

Expression of the Qa-2^k Phenotype Encoded by the Q5^k Gene on the Surface of Tumor Cells Derived from H-2^k Mice

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Summary

Immunological and biochemical characteristics of the Qa-2 murine nonclassical histocompatibility class 1 antigen expressed on tumor cells derived from H-2^k (Qa-2⁻) mice were studied. It was found that the Qa-2 antigen on normal H-2^b lymphocytes reacted with Qa-2-specific monoclonal antibodies (mAbs) 34-1.2, 59 (both specific to the $\alpha 1/\alpha 2$ region) and 141-15.8 (specific to the $\alpha 3$ domain), and the Qa-2 antigen on H-2^k tumor cells (Qa-2^k antigen) reacted with mAbs 59 and 141-15.8, but not with 34-1.2. The normal Qa-2 antigen was susceptible to treatment with phosphatidylinositol-specific phospholipase C, but the Qa-2^k antigen was insensitive to it. By Northern hybridization, polymerase chain reaction (PCR) studies on cDNA, Southern hybridization, Western blotting, and nucleotide sequence analysis, the Q5^k gene was identified as the gene encoding the Qa-2^k antigen expressed on BW5147 lymphoma cells derived from a mouse of AKR strain (H-2^k, Qa-2⁻). The nucleotide sequence of PCR-amplified BW5147 Q5^k cDNA showed complete agreement with the reported sequence of exons 1-5 of the Q5^k gene of C3H/He. It also showed complete deletion of the region corresponding to exons 6 and 7, and a very short coding region in exon 8, resulting in very short cytoplasmic domain of the product compared with regular class 1 antigens. These characteristics were expected from the reported Q5^k genomic sequence. These results revealed that the Qa-2^k antigen was distinct from the normal Qa-2 antigen expressed on H-2^b lymphocytes although it cross-reacted with some Qa-2-specific mAbs.

Murine Qa and TL antigens are nonclassical class I histocompatibility antigens encoded by genes in the Qa/Tla region, which is located telomeric to the H-2 gene region of chromosome 17. H-2 genes are expressed on virtually all somatic cells, and expression of Qa and TL antigens is restricted to selected subpopulations of bone marrow-derived cells. H-2D, K, and L antigens have various allotypes that are caused by polymorphism of each gene. In contrast, it is thought that the Qa and TL antigens are highly conservative and that there are only positive and negative phenotypes. Such phenotypes of antigen encoded by genes in the Qa-2,3 region, which exists adjacent to the H-2 region, correlate highly to H-2 allotypes. For example, all H-2^k mice are Qa-2,3 negative and all H-2^b mice, except some artificial recombinant mice, are Qa-2,3 positive. Recent analyses of the Qa-2,3 region revealed that the region of C57BL/10 mice (H-2^b) contained 10 class I genes (1). Among them, Q7^b and Q9^b, (Q9^b differs from Q7^b by only one nucleotide) were

shown to code for the Qa-2 phenotype (2, 3). Also in BALB/c mice (H-2^d, Qa-2⁺), which have seven class 1 genes in this region, the Q7^d gene, which corresponds to Q7^b of C57BL/10, was shown to code for the Qa-2 antigen (4). In C3H/He mice (H-2^k), the gene region including Q7, and a large part of Q9, is deleted (5). This deletion is thought to cause the Qa-2-negative phenotype of C3H/He lymphocytes. As described elsewhere, however, we demonstrated that most H-2^k tumor cells reacted with a mAb specific to Qa-2 antigen, and that the tumor Qa-2 antigen gave rise to immunological responses in the H-2^k host mice. It was supposed that the Qa-2 molecules on H-2^k tumors are encoded by one of the genes in the Qa-2,3 gene regions of the H-2^k mice. To further study the expression of the Qa-2 antigen on the tumor cells, it is essential to identify the gene that is silent in normal cells and expressed in the tumor cells. In this report we characterized the Qa-2 antigen expressed on H-2^k tumor cells and identified its gene in BW5147 cells.

Materials and Methods

Mice. C3H/He, AKR, B6, and B6.K1 mice were used. The former two have H-2^k and Qa-2^{*,3-} phenotypes. The latter two are H-2^b mice that are congenic with respect to Qa antigens, B6 being Qa-1^{-2*3+}, and B6.K1 being Qa-1⁺²⁻³⁻.

Cells. BW5147 is an in vitro cell line derived from a T cell lymphoma of the AKR mouse. MM2 and FM3A are ascites cell lines of virally induced mammary carcinomas of C3H/He. MH134 is an ascites cell line of a chemically induced hepatoma of C3H/He. The in vivo cell lines were maintained by serial passages in the peritoneal cavities of C3H/He. It has been shown that these cell lines reacted with a Qa-2-specific mAb 141-15.8. L^{Q7b/Kb} and L^{Kb} cells, which were established by transfection of Q7^b/K^b hybrid gene DNA, and H-2K^b genomic DNA, respectively, will be reported elsewhere. L^{Q7b/Kb} cells express α 1 and α 2 domains of the Q7^b gene product and α 3, transmembrane, and cytoplasmic domains of the H-2K^b. L^{Kb} cells express H-2K^b antigen. Both cells, as well as nontransfected L cells, express H-2^k antigens.

Antibodies. mAb 141-15.8, which has specificity to the α 1 or α 2 domain of the Qa-2 antigen with some cross-reactivity to H-2 antigens (K^d, D^d, L^d, K^b, r, s, p, and q) (7, 8), was obtained from the American Type Culture Collection, Rockville, MD. mAb 34.1.2, which recognizes the α 3 domain of the Qa-2 antigen and did not have cross-reactivity to classical class 1 antigens (8), was from Australian Monoclonal Development, Arlurmon, NSW. A hybridoma clone producing Qa-2-specific mAb 59, was newly established by Y. Matsudaira and T. Takahashi by fusing NS-1 cells with lymphocytes from B6.K1 immunized with B6 lymphocytes. Thy-1.1-specific mAb was purchased from Meiji Institute of Health Science, Tokyo, Japan. Thy-1.2-specific mAb was from Cedar Lane Laboratories, Westbury, NY. A mouse mAb of the IgG2b class specific to a bacterial component (NusA protein) was kindly provided by Dr. Y. Nakamura of this laboratory and used as a mAb not related to tumor cell surface antigens. F(ab')₂ of goat antibody specific to mouse Ig and its FITC derivative were obtained from Tago, Inc., Burlingame, CA. Fab' fragment of the antibody was obtained by reducing the F(ab')₂ with 2-ME and fractionating by Sephacryl S-200 column chromatography.

Treatment of Cells with Phosphatidylinositol-specific Phospholipase C (PI-PLC).¹ PI-PLC derived from *Bacillus thuringiensis* (9) was purchased from Sapporo Beer Co. (Tokyo, Japan). Cells (2×10^6) were incubated at 37°C for 60 min in 30 μ l of DMEM (pH 7.5), containing 0.1 mU of PI-PLC and 1 mg/ml of OVA. After the incubation, the cells were washed twice with DMEM and used for detection of cell surface antigens.

Immunofluorescence Analysis. Detection of cell surface Qa-2 and Thy-1 antigens was carried out by flow cytometry (7). Briefly, the cells were reacted with Qa-2-specific mAb and then with the FITC-labeled F(ab')₂ fraction of goat antibody specific to mouse Ig. In the cases of lymphocytes, T cells were enriched by passing through a nylon wool column, and cell surface Ig of contaminating B cells were blocked by treating with Fab' fragment of the antibody. The FITC-labeled cells were fixed with 1% paraformaldehyde and analyzed using the FACS III[®]; (Becton Dickinson & Co., Mountain View, CA).

Preparation of cDNA. Total cellular RNA was extracted from tumor cells and thymocytes by the guanidine isothiocyanate method (10), and enriched for mRNA by isolating poly(A)⁺ RNA on oligo(dT)-cellulose (Boehringer Mannheim Diagnostics GmbH,

Mannheim, Germany) (11). Synthesis of cDNA from the mRNA was carried out using a cDNA synthesis kit (cDNA Plus; Amersham Corp., Arlington Heights, IL) with oligo-dT as a primer.

Oligonucleotide Primers and Probes. Based on the reported nucleotide sequences of genes in the Qa-2,3 region of C3H/He (5), 11 20 mer oligonucleotides were prepared on a DNA synthesizer (model 391; Applied Biosystems Inc., Wilmington, NC) and used as primers for PCR, and also for DNA sequencing. Their sequences and specificities are shown in Table 1 and Fig. 1. Oligonucleotides 1-6 were complementary to specific antisense sequences found in the first exons of each of the H-2D^k, Q1^k, Q2^k, Q4^k, Q5^k, and Q10^k genes, respectively. Nos. 10 and 11 were complementary to a Q5^k-specific sense sequence in exons 8 and 7, respectively. Nos. 7-9 were complementary to sequences common to all class 1 genes. Oligonucleotides complementary to Nos. 1-6 (Nos. 12-17, respectively) were also synthesized and used as probes in Northern hybridization experiments.

PCR. PCR was carried out in 100 μ l reaction mixture containing 50 ng of DNA, 50 pmol each of primers complementary to sense and antisense sequences, 20 mM Tris-chloride (pH 8.3), 1.5 mM MgCl₂, 25 mM KCl, 0.05% Tween 20, 0.1 mg/ml gelatin, 50 μ M each of dNTP, and 2 U of Taq DNA polymerase (Perkin-Elmer/Cetus Corp., Norwalk, CT). The reaction mixture was overlaid with 100 μ l of mineral oil to prevent evaporation. The thermal cycler (Atto, Tokyo, Japan) was run for 40 cycles (each for 1 min at 94°C, 1.8 min at 55°C, and 2 min at 72°C).

Cloning and Sequencing of PCR-amplified DNA. DNA amplified by PCR was sequenced either directly or after cloning into the SmaI site of pUC119. For the cloning, competent *Escherichia coli* MV1184 cells were transformed with the plasmid DNA and grown on a plate containing Xgal, IPTG, and ampicillin. White colonies were picked and grown in 2 \times YT broth. The cells were then infected with M13K07 helper phage. The resulting single-strand DNA, as well as the PCR-amplified DNA, was sequenced by the dideoxy method (12) using a DNA sequencing kit (Sequenase version 2.0; U.S. Biochemical Corp., Cleveland, OH), and synthetic oligonucleotides were used as PCR primers or M13 universal primer.

Preparation of Genomic DNA and Southern Hybridization. Splenocytes from B6, C3H/He, AKR, and BW5147 cells were incubated at 60°C overnight in 0.03% SDS solution containing 0.1 mg/ml proteinase K and then extracted with phenol. The resulting aqueous phase was dialyzed overnight against 10 mM Tris-chloride (pH 7.5) containing 1 mM EDTA and used as the DNA solution. 10 μ g each of genomic DNA was digested with HindIII or with BamHI, subjected to electrophoresis through 0.8% agarose gel, and transferred onto a nylon filter (Hybridon-N⁺; Amersham Corp.). The filter was dried at 37°C and incubated for 1 h at 60°C in 6 ml of hybridization buffer (6 \times SSC, 5 \times Denhardt's solution, 0.5% SDS, 50% formamide, 20 mM sodium phosphate buffer, pH 6.5) containing 1 mg of salmon sperm DNA. The DNA probe that had been labeled with ³²P by the random primer method was then added to the incubation. Hybridization was carried out at 42°C overnight. The filter after the hybridization was washed twice at room temperature for 15 min in 2 \times SSC containing 0.1% SDS and twice at 37°C for 30 min in 0.2 \times SSC containing 0.1% SDS. The filter was then rinsed with 0.1 \times SSC and exposed to x-ray film using an intensifier screen.

Northern Hybridization. Poly(A)⁺ RNA was prepared as described above from AKR thymocytes and BW5147 cells, denatured with glyoxal, subjected to electrophoresis through a 1.2% agarose gel containing 20 mM phosphate buffer (pH 7.4), and transferred onto a nylon filter. The filter was dried at 37°C and incubated for

¹ Abbreviations used in this paper: GPI, glycosylphosphatidylinositol; PI-PLC, phosphatidylinositol-specific phospholipase C; TTBS, Tween 20-containing Tris-buffered saline.

1 h at 60°C in 2 ml of the hybridization buffer containing 100 µg salmon sperm DNA. Oligonucleotide probes, 5'-terminus of which had been labeled with ³²P, were then added to the incubation. After incubating at 42°C overnight, the filter was washed twice with 2× SSC for 15 min at room temperature, and twice with 2× SSC containing 0.1% SDS for 30 min at 50°C. The filter was then rinsed with 0.2× SSC and exposed to x-ray film using an intensifier screen.

Synthesis of Q5^k Protein and Western Blotting. DNA corresponding to exons 1–4 of the Q5^k gene that had been amplified from BW5147 cDNA was cloned into pUC119 and sequenced as described above. A colony was selected that had the insertion in the right direction and in frame downstream of the lacZ promoter. This colony was grown in L broth containing ampicillin until the optical density at 600 nm reached 0.3. IPTG was added to the culture to 50 µM and culturing continued until the optical density became 0.75. The cells were harvested, precipitated with 10% TFA, washed with acetone, and dissolved by heating for 30 min at 100°C in 25 mM Tris-hydrochloride (pH 6.8) containing 2% SDS, 5% glycerol, 0.0125% bromophenol blue, and 2.5% 2-ME. The dissolved material was subjected to electrophoresis through a 12% polyacrylamide gel containing 0.1% SDS and transferred onto polyvinylidene difluoride membrane (Immobilon; Millipore Continental Water Systems, Bedford, MA) by electroblotting in a solution composed of 25 mM Tris base, 192 mM glycine, 20% methanol, and 0.02% SDS. The membrane was then rinsed with 0.15 M sodium chloride containing 0.2% Tween 20 and buffered with 10 mM Tris hydrochloride (pH 7.6) (TTBS), incubated for 2 h at 37°C in TTBS containing 10% goat and 10% horse serum. The membrane was rinsed with water and incubated overnight at room temperature in 200× diluted mouse ascites containing mAb 59 in 5 ml of the same medium. After incubation, the filter was rinsed three times with TTBS and reacted for 1 h at 37°C with peroxidase-labeled anti-mouse IgG (Amersham Corp.) in saline buffered with 10 mM Tris-borate (pH 7.6). The membrane was again washed three times with TTBS and treated for coloration in 50 ml of TTBS containing 40 mg of 3,3'-diaminobenzidine tetrahydrochloride (Wako Chemicals, Osaka, Japan) and 15 µl of 30% hydrogen peroxide.

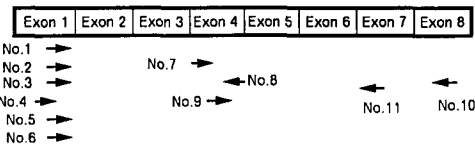


Figure 1. Synthetic deoxyoligonucleotide primers used in this study. (Arrows) Positions and 5' to 3' orientations of the primers.

Results

Characteristics of the Qa-2 Antigen Expressed on Tumor Cells Derived from H-2^k Mice. As reported elsewhere, we demonstrated that most tumor cell lines (seven of nine cell lines tested) derived from H-2^k (Qa-2⁻) mice express the Qa-2 antigen detected by the membrane immunofluorescence method using anti-Qa-2 antiserum or Qa-2-specific mAb 141-15.8. It was found, however, that the Qa-2 antigen was not detected on these cells in general when another Qa-2-specific mAb 34-1.2 was used. Qa-2 specificity of the newly obtained mAb 59 was examined and compared with those of mAbs 141-15.8 and 34-1.2 (Table 2). It is evident that mAb 59 recognizes the α1 or α2 domain of the Qa-2 molecule, which is the product of the Q7^b gene, and does not cross-react with at least the H-2^k and H-2^b antigens. Unlike mAb 34-1.2, mAb 59 reacted not only with H-2^b lymphocytes but also with various H-2^k tumor cells such as MM2 and BW5147. Distribution of mAb 59-reactive antigen among various tumor cells derived from H-2^k mice was essentially identical to that of the antigen reactive to mAb 141-15.8 (data not shown). Such reactivity to Qa-2-specific mAbs indicates that these tumor cells express a Qa-2 antigen that is closely related but not identical to the Qa-2 antigen detected on normal lymphocytes of H-2^b mice, and that the α1 or α2 domain of the molecule is responsible for at least a part of the difference. Table 2 also shows that the Qa-2 antigen was

Table 1. Synthetic Deoxyoligonucleotides Used as Primers and Probes

No.	Sequence (5'–3')	Specificity
1	TGGCCCCGGCTCAGACCCGC	H-2D ^k
2	TGACCCTGACCAAAACCGGA	Q1 ^k
3	CCCGGACCCAGAACCGAGCC	Q2 ^k
4	AGTCGCCAGACCCTGATCG	Q4 ^k
5	CTCTGACCCAGACCCGCGCT	Q5 ^k
6	CCCCGACCCAGACCCAGGCA	Q10 ^k
7	GCTGGGCCCTGGGCTTCTAC	pan class 1
8	AGGGTGAGGGGCTCAGGCAG	pan class 1
9	CTGGCAGTTGAATGGGGAGG	pan class 1
10	ATCGTCTGTCAGTCCCA	Q5 ^k
11	GCTCTAGGAGCTGTCCCTGC	Q5 ^k
12–17*		

* Complementary to nos. 1–6, respectively.

Table 2. Detection of the Qa-2 Antigen on Various Cells Using Anti-Qa-2 Polyclonal and Monoclonal Antibodies

Cell	Qa-2-specific mAb			Nonrelated mAb
	34-1.2	59	141-15.8	
B6 lymphocyte		156.1	59.0	22.3
B6.K1 lymphocyte		30.7	25.0	30.9
C3H/He thymocyte	16.7	17.4	16.6	16.8
AKR thymocyte	18.1	19.2	19.5	18.0
MM2	58.9	101.3	102.8	64.8
BW5147	46.4	80.4	102.3	45.2
L ^{Q7b/Kb}	192.0	180.2	42.1	48.4
L ^{Kb}		81.2	68.2	71.5

Data are mean fluorescence intensity.

not detected on thymocytes and splenocytes of H-2^k mice (C3H/He and AKR) when either one of these mAbs was used.

It has been reported that the Qa-2 antigen expressed on lymphocytes obtained from H-2^b mice is susceptible to digestion with PI-PLC (13), showing that the molecule is anchored to the cell membrane via a glycosylphosphatidylinositol (GPI) moiety. It is known that the GPI structure is linked to an aspartic acid of the cell surface protein molecule (14). With regard to the Qa-2 molecule, the GPI anchor has been supposed to be linked to an aspartic acid residue at position 295 coded by a CAC in exon 5 of Q7^b gene (15). In H-2 antigen molecules, which are resistant to treatment with PI-PLC, this aspartic acid residue is replaced by valine. DNA sequences of genes of the Qa-2,3 region reported so far show that all putative Q gene products other than Q7 and Q9 (16) have valine at this position. From these observations, it was supposed that the Qa-2 antigen expressed on H-2^k tumor cell surfaces might be resistant to PI-PLC treatment. As shown

Table 4. Susceptibility of Thy-1 Antigens to PI-PLC Treatment

Cell	PI-PLC treatment				Nonrelated mAb
	Anti-Thy-1.1		Anti-Thy-1.2		
	-	+	-	+	
AKR thymocyte	193.0	98.0			34.5
B6 thymocyte			176.0	79.7	40.1
BW5147	176.0	95.1			

Data are mean fluorescence intensity.

in Table 3, Qa-2 molecules detected by mAb 141-15.8 on various tumor cells derived from H-2^k mice were resistant to PI-PLC treatment. In a control experiment, susceptibility of the Thy-1 antigen on BW5147 cells was tested. The Thy-1 antigen is also known to be one of the cell surface molecules having a GPI anchor (17). As shown in Table 4, the Thy-1 antigen expressed on BW5147 cells was susceptible to PI-PLC treatment. This result showed that the lack of a GPI anchor on the Qa-2 antigen of BW5147 cells was not due to a defect in the cellular mechanism to synthesize GPI anchors, but due to biochemical differences of the Qa-2 molecules themselves.

From these observations, it is evident that the Qa-2 antigen expressed on tumor cells derived from H-2^k mice (Qa-2^k antigen) is distinct immunologically and biochemically from that expressed on H-2^b normal lymphocytes. The Qa-2^k antigen can be tentatively defined as a PI-PLC-resistant molecule expressed on tumor cells derived from H-2^k mice, which is reactive to mAbs 59 and 141-15.8, but not to mAb 34-1.2.

Identification of the Gene Encoding the Qa-2^k Phenotype of BW5147 Cells. Among the Qa-2^k-positive tumor cells, BW5147 cells grown in vitro were selected for further anal-

Table 3. Susceptibility of Qa-2 Antigens to PI-PLC Treatment

Cell	PI-PLC Treatment			
	mAb 141-15.8		Nonrelated mAb	
	-	+	-	+
B6 lymphocyte	152.0	59.7	30.6	29.4
BW5147	85.1	96.4	35.8	36.2
MM2	88.2	98.0	73.2	74.6
FM3A	108.0	109.3	71.1	72.3
MH134	101.2	102.9	67.9	72.5

Data are mean fluorescence intensity.

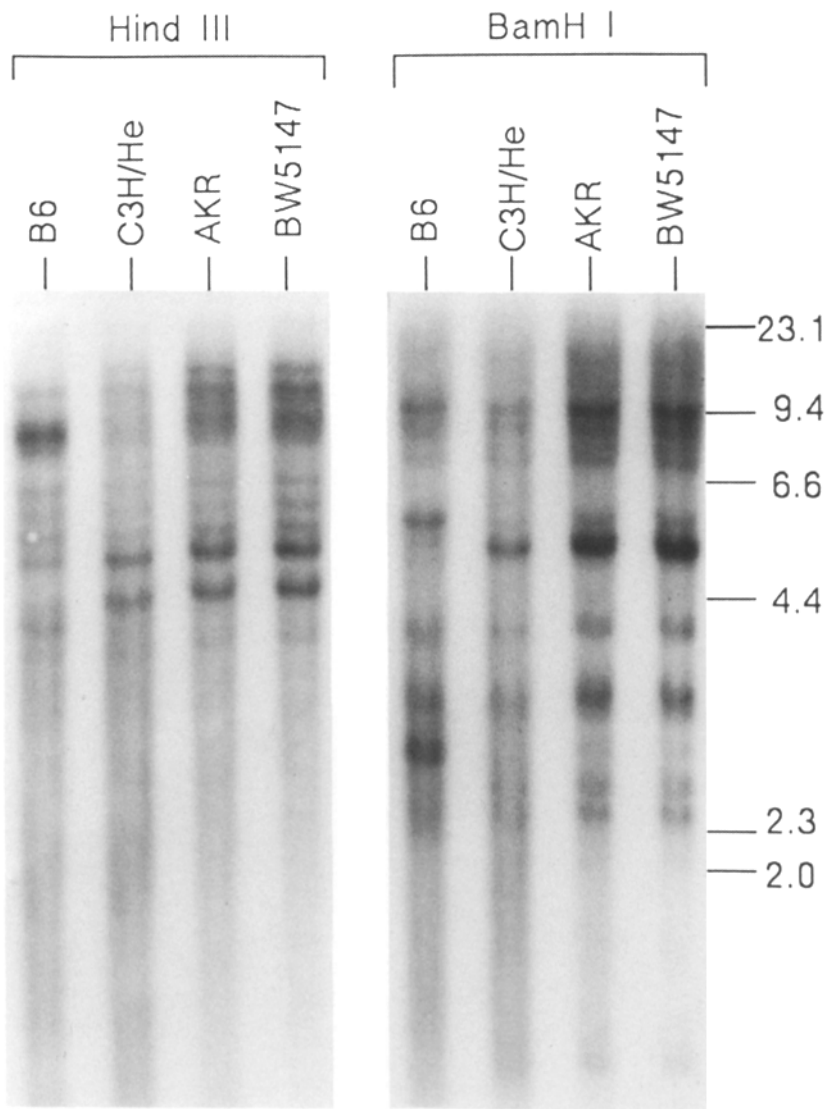


Figure 2. Southern blot analysis of B6, C3H/He, AKR, and BW5147 genomic DNA digested with HindIII or BamHI. DNA amplified by PCR from cDNA of BW5147 cells using primers nos. 5 and 8 was labeled with ^{32}P and used as the probe.

yses because expression of Qa-2^k was fairly strong, and also because the cells could be obtained without contamination of cells from the host animals. Fig. 2 shows the results of Southern hybridization analyses in which HindIII or BamHI fragments of genomic DNAs were hybridized with a ^{32}P -labeled DNA probe of about 800 bp that could detect the DNA region containing exons 2–4 of all class 1 genes. The patterns of autoradiograms obtained using DNAs from C3H/He, AKR, and BW5147 cells were essentially identical and they were distinct from that obtained using B6 DNA. From these results, we assumed that the arrangement of class 1 genes was conserved among genomes of these H-2^k mice (C3H/He and AKR) and that the lymphoma cell line was derived from AKR. It was reported that the Qa-2,3 region of the C3H/He genome contained Q1^k , Q2^k , Q4^k , Q5^k , and Q10^k genes (5, 18). The Q3^k gene was a pseudogene, and genes corresponding to Q6, Q7, Q8, and a large part of Q9 were deleted. The Q5^k gene seemed to be a hybrid gene composed of a large 5' part of the Q5 gene and a short

3' part of the Q9 gene. We tried to identify the gene that was silent in AKR and transcribed in BW5147 by Northern hybridization and also by amplification of cDNA by PCR using primers and probes specific for each of the Q1^k , Q2^k , Q4^k , Q5^k , and Q10^k genes (Table 1 and Fig. 1). A primer and probe specific to H-2D^k gene was also used as a positive control.

Fig. 3 shows the results of Northern blot analysis. H-2D^k -specific probe (no. 12) showed a specific hybridizing band at 1.5 kb with both AKR thymocyte RNA and BW5147 RNA. In contrast, Q5^k -specific probe (no. 16) hybridized to approximately 1.2-, 1.5-, and 4.0-kb species in BW5147 RNA, whereas no hybridization was detected with regard to AKR RNA. Also, hybridization either to AKR RNA or BW5147 RNA was not detected when probe nos. 13, 14, 15, and 17 were used (data not shown). These results indicated that Q5^k gene, the transcription of which was not detected in H-2^k thymocytes, was specifically transcribed among Q genes in BW5147 cells. In agreement with the

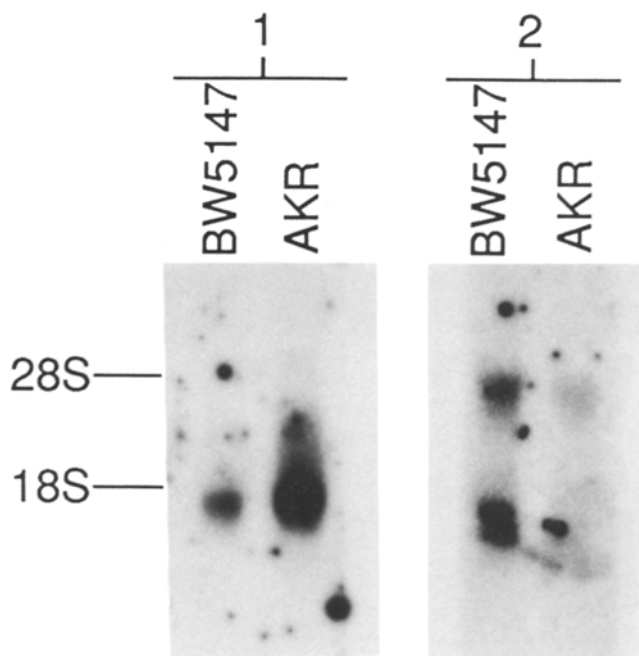


Figure 3. Northern blot analysis of poly(A)⁺ RNA of BW5147 cells and AKR thymocytes. 3 μg of poly(A)⁺ RNA was used in each slot. H-2D^k-specific probe (no. 11) and Q5^k-specific probe (no. 15) were ³²P-labeled and used in 1 and 2, respectively.

present observation, transcripts of 4.0, 1.5, and 1.2 kb have been reported for both Q7 and Q9 genes (2, 19, 20, 21). They were supposed to represent unprocessed transcripts, normally spliced message, and a truncated form, respectively. The transcription of Q5^k gene in BW5147 cells was supported by the results of PCR. Using primers specific to H-2D^k, Q1^k, Q2^k, Q4^k, Q5^k, and Q10^k (nos. 1-6, respectively) and a primer (no. 8) that was complementary to a class 1 common sense sequence in the 3'-terminal region of exon 4, the PCR was carried out. As shown in Fig. 4, amplification of BW5147 cDNA was successful only when primer nos. 1 and 5, which were complementary to H-2D^k and Q5^k exon 1 antisense sequences, respectively, were used together with primer no. 8. Amplification was not detected when primer nos. 2-6 were used together with primer no.

8, even when the annealing temperature was lowered to 45°C. The DNA amplified using primer nos. 5 and 8 was sequenced directly, and also after cloning into pUC119 using primer nos. 5, 7, 8, and universal primer M13. The sequence was found to be identical to the reported nucleotide sequence of exons 1-4 of the Q5^k gene of C3H/He. Amplification of genes other than Q5^k was not detected during direct sequencing. Amplification of the H-2D^k sequence from AKR cDNA was also detected using primer nos. 1 and 8, showing that synthesis of AKR cDNA was successful. However, none of the Q-specific sequences could prime amplification of the AKR cDNA. Amplification of BW5147 cDNA was observed also when primer no. 9, which was complementary to a class 1 common antisense sequence in exon 4, and primer no. 10, which was complementary to a Q5^k-specific sense sequence in exon 8, were used. The nucleotide sequence of the DNA amplified using these primers was determined directly using primer nos. 9 and 10. A part of the resulting sequence and the deduced amino acid sequence are shown in Fig. 5. Contaminating sequences were not detected in this case also. It showed substantial differences from the reported sequences of regular class 1 cDNA. Nine additional nucleotides were found between exons 4 and 5. This addition was expected from the reported genomic DNA sequence of the Q5^k gene of C3H/He because the 5'-terminal sequence of intron 4 of Q5^k was GC, whereas those of other class 1 genes in general were splice donor GT, and the GT 9 bases downstream of the GC could be used as a splice donor. In the Q7^d gene of BALB/c mice, the latter GT was reported to be a splice donor and addition of nine bases, which coded for additional GRW, was also observed (5). The sequence of exon 5, which coded for the transmembrane domain, was identical to the reported sequence of exon 5 of C3H/He Q5^k, but exons 6 and 7 were deleted and only two bases in exon 8 were included in the coding region. This deletion was also expected from the genomic sequence because the 5'- and 3'-terminal sequences of intron 6 were CA and TG, respectively, whereas those of most other class I genes were splice donor GT and splice acceptor AG. These changes may result in a truncated mRNA ending at exon 6, which may be labile, and a mRNA deleting exons 6 and 7. A GTC coding for valine in exon 5 was conserved, which corresponds to the CAC coding for aspartic

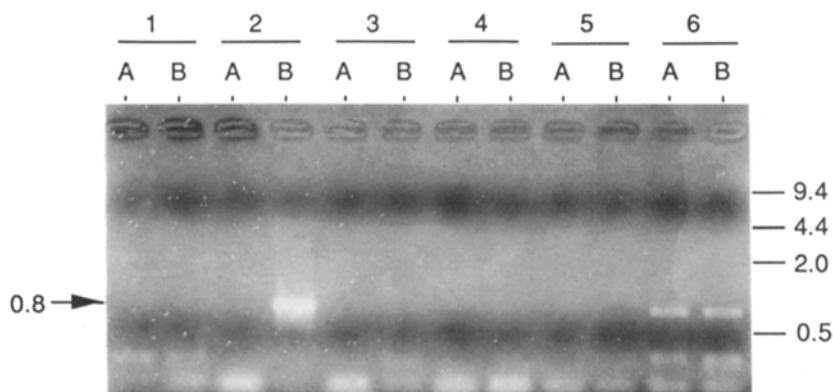


Figure 4. Amplification of cDNA by PCR. A, AKR cDNA; B, BW5147 cDNA. Probes specific for Q10^k (1), Q5^k (2), Q4^k (3), Q2^k (4), Q1^k (5), and H-2D^k (6) were used. A 10 μl quantity of the resulting reaction mixture was subjected to electrophoresis through a 1% agarose gel and DNAs were visualized by ethidium bromide. The positions of marker deoxypolynucleotides with known sizes in kilobase pairs are given on the right.

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          exon 4 | exon 5
G AGA TGG GGC AAG GAG GAG CCT CCT TCA TCC ACC AAG ACT AAC ACA GTA ATC ATT GCT GTT
  R   W   G   K   E   E   P   P   S   S   T   K   T   N   T   V   I   I   A   V

CCG GTT GTC CTT GGA GCT GTG GTC ATC CTT GGA GCT GTG GTG GCT TTT GTG ATG AAG AGG AGG
  P   V   V   L   G   A   V   V   I   L   G   A   V   V   A   F   V   M   K   R   R

          exon 5 | exon 8
AGA AAC ACA GGG TGA AGA CAG CTG CC
  R   N   T   G   end

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Figure 5. Deoxynucleotide sequence of the region of Q5^k cDNA encoding the COOH-terminal part of the Q5^k protein and its deduced amino acid sequence.

acid at position 295 of Q7^b, in the transmembrane domain. The lack of aspartic acid residue at this position could be responsible for the resistance of the molecule to PI-PLC treatment (15). When PCR was carried out with BW5147 genomic DNA using primer no. 11, which was specific to exon 7 sequence and primer no. 9, amplification of DNA with length expected from the genomic sequence (1,020 bp) was observed, whereas amplification of the cDNA was not detected when primer no. 11 was used together with primer nos. 7 or 9. These results indicated that deletion of exons 6 and 7 did not take place in the genomic DNA.

To determine whether the Q5^k gene of BW5147 codes for the immunologically defined Qa-2 antigen, Western blotting analysis was carried out on the peptide encoded by the DNA sequence amplified by PCR using primers nos. 5 and 9. The amplified DNA was ligated into the SmaI site of pUC119 and a clone was selected by DNA sequencing in which Q5^k exons 1-4 were fused in frame in the right direction

downstream of the lacZ initiation codon. The plasmid was introduced into competent *E. coli* MV1184 cells and the expression of the lacZ-Q5^k fusion protein was induced by culturing the cells in the presence of IPTG. As shown in Fig. 6, the resulting cells showed the induced synthesis of a peptide with molecular mass of about 35 kD, which was the value expected from the fusion protein. This 35-kD protein reacted with anti-Qa-2 mAb 59. The protein was not detected under identical conditions when anti-Qa-2 mAb 34-1.2 was used.

From these results, we concluded that the Qa-2^k antigen on the surface of BW5147 cells, which was detected by mAb 59 but not by mAb 34-1.2, and which was resistant to treatment with PI-PLC, was the product of the Q5^k gene which was inactive in normal tissues and cells of adult AKR mice.

Discussion

The results presented in this communication clearly show that the antigen detected by mAbs with Qa-2 specificity on various tumor cells derived from Qa-2^k mice was distinct from that detected on normal H-2^b lymphocytes, the former being Q5 gene product and the latter Q7 or Q9 gene product. Therefore, despite the cross-reactivity, the tumor Qa-2 antigen is not an illegitimately expressed normal allogeneic antigen. Such cross-reactivity to normal antigens may be a feature of tumor antigens coded for by genes that belong to the same highly homologous multigene family with the normal antigen genes. Biochemical characteristics of the tumor Qa-2^k antigen described here could be expected from the reported genomic DNA sequence of the Q5^k gene. Resistance of the tumor Qa-2 antigen to PI-PLC treatment may be attributed to a change of one nucleotide in the exon 5 which will result in a D to V change. Deletion of exons 6 and 7 in the cDNA seems to take place during splicing of mRNA, because the result of PCR indicated that BW5147 genomic DNA contained these exons, and the deletion may be caused by changes in the splice site in the intron 6. A very short cytoplasmic domain of the tumor Qa-2 antigen is the result of a stop codon in the exon 8 close to 5'-terminus of the exon 5, whereas an even shorter cytoplasmic domain of the Q7^b gene product caused by a stop codon exists in the exon 5. Elongation of the exon 4 by nine nucleotides compared with Q7^b and most other class 1 genes could also be attributed to the change in the splice donor site in the intron 4. Therefore, the Q5^k cDNA sequence matched completely to that expected from the reported genomic DNA sequence.

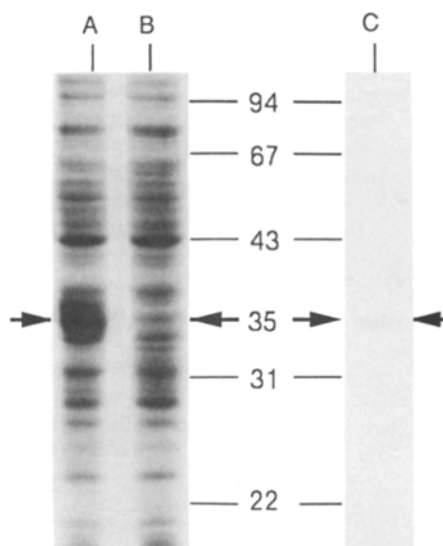


Figure 6. Reactivity to Qa-2-specific mAb of the fusion protein composed of the $\alpha 1$ to $\alpha 3$ domains of the Q5^k protein and lacZ. (A) Lysate of *E. coli* transformed with the plasmid containing exons 1-4 of Q5^k cDNA and cultured in the presence of IPTG was electrophoresed and stained with coomassie blue; (B) *E. coli* transformed with pUC119 was treated in the same way; and (C) Western blotting of A using Qa-2-specific mAb 59. Positions of standard proteins with known molecular masses in kilodaltons are shown. (Arrows) The position of the protein induced with IPTG.

In agreement with the results of Northern blot analyses in which Q5^k transcripts were not detected in H-2^k thymocytes, antigens reactive to Qa-2-specific mAbs were not detected on H-2^k thymocytes. also, Qa-2-specific antibody was detected in C3H/He mice immunized with Qa-2-positive syngeneic tumor cells and in B6 mice immunized with a

Q5^k-specific synthetic peptide (unpublished results). These findings indicate that the mice are not tolerant to the Q5 gene product and therefore the Q5 gene may not be expressed in the normal adult H-2^k and H-2^b mice. Mechanism of Q5 gene expression in tumor cells will be a point of interest in future studies.

We are deeply grateful to Drs. Y. Nakamura, K. Kawakami, and K. Ito of this department for expert advice in the DNA and RNA studies; Miss T. Sugimoto for assistance during preparation of the manuscript; and Professor J. Hershey of the Medical School, University of California for critical reading of the manuscript.

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Received for publication 24 June 1991 and in revised form 21 November 1991.

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