THE TUMOR-REJECTION ANTIGENS OF THE 1591 ULTRAVIOLET FIBROSARCOMA

Potential Origin and Evolutionary Implications

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The nature of tumor-specific transplantation antigens has been an object of interest ever since the initial demonstrations that primary tumor hosts can make an immune response capable of providing protection against the growth of the tumor (1–4). The analysis of these products has provided significant insight into tumor biology and has led to the development of potential immunological approaches to the treatment of cancer.

Tumor-specific antigens are often found to be associated with tumors induced with carcinogenic agents such as UV-light or 5-methylcholanthrene (4–6). Initially these tumor antigens were examined using in vivo tumor rejection assays. Such studies suggested that in some cases these antigens were immunologically unique. For example, analysis of a panel of highly antigenic UV-induced fibrosarcomas indicated that immunization with one tumor would provide little protection against the growth of a second syngeneic tumor (7, 8). The cumulative analysis of these and other tumors suggested that the repertoire of potential antigens might be quite large (9, 10). The precise molecular mechanisms by which such a diversity of antigens might be generated remained unclear.

To define more precisely the biochemical characteristics and biological functions of tumor-specific antigens, Schreiber and collaborators undertook the detailed analysis of the antigenic products of a single C3H/HeN-derived, UV-induced fibrosarcoma, 1591. In the course of these studies, these workers demonstrated that 1591 expresses several genetically distinct tumor antigens responsible for the growth behavior of the tumor (11, 12). In addition, two tumor-reactive mAbs were isolated which allowed the biochemical analysis of a subset of these tumor antigens (13). Intriguingly, these mAbs appeared to crossreact with various allogeneic MHC haplotypes and were able to immunoprecipitate molecules exhibiting the biochemical features of class I MHC antigens (13, 14). These data were particularly exciting since they suggested that the polymorphism observed among tumor antigens might be analogous to that which has been defined for MHC antigens.

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Using the tumor-reactive mAbs in conjunction with class I-specific DNA probes, Stauss et al. (15) isolated from a 1591 genomic library three genes that account for all of the apparently allogeneic class I reactivities associated with the 1591 tumor. The sequences of these three genes were determined, and the CTL recognition and growth behavior of transfectants were examined (16, 17). The conclusion of these studies was that two of these genes, A149 and A166, were very similar and encoded products exhibiting significant structural and functional homology with the H-2L^d product, while the third tumor gene, A216, encoded the target antigen of a tumor-specific CTL clone. None of these genes appeared to exist intact in the C3H genome, as determined by genomic Southern blot analysis (15). Furthermore all three genes appeared to be genetically linked, since all genomic polymorphisms were lost in parallel after selection for the loss of a single antigen. It was postulated that these novel genes might have been generated by multiple recombination events among endogenous C3H class I genes (4, 15–17).

In this report, we extend the analysis of these three novel genes and examine further the nature of genetic polymorphisms in the 1591 tumor. Although an examination of a complete set of C3H H-2- and Qa-linked class I sequences failed to identify a likely progenitor for any of the novel tumor genes, it was discovered that two of these genes, A149 and A166, are extremely similar to the H-2L and H-2D genes of the H-2^q haplotype. Furthermore, genomic Southern blot analysis suggests that the homology between the H-2^q haplotype and the 1591-associated MHC polymorphism extends beyond the A149 and A166 genes. In addition, non-MHC genetic polymorphism is detected in the 1591 tumor. We believe that these data are most consistent with an allogeneic origin for the novel MHC antigens expressed by the 1591 tumor.

Materials and Methods

Cell Lines and Animals. The following UV-induced tumor cell lines were the kind gift of Hans Schreiber (Department of Pathology, Unversity of Chicago, Chicago, IL): 1591 and the CP28$^-$ antigen loss variant; uv 802, uv 918, uv 948, uv 4184, puva 2210, puva 3312a. L cell transfectants of H-2K^d, D^d, L^d, A149, A166, A216 were generated previously (15). The H-2^{q,r,s} LF cell lines were generated from fibroblasts grown out of murine lung explants. The KB29 and LR335 C3H UV-induced tumor lines were the gift of Raymond Daynes (Department of Pathology, University of Utah, Salt Lake City, UT). The BL-5 cell line was the gift of Miriam Lieberman (Stanford University, Stanford, CA). The Swiss 3T3 line was obtained from American Type Culture Collection (Rockville, MD). C3H/HeJ, BALB/cJ, SJL/J, RIIIS/J, and A.SW/J mice were purchased from The Jackson Laboratory (Bar Harbor, ME). B10.AKM was a generous gift from Chella David (Mayo Clinic, Rochester, MN).

DNA Sequences. 1591 tumor genes were cloned from a genomic library (15). C3H/HeN H-2 genes (18) and Qa genes (our unpublished data) were isolated from among λ and cosmid genomic libraries. All were sequenced by the dideoxy chain termination method using internal oligonucleotide primers as previously described (16, 18).

Southern Blots. Mouse genomic DNA was isolated from liver. Tumor DNAs were isolated from cells or were a gift from Hans Schreiber. Restriction endonucleases were purchased from New England Biolabs (Beverly, MA). 10 μg of digested DNA was used per lane. Probes were labeled by the random oligonucleotide priming method of Feinberg and Vogelstein (19) to a specific activity of >10^8 cpm. Some filters were stripped after hybridization with 0.5 M NaOH and reprobed.

Radioimmune Assay. H-2 expression was determined in a cellular radioimmune assay. Briefly, 2.5 × 10^6 cells were incubated in duplicate wells in microtiter plates with specific anti H-2

monoclonal antibodies. The following mAbs were used in this study: CP28 and CP3F4 (13); 30-5-7, 28-14-8, 34-4-20, and 34-1-2 (20, 21). After washing, cells were incubated with ^{125}I-labelled Protein A. Unbound Protein A was washed out and the amount of radioactivity measured.

β-Glucuronidase Assay. Cell lysate extracts were assayed for β-glucuronidase activity as described (22). Briefly, cells were lysed, extracts were obtained after centrifugation and diluted 1/10, four 1-ml samples were heat inactivated at 70°C for 0, 5, 10, and 20 min, respectively, and activity was measured by fluorimetry.

Results

Sequence Comparisons between 1591 and C3H Class I Genes. The UV-induced, C3H/HeN-derived fibrosarcoma, 1591, appears to express at least three H-2-like products not found on normal C3H tissue. Previously, three class I genes encoding these tumor products were identified within a collection of class I-hybridizing clones isolated from a 1591 genomic library (15). Together, these genes, designated A149, A166, and A216, direct the expression of all 1591-associated serological and CTL-defined reactivities after transfection into C3H L cells. In addition, each of these three genes accounts for a polymorphic class I restriction fragment detected by genomic Southern blot analysis of 1591 DNA, suggesting that the sequences encoding the 1591 tumor-specific class I antigens do not exist intact within the ancestral C3H genome (15). The mechanisms underlying the presence of these three genes in 1591 were not defined, although it was suggested that they might be derived by mutation or recombination from endogenous C3H class I genes.

As a first step towards elucidating the molecular origin of these genes, we determined their DNA sequences (16). Each of the three tumor genes exhibits strong homology with previously sequenced H-2 genes. In particular, A216 is highly homologous to H-2K^k throughout its 3′-coding and untranslated region, while A149 and A166 are homologous to each other and to both H-2L^d and H-2D^b (16).

If the 1591 class I genes are indeed derived by some simple recombinational pathway from endogenous C3H class I genes, it should be possible to identify potential parental genes on the basis of sequence homology. Recently, we have completed the DNA sequence analysis of all of the H-2 and Q region-linked genes from C3H/HeN (Watts, S., unpublished data). In an attempt to identify regions of shared identity between any of these C3H genes and the H-2-like genes from the 1591 tumor, sequences from each of these genes were aligned in a pairwise manner, and the number of point differences was determined (Table I). An average of 73 differences exist between these genes in exons 2 and 3, out of 543 positions compared. The most homologous pair examined was A149 and Q2^k, which differed by a total of 36 bases in these regions. The longest stretch of identity observed in these comparisons was 129 bp between the third exon sequences of A216 and H-2D^k. However, these two sequences differ at a total of 52 positions over the regions compared. Less diversity is found within exon four, but here also none of the C3H sequences are identical to the A149, A166, or A216 sequences. Therefore, unless it is assumed that A149, A166, or A216 were generated as a patchwork from multiple parental genes, it is difficult to identify a 5′ parent for any of the tumor genes from among this set of sequences. Although it remains possible that A149, A166, and A216 were derived from Tla region genes, the overall structure and sequence of the Tla genes that have been analyzed to date tend to be even more distinct from that of H-2 genes (Table

TABLE I

Nucleotide Differences among 1591 and C3H/HeN Class I Genes*

	Exon 2‡			Exon 3			Exon 4	
	A149	A166	A216	A149	A166	A216	A149	A216
H-2K^k	20	27	26	23	27	20	12	4
H-2D^k	26	23	31	21	22	21	4	10
K1^k	38	34	42	53	53	58	10	15
Q1^k	29	30	38	54	53	60	6	13
Q2^k	17	19	34	38	38	42	9	16
Q4^k	28	26	33	30	31	36	13	17
Q5^k	52	55	56	46	45	35	17	28
Q10^k	33	34	44	26	29	52	11	17
Tla^a	88	87	88	71	69	71	24	29
Tla^b	80	79	80	69	67	70	22	28
Tla^c	81	80	81	68	66	69	23	29
H-2K^b	27	30	17	28	31	19	9	2
H-2D^b	11	15	31	14	12	25	0	8
H-2K^d	36	31	28	17	17	22	11	14
H-2D^d	33	31	11	22	22	24	7	7
H-2L^d	1	5	29	8	10	21	0	9
H-2D^p	32	32	22	24	23	22	4	9
A149	–	8	31	–	8	21	–	8
A166	–	–	29	–	–	20	0	8

* Sequences (Watts, S., unpublished data; references 16, 18, 23–29). Only five Q region genes have been indentified from a C3H/HeN genomic library (Watts, S., unpublished data).

‡ Sequences were aligned only in exons 2, 3, and 4. Exon lengths used were as follows: exon 2, 270 bases; exon 3, 276 bases; exon 4, 276 bases.

I). In particular, we have compared the DNA sequences of the 1591 class I genes to those of three Tla region genes from different serologically defined haplotypes: Tla^a (23), Tla^b (24), and Tla^c (25). At least 146 differences are observed between the 1591 and Tla genes within the exon 2 and 3 sequences compared. Furthermore, no Tla-containing clone isolated from genomic C3H libraries appears to be homologous to any of the novel tumor genes at the level of restriction mapping (Watts, S., unpublished data). Given the sum of these data, it is difficult to conclude that the three 1591-derived class I genes were generated by any limited mutational or recombinational mechanism from the class I genes of C3H/HeN.

Identification of Potential Phylogenetic Origins of the Novel 1591 Class I Genes. Since no likely progenitor loci for the 1591 genes have been found within the C3H MHC, it is important to consider whether any alternative phylogenetic origins might be discerned. One possible indication of an allogeneic origin for the novel MHC products of 1591 could be inferred from the pattern of serological crossreactivity exhibited by the tumor (see Table II). In particular, the C3H anti-1591 mAb, CP3F4, recognizes not only the A166 antigen but also H-2K^d and a product expressed by the H-2^q haplotype, while CP28 reacts with A216, H-2D^d, and an H-2^s product, probably H-K^s. Although the sequences of A166 and A216 indicate that these molecules are clearly distinct from H-2K^d and H-2D^d, these novel antigens might, in principle, be related to H-2^q and H-2^s haplotype products. This possibility is supported by

TABLE II

Serological Reactivities of several H-2-specific mAbs by RIA

Antigens	Cell line	Monoclonal antibody					
		CP28	CP3F4	30-5-7	28-14-8	34-1-2	34-4-20
		cpm					
A149	H12-23.1	831	114	646	28,169	3,420	126
A166	H12-9.2	220	8,114	34,633	19,082	16,139	131
A216	H12-20.1	28,613	143	121	155	22,573	137
1591	1591 28+	5,985	3,484	9,571	13,775	9,411	115
H-2K^d	H40-5	111	3,336	127	102	17,902	97
H-2D^d	K8-30P	9,208	129	68	83	12,085	12,329
H-2L^d	K2a-7	171	139	18,852	18,723	1,206	14,133
H-2^{m*}	B10.AKM LF	339	8,299	13,879	13,744	10,431	396
H-2^{q‡}	Swiss 3T3	298	18,753	27,383	27,568	26,103	7,061
H-2^k	Ltk$^-$	148	103	95	84	65	98
H-2^b	BL-5	148	147	110	9,598	1,857	194
H-2^r	RIIIS/J LF	90	100	97	94	7,601	12,707
H-2^s	A.SW LF	12,646	219	103	87	13,991	128

* H-2K^{k}D^{q}L^q.

‡ H-2K^q verified by reactivity with 16-1-11 mAb (30).

the observation that H-2^q haplotype cells are also recognized by several mAbs (28-14-8, 30-5-7, and 34-1-2) that react with the A149 and/or A166 antigens. In addition, C3H anti-1591 CTL populations exhibit strong reactivity with H-2^q haplotype cells (31). Therefore, it is important to determine to what extent structural homology between H-2^q and H-2^s products and the 1591 tumor antigens might be responsible for the observed serological similarities.

In fact, like 1591, B10.AKM cells (K^k, D^q, L^q) appear to express at least two distinct H-2D region class I products that are highly homologous to H-2L^d (32). Like A149, H-2L^q (also referred to as H-2R^q) reacts with the mAb 28-14-8. A166 and H-2D^q are both recognized by the antibodies 28-14-8 and 30-5-7 (33). The limited peptide sequence analysis that has been performed on these H-2^q haplotype products indicates that they are nearly identical to A149 and A166, respectively (32). In addition, the sequence of a cDNA clone derived from SWR/J liver is 99.4% identical to A149 over 1,368 bases compared (Jay, G., personal communication). On the basis of these observations, we previously proposed that A149 and A166 are functionally equivalent to the H-2D region products of the H-2^q haplotype (31). Recently, the class I genes encoding both the H-2L^q and D^q antigens were isolated from a B10.AKM genomic library (33). The limited DNA sequence derived from the 5′ regions of these clones confirms the structural identity noted between the 1591 and H-2^q products. However, several point differences are observed between the intronic sequences of these genes, suggesting that the 1591 genes might not be directly derived from the B10.AKM H-2D^q and L^q alleles. Nonetheless, given the range of divergence that might be attributable to DNA sequencing differences, point mutation events in the course of tumor growth or molecular cloning, or subtle allelic variation between B10.AKM and other H-2^q-related haplotypes, the level of homology observed is striking. Therefore, the sum of these data indicates that the 1591 tumor

bears two H-2-like antigens that exhibit extensive structural and functional homology to the H-2D and H-2L products of the H-2^q haplotype.

Genomic Southern Blot Analysis of Class I Polymorphisms in the 1591 Tumor. Given the strong structural homology that exists between A149 and A166 and the H-2D region genes of the H-2^q haplotype, it was of interest to determine whether additional homologies exist between 1591 and the H-2^q haplotype. Such homologies might be identified as non-C3H restriction fragments shared between 1591 and H-2^q haplotype genomic DNA. Fig. 1 shows the results of Southern blot analysis using both

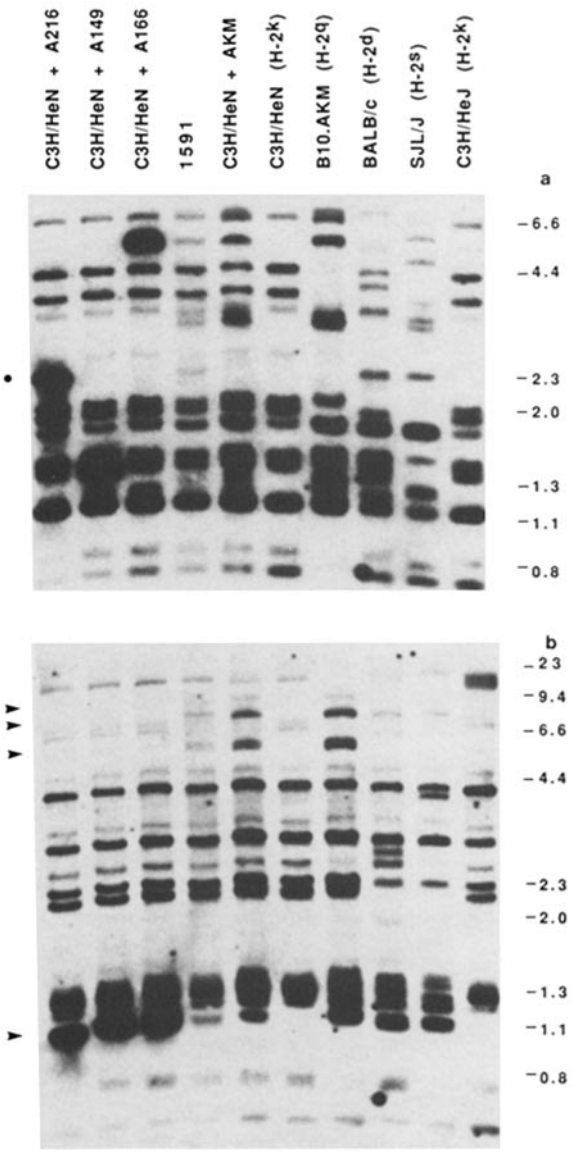

FIGURE 1. A Southern blot of genomic DNA digested with Pst I was hybridized to a 5′ exon 3-specific probe (Aat II–Xba I from A149) (*a*) or a coding region probe corresponding to the 1.1-kb polymorphic Pst I fragment of A149 (*b*). (*a*) Reprobing of the filter in *b*. C3H/HeN DNA was derived from the C3H-derived UV-induced tumor, 2240, which shows no polymorphism when compared with bona fide C3H/HeN DNA digested with Pst I. Arrows indicate polymorphic restriction fragments in 1591 relative to C3H that are reconstituted by cloned tumor genes (lanes *1–3*) and B10.AKM DNA (lane *5*). The dot in *a* indicates the 2.2-kb polymorphic restriction fragment reconstituted with A216 DNA.

5′ and 3′ class I coding region probes. In these blots Pst I digests of genomic DNA from various MHC haplotypes as well as 1591 and a C3H/HeN control are compared. In addition, polymorphic restriction fragments detected in 1591 are reconstituted by the addition of cloned class I genes or B10.AKM DNA to C3H/HeN DNA. It is clear from these blots that 1591 bears at least three 3′ and one 5′ hybridizing class I fragments that are neither found in C3H/HeN DNA nor accounted for by the A149, A166, or A216 genes. At lower stringency, seven such 5′ fragments can be discerned (data not shown). Intriguingly, each of these novel fragments, as well as those corresponding to A149 and A166, is also found in the B10.AKM DNA. However, it is important to note that B10.AKM does not contain a 2.2-kb restriction fragment equivalent to that reconstituted with A216 DNA. This result is consistent with the observation that H-2^q haplotype cells do not express an A216-like product, as measured by their lack of reactivity with the CP28 mAb (Table II). In contrast, BALB/cJ and SJL/J DNA both appear to bear a 2.2-kb restriction fragment, and both H-2^d and H-2^s haplotypes are CP28$^+$. Comparable results are seen with the restriction enzymes Bam HI, Pvu II, and Taq I (data not shown). In each of these cases, between one and four non-C3H restriction fragments are found in the 1591 DNA, and in every example these restriction fragments can be reconstituted by the addition of B10.AKM DNA. Furthermore, in no case is a restriction fragment seen in the B10.AKM digests that is not also found in 1591. Therefore, except for the restriction fragments corresponding to the A216 gene, the genomic Southern blot pattern of 1591 resembles that of an admixture of C3H/HeN and B10.AKM DNA. A possible origin of the CP28-reactive A216 is discussed below.

Analysis of Non-MHC Genetic Background in the 1591 Tumor. Since 1591 appeared to be heterozygous with respect to the class I genes of the MHC, it was of interest to determine whether any heterozygosity might be detected elsewhere in the genome. To this end the polymorphic locus RP2, located on the 7th chromosome, was examined (34). Most common inbred strains of mice, such as C3H, C57BL/10, and BALB/c, carry an allele at this locus that generates a 1.9-kb Hind III fragment. Several Swiss-derived strains such as SJL and SWR exhibit a 2.2-kb allele. In Fig. 2 it is readily apparent that 1591 carries both alleles. Two bands are present in every preparation of 1591 tested, including the 1591 CP28$^-$ antigen loss variant. The band is not present in the DNA of 2240, a C3H/HeN UV-fibrosarcoma derived in parallel with 1591.

The genetic constitution of the 1591 tumor at the β-glucuronidase locus was also examined. C3H is unique among inbred strains of mice in having a β-glucuronidase allele whose product is relatively unstable at elevated temperatures (22). Therefore the presence of non-C3H genetic material would likely be reflected as enhanced temperature stability of β-glucuronidase activity over that of bona fide C3H cells. In fact as shown in Fig. 3, the relative stability at 70°C of β-glucuronidase activity found in 1591 whole-cell extracts is intermediate between that of C3H-derived Ltk$^-$ and KB29 fibroblast lines and B10, B10.AKM, or Swiss-derived fibroblast lines. Comparable results are found at 65°C (Data not shown). Thus, 1591 appears to be heterozygous at the GUS locus as well.

Detection of Class I Alterations in Distinct UV-induced Tumors. The data described above suggest that the novel class I antigens detected on the 1591 tumor might represent an artifact of genetic heterozygosity, and therefore, might not be directly related

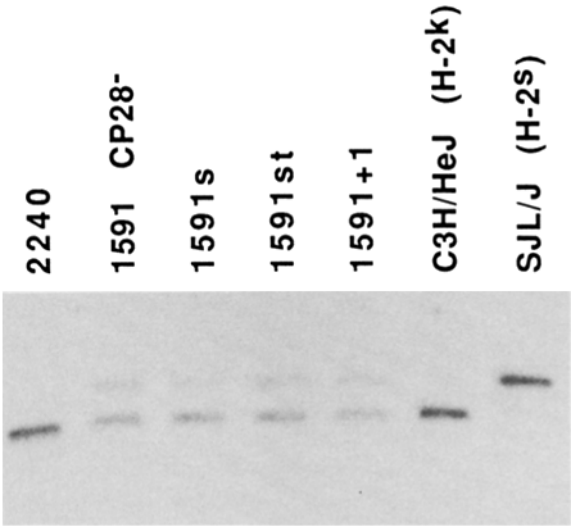

FIGURE 2. Comparison of RP2 alleles in C3H/HeN-derived 2240 DNA, four independent 1591 preparations including the 1591 CP28 antigen loss variant, C3H/HeJ, and SJL/J DNA. This preparation of CP28$^-$ DNA lacks all 1591-associated class I polymorphism (data not shown).

to the UV etiology of the tumor. Nonetheless, given the extreme antigenicity of many UV tumors like 1591, it is intriguing to postulate that class I alterations might occasionally be responsible for the expression of antigenic products. To estimate the extent to which class I alterations might in fact occur in other UV tumors, a panel of 16 C3H-derived, UV-induced tumors has been screened for the presence of class I polymorphisms. In fact, two of these tumors, LR335 and 2240, each appear to bear a distinct polymorphic Taq I fragment (Fig. 4). Using Pst I on the other hand, each of these tumors appears identical to C3H (data not shown). In the case of LR335, the novel Taq I fragment appears to be derived from a restriction fragment of a size contributed by H-2D^k. Given the limited extent of class I polymorphism in these tumors, each of these examples could represent a bona fide MHC mutation or recom-

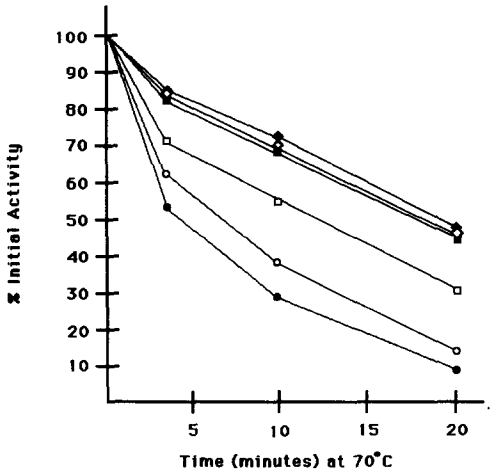

FIGURE 3. The 1591 tumor is heterozygous at the β-glucuronidase locus. Aliquots of cell extracts were incubated at 70°C for the times shown. Cell lines examined were C3H Ltk$^-$ fibroblasts (●) and KB29 fibrosarcoma (○), Swiss 3T3 (■) and B10.AKM heart-lung fibroblasts (◇), C57BL/10-derived BL-5 fibroblasts (◆), and 1591 fibrosarcoma (□). Results are presented as percent initial β-glucuronidase activity remaining at each time point.

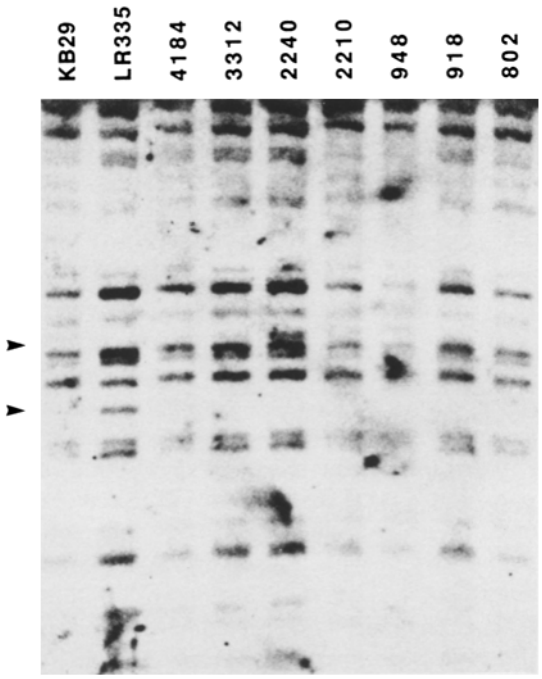

FIGURE 4. A Southern blot of Taq I-digested DNA from several C3H-derived, UV-induced tumors. Arrows indicate polymorphic restriction fragments in 2240 (*top*) and LR335 (*bottom*). The probe is the same as in Fig. 1 *a*.

bination event. However in neither of these cases is it possible to determine whether the mutation existed in the genome of the autochthonous host or whether it arose de novo. Furthermore, it would be difficult to determine whether these class I polymorphisms are relevant to the antigenicity of the tumors in the absence of extensive additional analysis.

Discussion

Previously we cloned and determined the DNA sequence of three H-2-like genes that encode tumor-specific rejection antigens expressed by the UV-induced, C3H/HeN-derived fibrosarcoma 1591. These genes, called A149, A166, and A216, do not appear to exist intact within the MHC of the parental C3H strain, since each accounts for a polymorphic restriction fragment in 1591 genomic DNA (15, 16).

In this report we present evidence that sheds light on the potential origin of these novel genes. First we show that analysis of the DNA sequences of a complete set of C3H/HeN H-2 and Qa genes fails to identify a convincing progenitor for any of the novel tumor genes. In contrast, two of the tumor genes, A149 and A166, exhibit striking structural similarity to the H-2L^q and H-2D^q antigens, respectively. This structural homology is consistent with a strong functional homology that has been previously described (31). Furthermore, the similarity between some of the novel class I genes from 1591 and class I genes from the H-2^q haplotype appears to extend beyond those genes that have been sequenced. In fact, H-2^q haplotype class I genes appear to be able to account for all of the class I polymorphism detected by genomic Southern blot in 1591, except for those bands associated with the A216

gene. Intriguingly, the complete set of class I polymorphisms in 1591 appears to be linked to a single chromosome, since all of the novel restriction fragments are lacking from the 1591 CP28$^-$ tumor variant, which was selected for the loss of the A216 product alone (13, 15, 17; data not shown). Finally, we demonstrate that genetic heterozygosity exists at two loci outside of the MHC of this tumor. We believe that the most parsimonious mechanism to account for all of these observations is that the set of polymorphic class I genes in 1591 was originally acquired as a haplotype from some unidentified allogeneic donor exhibiting homology to H-2^q.

Given the homology observed between A149 and A166 and H-2^q haplotype antigens, it is of interest to determine whether A216 exhibits homology with any known class I antigen. As shown in Table II, the CP28 mAb recognizes not only A216, but also H-2D^d and an unidentified H-2^s haplotype product. This antibody does not recognize H-2^q to any significant extent. Intriguingly, a 2.2-kb Pst I fragment, similar to that accounted for by A216 in the 1591 DNA, can also be detected in SJL/J DNA. Comparison with the congenic recombinant line B10.S(8R) (K^k, D^s) indicates that this band maps to the K-end of the MHC (Linsk, R., unpublished observation). Therefore, of the class I genes and products examined by serology and genomic Southern blotting, H-2K^s appears most consistent with that determined for A216. Intriguingly, out of 24 human and mouse class I products examined at the level of primary structure, only A216 and H-2D^d are structurally consistent with the limited peptide sequence data available for the H-2K^s antigen (35, 36).

The apparent similarity between A216 and H-2K^s is especially intriguing since both the H-2^q and H-2^s haplotypes are found within Swiss mice. Swiss mice are an outbred population of mice descended from a founder population of nine mice in 1926 (37). In addition, several inbred strains have been derived from these founders, including SWR/J (H-2^q), SJL/J (H-2^s), and NFS/N (K^s, D^q) (38). Recent isozyme analysis of several populations of Swiss mice indicates that these populations remain a reservoir of genetic variability (39). Indeed, within these populations can be found individuals that bear each of the isozyme alleles that have been shown to be shared by 1591 and C3H (13, 39–41). For example, SJL/J and SWR/J each differ from C3H/HeJ at only one of the seven isozyme loci at which 1591 was previously shown to be identical to C3H. In addition both of these inbred strains exhibit an allele at the RP2 locus that appears identical to that observed in 1591, while the strain NFS/N potentially bears an MHC haplotype compatible with that observed in the tumor (38). Therefore, within the population of Swiss mice, individuals are likely to exist whose genetic constitutions would be consistent with their serving as donors of the apparently allogeneic information in 1591. Given the existence of this pool of genetic information, as well as the homology observed between A149 and A166 and two D region genes of an established MHC haplotype, it is difficult to conclude that the novel 1591 class I genes are indeed derived from endogenous C3H genes in the absence of further positive data.

If the conclusions described above are correct, they suggest that the results of the original 1591 sequencing studies might prove to be informative about the evolution of the H-2^q haplotype. In particular, the extreme sequence homology (>>99%) observed throughout the 3′ coding and untranslated regions of the H-2L^q and D^q genes, i.e., from the 1591 tumor sequences (16), might indicate that these two genes are derived by the diversification of two recently duplicated class I genes. Gene dupli-

cation has been previously proposed to account for the pattern of structural homology observed between pairs of Q-region genes in the C57BL/10 mouse (42). However, no clear examples of gene duplication have yet been identified within the H-2 region. The BALB/c mouse contains five H-2D genes, with H-2D^d and three other loci proximal to H-2L^d. It has been proposed, based on restriction map homology and crossreactivity of flanking probes with Qa-containing clones, that unequal homologous recombination between two distinct haplotypes could have accounted for this expanded H-2D region (43). Patterns of crossreactivity of these flanking probes with H-2^q genomic DNA indicated that the organization of the H-2D^q region might be similar to that of BALB/c (43), but without a complete physical map of the q haplotype the parallels between the BALB/c molecules and the highly homologous H-2L^q and H-2D^q are unclear.

If H-2L^q and H-2D^q are derived by relatively recent duplication, then they provide an example of how H-2 genes diversify, namely, by point mutation. It has been proposed that selective pressures might exist on class I molecules for clustered sequence changes via gene conversion which could substantially alter the immunological function of a particular restriction element (44–46). Most of the H-2K^{bm} mutants (47), as well as the recently characterized H-2K^{km2} mutant (48), must have been generated by gene conversion as they involve multiple amino acid substitutions. A gene conversion model has also been invoked for the single amino acid substitutions in H-2K^{bm5}, H-2K^{bm16} (47), and H-2K^{km1} (49) since the mutant sequence is shared by other class I genes in the respective haplotypes, although point mutations cannot be ruled out. It is important to remember that the H-2K^{bm} mutants were detected by a change in histocompatibility significant enough to elicit relatively swift graft rejection. Single point mutations might not result in the expression of new molecules sufficiently antigenic to permit detection by standard skin graft rejection (50). Examination of nonimmunogenically screened closely related class I genes would be expected to reveal this more basic mechanism of divergence. It is therefore not surprising that all of the sequence substitutions between the two H-2^q-like molecules from the 1591 tumor seem to have arisen by point mutation. There are several closely spaced differences in the polymorphic regions in exons 2 and 3, but no donors exist for these among the Qa sequences available to date (data not shown). While gene conversion may play a significant role in the evolution of this multigene family, the impact of point mutation cannot be underestimated.

The results presented here do not imply that genetic contamination is generally responsible for the phenomenon of UV tumor antigenicity. On the contrary, a large number of experiments over several years in several labs have consistently demonstrated that UV-induced tumors are highly antigenic and are rejected by immunocompetent syngeneic hosts (4, 7, 51, 52). Similarly, the results here do not negate the value or relevance of past studies that have examined the interaction between the immune system of UV-irradiated hosts and tumor transplants. These studies have demonstrated that many UV-induced tumors, including 1591, are specifically tolerated by UV-irradiated hosts, and that this tolerance appears to be mediated by specific suppression (8, 51, 53–58).

Unfortunately, it is not yet possible to resolve the origin of the diverse antigenicity associated with UV tumors. Most likely, these antigens reflect the random mutagenic effects of UV radiation. Recent insights into the mechanism of antigen pre-

sentation suggest that any mutant cellular peptide can become antigenic if it can bind to self-MHC restriction elements (59). The mutant gene encoding such an antigenic product has recently been isolated from an antigenic variant of the P815 mastocytoma (60). In addition, tumor-specific antigens have recently been biochemically isolated from several chemically induced tumors (6, 61–64). In none of these cases have the antigenic products proven to be mutated class I molecules.

In fact, it remains possible that class I mutation might be one mechanism by which tumor antigenicity arises. The genomic Southern blots shown in Fig. 4 show that polymorphisms can be found in some UV-induced tumors. However, in no cases except 1591 have mutated class I genes been directly implicated in the antigenicity of a UV- or chemically induced tumor. If a tumor-specific class I mutant were in fact to be defined in such an antigenic tumor, the question of its significance would remain unresolved. As we have recently discussed, the relevance of class I alterations associated with tumor progression remains controversial. It is unclear that the expression of a novel MHC antigen by a tumor would have any functional significance beyond that associated with the expression of any strong antigen (65).

Summary

Previously, we cloned and sequenced the three novel MHC class I genes expressed by the C3H UV fibrosarcoma, 1591. We have extended the analysis of the polymorphic nature of these genes relative to the C3H strain. Scattered nucleotide differences among the tumor genes as compared with the C3H H-2 and Qa sequences make it highly unlikely that the novel tumor genes were generated by recombination between endogenous C3H sequences. Given that two of the tumor clones, A149 and A166, are remarkably similar in amino acid and DNA sequence to H-2L^q and H-2D^q, respectively, we also examined the 1591 RP2 and GUS loci for evidence of polymorphism. Compared with C3H and B10.AKM, 1591 appears to be heterozygous at each of these loci, consistent with an H-2^q origin for the two novel 1591 class I genes. Interestingly, the third tumor gene, designated A216, shares certain characteristics with the H-2K^s antigen, reminiscent of the naturally occurring combination of H-2K^s, H-2D^q, and H-2L^q antigens found in some Swiss mouse strains. As a result, we propose that the non-C3H/HeN characteristics displayed by the 1591 tumor point to a non-C3H origin for the novel tumor class I genes of 1591.

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