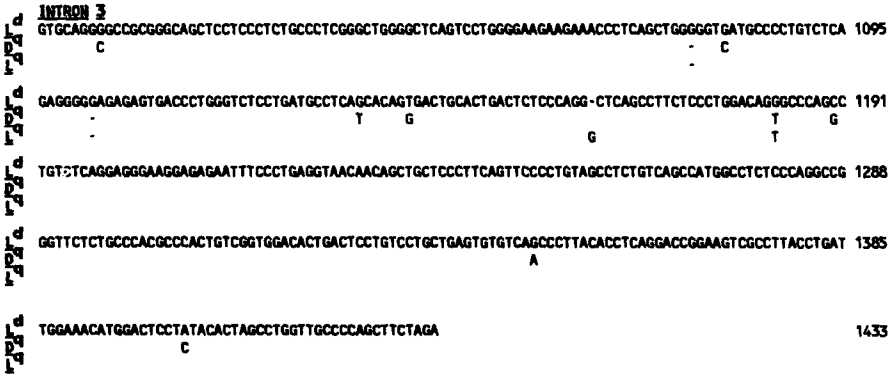

FIGURE 3. DNA sequence and implied amino acid sequence comparisons of the 5′ region of the D^q and L^q genes with the L^d gene sequence (37). Although the entire regions were sequenced in both directions for the D^q and L^q genes, only differences from the L^d gene are shown for those genes. Gaps are indicated by dashes.

specific antigens. The differences observed in the introns could be due to sequence error, since these areas are GC rich. Furthermore, the 3′ regions of the *A166* and *A149* genes contain sequences identical to the L^d gene-derived oligonucleotide probes mentioned above. Thus, these genes, reported to encode tumor-specific antigens, are probably identical (or nearly identical) to the D^q and L^q genes. These observations raise questions concerning the origin of the fibrosarcoma 1591.

Functional Analysis Using the D^q and L^q Gene Transfectants as Stimulators of and Targets for CTL. The expression of the D^q and L^q genes in mouse L cells allows for the investigation of their recognition by CTL. Since the L cell transfectants express their endogenous class I gene products, K^k and D^k, on the cell surface in addition to the exogenous gene product, and B10.AKM mice express K^k on the surface of their cells, CTL were raised in C3H ($H\text{-}2^k$) mice to eliminate reactivity to these antigens. Thus, after priming C3H mice with B10.AKM cells in vivo, CTL cultures were set up in vitro by incubating in the presence of irradiated B10.AKM splenocytes, or irradiated L cells expressing the D^q, L^q, or L^d molecules. A fifth group of effector cells were cultured in the absence of a secondary stimulator. The five effector groups were tested for cytotoxicity on five different ^{51}Cr-labeled targets: Con A-activated B10.AKM splenocytes, the D^q, L^q, and L^d gene L cell transfectants, and the untransfected L cell (DAP-3). The results of these assays are shown in Fig. 4. Unexpectedly, the CTL generated by secondary stimulation with B10.AKM splenocytes or the D^q or L^q gene transfectant recognized the L^d gene transfectant as a target (Fig. 4 *D*) almost as well as they recognized the secondary stimulating cell. These data suggest a significant amount of crossreactivity among the CTL generated in this manner. Reciprocally, CTL generated by secondary stimulation with the L^d gene transfectant also recognized the targets expressing D^q or L^q gene products (Fig. 4, *A-C*). This recognition of the D^q region class I gene products by the CTL generated by secondary stimulation with the L^d gene transfectant was not due to residual reactivity of the CTL from the primary stimulation with B10.AKM cells, since the effector population that was cultured in the absence of secondary stimulation did

TABLE III

Homologies of D Region Class I Genes

Genes compared		Gene regions						Total
		L	i1	α1	i2	α2	i3	
D^q vs.	*A166*	100*	97.0	100	98.4	100	99.1	99.1
	A149	100	96.0	96.7	97.4	97.1	97.9	97.3
	L^q	100	95.5	96.7	96.6	97.1	98.3	97.2
	L^d	100	96.0	97.0	97.4	96.4	97.9	97.2
	D^b	100	95.0	94.4	95.3	95.3	98.4	96.3
	D^k	93.2	91.3	89.6	94.0	90.6	96.2	92.8
	D^p	100	93.4	86.7	91.6	92.0	96.1	92.7
	D^d	95.3	91.6	89.3	90.2	92.0	95.6	92.4
L^q vs.	*A149*	100	98.9	100	100	100	99.4	99.7
	L^d	100	98.9	99.6	100	97.1	99.4	99.1
	A166	100	98.4	96.7	98.9	97.1	98.3	97.9
	D^b	100	99.5	95.6	98.9	94.2	99.0	97.5
	D^q	100	95.5	96.7	96.6	97.1	98.3	97.2
	D^k	93.2	95.3	90.7	96.5	92.0	96.1	94.1
	D^d	95.3	94.5	88.1	93.4	92.0	95.5	93.0
	D^p	100	91.6	87.0	92.5	91.7	96.7	92.8
L^d vs.	*A149*	100	100	99.6	100	97.1	100	99.4
	L^q	100	98.9	99.6	100	97.1	99.4	99.1
	A166	100	98.9	97.0	98.9	96.4	97.7	97.8
	D^b	100	98.9	95.9	98.9	94.2	98.6	97.5
	D^q	100	96.0	97.0	97.4	96.4	97.9	97.2
	D^k	93.2	95.8	91.1	96.5	90.9	95.5	93.9
	D^p	100	94.2	87.4	92.5	92.0	96.3	93.2
	D^d	95.3	91.3	88.5	92.9	94.6	94.9	92.9

* Numbers indicate percent homology in that exon or intron. The sequences for the D^d and D^p genes lack the first nine nucleotides of the leader (L) exon, and thus, comparisons with those genes were formulated on the basis of the last 64 nucleotides of that gene region only. L, leader exon; i1, intron 1; α1, α1 exon; i2, intron 2; α2, α2 exon; i3, intron 3 (only that part of intron 3 shown in Fig. 3 was analyzed). The sequences for the L^d, *A166*, *A149*, D^b, D^d, D^k, and D^p genes were obtained from references 37, 17, 17, 14, 38, 14, and 39, respectively.

not recognize any of the targets. In fact, lysis of all targets with this unstimulated population of effectors (E/T ratios ranging up to 40:1) was <2% specific lysis (data not shown). Furthermore, the CTL generated by secondary stimulation with either the D^q or L^q gene transfectant crossrecognize the other transfectant as a target almost as well as they recognize the secondary stimulator cell (Fig. 4, *B* and *C*). Collectively, these data suggest that the products of the D^q, L^q, and L^d genes are closely related functionally.

Discussion

The data presented here demonstrate that the D^q region of B10.AKM has a very similar gene organization to that of the D^d region of BALB/c. This alignment of the D^q, $D4^q$, and L^q genes isolated from B10.AKM with the analogous genes in BALB/c is based on the restriction endonuclease pattern similarities as resolved by FIGE. This analogy was strengthened by the demonstration that, like the L^d gene

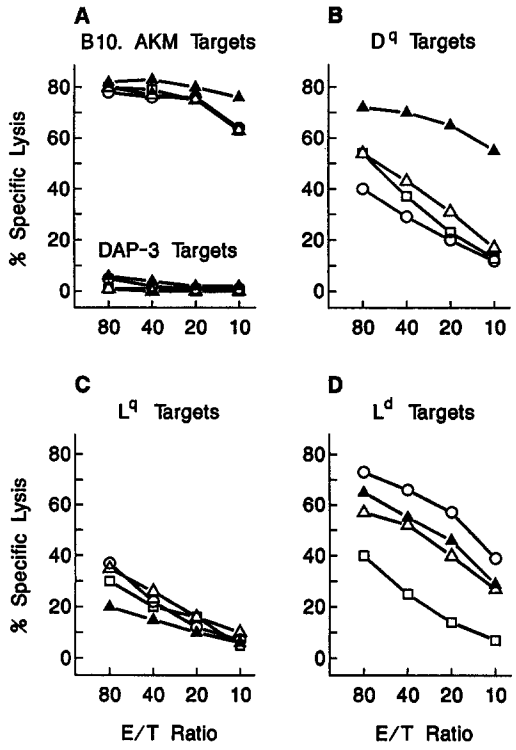

FIGURE 4. CTL recognition of the D^q, L^q, and L^d molecules. Splenocytes from C3H mice primed in vivo by B10.AKM cells were restimulated in vitro by irradiated B10.AKM cells (*open squares*), D^q gene transfectants (*closed triangles*), L^q gene transfectants (*open triangles*), L^d gene transfectants (*open circles*), or were cultured without secondary stimulation. The different pools of CTL were then assayed for activity in a ^{51}Cr-release assay on Con A-stimulated B10.AKM splenocytes (*A*), D^q gene transfectants (*B*), L^q gene transfectants (*C*), L^d gene transfectants (*D*), or the untransfected parent L cell line, DAP-3 (*A*). The lysis by splenocytes cultured without restimulation was <2% on all targets (data not shown).

(13), the L^q gene is linked on its 5′ side to the *D4* gene. This *D4* gene isolated from B10.AKM was identified as such by its hybridization with a $D4^d$ gene-derived oligonucleotide probe. It should be noted that the maps of cosmids clones containing the D^q gene and that of the cosmid clone containing the $D4^q$ and L^q genes are not contiguous, and therefore, the genomic segment between the D^q and $D4^q$ genes has yet to be characterized. Thus, it remains to be determined whether the D^q region also contains "alleles" of the $D2^d$ and $D3^d$ genes defined in BALB/c.

Despite the similarity of the D region organization in BALB/c and B10.AKM, the distribution of L^d-like genes differs. BALB/c expresses at least two D region class I genes: the prototypic L^d gene and the more distantly related D^d gene (Table III). On the other hand, the D^q and L^q genes from the B10.AKM D region are both closely related to each other and to the L^d gene (Table III). However, of the L^d-like genes compared in Table III, D^q is the least homologous to the L^d gene; moreover, of the two D^q region class I genes, D^q has diverged more from L^d. The similar gene organizations in the D regions of BALB/c and B10.AKM may have arisen by misalignment of a single D region class I gene with a linked (telomeric) Q region gene followed by a nonequal crossover event as proposed by Stephan et al. (13). This "duplication" event would yield two D region class I genes flanking Q region class I genes. Recent sequence analysis of the *Q4* gene suggesting that it is comparatively homologous to D region class I genes lend more credence to this model (40). The paradox of the apparent similarity in the D region gene organization of these two strains

but dissimilarity in the relationship of their genes could be explained by two different models.

Model 1. If the putative "duplication" event occurred independently in the ancestry of BALB/c and B10.AKM, then the dissimilarities of the D^q and D^d regions could be readily explained. It could be postulated that the ancestral D^q region genes that underwent duplication were derived from the same primordial *L*-like gene. This would explain the stretches of identical sequence in the α2 exons of the D^q and L^q genes that differ from the L^d gene sequence. In other words, it seems more likely that the D^q gene resulted from a duplication of an ancestral L^q gene that had accumulated two clusters of mutations via gene copy events than the same two gene copy events occurring in both of the ancestral D^q and L^q genes independently. By contrast, the ancestral D^d region genes could have arisen from a duplication event in which two allelic D region genes (e.g., one L^d-like and one D^d-like) were involved. However, to explain the similarities of the 5′ flanking regions of the L^q and L^d genes, one must postulate that the recombination events resulting in the duplication occurred in nearly identical locations in the two ancestral strains.

Model 2. If a duplication event occurred in a common ancestor of BALB/c and B10.AKM, then our data suggest that the D^q region is more similar to the ancestral haplotype in which the duplication occurred. This proposal is based on the striking homologies observed in the comparison of the D^q and L^q genes. The dissimilarity of the D^d gene from the L^d gene in the D^d region (and from the D^q and L^q genes in the D^q region) may have resulted from either a series of gene copy events or an intra-D region recombination event. The occurrence of these types of events in the MHC has been well documented (5, 41). Given the validity of this model, we would propose that a progenitor *L* gene underwent a duplication event, which gave rise to two *L*-like genes in the ancestral D^q region. These genes went on to evolve to become the D^q and L^q genes as isolated from B10.AKM. Furthermore, the ancestral D^d region could have evolved from the ancestral D^q region via either the introduction of substantial changes into its *D* gene either by gene copy events or the exchange of the *D* gene for another D allele (intra-D region recombination). Verification of either model will come from the analysis of D region organization of additional haplotypes with particular emphasis on those known to encode multiple products.

The results reported here further substantiate the existence of a cline of D region class I genes closely related to the L^d gene among mice of several H-2 haplotypes. At least two hypotheses can be invoked to explain this phenomenon. First, these genes could be more closely related because of some selective pressure to conserve their amino acid sequence. Second, this cline of genes may be more closely related to L^d than to other D region class I genes, because they represent comparatively recent ancestors of L^d that have not accumulated mutations. By examining whether nucleotide substitutions as compared with the L^d gene result in amino acid changes (replacements) or not (silent) in the encoded molecule, one can attempt to distinguish between the above alternatives. Since the selective pressure should be exerted on the amino acid sequence and not on the nucleic acid sequence, one would expect to observe a greater than random percentage (24%) of silent substitutions if the first hypothesis is correct. In Table IV, the nucleic acid sequences from the 5′ portions of various D region class I genes were compared with that portion of the L^d gene, and the silent and replacement substitutions were distinguished. Interestingly, the

L^q and D^b genes, and not the D^q gene, have a greater number of silent substitutions than randomly expected (the number in parentheses). As expected, in the D region class I genes (D^d and D^k) that are not as closely related to the L^d gene, the number of silent substitutions in comparing these genes with the L^d gene approximates the random percentage of the total. This higher level of divergence for the *D* genes as compared with the *L* genes in B10.AKM and BALB/c may be due to their more centromeric location on the chromosome, which may make them more accessible to gene copy events than the more telomeric *L* genes. Perhaps the presence of less polymorphic Q region-like genes centromeric of the L locus (*D2, D3,* and *D4*) insulates the L locus from change via gene copy events. Alternatively, this divergence may reflect conservation of one L^d-like gene (namely L^q or L^d) due to some selective force; thus, the possession of two L^d-like genes in the genome allows one of those genes to diverge more rapidly. In our analysis of silent vs. replacement substitutions in Table IV, the D^b gene demonstrates a significantly higher level of silent substitutions as compared with L^d than randomly expected suggesting that some selective force has preserved the amino acid structure of the product encoded by this gene. Since only one class I gene, the D^b gene, has been found in H-2^b mice, conservation of the sequence may be more important than in mice that possess two L^d-like genes in their D regions. On the other hand, B10.AKM and BALB/c, which express the closely related L^q and L^d genes, also express the D^q and D^d genes. The

TABLE IV

Comparison of D Region Class I Genes with the L^d Gene: Silent vs. Replacement Substitutions

Gene compared	Exon	Silent	Replacement	Total
L^q *(A149)*	α1	1 (0.24)*	0	1
	α2	2 (1.92)	6	8
	α1 + α2	3 (2.16)	6	9
Dq (A166)	α1	1 (1.92)	7	8
	α2	1 (2.4)	9	10
	α1 + α2	2 (4.32)	16	18
D^b	α1	4 (2.64)	7	11
	α2	8 (3.84)	8	16
	α1 + α2	12 (6.48)	15	27
D^d	α1	7 (7.44)	24	31
	α2	4 (3.6)	11	15
	α1 + α2	11 (11.04)	35	46
D^k	α1	6 (5.76)	18	24
	α2	5 (6)	20	25
	α1 + α2	11 (11.76)	38	49

* Number given in parentheses is the expected number of silent substitutions of that total. Based on random substitution, the number of silent substitutions would be expected to be 24% of the total substitutions. Levels of silent substitution >24% would suggest selection for maintenance of the amino acid sequence. The nucleotide sequence in the α1 and α2 exons is the same for the L^q and *A149* genes, and for the D^q and *A166* genes

latter possess fewer or approximately equal numbers of silent substitutions than randomly expected for the total numbers of substitutions when compared with the L^d gene, suggesting an absence (or minimal contribution) of selection for an L^d-like structure in the evolution of these genes. Collectively, our results suggest that selection makes a significant contribution to the conservation of some of these L^d-like genes.

As many as five different class I antigens have been reported to be determined by genes mapping to the D^q region. Using sequential immunoprecipitation, peptide mapping and partial amino acid sequencing, four D^q-region products designated D^q, L^q, R^q, and $R^{q'}$ were defined (8, 9, 34). Using cocapping techniques, five different D^q region products designated D^q, $L1^q$, $L2^q$, $L3^q$, and $L4^q$ were defined (42). By all available serological and chemical criteria, the previously defined D^q molecule is identical with the product of the D^q gene defined in this study. However, determining the historical correlate of the product of our cloned L^q gene is more difficult. The equivocal typing of the L^q gene transfectant with mAb 30-5-7 raises questions as to whether the product of the cloned L^q gene corresponds to either the previously defined L^q or R^q antigens (8, 9). However, the published amino acid sequence of R^q is clearly most like that of the implied sequence reported here for the L^q gene. Several phenomena could account for the remainder of the previously defined antigenic heterogeneity of products encoded by the D^q region. For example, additional antigens could be encoded by genes mapping between the D^q and L^q genes. Since the D^q region appears to be similar to the D^d region, alleles of the $D2^d$, $D3^d$, and $D4^d$ genes could be detected. Consistent with this prediction, a putative $D4^q$ gene has already been detected centromeric of the L^q gene. It has yet to be determined even in the d haplotype, whether products of the *D2, D3,* and *D4* genes are expressed on the cell surface or elsewhere (13, 16). Alternatively, some of the previously defined heterogeneity could be explained by differential RNA splicing of the D^q or L^q genes. For example, the previously defined L^q antigen was identical to the D^q antigen in NH_2-terminal sequence analyses, yet showed minor differences in a tryptic peptide map comparison (9). Such a result is consistent with the possibility that alternative splicing of the D^q gene leads to alternative antigenic forms. Another mechanism for generating antigenic heterogeneity was suggested by our recent studies of the L^d molecule. L^d molecules synthesized in the absence of β_2-microglobulin (β_2m) show antigenic aberrancies implying their $\alpha1/\alpha2$ binding site is not folded correctly (43). Since the D^q and L^q molecules are structurally homologous to the L^d molecule, they may also have alternative β_2m-dependent conformations. Indeed, the antigenic composition of the previously defined $R^{q'}$ (34) molecule suggests that it may be a non-β_2m-conformed product of the L^q gene. In any case, further molecular studies of products of the D^q region could provide important insights into the mechanisms generating antigenic heterogeneity of class I molecules.

Based on the results of others working in allogeneic CTL systems, the strong broad crossreactivity of our CTL raised against a particular L^d-like antigen on other L^d-like antigens was somewhat unexpected. The results from investigators using limiting dilution and clonal analyses of allogeneic CTL demonstrated that only 10–20% of the total are crossreactive on cells of a third strain (44, 45). In other words, allogeneic CTL that are crossreactive against a third party are comparatively rare.

Even when allogeneic CTL were raised against the K^b molecule and tested against various K^b mutant molecules, crossreactive CTL that recognized all or most of the mutants were comparatively infrequent (46). In our CTL analyses, it is noteworthy that all of the L^d-like molecules used here (L^d, L^q, and D^q) served, not only as targets for the highly crossreactive CTL, but also as secondary stimulators for their production. Moreover, in most cases, they functioned equally well in both capacities with two exceptions. (*a*) The D^q gene transfectant was killed most efficiently by CTL stimulated in vitro with the same transfectant; and (*b*) the L^q gene transfectant served as a poorer target in general for all of the different CTL populations than did the other transfectants. The L^d-like molecules, L^d, L^q, and D^q, differ from each other more than the K^b and K^b mutant molecules differ among themselves (5). Despite having more numerous amino acid substitutions, all of the L^d-like molecules analyzed here are recognized by the crossreactive CTL. Thus, many of the substitutions in the L^d-like molecules must reside in amino acid residues that are less important for CTL recognition (compare reference 5). The substitutions in the K^b mutant molecules were selected by skin graft rejection and thus were selected to be important in CTL recognition. Taken together, these results suggest that the L^d-like molecules are not only related by their conserved nucleic acid and amino acid sequences but are also functionally related.

The striking homologies of the D^q and the L^q genes to previously reported tumor-associated transplantation antigens (TATA) was completely unexpected. Historically, there has been a morass of studies that were interpreted as evidence that TATA resulted from the expression of "alien" class I molecules. Two hypotheses were proposed to explain this putative phenomenon leading to alien class I expression. In the first model proposed by Bodmer (47), it was hypothesized that each H-2 haplotype expresses only a subset of its total class I genes, and that during oncogenesis, normally repressed class I genes undergo derepression. Numerous studies were reported to support such a derepression model (48). In a more recent model, it was proposed that the mechanisms that generate polymorphism of class I genes in germ cells (such as gene conversion and intragenic recombination) also operated in somatic cells and result in TATA (17). Because most of the evidence supporting each of these models could also be accounted for by the misinterpretation of serological data or tumor cell contamination, the association of class I and TATA remained highly controversial. However, the molecular approaches recently applied to the study of the C3H ($H\text{-}2^k$) fibrosarcoma 1591, appeared to substantiate the link between TATA and class I antigens (17). Three genes designated *A216, A166,* and *A149* encoding putative TATA were cloned from a 1591 library. From sequence comparisons, the *A216* gene looks very much like a K region gene but was clearly not K^k, whereas *A166* and *A149* look very much like D region genes but were clearly not the D^k gene. Furthermore, both *A166* and *A149* were found to be highly homologous to the L^d gene, a gene not expressed by $H\text{-}2^k$ strains. Thus, these authors proposed that *A216, A166,* and *A149* genes could have arisen by either deregulation or by an intragenic recombination event, mechanisms previously proposed to result in the formation of TATA. The data we report here strongly suggest an alternative explanation for the origin of these genes in 1591 cells. In the coding sequences compared, the D^q gene is identical to the *A166* gene, and the L^q gene is identical to the *A149* gene. Furthermore, all of the previously published serological and functional characterizations of the

A166 and *A149* gene products are consistent with their identity to the D^q and L^q molecules (8, 9, 17). However, the D^k region was previously shown to contain a single gene, D^k (13, 14). Thus, these findings strongly suggest that *A166* and *A149* are more likely to have resulted from contamination than a novel genetic mechanism. Such a contamination could have resulted either at the level of the original C3H mouse, from which 1591 tumor was isolated, or from cell fusion during passage of this tumor in mice expressing the D^q and L^q genes. Consistent with there being such a contamination, R. Goodenow and R. Linsk (personal comunication) have detected non-C3H isozymes in 1591 cells. Thus, the whole question regarding the association of class I antigen and TATA requires re-examination using tumor cells of a well-defined origin.

Summary

Two phenomena appear to distinguish the D region class I genes from those in the K region in the murine MHC: (*a*) haplotype disparity in the number of expressed D region class I molecules has been observed; and (*b*) clines of closely related D region class I molecules among and within mice of different H-2 haplotypes can be defined. Both of these observations have been based on serological and peptide mapping analyses of these molecules. Recent reports using molecular biological approaches have corroborated these findings. Since the mouse strain B10.AKM expresses multiple D region class I antigens, all of which are closely related to the prototypic L^d molecule, we investigated the D^q region of B10.AKM using molecular approaches. Three D region class I genes were isolated from genomic B10.AKM bacteriophage and cosmid libraries. Based on alignment of those genes with the BALB/c D region class I genes by analogous restriction endonuclease sites and by hybridization of one of those genes with a $D4^d$ gene-derived oligonucleotide probe, we have designated these genes as D^q, L^q, and $D4^q$. As determined by DNA-mediated gene transfer to mouse L cells followed by serological analyses, the D^q and L^q genes encode previously characterized D^q region class I antigens. The nucleic acid sequence comparisons of the D^q and L^q genes demonstrated a higher level of homology with the L^d and D^b genes than with other D region class I genes. In addition, CTL stimulated with a D^q, L^q, or L^d gene transfectant showed strong crossreactions with the other transfectants as targets, suggesting that the products of these genes are also functionally related. Thus, these studies suggest that the L molecule represents a prototypic structure shared by several D region gene products, and furthermore, that duplication of an L^d-like progenitor gene resulted in two D^q region class I genes, D^q and L^q. Unexpectedly, the sequences determined for the D^q and L^q genes are nearly identical to the sequences of two genes, *A166* and *A149*, respectively, which were reported to encode the tumor-specific antigens; these novel class I genes were isolated from an H-2^k fibrosarcoma, 1591. This raises the distinct possibility that these purported tumor-specific class I genes were introduced into this tumor by contamination.

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