STRUCTURE AND FUNCTION OF THREE NOVEL MHC CLASS I ANTIGENS DERIVED FROM A C3H ULTRAVIOLET-INDUCED FIBROSARCOMA

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The class I antigens encoded within the MHC of the mouse are cell surface glycoproteins that play an essential role in immune recognition and regulation. CTL exhibit an absolute requirement for associated recognition of foreign antigens in the context of self MHC components (1). In addition, CTL can recognize non–self MHC molecules. This specific recognition of non–self MHC, known as allorecognition, is the cellular basis of the well known phenomenon of allograft rejection.

Class I antigens also appear to be involved in the immune elimination of certain tumors (2, 3). Many tumors are highly immunogenic and are rejected when transplanted into normal, histocompatible mice (2–6). The ability of such tumors to arise in the autochthonous host might therefore represent the consequence of a compromised immune system that cannot limit neoplastic progression. In fact, many carcinogenic treatments, such as UV irradiation, are also immunosuppressive (5), and this might contribute to the outgrowth of otherwise immunogenic neoplastic cells. Consequently, ~75% of the tumors that arise in UV-treated mice are rejected in normal syngeneic recipients but progress in UV-irradiated animals (5, 6). Interestingly, the immune suppression associated with UV irradiation appears to be specific for tumors arising in UV-irradiated hosts (7) and can be mediated by specific Ts (8). To understand the complex biology of these tumors, it is necessary to identify and characterize the molecules responsible both for their immunogenicity and for their ability to escape the immune system in UV-treated animals.

One UV-induced tumor that has been extensively studied is the C3H fibrosarcoma, 1591 (5). It has recently been shown (9, 10) that 1591 expresses at least three novel class I antigens not normally found on C3H tissue. These novel antigens have been identified by mAbs derived from normal C3H mice immunized with the tumor and are also recognized by allospecific anti-H-2 mAbs (9,

10). Certain tumor variants lacking the novel antigens can grow progressively in immunocompetent hosts, suggesting that these molecules may play an important role in the biology of this tumor (11). The isolation of the genes encoding the novel antigens, termed A149, A166, and A216, has been previously described (12). Since these genes account for several of the polymorphic restriction fragments observed in the class I genes of 1591 when compared with C3H by genomic Southern blot analysis, we have previously proposed (12) that these genes were generated by recombination events among the endogenous class I genes of C3H. To determine the origin of these novel genes and the role of their products in the neoplastic process, it is necessary to characterize their structure and examine the extent to which each molecule can be recognized by the immune system.

In this report, we present the complete DNA sequence of these three novel class I genes, and describe in detail the recognition of their products by alloreactive and tumor-specific CTL. We find that these genes are highly homologous to those of previously characterized class I antigens, such as H-2K^k (13) and H-2L^d (14), and that the structural conservation seen among these gene products is reflected in their functional homology as well. Since the class I diversification events by which these novel tumor genes were generated probably parallel events that occur in the germline, the identification and analysis of the parental C3H genes from which they were derived might be relevant to the study of class I evolution. Furthermore, the discrete pattern of structural and functional homology seen among A149, A166, and H-2L^d suggests that functional analysis of these novel H-2L products should provide significant insight not only into the role that these molecules played in the progression of the 1591 tumor, but also into the structural basis of T cell recognition.

Materials and Methods

Materials.   Restriction endonucleases were obtained from New England Biolabs, Beverly, MA. *Escherichia coli* DNA polymerase Klenow fragment was obtained from Boehringer Mannheim Biochemicals, Indianapolis, IN. *E. coli* strain JM103, phage M13mp18, and mp19 were the gift of Dr. Chuck Staben (Department of Biochemistry, University of California, Berkeley). Oligonucleotide primers homologous to class I sequence were synthesized by Lynn Williams at the University of Southern California Medical Center, and by Bruce Malcolm at the Department of Biochemistry, University of California, Berkeley.

Cell Lines and Animals.   The C3H origin of the 1591 tumor has been previously documented (9, 10, 12). The 1591 tumor and the 28$^-$ tumor variant (9) were the kind gift of Dr. Hans Schreiber (Department of Pathology, University of Chicago). L cell transfectants were generated previously (12). Mice were bred and maintained in the colony at the University of Texas Health Science Center, Dallas.

DNA Sequence Analysis.   The genes were sequenced in parallel by the dideoxy chain termination method (15) using a set of 40 synthetic oligonucleotide primers complementary to class I sequences. We chose primers that hybridize at 200–250 bp intervals in both orientations, according to the DNA sequence of the *H-2D^d* gene (16). Up to 350 bp were read per sequencing reaction on 5% urea/7% polyacrylamide gels.

Generation of Cytotoxic T Lymphocytes.   To generate CTL activity against the 1591 tumor or L cells transfected with tumor-derived class I genes, (C3H × dm2)F$_1$ animals were inoculated intraperitoneally with 10^7 tumor or transfected L cells. ~3 wk later, spleens were removed from the primed animals and these cells were cultured in vitro with either x-irradiated (3,000 rad) tumor cells, L cells transfected with the tumor genes, or

TABLE I

Summary of the Class I Specificities Displayed on the UV Tumor 1591

Cell line or transfectant	Antibodies					
	α1 34-1-2	α2				α3 28-14-8
		34-4-20	CP3F4	30-5-7	CP28	
1591	+	−	+	+	+	+
A216	+	−	−	−	+	−
A166	+	−	+	+	−	+
A149	±	−	−	−	−	+
H-2L^d	±	+	−	+	−	+
H-2D^d	+	+	−	−	+	−
H-2D^{dm1}	+	+	−	+	−	−
H-2K^d	+	−	+	−	−	−

Summary of the class I specificities displayed on the UV tumor, 1591. Specificities and tentative domain assignments of antibodies 34-1-2, 34-4-20, 30-5-7, and 28-14-8 were previously determined (19–22). CP28 and CP3F4 are 1591-specific mAbs (9), which have been mapped to the α2 domain (M. McMillan, University of Southern California, Medical Center, personal communication). Reactivities were previously measured by cell-binding RIA of 1591 tumor cells or L cell transfectants (12). 34-1-2 reactivities are unpublished data (H. Stauss). +, strong reactivity; ±, weak reactivity; −, no reactivity.

BALB/c spleen cells. 5 d later, the cultures were tested for cytolytic activity against ^{51}Cr-labeled target cells. CTL clones were generated as described previously (17). All of the clones are specific for H-2L^d and were derived from either CBA anti-A.AL or (C3H × dm2)F$_1$ anti-BALB/c cultures. The clones are maintained in the presence of rat Con A supernatant and irradiated spleen feeder cells.

Target Cells. The target cells consisted of either L cells expressing transfected class I genes or 1591 tumor cells. Targets were labeled with ^{51}Cr and between 2×10^3–10^4 cells were added to wells containing effector cells. After 4 h of incubation, the culture supernatants were tested for radioactivity. Specific release = (experimental release − spontaneous release)/maximum release.

CTL Competition Assay. This assay has been described previously (18). Briefly, varying numbers of unlabeled L or tumor cells were added to a mixture of effector and labeled target cells in the cytolytic assay. Data are presented as: % inhibition = [1 − (specific release in the absence of inhibitor cells − specific release in the presence of inhibitor cells)/specific release in the absence of inhibitor cells] × 100.

Results

Expression of the Novel Antigens by L Cell Transfectants, and Serological Characterization of Novel Gene Products.   The 1591 tumor is recognized by several allo- and tumor-specific mAb unreactive with products expressed on normal C3H tissues (9, 10). As summarized in Table I, these include the syngeneic anti-1591 mAbs CP28 (9) and CP3F4 (9) and the H-2L crossreactive antibodies 34-1-2 (19, 20), 30-5-7 (19, 21), and 28-14-8 (19, 21). Genomic λ clones A149, A166, and A216 were isolated from a 1591 genomic library on the basis of crosshybridization to the class I–specific cDNA clone, pH-2IIa, and identified as encoding the 1591 tumor-specific epitopes by transfection into C3H L cells and RIA screening of transfectants with a panel of tumor-reactive antibodies, as previously described (12). The serological reactivities of the individual novel class I gene products, as
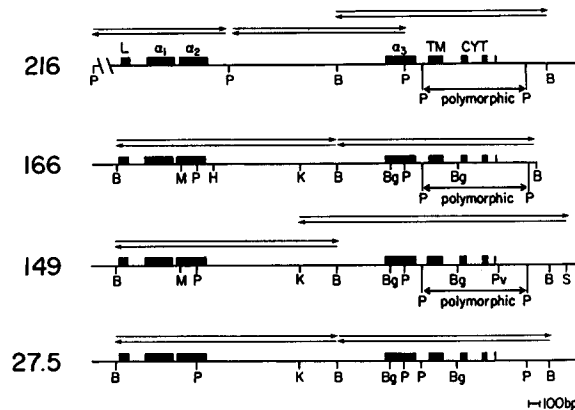
FIGURE 1.   Sequencing strategy. Unique 2–3 kb restriction fragments of A216, A149, A166, and λ27.5 (represented by arrows) were subcloned into the polylinker of M13mp18 or mp19. Subclones of different genes could be distinguished by the presence of polymorphic restriction enzyme sites: B, Bam HI; P, Pst I; K, Kpn I; S, Sal I; M, Mbo I; H, Hae II; (not all sites shown). Genes were sequenced in parallel using either the M13 universal primer or one of a set of class I crosshybridizing primers synthesized according to the sequences of H-2D^d (16) or H-2K^k (13) (P-40, C4243). Primers are numbered according to the H-2D^d sequence. P primers give sequence in the published orientation. C primers give complementary sequence. The following primers were used: P-40, P249, P437, P493, P708, P989, P1210, P1509, P1810, P1964, P2476, P2639, P2975, P3290, P3494, P3999, C286, C552, C580, C761, C1034, C1240, C1540, C1803, C2000, C2222, C2400, C2727, C3066, C3226, C3390, C3580, C3760, C3983, and C4243.

well as other related class I antigens, are summarized in Table I. All of the allo- and tumor-specific class I serological reactivities seen on the 1591 tumor can be accounted for by the reactivities of the three individual novel class I antigens. We have described two of these antigens as H-2L-like because they are recognized by at least two mAbs crossreactive with the H-2L^d antigen from BALB/c. A149, A166, and H-2L^d share the determinants recognized by the mAbs 28-14-8 and 34-1-2. In addition, both A166 and H-2L^d are recognized by the antibody 30-5-7. The antibody 34-4-20 (22), on the other hand, recognizes neither H-2L-like tumor antigen, although it recognizes both H-2L^d and H-2D^d. The A166-specific mAb, CP3F4, crossreacts with H-2K^d, but it recognizes neither A149 nor H-2L^d transfectants. The product of the A216 gene is identified by the anti-1591 mAb CP28. In addition, expression of the A216 and A149 products in BALB/c 3T3 cells indicates that these C3H-derived antigens retain several H-2^k haplotype reactivities (data not shown).

DNA Sequence of the Novel Class I Genes Derived from the 1591 Tumor.   The nucleotide sequence of genes A149, A166, A216, and the H-2L^d clone λ27.5 (14) were determined using the sequencing strategy described in Fig. 1. Briefly, 1–3-kb unique restriction fragments diagnostic for each of the λ clones were subcloned into the polylinker of M13mp18 or mp19. Templates derived from these subclones were sequenced in parallel by the dideoxy-chain termination method, using a set of 40 crosshybridizing synthetic oligonucleotides as primers (see Fig. 1). Sequence differences between the genes could thus be confirmed directly on the same gel.

The DNA sequences of the three novel class I genes are quite homologous to

EXON 1-LEADER PEPTIDE

```
        ATG GGG GCG ATG GCT CCG CGC ACG CTG CTC CTG CTG CTG GCG GCC GCC CTG GCC CCG ACT CAG ACC CGC GCG G          73
        Met Gly Ala Met Ala Pro Arg Thr Leu Leu Leu Leu Leu Ala Ala Ala Leu Ala Pro Thr Gln Thr Arg Ala G
```

INTRON 1

```
        GTGAGTGGGGTCGGGAGGGAAACGGCCTCTGCGGGGACAGGGGCGGCACGGGGGAAGCCCGGTCCCCGTCGCCCACCGGACCCTCCGCCCCTTCTCCAC          173
A166                                                                                           T
```

```
        CTGAGCCCCGCCGCCCAGATCCCCTCCCGGCCTGCGCAGCCGCCCGGGGTTTGGTGAGGAGGTCGGGGTCTCACCGCGCGCCGGCCCCAG          262
A166                                                                                         T
```

EXON 2-ALPHA1 DOMAIN

```
        GC CCA CAC TCG ATG CGG TAT TTC GAG ACC GCC GTG TCC CGG CCC GGC CTC GGG GAG CCC CGG TAC ATC TCT GTC GGC          339
        ly Pro His Ser Met Arg Tyr Phe Glu Thr Ala Val Ser Arg Pro Gly Leu Gly Glu Pro Arg Tyr Ile Ser Val Gly      26
```

```
        TAT GTG GAC AAC AAG GAG TTC GTG CGC TTC GAC AGC GAC GCG GAG AAT CCG AGA TAT GAG CCG CAG GCG CCG TGG ATG          417
        Tyr Val Asp Asn Lys Glu Phe Val Arg Phe Asp Ser Asp Ala Glu Asn Pro Arg Tyr Glu Pro Gln Ala Pro Trp Met      52
```

```
        GAG CAG GAG GGG CCG GAG TAT TGG GAG CGG ATC ACG CAG ATC GCC AAG GGC CAG GAG CAG TGG TTC CGA GTG AAC CTG          495
        Glu Gln Glu Gly Pro Glu Tyr Trp Glu Arg Ile Thr Gln Ile Ala Lys Gly Gln Glu Gln Trp Phe Arg Val Asn Leu      76
A166                                                                              A  A T                      G
                                                                                 Asp Asn                      Ser
A149                                           A
```

```
        AGG ACC CTG CTC GGC TAC TAC AAC CAG AGC GCG GGC G          532
        Arg Thr Leu Leu Gly Tyr Tyr Asn Gln Ser Ala Gly G        90
A166     GC    G  C
         Ala     Ala
```

INTRON 2

```
        GTGAGTGACCCCGCGTCGGAGGTCAGGCCCCTCCACTTCCCGACACAGGGACGCTGACGTCCCGGGTCCCAAGTCCGAGGTTCGGGAACAGAACGGACCC          632
A166                       G
```

```
        GGAACCGGTTTCCCTTTCAGTTTGGAGGAGTCCGCGGCCGGGCGGGCGGGGCCGGGGCGGGTGAGCGGGGCTGACCGCGGGGTCCCGCAG          722
A166                                                                                  G
```

EXON 3-ALPHA2 DOMAIN

```
        GC ACT CAC ACA CTC CAG TGG ATG TAC GGC TGT GAC GTG GGG TCG GAC GGG CGC CTC CTC CGC GGG TAC GAG CAG TTC          799
        ly Thr His Thr Leu Gln Trp Met Tyr Gly Cys Asp Val Gly Ser Asp Gly Arg Leu Leu Arg Gly Tyr Glu Gln Phe      116
A166     T        G A     C                                T
         Ser        Ile  Arg                              Leu
A149              G A     C                                    T                                            A
                   Ile  Arg                                  Trp                                          Tyr
```

```
        GCC TAC GAC GGC TGC GAT TAC ATC GCC CTG AAC GAA GAC CTG AAA ACG TGG ACG GCG GCG GAC ATG GCG GCG CAG ATC          877
        Ala Tyr Asp Gly Cys Asp Tyr Ile Ala Leu Asn Glu Asp Leu Lys Thr Trp Thr Ala Ala Asp Met Ala Ala Gln Ile      142
A166                                CT
                                   Leu
A149     A
```

```
        ACC CGA CGC AAG TGG GAG CAG GCT GGT GCT GCA GAG TAT TAC AGG GCC TAC CTG GAG GGC GAG TGC GTG GAG TGC CTC          955
        Thr Arg Arg Lys Trp Glu Gln Ala Gly Ala Ala Glu Tyr Tyr Arg Ala Tyr Leu Glu Gly Glu Cys Val Glu Trp Leu      178
A166                                          G                    C        A
                                            Gly                   His      Lys
A149                                                               C        A
                                                                 His      Lys
```

```
        CAC AGA TAC CTG AAG AAC GGG AAC GCG ACG CTG CTG CGC ACA G          996
        His Arg Tyr Leu Lys Asn Gly Asn Ala Thr Leu Leu Arg Thr G        192
```

INTRON 3

```
        GTGCAGGGGCCGCGGGCAGCTCCTCCCTCTGCCCTCGGGCTGGGGCTCAGTCCTGGGGAAGAAGAAACCCTCAGCTGGGGGTGATGCCCCTGTCTCAGAG          1098
A166             C                                                                      C
```

```
        GGGGAGAGAGTGACCCTGGGTCTCCTGATGCCTCAGCACAGTGACTGCACTGACTCTCCCAGGCTCAGCCTTCTCCCTGGACAGGGCCCAGCCTGTCTCA          1198
A166                       T     G
```

```
        GGAGGGAAGGAGAGAATTTCCCTGAGGTAACAACAGCTGCTCCCTTCAGTTCCCCTGTAGCCCTCTGTCAGCCATGGCCTCTCCCAGGCCGGGTTCTCTG          1298
        CCCACGCCCACTGTCGGTGGACACTGACTCCTGTCCTGCTGAGTGTGTCAGCCCTTACACCTCAGGACCGGAAGTCGCCTTACCTGATTGGAAACATGGA          1398
        CTCCTATACACTAGCCTGGTTGCCCCAGCTTCTAGAATTTTCCAGAGAATACATTCTCCCAGATCCCTCCCTGTCTGTGGGGTTTGCACCCCTTCGACAA          1498
A166         C
        CCTAATTCTCTCTATTCCTACAGTGGTGAATGGTCACATGAGCTCTTATGGGGTACCCTGGAGGAATATAAATCGAGAATTTTCTTTTTTGTTTTCTCTC          1598
A166                                                                                                    --
A149                                                                                                    --
        TCTCTTTCTCTCTCTCTCTCTCTCTCTTTTCCGATTTTTTTGAGACACCGGGTCCCTCTGTATAGCCCTGGCTGTCCTGGAACTCACTTTGTAGCCCA          1698
A166                                                        A
        AAGGTGGTCCTCAAATTCACATGCCTCTGCCTCCTGAGTGCTGGGATTATGGACTGCCTGGGCCTTTCTTTTTTCTTTAC-------TTTTTTTTTTTTT          1791
A166                                                                                        TTTTTTT
A149                                                                                        -------
        TTTGAGGCCTTATTTTTTTTTTAGTCAGTTTTTGTCTGCACTGGAGTGATCCTGTTTCTCCATGCCCTTGTATTATCATTTGTATCAGTCTCCACAGGTG          1891
A166             C  C
        CCAGAGGAGACTGCTAACCTGGATAATTATGCAAGTTAAATCAGGGTTCTCATTAAAGGAGAGATTCTTGTGAACTTAGAGTGTTTCCTGTCAGAACTAG          1991
A166                      GA
        ACATCCAGAAGCCTCCTGCTCTTCCTCTGCCCCCACAAGTTACAGTG---CCCCCCCCCCAGTGAATCAGGACTTGGACTCTGAGAGACAGGGTCTTCTG          2089
A166                                                  CC
A149                                                  --
        CAATCCAGGGCTGGATTGAGAGGGAAGACCACACACCCTGTGAGCTCACTGTGTTCCAGTGAGTGCTGCACTGGGGTCCACAGCTCACTCCAGGGATCCT          2189
        GTTGTGACACACCTGTACCTTGTCCTCCAGAGTCAGGGGCAGGGAGTCATTTTCTCTGGCTACAGACTTTGTGATGGCTGTTCACTCGGCACTGACAGTTA          2289
        ACGTTGGTCAGCAAGATGACCACAATGGTTGAGTCTCAGTGGTGGGACCCTTCCAGTAGCATATGCCCCTAATTTTGATATGAACTCAAACAGATATTAA          2389
        ATTACTTATTTTCCATTCCCTATTCCCATTCTGTGACTATCTCTCTCATGCTATTGAACATCACATAAGGATGGCCATGTTCACCCACTGGCTCATGTGGA          2489
        TTCCCTCTTAGCTTCTTTGTCCCAAAAGAAAATGTGCAGTCCTGTGCTGAGGGGACCAGCTCTGCTTTTGGTCACTAGTGCAATGACAGTGTAGTGTCAA          2589
        ATAGACACATAGTTCACTCTCATCATTGATTTAACTGAGTCTTGTGTAGATTTCAGTTTGTCTTGTTAATTGTGGAATTTCTTAAATCTTCCACACAG          2667
A166                                                                                                   G
```

FIGURE 2. DNA Sequence of A149, A166, and H-2L^d. Unless otherwise specified, the sequence of all three genes is identical. Coding regions are shown as triplets, translated amino acid sequences are shown below. Numbering shown is for H-2L^d. Protein sequence numbering begins at the first glycine in α1 domain. Deletions are represented by −. The Pst I sites defining the 1-kb polymorphic Pst I fragment are underlined.

EXON 4-ALPHA3 DOMAIN

```
AT TCC CCA AAG GCA CAT GTG ACC CAT CAC CCC AGA TCT AAA GGT GAA GTC ACC CTG AGG TGC TGG GCC CTG GGC TTC          2764
lu Ser Pro Lys Ala His Val Thr His His Pro Arg Ser Lys Gly Glu Val Thr Leu Arg Cys Trp Ala Leu Gly Phe          218

TAC CCT GCT GAC ATC ACC CTG ACC TGG CAG TTG AAT GGG GAG GAG CTG ACC CAG GAC ATG GAG CTT GTG GAG ACC AGG          2842
Tyr Pro Ala Asp Ile Thr Leu Thr Trp Gln Leu Asn Gly Glu Glu Leu Thr Gln Asp Met Glu Leu Val Glu Thr Arg          244

CCT GCA GGG GAT GGA ACC TTC CAG AAG TGG GCA TCT GTG GTG GTG CCT CTT GGG AAG GAG CAG AAT TAC ACA TGC CGT          2920
Pro Ala Gly Asp Gly Thr Phe Gln Lys Trp Ala Ser Val Val Val Pro Leu Gly Lys Glu Gln Asn Tyr Thr Cys Arg          260

GTG TAC CAT GAG GGG CTG CCT GAG CCC CTC ACC CTG AGA TGG G                                                        2965
Val Tyr His Glu Gly Leu Pro Glu Pro Leu Thr Leu Arg Trp G                                                        294
```

INTRON 4

```
GTAAGGAGGGTGTGGGTGCAGAGCTGGGGTCAGGGAAAGCTGCAGCCTTCTGCAGACCCTGAGCTGGTCAGGGATGAGAGCTGGGGTCATAACCCTCACC           3081
TTCATTTCCTGTACCTGTCCTTCCCAG
```

EXON 5-TRANSMEMBRANE DOMAIN

```
AG CCT CCT CCG TCC ACT GAC TCT TAC GTG ATC GTT GCT GTT CTC GGT GTC CTT GGA GCT ATG GCC ATC ATT GGA             3185
lu Pro Pro Pro Ser Thr Asp Ser Tyr Met Val Ile Val Ala Val Leu Gly Val Leu Gly Ala Met Ala Ile Ile Gly          320

GCT GTG GTG GCT TTT GTG ATG AAG AGA AGG AGA AAC ACA G                                                            3225
Ala Val Val Ala Phe Val Met Lys Arg Arg Arg Asn Thr G                                                            333
```

INTRON 5

```
GTAAGAAAGGGCAGGGTCTGAGTTTTCTCTCAGCCTCCTTTAGAAGTGTGCTCTGCTCATTAATGGGGAACACAGCCACACCCCACATTGCTACTGTCTC           3325
TAACTGGGTCTGCTGTCAGTTCTGGGAATTTCCAGTGTCAAGATCTTCCTTGAACTCTCACAGCTTTTCTTTTCACAG                                 3403
```

EXON 6-CYTOPLASMIC DOMAIN

```
GT GGA AAA GGA GGG GAC TAT GCT CTG GCT CCA G                                                                     3436
ly Gly Lys Gly Gly Asp Tyr Ala Leu Ala Pro G                                                                     344
```

INTRON 7

```
GTTAGTGTGGGGACAGGATGGTCTGGGGGACATTGGATTGAAGTTGGAGATGATGGGAGCTCTGGGAATCCATAATAGCTCCTCCAGAGAAATCTTCTAG          3536
GGGCCTGAGTTGTGCCATGAAGTGAATACATTCATGTACATATGCATATACATTTGTTTTGTTTTACCCTAG                                       3620
```

EXON 7-CYTOPLASMIC DOMAIN

```
GC TCC CAG AGC TCT GAA ATG TCT CTC CGA GAT TGT AAA G                                                             3653
ly Ser Gln Ser Ser Glu Met Ser Leu Arg Asp Cys Lys A                                                             367
```

INTRON 8

```
GTGACACTCTAGGGTCTGATTGGGGAGGGGCAATGTGGACATGATTGGGTTTCAGGGACTCCCAGAATCTCCTGAGAGTGAGTGGTGGGTTGCTGGAATG          3753
TTGTCTTCACAGTGATGGTTCATGACTCTCATTCTCTAG                                                                         3792
```

EXON 8-TERMINATION CODON and 3' UNTRANSLATED REGION

```
CG TGA AGACAGCTGCCTGGACTGTACTGAGTGACAGACGATGTGTTCAGGTCTCTCCTGTGACATCCAGAGCCCTCAGTTCTCTTTACACAACATTG            3892
la Trm                                                                                                          368

TCTGATGTTCCCTGTGAGCTTGGGTTCAGTGTGAAGAACTGTGGAGCCCAGCCTGCCCTGCACACCAGGACCCTATCCCTGCACTGCCCTGTGTTCCCT           3992
TCCATAGCCAACCTTGCTGCTCCAGTCAAACACTGGGGGACATCTGCATCCTGTAAGCTCCATGCTACCCTGAGCTGCAGCTCCTCACTTCCACACTGA           4092
                                                                             C
```
A166

```
GAATAATAATTTGAATGTGCGTGGCTGGAGAGATGGCTCAGCGCTGACTGCTCTTCCAAAGGTCCTGAGTTCAAATCCCAGCAACCACATGGTGGCTCA           4192
```

FIGURE 2.

those of other known class I genes (Figs. 2 and 3). Intron-exon boundaries of A149 and A166 were assigned by comparison with the published sequences of H-2D^d (16), H-2L^d (14), and H-2D^b (23), and A216 by comparison with H-2K^k (13). All three genes appear to comprise eight exons, the first encoding the leader peptide; three exons encoding the three external domains, α1, α2, and α3; an exon encoding the hydrophobic transmembrane region; and three exons accounting for the cytoplasmic domain of the antigen and the 3'-untranslated trailer sequence. All splice junctions assigned for H-2L^d (14) and H-2K^k (13) appear to be represented. Like H-2L^d, A149 and A166 each contain three attachment sites for N-linked glycosylation; like H-2K^k, A216 contains two. The single amino acid substitution of a leucine for a cysteine at residue 121 in the α2 of A166, does not involve a cysteine normally involved in a disulfide bond.

Since the A216 gene encodes a product recognized by mAbs crossreactive with H-2K^k, and since the A216-specific antibody CP28 crossreacts with H-2D^d, we have compared the DNA sequence of A216 with the published sequences of these class I genes. The overall homology between A216 and H-2D^d is comparable to that seen between other H-2 genes, such as H-2L^d or H-2D^d. Comparison

EXON 1-LEADER PEPTIDE

ATG GCA CCG TGC ATG CTG CTC CTG CTG TTG GCG GCC GCC CTG GCC CCG ACT CAG ACC CGC GCG G   64
Met Ala Pro Cys Met Leu Leu Leu Leu Ala Ala Ala Leu Ala Pro Thr Gln Thr Arg Ala G

INTRON 1

GTGAGTACCGGGCCGGGAGGGAAACGGCCTCTGAGGGGAGGGGCGGCATCGGGGAAGCCGGTCCTCGGGTCGCCCACCGGACCCTCCGCCCGTTCTCCAT   164
CCCCGTCCCGAGCCCCGAGCCCTGCTCCCCTCTCAGCCCGCGCAGCCGCCCGGGGTCTGGTGAGGAGGTCGGGGTCTCACCGCGCGCCGCCCCCAG   260

EXON 2-ALPHA1 DOMAIN

GC CCA CAT TCG CTG AGG TAT TTC GTC ACC GCC GTG TCC CGG CCC GGC TTC GGG AAG CCC CGG TAC ATG GAA GTC GGC   337
ly Pro His Ser Leu Arg Tyr Phe Val Thr Ala Val Ser Arg Pro Gly Phe Gly Lys Pro Arg Tyr Met Glu Val Gly   26

TAC GTG GAC GAC ACG GAG TTC GTG CGC TTC GAC AGC GAC GCG CAG AAT CCG AGA TAT GAG CCG CGG GCG CGG TGG ATA   415
Tyr Val Asp Asp Thr Glu Phe Val Arg Phe Asp Ser Asp Ala Gln Asn Pro Arg Tyr Glu Pro Arg Ala Arg Trp Ile   52

GAG CAG GAG GGG CCG GAG TAT TGG GAG CGG AAC ACA CAG AAC GCC AAG CGC AGT GAG CAG AGT TTC CGA GTG AAC CTG   493
Glu Gln Glu Gly Pro Glu Tyr Trp Glu Arg Asn Thr Gln Asn Ala Lys Arg Ser Glu Gln Ser Phe Arg Val Asn Leu   78

AGG ACC GCG CTG CGC TAC TAC AAC CAG AGC GCG GGC G   530
Arg Thr Ala Leu Arg Tyr Tyr Asn Gln Ser Ala Gly G   91

INTRON 2

GTGAGTGACCCCGGGTCGGAGGTCACGACCCCTCTACTTCCCGACAGGGACCTTGACGTCCCGGGTCCCAAGTCCGAGGTTCGGGAACAGAACGGACCCG   630
GAGCCGGTTTCTCTTTCACTTTGGAGGAGTCCGCGGCCGGGCGGGCGGGGCCGGGGCGGGTGAGCGGGGCTGACCCGGTGGTCCCGAG   718

EXON 3-ALPHA2 DOMAIN

GC TCT CAC ACG ATC CAG CGG ATG TAT GGC TGT GAC GTG GGG TCG GAC AGG CGC CTC CTC CGC GGG TAC GAG CAG TTC   795
ly Ser His Thr Ile Gln Arg Met Tyr Gly Cys Glu Val Gly Ser Asp Arg Arg Leu Leu Arg Gly Tyr Glu Gln Phe   116

GCC TAC GAC GGC TGC GAT TAC ATC GCC CTG AAC GAA GAC CTG AAA ACG TGG ACG GCG GCG GAC ATG GCG GCG CTG ATC   873
Ala Tyr Asp Gly Cys Asp Tyr Ile Ala Leu Asn Glu Asp Leu Lys Thr Trp Thr Ala Ala Asp Met Ala Ala Leu Ile   142

ACC AAA CAC AAG TGG GAG CAG GGT GGT GAA GCA GAG AGA TTC AGG GCC TAC CTA GAG GGC GTA TGC GTG GAG TGG CTC   951
Thr Lys His Lys Trp Glu Gln Gly Gly Glu Ala Glu Arg Phe Arg Ala Tyr Leu Glu Gly Val Cys Val Glu Trp Leu   168

CTC AGA TAC CTG AAG AAC GGG AAC GCG ACG CTG CCG CGC ACA G   994
Leu Arg Tyr Leu Lys Asn Gly Asn Ala Thr Leu Val Arg Thr G   183

INTRON 3

GTGCAGGGCCGCGGGCAGCTCCTCCCTCTGCCCTCGGGCTGGGGCTCAGTCCTGGGGAAGAAGAAACCCTCAGCTGGGGTGATGCCCCTGTCTCAGAGGG   1094
GAGAGAGTGACCCTGGGTCTCCTGATCGCTCATCACAGGGACTGCACTGAGTCTCCCAGGGCTCAGCCTTCTCCCTGGACAGTGCCCAGGCTGTCTCAGG   1194
GAAGGAGAGAATTTCCCTGAGGTAACAACAGCTGCTCCCTCTAGTTCCCCTGCAGCCCTCTGTCAGCCATGGCCTCTCCCAGGCCGGGTTTCTCTGCCCAT   1294
GCCCAGTGTCTGTGGCACACTGACTCCTGTCCTGCTGAGTGTGTCAGCCCTTACACCTCAGGATCTGGAAGTCTCCTTTCCTTGATTGGAGACATGGACTC   1394
CCCTACACTAGGACGGTTGACCTAGGTTCTAGAGTTTTCCAAAGAATACATTCTCACAGATCCCTCCCTGTCTGTGGGGTTTTGCACCCCTTAACAACCC   1494
AGGTCTATCTATTCCTACAGTGGTGAATGGTCACATGAGCCCTTATGGGCTACCCTGGAGGAATATAAATCGTGGATTTTCTTCTCTCTCTCCTCTCCTC   1594
TCTCTCTCTCTCTCTCTCTCTCTTTCCGTTTTTTTTTTTTGTTTTTGTTTTTTTTTTTTTGGTTTTGGTTTTTTTTTTTTTTTTCCTAGACGGGGTTTCTC   1694
TGTATAGCCCTGGCTGTCCTGGAACTCACTTTGTAGACCAGGTTGGCCTTGAACTCAGAAATCTGCCTGCCTCTGGCTCCCGAGTGCTGGGATTAAAGCA   1794
TGTGCCACCACTCCTGGCCTTTCTTTTTTCTTAACTTTTTTTTTTGGTGGGGGGGGGGGATGCTTATTTTGTTTCTAGTCAGCTTTTGTCTGCCATTGGAG   1894
TGATCCAGTCTCTCCCATGCCCTCCTATTATCATTTGTTTCAGTCTCCACAGGTGCCAGGGAAGGCTGCTAACCTGGATAATTAGACAAGTTAAATCAGGG   1994
TTCGAATTAAAGAAGAGATTCTTGTGAACTTAGACTGTTTCCTGTCAGAACTAAACATGCAGAAGCCTCCTGCTCTTCCTCTGCCCCACAAGTTACAGTG   2094
CTCCCTCCCGCTAGTAAAATCAGGACTTGGACTCTGAGAGGGCAGGGTCTTCTGCAATCCAGGGCTGAGTCAGAGGGAAGACCACACACCCTGTGAGCCCA   2194
CTGTGTTCCAGTGAGTGCTGCACTGGGGTCCACAGCACACTCCAGGGATCCTGTGTGACACACCTGTACCTTGTCCCCCAGAGTCAGGGGCTGGGAGTCA   2294
TTTTCTCTGGCTACACACTTAGTGATGGCTGTTCACTTGGACTGACAGTTAATGTTGGTCAGCAAGGTGACTACAATGGTTGAGTACCAACGGTGTCACC   2394
TTCCAGGATCATACAGCCCTAATTTTAATATGAACTCAAACACATATTAAATTAGTTATTTTCCATTCCCTCCTCCATTCTTTGACTACCTCTCTCATGC   2494
TACCTCTCATCACATAAGGATGGCCATGTTTACCCACTGGCTCATGTGGATTCCCTCTTAGCTTCTGAGTCCCAAAAGAAAATGTGCAGTCCTGTGCTGA   2594
GGGGACCAGCTCTGCTTTTGGTCACTAGTGCGATGACAGTTGAAGTGTCAAACAGACACATAGTTCACTGTCATCATTGATTTAACTGAGTCTTGGGTAG   2694
ATTTCAGTTTGTCTTGTTAATTGTGTGATTTCTTAAATCTTCCACACAG   2743

EXON 4-ALPHA3 DOMAIN

AT TCC CCA AAG GCC CAT GTG ACC CAT CAC AGC AGA CCT GAA GAT AAA GCC ACC CTG AGG TGC TGG GCC CTG GGC TTC   2820
sp Ser Pro Lys Ala His Val Thr His His Ser Arg Pro Glu Asp Lys Ala Thr Leu Arg Cys Trp Ala Leu Gly Phe   208

TAC CCT GCT GAC ATC ACC CTG ACC TGG CAG TTG AAT GGG GAG GAG CTG ACC CAG GAC ATG GAG CTT GTG GAG ACC AGG   2898
Tyr Pro Ala Asp Ile Thr Leu Thr Trp Gln Leu Asn Gly Glu Glu Leu Thr Gln Asp Met Glu Leu Val Glu Thr Arg   234

CCT GCA GGG GAT GGA ACC TTC CAG AAG TGG GCA TCT GTG GTG GTG CCT CTT GGG AAG GAG CAG TAT TAC ACA TGC   2976
Pro Ala Gly Asp Gly Thr Phe Gln Lys Trp Ala Ser Val Val Val Pro Leu Gly Lys Glu Gln Tyr Tyr Thr Cys His   260

GTG TAC CAT CGA GGG CTG CCT GAG CCC CTC ACC CTG AGA TGG G   3019
Val Tyr His Lys Gly Leu Pro Glu Pro Leu Thr Leu Arg Trp G   275

INTRON 4

GTAAGGAGGGTGTGGGTGCAGAGCTGGGGTCAGGGAAGCTGGAGCTTTCTGCAGACCCTGAGCTGCTCAGGGCTGAGAGCTGGGGTCATGACCCTCACCT   3119
TCATTTCTTGTACCTGTCCTTCCCAG   3145

EXON 5-TRANSMEMBRANE DOMAIN

AG CCT CCT CCA TCC ACT GTC TCC AAC ACG GTA ATC ATT GCT GTT CTG GTT GTC CTT GGA GCT GCA ATA GTC ACT GGA   3222
lu Pro Pro Pro Ser Thr Val Ser Asn Thr Val Ile Ile Ala Val Leu Val Val Leu Gly Ala Ala Ile Val Thr Gly   300

GCT GTG GTG GCT TTT GTG ATG AGG AGG AGG AGA AAC ACA G   3262
Ala Val Val Ala Phe Val Met Arg Arg Arg Arg Asn Thr G   314

INTRON 5

GTAGGAAAGGGCAGGGTCTGAGTTTTCTCTCAGCCTCCTTTAGAGTGTGCTCTGCTCATCAATGGGGAACACAGCCACACCCCACATTGCTACTGTCTGT   3362
AACTGGGTCTGCTGTCAGTTCTGGGAACTTCCTAGTGTCAAGATCTTCCTGGAACTCTCACAGCTTTTCTTCTCACAG   3440

EXON 6-CYTOPLASMIC DOMAIN

GT GGA AAA GGA GGG GAC TAT GCT CTG GCT CCA G   3573
ly Gly Lys Gly Gly Asp Tyr Ala Leu Ala Pro G   325

FIGURE 3.   DNA sequence of the A216 gene. Coding sequences are shown as triplets with the translated amino acid sequences below. Protein sequence numbering begins at first glycine in α1 domain. The Pst I sites defining the 950-bp polymorphic Pst I fragment are underlined.

INTRON 6

GTTAGTGTGGGGACAGAGTTGTCCTGGGGACATTGGAGTGAAGTTGGAGATGATGGGAGCTCTGGGAATCCATAATAGCTCCTCCAGAGAAATCTTCTA   3673
GGTGCCTGAGTTGTGCCATGAAATGAATATGTACATGTACATATGCATATACACTTGTTTTGTTTTACCCTAG   3746

EXON 7-CYTOPLASMIC DOMAIN

GC TCC CAG ACC TCT GAT CTG TCT CTC CCA GAT TGT AAA G   3785
ly Ser Gln Thr Ser Asp Leu Ser Leu Pro Asp Cys Lys G   338

INTRON 7

GTGACACTCTAGGGTCTGATTGGGGAGGGGCAATGTGGACATGATTGGGTTTCAGGAACTCCCAGAATCCCCTGTGAGTGAGTGATGGGTTGTTCGAATG   3885
TTGTCTTCACAG   3897

EXON 8-CYTOPLASMIC DOMAIN

TG ATG GTT CAT GAC CCT CAT TCT CTA GCG TGA   3929
al Met Val His Asp Pro His Ser Leu Ala Trm   347

3'-UNTRANSLATED REGION

AGACAGCTGCCTGGAGTGGACTTGGTGACAGACAATGTCTTCTCATATCTCCTGTGACATCCAGAGCCCTCAGTTCTCTTTAGTCAAGTGTCTGATGTTC   4029
CCTGTGAGCCTATGGACTCAATGTGAAGAACTGTGGAGCCCAGTCCACCCCTCCACACCAGGACCCTGTCCCTGCACTGCTCTGTCTTCCCTTCCACAGC   4129
CAACCTTGCTGGTTCAGCCAAACACTGAGGGACATCTGCAGCCTGTCAGCTCCATGCTACCCTGACCTGCAACTCCTCACTTCCACACTGAGAATAATAA   4229
TTTGAATGTAACCTTGATTGTTATCATCTTGACCTAGGGCTGATTTCTTGTTAATTTCATGGATTGAGAATGCTTAGAGGTTTTGTTTGTTTGTTTGATT   4329
GATTTGTTTTTTTGAAGAAATAAATGATAGATGAATAAACTTCCAGAATCTGGGTCACTATGCTGTGTGTATCTGTTGGGACAGGATGAAACTGTAGCAG   4429
CTGAGTGTGAACAGGCTGTGCCGAGGTGGGCTCAGTCCGTTTTGATCTGTGATGGGGCCATACCTCCACTGTGTCACCTCTGGGCTCTGTTCCCTCTATC   4529
ACTATGAGGCACATGCTGAGAGTTTGTGGTCACAAAGACACAGGGAAGGCCTGAGCCTTTGCCTGTCCCCAGGATTATGAGCCCCCAGGGCTAAAGATCA   4629
GACACTCAGCCAGGGATCC   4662

of A216 and the H-2K^k from AKR revealed similar homology of 85–90% throughout the 5' ends of these genes. Striking homology of >99%, however, was seen between the 3' ends of these two genes, from a point ~300 bp 5' of exon 4, and continuing for 1,800 bp into 3'-untranslated sequences. A 1-kb polymorphic Pst I restriction fragment not found in the C3H genome (12) maps within this region, suggesting that the 3' end of this gene was generated by a recombination event between two class I genes homologous to H-2K^k in this region. Preliminary screening of the class I genes from a normal C3H library by restriction mapping and with A216-specific oligonucleotide probes has identified several potential 3' parents, including H-2K^k (S. Watts, University of California, Berkeley, unpublished results). Thus it is possible that H-2K^k is itself a parent, and that the minimal degree of divergence, i.e., eight point differences and two small clustered changes, seen between the 3' coding region of A216 and the H-2K^k sequence might reflect sequencing differences or strain substitutions between the C3H and AKR H-2K alleles. Further sequence analysis of the normal C3H genes will be required to unambiguously identify the parental genes.

Since the molecules encoded by A149 and A166 share serological determinants with each other and H-2L^d, we sequenced these genes in parallel. The complete and revised sequence of H-2L^d, as well as the sequences of the two C3H-derived H-2L-like genes, is presented in Fig. 2. The DNA sequences of the H-2L-like tumor genes are nearly identical to each other, to H-2L^d, and to the corresponding regions of an H-2D^b cDNA clone (23) for ~3,000 bp from within the large intron until the end of the 166 clone (Fig. 2). Within this region A149 is identical to H-2L^d, and A166 exhibits only two bases that are polymorphic. The only region of these genes for which <99.9% homology is found is within the exons encoding the first and second external domains, α1 and α2, which are believed to be the regions primarily responsible for T cell recognition (24). Within the first 1,000 bp of these genes, A149 and A166 are 99.1 and 98.6% homologous to H-2L^d, respectively.

The two Pst I sites defining one of the polymorphic restriction fragments seen in A149 and A166, but not in the C3H genome, map within the region of near

α₁ DOMAIN

```
                    30                60                90
                     I                 I                 I
H-2L⁴  GPHSHRYFETAVSRPGLGEPRYISVBYVDNKEFVRFDSDAENPRYEPGAPWHEDEGPEYHERITQIAKGQEGHFRVHLRTLLGYYHQSAG
A149   --------------------------------------------------------------------------------------
A166   ------------------------------------------------DN-----S---A-A-----
A216   ----L---V-------F-K---NE----BT---------Q----R-R-I---------N--N--RS--S-------A-R-------
H-2D⁴  -S--L---V------f-----NE------T----------------R-R-I---------E-RR--N--S---D--A-R-------
H-2Kᵏ  ----L---N---------K--F------BTQ--------------RVR--EQVE-----N--I---N-I------A-R-------
H-2K⁴  ----L---V-----------F-A----DTQ-------D--F--R-----------EQ--R--SD-----S--AQR----K-
```

α₂ DOMAIN

```
                    120               150               180
                     I                 I                 I
H-2L⁴  GTHTLQNNYGCDVGSDGRLLRGYEGFAYDGCDYIALMEDLKTWTAADNAAQITRRKNEGABAAEYYRAYLEGECVEHLHRYLKNBNATLLRT
A149   ----I-R--------W-------Y-----------------------H-K---------------------
A166   -S--I-R-----L--------------L--------------G---H-K---------------------
A216   -S--I-R--------R------------------------L--KH---G-E--RF-----V----L---------P--
H-2D⁴  -B-----A---E--------W---------------------RD-----------R-------------
H-2Kᵏ  -S--F-R---E----W------Y-------------------L--KH----D--RD----T-----R--QL----P--
H-2K⁴  -S--F-R-F-------W-----Q-----R-------------T--L---------D-------------R--EL--E----
```

FIGURE 4.   Comparison of amino acid sequences of tumor antigens with other class I antigens. Sequences are shown from the first and second external domains, α1 and α2. Sequences compared are A149, A166, A216, H-2L^d, H-2D^d (16), H-2K^d (25), H-2K^k (13), and H-2D^{dm1} (26).

identity to H-2L^d at positions 3009 and 4047, suggesting that these H-2L coding sequences exist in unexpressed form within the C3H MHC requiring a specific recombination event to be activated. Since both A149 and A166 contain the same polymorphic Pst I fragment, it is possible that they were generated by duplication and divergence from a single parental recombinant gene. However, analysis of 5′-flanking sequences and restriction enzyme site polymorphisms indicate that these two genes are derived from different 5′ parents (data not shown).

Structural Basis for Conserved Serological Determinants on Novel H-2L-like Antigens.   The data concerning the serological recognition of these novel gene products can be analyzed in light of the predicted amino acid sequences (Figs. 2–4). The identity between the three L-like antigens in the α3 domain, and the extreme homology between the antigens in the α1 domain are consistent with the shared reactivities with 28-14-8 and 34-1-2, which have been shown to map to the α3 and α1 domains, respectively (20, 21). Since 34-1-2 binds A166 with higher affinity than either A149 or H-2L^d (Table I), it is intriguing that three of the five amino acid substitutions that A166 bears in the α1 domain with respect to H-2L^d, render A166 more homologous to either H-2K^d (25) or H-2D^d, both of which are strongly reactive with 34-1-2.

The lack of reactivity with the α2-specific antibody, 34-4-20 (M. McMillan, University of Southern California, Los Angeles, CA; personal communication), seen for both tumor antigens, correlates with the presence of isoleucine and arginine at positions 95 and 97 within α2 of both molecules. H-2D^d and H-2L^d, which are recognized by this antibody, both have leucine and tryptophan at these two positions. The pattern of reactivity of the antibody, 30-5-7, appears more complicated. Previously, exon-shuffling experiments (21), as well as analysis of the 30-5-7 reactive H-2D^{dm1} molecule (26) mapped the epitope recognized by

FIGURE 5. Cytolytic T cell activity from tumor primed mice. (C3H × dm2)F₁ mice were primed with 1591 cells and their spleen cells were re-challenged with x-irradiated 1591 cells (A) or BALB/c spleen cells (B). CTL generated were tested against 1591 cells (△), the 28-tumor variant (▽), or L cells transfected with A216 (□), A149 (◻), A166 (○), H-2Lᵈ (●), or tk (◇).

this antibody to the C-terminal two-thirds of the α2 domain. However, it is likely that other regions will be involved in the formation of a functional antigenic epitope, since A149, which is not 30-5-7-reactive, is quite conserved with H-2Lᵈ and A166, both of which are recognized. For example, the amino acid substitution of a tryptophan for a glycine at position 107 of A149 might somehow interfere with the formation of a functional determinant by the rest of the domain. Oligonucleotide-directed mutagenesis experiments should help resolve this issue.

The tumor-specific mAb CP3F4 recognizes the antigens A166 and H-2Kᵈ. Since the only amino acid residues unique to these molecules are a serine and an alanine at positions 77 and 81 in the α1 domain, it is impossible to identify any specific set of amino acids influencing the epitope recognized by this antibody without additional mutagenesis experiments. Similarly, although the tumor-specific antibody CP28 recognizes both A216 and H-2Dᵈ (9), the available data do not reveal any obvious pattern of unique structural homology between these molecules.

Recognition of Novel Antigens by Allospecific CTL. The 1591 tumor is highly immunogenic and induces a strong CTL response when transplanted into normal syngeneic mice (27, 28). To distinguish the components of the CTL response directed against each of the novel class I antigens, we have tested L cells transfected with the three individual genes for their ability to function as targets for anti-1591 CTL. Since primary in vitro anti-1591 responses could not be detected (data not shown), CTL populations directed against the tumor were generated by immunization of (C3H × H-2ᵈᵐ²)F₁ mice with 10⁷ 1591 tumor cells. 3 wk later the spleen cells of these mice were placed in culture with irradiated 1591 stimulator cells. After a 5-d culture period, the effector cells were tested against ⁵¹Cr-labeled L cell targets, as well as 1591 and the 1591 (28⁻) variant, selected in vitro for the loss of the three novel class I products (11). Both 1591 and L cells expressing each of the novel gene products were recognized and effectively lysed by the anti-1591 CTL (Fig. 5a), as were BALB/c 3T3 cells

TABLE II

Activity of Anti-H-2L^d CTL Clones against 1591 Class I Molecules

Clone	Target Cells							
	Ltk	L^d	D^d	A149	A166	A216	1591	28$^-$
L9.4	0	19	6	5	ND	1	7	2
L9.24	2	31	2	5	1	2	0	1
L13.D4	3	39	4	6	2	0	8	3
L13.D17	0	41	2	41	0	0	28	1
46(5)5.6	1	30	1	22	3	2	17	0
L11.15	0	45	4	20	6	3	12	10
L13.D.8	0	18	3	23	ND	0	ND	ND

CTL clones tested against panel of target cells listed above by specific chromium release assay. E/T ratio, 20.

transfected with the A216 gene (data not shown). L cells expressing the H2L^d antigen were also recognized, but to a lesser extent. These data indicate that all three novel class I products function as targets of the CTL generated against the tumor. In addition, residual reactivity can be detected against the 28$^-$ tumor variant lacking these novel antigens (reference 9, Fig. 5a). This residual reactivity probably reflects the recognition of antigenic molecules distinct from the novel class I products on this tumor. Wortzel et al. (28) have shown that 1591 expresses at least four genetically distinguishable sets of novel tumor antigens, termed A, B, C, and D, as defined by cloned CTL. Three of these antigens, B, C, and D, remain on the 28$^-$ tumor variant (9), and are therefore not related to the novel class I molecules discussed in this report. However, given that none of the tumor-reactive mAbs block the anti-A CTL (29), it is not yet possible to determine the relationship between any of the novel class I antigens and the A target molecule.

Since some anti-1591 CTL activity was directed towards H-2L^d (27.5 cells) (Fig. 5a); we cultured spleen cells from the 1591-immunized (C3H × H-2^{dm2})F$_1$ mice with BALB/c (H-2^d) spleen cells. H-2^{dm2} is a meiotic mutant of BALB/c that lacks the H-2L^d antigen. Therefore, this procedure should generate a primary anti-H-2L^d response, as well as amplify any anti-H-2L^d crossreactive component that arose from the priming with 1591 cells. The effector cells generated lysed 1591 tumor cells, L cells expressing the A149 antigen, and H-2L^d (Fig. 5b). L cells expressing the A166 product were also killed, but much less efficiently. In contrast to primed cells challenged in vitro with 1591 cells (Fig. 5a), effector cells challenged in vitro with BALB/c spleen cells did not lyse target cells expressing A216 or the 28$^-$ variant tumor line. This indicates that the A216 antigen does not cross react with H-2L^d at the T cell level. It is likely that the differential recognition of A149 and A166 by the anti-H-2L CTL reflects some of the five amino acid differences that exist between the α1 domains of these molecules.

To further study the relationships among H-2L^d, A149, and A166, we tested bulk-cultured anti-H-2L^d CTL, as well as several anti-H-2L^d CTL clones, against these target cells (Table II). (C3H × dm2)F$_1$ anti-BALB/c (anti-H-2L^d) CTL displayed lytic activity against H-2L^d and A149 transfectants, but not L cells transfected with A166 or A216 (Fig. 6, left). Activity was also noted against the
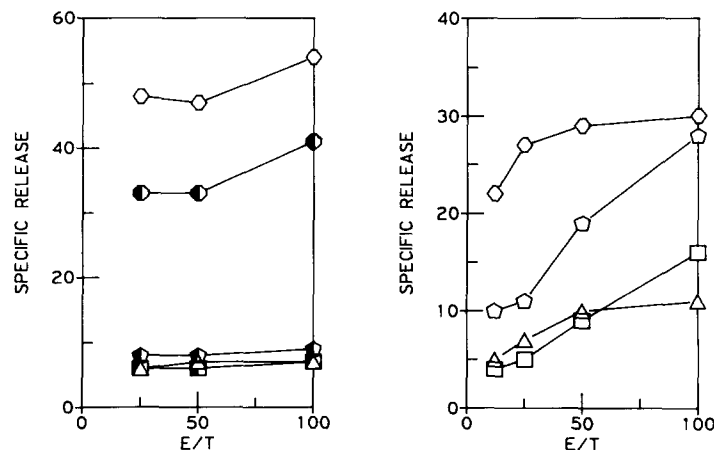
FIGURE 6. Anti-H-2L^d CTL activity vs. 1591 class I genes. (C3H × dm2)F$_1$ anti-BALB/c CTL were tested against 1591 cells (○), 28$^-$ tumor variant (□), or L cells transfected with A216 (●), A149 (⊙), A166 (■), H-2L^d (o), or tk (△). Data from *left* and *right* panels are from two separate experiments.

1591 target cells (Fig. 6, *right*), but not the 28$^-$ variant. Four out of seven anti-H-2L^d clones recognized A149 transfectants, while none lysed A166 transfectants (Table II). Taken together with the data presented in Fig. 5b, these results indicate that A149 extensively crossreacts with H-2L^d at the T cell level, while A166 is much less crossreactive.

To further show that the A149 molecule, either expressed on the transfected L cells or the 1591 tumor, crossreacts with allospecific anti-H-2L^d CTL, we tested the ability of A149-expressing cells to block the cytolytic activity of an anti-H-2L^d CTL clone, L13D.17. This clone lysed H-2L^d (27.5) and A149 transfectants, as well as 1591 cells (Fig. 7, *a–c*). The lysis of any of these three targets could be blocked by adding an excess of 27.5, A149, or 1591 inhibitor cells. No inhibition occurred when Ltk$^-$ or the 28$^-$ variant inhibitor cells were added, as expected.

Thus, the above data show that the novel H-2L-like antigens expressed on the tumor can serve as targets for alloreactive anti-H-2L^d CTL. In addition, we wished to determine whether these antigens could also function as restriction elements for antigen-specific CTL. Therefore, we analyzed the reactivity of H-2L^d-restricted anti–vesicular stomatitis virus (VSV)[1] CTL with VSV-infected L cells expressing the class I molecules from the tumor. The H-2L-like A149 antigen can, in fact, function as a restriction element for these H-2L^d-restricted CTL (Fig. 8, *a–c*). No lytic activity against A216 or A166 transfectants was shown (Fig. 8c). Thus the heirarchy of recognition of the tumor antigens by the bulk and cloned anti-H-2L^d alloreactive CTL was comparable to that of the H-2L^d-restricted CTL, suggesting perhaps that the same class I structures recognized by allospecific CTL are involved in the function of class I restriction elements.
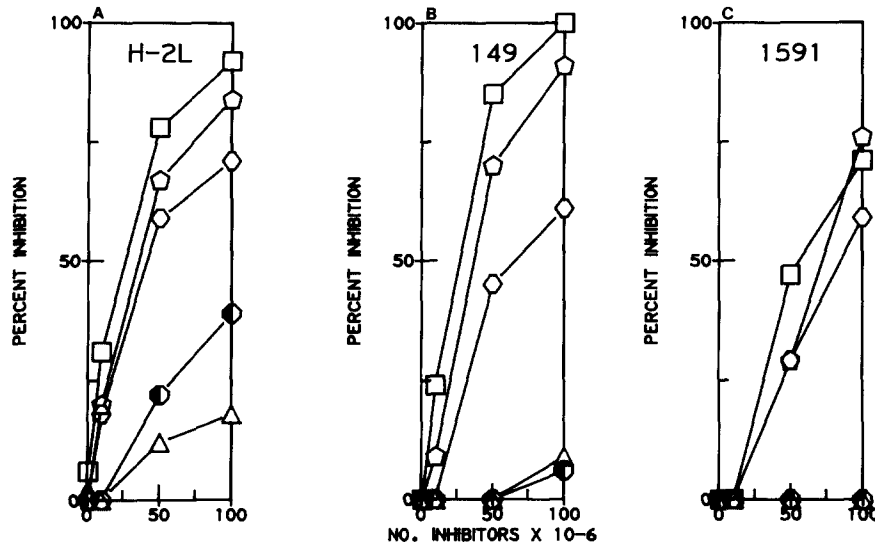
FIGURE 7. Percent inhibition of lysis mediated by the anti-H-2L^d CTL clone L13.D17 in the presence of unlabeled competitor cells. L13.D17 was tested against ^{51}Cr-labeled L cells transfected with H-2L^d (A), A149 (B), or 1591 cells (C). Unlabeled inhibitor cells are L cells transfected with H-2L^d (□), A149 (○), thymidine kinase (△), or 1591 tumor cells (o), or the 28$^-$ tumor variant (●). Specific release in the absence of inhibitor cells against H-2L^d L cells (E/T, 10:1), A149 L cells (E/T, 10:1), and 1591 (E/T, 40:1) was 49, 33, 17, respectively.
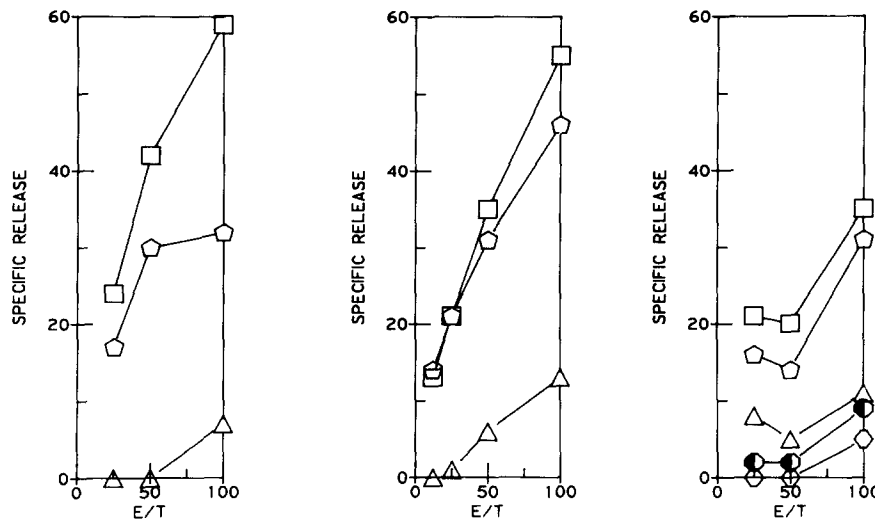
FIGURE 8. Anti-VSV CTL activity against 1591 class I antigens. Spleen cells from BDF$_1$ mice that were primed with VSV were challenged in vitro with VSV-infected spleen cells. CTL that were generated were tested against VSV-infected L cells transfected with H-2L^d (□), A149 (○), A166 (o), A216 (●), or tk (△). Data from three panels represent three separate experiments.

Discussion

In addition to the normal complement of H-2K^k and H-2D^k, the UV-induced C3H (H-2^k) fibrosarcoma, 1591, expresses at least three novel class I antigens

(12). Previous characterization of 1591 and 1591 tumor variants suggests that these novel class I antigens might function as the primary targets of a CTL response in normal histocompatible mice (9, 28). Since the loss of these products correlates with the ability of certain variants of this normally nonmetastatic tumor to escape immune rejection and progress to metastasis in immunocompetent hosts (9, 11), it is clear that they play an essential role in limiting tumor growth in the transplant situation. It is not obvious, however, what effect expression of these products exerted on the progression of the tumor in its original UV-irradiated host. While UV irradiation appears to induce a specific suppression of the ability of normal mice to mount an immune response against UV-induced tumors (5, 28), the immune elimination of other non-UV-induced tumors remains relatively unimpaired (30, 31). Most UV-tumors express unique tumor specific-antigens (9, 31), but the biochemical nature of these antigens and their relationship, if any, to UV-specific suppression remain obscure. The 1591 fibrosarcoma is the first UV-induced tumor for which a molecular description of any tumor antigens is available.

Before it is possible to define the specific immunobiological interactions that the novel class I antigens might mediate when expressed on the developing tumor, we believe that it is necessary to define the functional properties of these molecules in the context of a competent immune system. It is also essential to define the molecular origin of these antigens to determine whether their expression is, for example, a general response to the UV treatment.

To this end we have identified (12) the genes encoding the three novel class I antigens expressed on the 1591 tumor, determined the DNA sequences of the genes and the amino acid sequences of their predicted products, and characterized the functional recognition of these molecules on the 1591 tumor and on L cells transfected with the individual novel tumor genes. The three novel class I genes, A149, A166, and A216, encode polypeptides that are highly homologous to, yet distinct from, previously characterized class I antigens. Since the 1591 tumor does indeed appear to be derived from C3H (9, 12), and the novel genes account for several of the polymorphic restriction fragments observed in genomic Southern blots comparing the class I genes of 1591 with those of normal C3H tissue (12), these unique genes may have been generated by multiple recombination events among the endogenous class I genes of C3H.

In support of this hypothesis, the A216 antigen is extremely homologous to the AKR H-2K^k antigen throughout its C-terminal domain. The 5'-flanking information encoded on the λ clone containing this gene suggests strongly that the 5' coding information of this gene is derived from the equivalent of one of the cluster 8 genes defined from the BALB/c cosmid library (reference 32 and S. Watts, University of California, Berkeley, unpublished results). The A216 λ clone not only contains a fragment of a second class I gene in a head-to-head orientation, but hybridizes to a low copy probe specific for this cluster (31). Furthermore, this clone contains a 7.7-kb polymorphic Hind III fragment defined as representative of C3H (31), consistent with the C3H origin of this gene. Although the H-2K^k gene may serve as a 3' parent, the complete description of the events by which A216 was generated awaits further DNA sequence comparison with the appropriate C3H loci.

One of the most intriguing features of this tumor is that 1591 expresses two distinct, highly homologous, and immunologically crossreactive class I antigens. A149 and A166 are >98% homologous to each other and to the H-2L^d antigen from BALB/c. The genes encoding these three antigens are nearly identical for 2,000 bp containing the exons encoding the third external domain, α3, the transmembrane domain, and the cytoplasmic domain. Unique sequences within the 5' ends of the genes account for the discrete functional differences observed between their products. Since a polymorphic Pst I fragment not found in C3H is contained within this region of near identity, it is possible that A149 and A166 were derived by duplication of a recombinant precursor gene, followed by additional recombination events to generate the discrete differences between the two tumor genes. Alternatively, the polymorphic 1.0-kb Pst I fragments found in both A149 and A166 might have been generated independently by comparable recombination events involving two highly conserved parental genes. Additional polymorphic restriction fragments map to the 5' end of the gene, suggesting that multiple recombination events were required to generate each of these novel genes (data not shown). Identification and DNA sequence analysis of all of the class I genes involved in these complex recombination events will probably be necessary to define the precise recombinational pathway by which these novel genes were generated.

The extensive sequence homologies observed among A149, A166, and H-2L^d are also shared with the 3' coding region of the *H-2D*^{b} gene. Such unprecedented sequence conservation between class I genes from three different haplotypes is remarkable, and argues for an allelic relationship between these three sets of genes (33). In support of this hypothesis, a 5' flanking probe derived from A149 crosshybridizes to H-2L^d, and detects several class I genes from a normal C3H library, which map to the D end of the H-2 (e. g., *H-2D*^{k}). Thus, although C3H is not believed to express H-2L locus information, conserved H-2L coding sequences may exist in an unexpressed configuration within the appropriate region of the MHC.

The extreme structural conservation observed among A149, A166, and H-2L^d is reflected by significant functional homology. The three antigens share both public and private class I antigenic determinants, and most of the antigenic differences that have been observed among these molecules appear to correlate with discrete sequence substitutions. Previously, studies involving the serological characterization of exon-shuffled class I gene products have succeeded in localizing the structures recognized by several different allospecific mAb to individual protein domains (21, 34–37). Unfortunately, attempts to correlate such serological determinants with specific sets of amino acids have been complicated by the comparison of class I products that differ at multiple amino acid residues (38). However, given the ability of antibodies directed against discrete peptides to recognize the native conformation of a protein (39), it is essential to determine whether specific alloantibodies against H-2 react with conformational determinants, or whether it is possible to identify specific coding sequences that can function as independent epitopes.

While both A149 and A166 molecules are closely related to H-2L^d, both by sequence and serological criteria, only the former molecule crossreacts exten-

sively with H-2L^d at the T cell level. Thus, anti-1591 CTL react against H-2L^d, A149, A166, and A216, while anti-H-2L^d CTL crossreact primarily with A149. However, a distinct population of A166 crossreactive CTL could be shown by challenging the spleen cells from tumor immune animals in vitro with H-2L^d. A149 and A166 differ from H-2L^d at 6 and 8 amino acid residues, respectively, in the α2 domain. Four of the changes are shared by both A149 and A166, and the additional unique changes found in A166 are no more different, with respect to charge or side-chain bulk, than those seen in A149. In contrast, A166 has 5 amino acid changes in the α1 domain while A149 has none. Thus, these results could indicate that the vast majority of anti-H-2L^d CTL are controlled by determinants mapping to the α1 domain. This is surprising, however, in light of previous results that mapped some H-2L^d CTL determinants to the α2 domain. Specifically, Murre et al. (37) have observed that a domain shuffled molecule that contained the α1 of H-2D^d and the α2 of H-2L^d retained anti-H-2L^d CTL determinants. We (J. Forman, unpublished data) have noted that anti-H-2L^d CTL also readily recognize the H-2^{dm1} molecule, which has H-2L^d sequences only in the carboxy-terminal end of the α2 domain, but H-2D^d sequences in the α1 and the proximal half of the α2. Perhaps these observations can be reconciled with the data discussed in this report by postulating that specific intramolecular interactions with α1 structures are required to impart a recognizable conformation on α2-encoded determinants. Amino acid substitutions found in the α1 of A166 might somehow interfere with these conformational interactions. Such interactions might not be obvious from the analysis of the shuffled or dm1 molecules described above if the H-2D^d α1 domain was itself compatible with the α2 of L^d. This hypothesis can be tested by generating mutant A166 molecules in vitro, in which A166 residues are replaced with H-2L^d and D^d sequences.

Conformational interactions between class I domains have been implicated in previous studies involving the H-2K^b series of class I mutants (40, 41) where one to three amino acid changes in either the α1 or α2 domains usually result in the loss of most of the CTL epitopes. Further evidence for conformational interactions and the requirement for domain compatibility comes from studies involving the shuffling of exons that encode the α1 and α2 domains of class I molecules (34–36). In these studies it was found that virtually all of the CTL epitopes characteristic of the native molecules are absent from the hybrid molecules.

Detailed study of the structural bases of these conformational interactions might be uniquely accessible in the 1591/H-2L^d system for several reasons: (a) the structural differences that exist between A149, A166, and H-2L^d are quite discrete and can be easily manipulated by oligonucleotide directed mutagenesis; (b) all three antigens seem able to evoke a CTL response; (c) A149 and A166 each crossreact to some extent with H-2L^d with respect to both CTL and serological reagents; (d) discrete changes in crossreactivity will probably be observed after in vitro mutagenesis; and (e) H-2L^d has already been extensively characterized as a T cell target (17, 18, 35).

The crossreactivity of A149 with H-2L^d was also noted with regard to antigen-specific CTL. Thus, BDF$_1$ anti-VSV CTL, restricted to H-2L^d, also recognize VSV antigens in association with A149. These data indicate that the tumor not only expresses a molecule that can function in the restriction of virus-specific

CTL, but also that the functional homology of A149 to H-2L^d involves determinants recognized by both allospecific and H-2-restricted CTL.

Taken together, our present results indicate that all three novel class I antigens expressed on 1591 can function as potential target structures for the immune destruction of the transplanted tumor in histocompatible hosts. Of course, the immunogenicity of any one of these molecules could depend upon its relative level of expression on the surface of the tumor cell, as well as the immunological status and specific MHC background of the host. It is also difficult to predict the precise effects of the expression of the two highly homologous, but distinct, H-2L-like antigens. Clearly, this tumor could progress in the autochthonous host despite its extreme immunogenicity, and it is quite possible that the expression of these novel antigens was somehow related to the UV-induced immune suppression operative during tumor progression. Further immunobiological studies involving tumor variants and class I transfectants transplanted into UV-irradiated and normal hosts may elucidate the precise effect each of these molecules might mediate during tumor progression.

Summary

The UV-induced, C3H fibrosarcoma, 1591, expresses at least three unique MHC class I antigens not found on normal C3H tissue. Here we report the complete DNA sequence of the three novel class I genes encoding these molecules, and describe in detail the recognition of the individual products by tumor-reactive and allospecific CTL. Remarkably, although C3H does not appear to express H-2L locus information, this C3H tumor expresses two distinct antigens, termed A149 and A166, which are extremely homologous to each other and to the H-2L^d antigen from BALB/c. The gene encoding the third novel class I antigen from 1591, A216, is quite homologous to H-2K^k throughout its 3' end. Since all three of these genes account for polymorphic restriction fragments not found in C3H, it is likely that they were derived by recombination from the endogenous class I genes of C3H. The DNA sequence homology of A149, A166, and H-2L^d is especially significant given the functional conservation observed between the products of these genes. Limited sequence substitutions appear to correlate with some of the discrete serological differences observed between these molecules. In addition, both A149 and A166 crossreact, but to differing extents, with H-2L^d at the level of T cell recognition. Our results are consistent with the view that CTL recognize complex conformational determinants on class I molecules, but extend previous observations by comparing a set of antigens with discrete and overlapping structural and functional differences.

References

1. Zinkernagel, R. M., and P. C. Doherty. 1979. MHC-restricted cytotoxic T cells: studies on the role of polymorphic major transplantation antigens determining T cell restriction specificity, function, and responsiveness. *Adv. Immunol.* 27:51

2. Doherty, P. C., B. B. Knowles, and P. J. Wettstein. 1984. Immunological surveillance of tumors in the context of major histocompatibility complex restriction of T cell function. *Adv. Cancer Res.* 42:1

3. Goodenow, R. S., J. M. Vogel, and R. L. Linsk. 1985. Histocompatibility antigens on murine tumors. *Science (Wash. DC).* 230:777

4. Parmiani, G., G. Carbone, G. Invernizzi, M. A. Pierotti, M. L. Sensi, M. J. Rogers, and E. Appella. 1979. Alien histocompatibility antigens on tumor cells. *Immunogenetics.* 9:1

5. Fisher, M. S., and M. L. Kripke. 1977. Systemic alteration induced in mice by ultraviolet light irradiation and its relationship to ultraviolet carcinogenesis. *Proc. Natl. Acad. Sci. USA.* 74:1688

6. Kripke, M. L. 1977. Latency, histology, and antigenicity of tumors induced by ultraviolet light in three inbred mouse strains. *Cancer Res.* 37:1395

7. Roberts, L. K., and R. A. Daynes. 1980. Modification of the immunogenic properties of chemically induced tumors arising in hosts treated with concomitantly with ultraviolet light. *J. Immunol.* 125:438

8. Roberts, L. K. 1986. Characterization of a cloned ultraviolet radiation (UV)-induced suppressor T cell line that is capable of inhibiting anti-UV tumor immune responses. *J. Immunol.* 135:1908

9. Philipps, C., M. McMillan, P. M. Flood, D. B. Murphy, J. Forman, D. Lancki, J. E. Womack, R. S. Goodenow, and H. Schreiber. 1985. Identification of a unique tumor-specific antigen as a novel class I major histocompatibility molecule. *Proc. Natl. Acad. Sci. USA.* 82:5140

10. McMillan, M., K. D. Lewis, and D. M. Rovner. 1985. Molecular characterization of novel H-2 class I molecules expressed by a C3H UV-induced fibrosarcoma. *Proc. Natl. Acad. Sci. USA.* 82:5485

11. Wortzel, R. D., J. L. Urban, and H. Schreiber. 1984. Malignant growth in normal host after variant selection *in vitro* with cytolytic T-cell lines. *Proc. Natl. Acad. Sci. USA.* 81:2186

12. Stauss, H. J., R. L. Linsk, S. Watts, A. Fischer, D. Banasiak, A. Haberman, I. Clark, J. Forman, M. McMillan, H. Schreiber, and R. S. Goodenow. 1986. Isolation of the MHC genes encoding the tumor-specific class I antigens expressed on a murine fibrosarcoma. *J. Immunogenet. (Oxf.).* In press

13. Arnold, B., H.-G. Burgert, A. L. Archibald, and S. Kvist. 1984. Complete nucleotide sequence of the murine H-2K^k gene. Comparison of three H-2K alleles. *Nucleic Acids Res.* 12:9473

14. Moore, K. W., B. T. Sher, Y. H. Sun, K. A. Eakle, and L. Hood. 1982. DNA sequence of a gene encoding a BALB/c mouse H-2L^d transplantation antigen. *Science (Wash. DC).* 215:679

15. Sanger, F., A. R. Coulson, B. G. Barrell, A. J. H. Smith, and B. A. Roe. 1980. Cloning in single stranded bacteriophage as an aid to rapid DNA sequencing. *J. Mol. Biol.* 143:161

16. Sher, B. T., R. Nairn, J. E. Coligan, and L. E. Hood. 1985. DNA sequence of the mouse H-2D^d transplantation antigen gene. *Proc. Natl. Acad. Sci. USA.* 82:1175

17. Miyazaki, J. I., E. Appella, H. Zhao, J. Forman, and K. Ozato. 1986. Expression and function of a nonglycosylated major histocompatibility class I antigen. *J. Exp. Med.* 163:856

18. Forman, J., R. S. Goodenow, L. Hood, and R. Ciavarra. 1983. Use of DNA-mediated gene transfer to analyze the role of *H-2L*^{d} in controlling the specificity of anti-vesicular stomatitis virus cytotoxic T cells. *J. Exp. Med.* 157:1261

19. Ozato, K., T. H. Hansen, and D. Sachs. 1980. Monoclonal antibodies to mouse MHC antigens. *J. Immunol.* 125:2473

20. Stroynowski, I., S. Clark, L. A. Henderson, L. Hood, M. McMillan, and J. Forman. 1985. Interaction of α1 with α2 region in class I MHC proteins contributes determinants recognized by antibodies and cytotoxic T cells. *J. Immunol.* 135:2160

21. Evans, G. A., D. M. Margulies, B. Shykind, J. G. Seidman, and K. Ozato. 1982. Exon shuffling: mapping polymorphic determinants on hybrid mouse transplantation antigens. *Nature (Lond.).* 300:755

22. Ozato, K., N. M. Mayer, and D. Sachs. 1982. Monoclonal antibodies to mouse MHC antigens. A series of hybridoma clones producing anti-H-2^d antibodies and an examination of expression of H-2^d antigens on the surface of these cells. *Transplantation (Baltimore).* 39:113

23. Reyes, A. A., M. Schold, and R. B. Wallace. 1982. The complete amino acid sequence of the murine transplantation antigen H-2D^b as deduced by molecular cloning. *Immunogenetics.* 16:1

24. Reiss, C. S., G. A. Evans, D. H. Margulies, J. G. Seidman, and S. J. Burakoff. 1983. Allospecific and virus-specific cytolytic T lymphocytes are restricted to the N or C1 domain of H-2 antigens expressed on L cells after DNA-mediated gene transfer. *Proc. Natl. Acad. Sci. USA.* 80:2709

25. Kvist, S., L. Roberts, and B. Dobberstein. 1983. Mouse histocompatibility genes: structure and organisation of a K^d gene. *EMBO (Eur. Mol. Biol. Organ.) J.* 2:245

26. Sun, Y. H., R. S. Goodenow, and L. Hood. 1985. Molecular basis of the dm1 mutation in the MHC of the mouse: a D/L hybrid gene. *J. Exp. Med.* 162:1588

27. Flood, P. M., M. L. Kripke, D. A. Rowley, and H. Schreiber. 1980. Suppression of tumor rejection by autologous anti-idiotypic immunity. *Proc. Natl. Acad. Sci. USA.* 77:2209

28. Wortzel, R. D., C. Philipps, and H. Schreiber. 1983. Multiple tumour-specific antigens expressed on a single tumour cell. *Nature (Lond.).* 304:165

29. Philipps, C., H. J. Stauss, R. D. Wortzel, and H. Schreiber. 1986. A novel class I molecule as tumor specific antigen: correlation between the antibody-defined and CTL-defined target structure. *J. Immunogenet. (Oxf.).* In press.

30. Kripke, M. L., J. S. Lofgreen, J. Beard, J. M. Jessup, and M. S. Fisher. 1977. In vivo immune responses of mice during carcinogenesis by ultraviolet irradiation. *J. Natl. Cancer Inst.* 59:1227

31. Kripke, M. L. 1981. Immunologic mechanisms in UV radiation carcinogenesis. *Adv. Cancer Res.* 34:69

32. Winoto, A., M. Steinmetz, and L. Hood. 1983. Genetic mapping of the major histocompatibility complex by restriction enzyme site polymorphisms: Most mouse class I genes map to the *Tla* complex. *Proc. Natl. Acad. Sci. USA.* 80:3425

33. Weiss, E. H., L. Golden, K. Fahrner, A. L. Mellor, J. J. Devlin, H. Bullman, H. Tiddens, H. Bud, and R. A. Flavell. 1984. Organization and evolution of the class I gene family in the major histocompatibility complex of the C57BL/10 mouse. *Nature (Lond.).* 310:650

34. Allen, H., D. Wraith, P. Pala, B. Askonas, and R. A. Flavell. 1984. Domain interactions of H-2 class I antigens alter cytotoxic T-cell recognition sites. *Nature (Lond.).* 309:279

35. Bluestone, J. A., M. Foo, H. Allen, D. Segal, and R. A. Flavell. 1985. Allospecific

cytolytic T lymphocytes recognize conformational determinants on hybrid mouse transplantation antigens. *J. Exp. Med.* 162:268

36. Arnold, B., U. Horstmann, W. Kuon, H.-G. Burgert, G. J. Hammerling, and S. Kvist. 1985. Alloreactive cytolytic T-cell clones preferentially recognize conformational determinants on histocompatibility antigens: analysis with genetically engineered hybrid antigens. *Proc. Natl. Acad. Sci. USA.* 82:7030

37. Murre, C., E. Choi, J. Weis, J. G. Seidman, K. Ozato, L. Liu, S. J. Burakoff, and C. S. Reiss. 1984. Dissection of serological and cytolytic T lymphocyte epitopes on murine major histocompatibility antigens by a recombinant H-2 gene separating the first two external domains. *J. Exp. Med.* 160:167

38. Ozato, K., H. Takahashi, E. Appella, D. W. Sears, C. Murre, J. G. Seidman, S. Kimura, and N. Tada. 1985. Polymorphism of murine major histocompatibility class I antigen: assignment of putative allodeterminants to distinct positions of the amino acid sequence within the first external domain of the antigen. *J. Immunol.* 134:1749

39. Walker, L. E., T. A. Ketler, R. A. Houghton, G. Schulz, A. Chersi, and R. A. Reisfeld. 1985. Human major histocompatibility complex class I antigens: residues 61-83 of the HLA-B7 heavy chain specify an alloreactive site. *Proc. Natl. Acad. Sci. USA.* 82:539

40. Clark, S. S., S. Geier, S. G. Nathenson, and J. Forman. 1985. Analysis of associative recognition of determinants on class I H-2^{kb} mutants. *Immunogenetics.* 22:391

41. de Waal, L. P., W. M. Kasp, R. W. Melvold, C. J. M. Melief. 1983. Regulation of cytotoxic T lymphocyte response against Sendai virus analyzed with H-2 mutants. *J. Immunol.* 130:1090