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Immunology

Qa-region class I gene expression: Identification of a second class I gene, *Q9*, encoding a Qa-2 polypeptide

(gene transfection/major histocompatibility complex/regulation of expression)

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ABSTRACT **A feature of the expression of the tissue-specific class I antigen Qa-2 is the quantitative variation among mouse strains. Recently, the class I gene *Q7* has been shown to encode a protein product that is biochemically indistinguishable from the lymphocyte-bound Qa-2 molecule. Utilizing gene transfection, we have identified a second *Qa-2* subregion class I gene (*Q9*), in *H-2*^{b} mice, which encodes a polypeptide biochemically similar to the Q7 and the Qa-2 polypeptides. Furthermore, we have observed that cell lines transfected with the allelic forms of the *Q7* gene from C57BL/10 (Qa-2^{hi}) or BALB/C (Qa-2^{low}) display quantitative differences in cell-surface expression. Based on these studies, we suggest that gene dosage and allele-specific variation in cell-surface expression contribute to the strain-specific variation in the levels of Qa-2 antigen expression.**

The murine major histocompatibility complex-encoded H-2K, H-2D, and H-2L class I molecules are highly polymorphic, ubiquitously expressed cell-surface glycoproteins that serve fundamental roles in the rapid rejection of tissue/organ grafts and in antigen recognition by T cells (1). Serological, biochemical, and molecular approaches have determined that the chromosomal segment located telomeric to the murine H-2 major histocompatibility complex encodes at least three cell surface (Qa-1, Qa-2, and TL) and two soluble/secreted (Q10 and Qb-1) polypeptides, which are structurally related to H-2K, -D, and -L (2–4). Unlike their counterparts H2-K, -D, and -L, the Qa/TL polypeptides exhibit low polymorphism and are expressed in a tissue-specific fashion (5, 6). The function(s) of the Qa/TL molecules is unknown.

Twenty to thirty distinct class I genes have been localized to the *Qa/TL* chromosome segment of BALB/c and C57BL/10J (B10) mice (7, 8). Gene transfection studies have been utilized to identify those *Qa/TL* region genes that encode the biochemically and serologically defined Qa/TL proteins. Analysis of mouse L cell fibroblasts transfected with the *Q6*^{b}, *Q7*^{b}, *Q8*^{b}, or *Q9*^{b} genes led to the identification of intracellular polypeptides that are reactive with Qa-2 antiserum (9). Recently the *Q7*^{b} gene transfected into a mouse T-cell line and the *Q7*^{b} and *Q7*^{d} genes transfected into a mouse liver cell line have been shown to encode cell-surface proteins reacting with several serological reagents that detect Qa-2-specific molecules on lymphocyte cell surfaces (10, 11). Biochemical studies have determined that the *Q7* gene derived from either BALB/c (*Q7*^{d}) or B10 (*Q7*^{b}) mice encodes a M_r 40,000 cell-surface polypeptide that is biochemically and serologically indistinguishable from the Qa-2 polypeptide expressed on lymphoid cell surfaces (12). It has been demonstrated that the *Q7*^{d} and *Q7*^{b} genes encode both a membrane bound and a secreted soluble polypeptide, and the cell surface molecule is attached to the cell surface by a lipase-sensitive membrane anchor. These two properties are characteristic of the lymphocyte Qa-2 molecule (13, 14).

Two allelic forms of the *Qa-2* locus have been defined based on the ability of lymphocytes from mouse strains to express (Qa-2$^+$ or Qa-2^a) or not express (Qa-2$^-$ or Qa-2^b) serologically defined Qa-2 antigens. Furthermore, among mice that express Qa-2 antigens, there is quantitative variation in the levels of expression of Qa-2 (5, 6, 15–17). Utilizing gene transfection to analyze *Qa* region class I gene expression, we have obtained evidence indicating that gene dosage and transcriptional/posttranslational processing both contribute to the strain-specific variation in lymphocyte *Qa-2* expression.

MATERIALS AND METHODS

Serological Reagents. Anti-Qa-2 antiserum was produced by the immunization protocol of Flaherty (5). The monoclonal antibodies (mAbs) 20-8-4 (anti-K^d, -K^b, -r, -s, -Qa-2), 34-1-2 (anti-K^d, -D^d, -L^d, -K^b, -r, -s, -p, -q, -Qa-2), 28-14-8 (anti-L^d, -D^b), 11-4-1 (anti-K^k), and anti-Qam8 were purified on protein A-Sepharose as described (12). The mAb Qam2 was purchased from Accurate Scientific Company (Hicksville, NY). Normal mouse serum was purchased from Pel-Freeze. The Qam7 and D3262 mAbs were partially purified from ascitic fluid and coupled to CNBR-activated Sepharose 4B as described in detail (12). Protein A-Sepharose was purchased from Genzyme (Boston, MA).

Establishment of Transfected Cell Lines. Thymidine kinase-negative (TK$^-$) L cells (C3H fibroblastic, *H-2*^{k}) designated as L cells, hepatoma cells (Hepa-1), and primary liver cells (CRL-3A) were used in our previous studies (12).

Plasmid 27.1 was described earlier (12). Plasmid clones containing the *Eco*RI fragments carrying the B10 *Q7*^{b}, *Q8*^{b}, and *Q9*^{b} genes were provided by David Sherman, Gerry Waneck, and Richard Flavell (Biogen Research, Cambridge, MA) (18). Transfections were carried out by the calcium phosphate coprecipitation method (19) as described (12) using ≈1 μg of plasmid DNA encoding the class I gene, 15 ng of pSV2-neo plasmid (20), and 10 μg of carrier L TK$^-$ DNA per 5 × 10^6 cells. Both mixed populations of transfectants and cloned cell lines were analyzed. Individual clones were isolated by plating cells at limiting dilution. Under conditions used in our studies, >90% of the clones in the mixed populations of transfectants selected for G418 resistance were also cotransfected with the class I gene. Levels of *Q*-region class I gene expression were determined by a sensitive cell binding radioimmunoassay using the mAb 20-8-4 as described (21).

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Abbreviations: B6, C57BL/6J mice; B10, C57BL/10J mice; mAb, monoclonal antibody; TK, thymidine kinase; Endo-F, endoglycosidase F; PtdIns, phosphatidylinositol.

Radiolabeling and Immunoprecipitation. Con A-activated lymphoid cells from C57BL/6J (B6) mice were prepared as described (12, 14). L cells, Hepa-1 cells, and lymphoid cells were harvested, washed with phosphate-buffered saline, and radiolabeled either by lactoperoxidase-catalyzed iodination or metabolically with [^{35}S]methionine (14). The preparation of Nonidet P-40 cell lysates and cell-free medium from radiolabeled cells has been described in detail (12, 14). Cell lysates and cell-free medium were precleared and treated with the appropriate serological reagents or mAb coupled to Sepharose. Immune complexes were absorbed to protein A-Sepharose, washed, and analyzed by one- or two-dimensional polyacrylamide gel analysis (14). In some cases, the Q7^b and Q9^b polypeptides were enzymatically deglycosylated by using endoglycosidase F (Endo-F, New England Nuclear) prior to two-dimensional analysis (22).

Phospholipase C Treatment of Cells. Hepa-1 cells transfected with the *Q7^b* or *Q9^b* genes were radiolabeled by lactoperoxidase-catalyzed iodination, washed, and resuspended at 10^7 cells per ml in RPMI 1640 medium containing 2 mg of bovine serum albumin (fraction IV, Sigma) per ml, glutamine, vitamins, and nonessential amino acids. Purified phosphatidylinositol (PtdIns)-specific phospholipase C from *Baccillus thermongenious* was incubated with cells for 2 hr at 37°C (23). After incubation, the cells and cell-free medium were recovered by centrifugation, and the distribution of Q7 or Q9 molecules in cell lysates or cell-free medium was determined by immunoprecipitation and PAGE.

RESULTS

Establishment of *Q9^b* Gene Transfectants. Previous studies have established that the *Qa* subregion of the B10 (*H-2^b*) mouse encodes 10 class I genes (designated *Q1–Q10*), while the BALB/c (*H-2^d*) mouse contains only 8 *Qa* genes (8, 24). A comparison of the class I *Qa* sequences revealed that the BALB/c strain lacks the *Q3* gene and carries a hybrid *Q8/9* gene. It is likely that this hybrid gene has been created by a fusion of ancestral BALB/c genes equivalent to B10 *Q8^b* and *Q9^b* genes (8, 24). DNA restriction mapping and hybridization studies have shown that, within the *Q4* to *Q10* group of genes, alternating genes are more similar to each other than to the adjacent genes (8). This homology is strongest between the *Q6–Q7* gene pair and *Q8–Q9* gene pair. Accordingly, the BALB/c strain contains a single *Q6^d–Q7^d* gene pair, while the B10 strain contains two pairs: *Q6^b–Q7^b* and *Q8^b–Q9^b*. We have shown previously that *Q7^d* is the only BALB/c *Qa* region gene that can be expressed in transfected cells as a cell-surface product biochemically similar to classically defined Qa-2 antigen. We have also demonstrated that its allelic equivalent from the B10 strain, *Q7^b*, has identical serological and biochemical properties.

To assess coding properties of the *Q8^b–Q9^b* gene pair and their potential contribution to the Qa-2 phenotype in the B10 mouse, we have established cell lines transfected with *Q8^b* and *Q9^b* genes. Cloned *Q8^b* and *Q9^b* genes (18) were introduced stably into hepatoma cells by the calcium phosphate precipitation method using pSV-2neo plasmid as a selectable marker. The recipient cells, Hepa-1, were shown previously to be permissive for cell-surface expression of Qa-2 antigen (12). Expression of *Q* gene products was monitored with mAbs 20-8-4 and 34-1-2, which are diagnostic for Qa proteins (12, 15). *Q9^b* gene transfectants reacted with these reagents when tested by a highly sensitive cell-binding radioimmunoassay. *Q8^b*-transfected Hepa-1 cells tested negative in this assay. Screening of mixed populations of Hepa-1 transfectants was confirmed by three independent transfection experiments (data not shown). The results of these experiments indicate that *Q9^b*, but not *Q8^b*, encodes in Hepa-1 cells a cell-surface Qa-2-like product. This is consistent with DNA homology studies (18) that suggested that *Q9* gene is very similar to the *Q7* gene, while *Q8* gene is similar to the *Q6* gene.

To test if *Q9^b* gene expression is liver specific, we have transfected a rat-derived primary cloned liver cell line, CRL-3A, and, as a control, the fibroblastic L cell line. As observed for *Q7* genes (12), *Q9^b* expression was detectable on the cell surface of CRL-3A cells but not on L cells.

Biochemical Characterization of the Q9^b Protein Product. We have reported that *Q7^b* gene expression is sufficient to explain serological and biochemical heterogeneity of Qa-2 antigen(s) on the B6 spleen (12). Since transfection experiments identified the *Q9^b* gene as potentially contributing to Qa-2 phenotype, we have performed a series of experiments to compare Q7^b, Q9^b, and B6 spleen Qa-2 properties. Hepa-1 cells transfected with either the *Q7^b* or the *Q9^b* gene as well as activated T-cell populations from B6 mice were radiolabeled by lactoperoxidase-catalyzed iodination. Cell-surface molecules immunoprecipitated by anti-Qa-2 sera (B6.K1 anti-B6) were analyzed by two-dimensional gel electrophoresis (Fig. 1). The anti Qa-2 sera recognized a M_r 40,000 polypeptide on the surface of *Q9^b*-transfected Hepa-1 cells that is indistinguishable by charge or molecular weight from the Q7^b polypeptide or the Qa-2 polypeptide expressed on lymphoid cells. This is consistent with our initial observation and suggests that B6 Qa-2 antigens are composed of two superimposed patterns of Q7^b and Q9^b products. Treatment of the M_r 40,000 Q7^b or Q9^b polypeptide with the enzyme Endo-F reduced the M_r to ≈34,000. This reduction in molecular weight is consistent with the removal of two N-linked carbohydrate chains as predicted from *Q7^b* and *Q9^b* DNA sequences (18, 22). Interestingly, the deglycosylated Q7^b and Q9^b core polypeptides displayed reproducibly distinct isoelectric patterns (compare *B* and *D* in Fig. 1). Both Q7^b and Q9^b contain three isoelectric species of which two

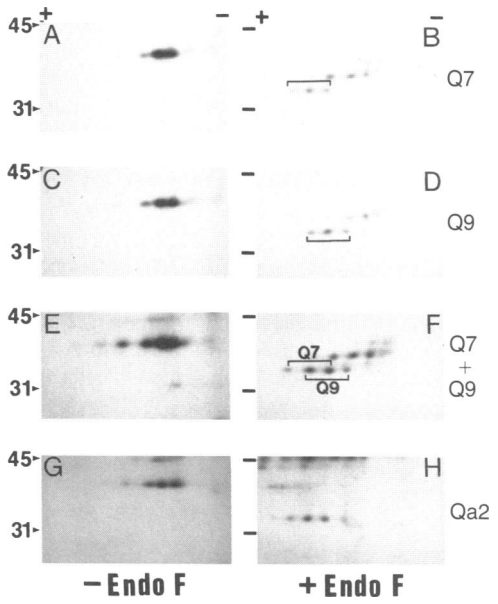

FIG. 1. Biochemical analysis of the Q7^b and Q9^b polypeptide. Cell lysates from I^{125}-radiolabeled Hepa-1 cells transfected with either *Q7^b* or *Q9^b* and B6 activated T cells were immunoprecipitated with anti-Qa-2 serum. Isolated molecules were treated with (*Right*) and without (*Left*) Endo-F and analyzed by two-dimensional gel analysis. Displayed is analysis of the fully glycosylated (*A*, *C*, *E*, and *G*) or Endo-F-deglycosylated (*B*, *D*, *F*, and *H*) Q7^b polypeptide (*A* and *B*), Q9^b polypeptide (*C* and *D*), a mixture of Q7^b and Q9^b (*E* and *F*), and lymphoid Qa-2 polypeptides (*G* and *H*). Only relevant regions of the autoradiogram are displayed. Positions of known molecular weight markers (shown × 10^{-3}) run in parallel are indicated.

major spots are common. In addition, $Q9^b$ has a unique acidic species, whereas $Q7^b$ has a unique basic species. When a mixture of the deglycosylated $Q7^b$ and $Q9^b$ species were analyzed, a series of four spots was observed (Fig. 1*F*). The middle two common spots displayed an increase in intensity, and the unique acidic ($Q9^b$) and basic ($Q7^b$) species remained less intense. Importantly, the isoelectric pattern observed in a mixing experiment was identical to the deglycosylated Qa-2 molecules identified in activated lymphoid cell populations of the B6 mice. Based on these observations, we propose that the Qa-2 molecules expressed by the Qa-2^{hi} strain B10 (and B6) are encoded by at least two *Q*-region class I genes: $Q7^b$ and $Q9^b$.

Serological Comparison of the Q7 and the Q9 Polypeptides. Previous studies have shown that the Q7 polypeptide is recognized by a battery of serological reagents that detect *Qa-2* subregion-controlled serological determinants (12). We wished to determine whether these reagents likewise detected the $Q9^b$-encoded polypeptide. Hepa-1 cells, expressing either the $Q7^b$ or $Q9^d$ polypeptides, were immunoprecipitated with various serological reagents and analyzed by one-dimensional PAGE (Fig. 2). Anti-Qa-2 serum as well as the mAbs 20-8-4, 34-1-2, Qam2, Qam7, Qam8, and D3262 recognized a M_r 40,000 polypeptide expressed on the surface of $Q7^b$- or $Q9^b$-transfected cells (Fig. 2, lanes A–H). Thus, based on the reagents utilized in this study, the $Q9^b$, $Q7^b$, and $Q7^d$ cell-surface products are serologically indistinguishable.

The $Q7^b$ and $Q9^b$ Genes Encode Lipase-Sensitive Cell-Surface and Secreted Soluble Polypeptides. The Q7 polypeptides expressed on Hepa-1 cells as well as the Qa-2 molecules expressed on lymphoid cells have been shown to be attached to the cell membrane via a PtdIns-bearing membrane anchor sensitive to cleavage with a phospholipase C (12, 13). We sought to determine whether the Q9 polypeptide was likewise attached to the cell surface by a lipase-sensitive anchor structure. Hepa-1 cells transfected with either $Q7^b$ or $Q9^b$ were radiolabeled by lactoperoxidase catalyzed-iodination and incubated in the presence of various concentrations of a PtdIns-specific phospholipase C purified from *Baccillus thuringiensis* (23). After incubation, both cell lysates and cell-free media were immunoprecipitated with the anti-Qa-2 reagents and analyzed by $NaDodSO_4$/PAGE (Fig. 3). Results showed that both the $Q7^b$ and $Q9^b$ molecules display identical sensitivity to the PtdIns-specific phospholipase C purified from *B. thuringiensis*.

The Q7 and Qa-2 polypeptides are synthesized as both cell-surface and soluble secreted forms (12, 14, 25). To explore whether the $Q9^b$ polypeptide was likewise synthesized in the secreted soluble form, Hepa-1 cells transfected

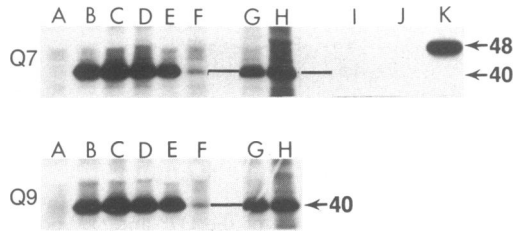

FIG. 2. Serological characterization of $Q9^b$ polypeptide. Cell lysates from I^{125}-labeled untransfected Hepa-1 cells or Hepa-1 cells transfected with the $Q7^b$ or $Q9^b$ genes were treated with various anti-Qa-2/H-2 serological reagents and analyzed by $NaDodSO_4$/PAGE. Displayed are immunoprecipitates from normal mouse serum (lanes a), anti-Qa-2 serum (lanes b), 20-8-4 (lanes c), 34-1-2 (lanes d), Qam8 (lanes e), Qam2 (lanes f), Qam7 (lanes g), and D3262 (lanes h). Also displayed are immunoprecipitates from untransfected Hepa-1 cells treated with H.11.4.1 [anti-H-$2K^k$ (control), lane i], 20-8-4 (lane j), and 28-14-8 (anti-H-$2D^b$, lane k).

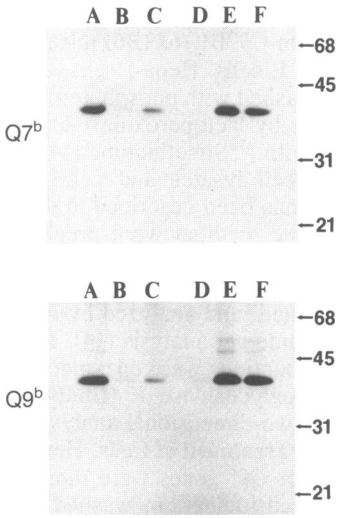

FIG. 3. Lipase sensitivity of $Q7^b$ and $Q9^b$. ^{125}I-radiolabeled Hepa-1 cells transfected with $Q7^b$ or $Q9^b$ were treated with *B. thuringiensis* PtdIns phospholipase C. After treatment, both cells and cell-free medium were harvested, and the distribution of $Q7^b$ or $Q9^b$ in cell lysates and cell-free medium was determined by immunoprecipitation and $NaDodSO_4$/PAGE. Displayed are the $Q7^b$ (*Upper*) and $Q9^b$ (*Lower*) recovered from the cell lysate (lanes A–C) or cell-free medium (lanes D–F) after treatment with PtdIns phospholipase C at 0 μg/ml (lanes A and D), 20 μg/ml (lanes B and E), and 5 μg/ml (lanes C and F). The positions of known molecular weight markers (shown $\times 10^{-3}$) are indicated on the right.

with either $Q7^b$ or $Q9^b$ genes were radiolabeled with [^{35}S]-methionine for 4 hr. Cell-free medium was immunoprecipitated with anti-Qa-2 serum and analyzed by two-dimensional PAGE (see Fig. 4). Both $Q7^b$- and $Q9^b$-transfected Hepa-1 cells synthesized and secreted a M_r ≈39,000 soluble polypeptide in addition to the cell-bound products. We have documented (12) that the *Q7* gene product in transfected L cells is exclusively expressed as a secreted soluble molecule. Similar to $Q7^b$ and $Q7^d$, the $Q9^b$ gene, when transfected into L cells, synthesized a soluble secreted M_r 39,000 polypeptide (Fig. 4). Analogous to what was observed with the $Q7^b$ and $Q9^b$ cell-surface proteins, the secreted soluble $Q7^b$ or $Q9^b$ polypeptides could not be distinguished by charge or molecular weight. Hence, it is likely that Q9 soluble prod-

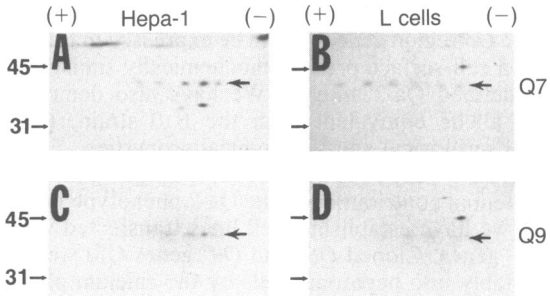

FIG. 4. Biosynthesis of $Q7^b$ and $Q9^b$ polypeptides in Hepa-1 and L cells. L-cell fibroblasts or Hepa-1 cells transfected with the $Q7^b$ or $Q9^b$ genes were radiolabeled with [^{35}S]methionine, and the cell-free medium was treated with anti-Qa-2 reagents. Immunoprecipitates were analyzed by two-dimensional electrophoresis. Only selected regions of the appropriate fluorograph are shown. Displayed are the anti-Qa-2-reactive polypeptides found in cell-free medium derived from Hepa-1 cells transfected with $Q7^b$ (*A*), Hepa-1 cells transfected with $Q9^b$ (*B*), L cells transfected with $Q7^b$ (*C*), or L cells transfected with $Q9^b$ (*D*). The arrows mark specific anti-Qa-2-reactive polypeptides not found in the control (normal mouse serum, not shown) immunoprecipitates. The positions of known molecular weight markers (shown $\times 10^{-3}$) are indicated.

ucts contribute to the soluble Qa-2 forms secreted from B6-activated T cells (12).

$Q7^b$ and $Q7^d$ Display Quantitative Variation in Cell-Surface Expression. Differences in gene dosage between BALB/c and C57BL strains do not fully explain the observed 6-fold variation in the levels of the *Qa-2* expression between these strains (16). Therefore, we have reasoned that the existence of the quantitative variation of the Qa-2 phenotype may be partially caused by variation in the levels of expression of individual *Q* genes encoding different allelic forms of Qa-2 polypeptides in different strains. To examine this possibility, we have determined whether *Q7* genes isolated from a Qa-2^{hi} (*$Q7^b$*) or a Qa-2^{low} (*$Q7^d$*) strain display quantitative variation in the levels of cell-surface expression. Hepa-1 cells were transfected with increasing amounts of *Eco*RI DNA fragments containing either *$Q7^b$* or *$Q7^d$* genes (see Fig. 5*a*). Both *Eco*RI segments contained equivalent 5′ and 3′ flanking regions. One microgram of the transfecting class I DNA was sufficient in both cases to observe maximal levels of Qa-2 expression in the *$Q7^d$* and *$Q7^b$* transfectants. In all parallel transfections, the *$Q7^b$* cells expressed ≈3-fold higher levels of cell-surface products than did the *$Q7^d$* cells. *$Q9^b$*-transfected Hepa-1 cells expressed Qa-2 levels comparable to *$Q7^b$* transfectants (data not shown). To test these results at the clonal level, 20 clones were derived from the *$Q7^d$*- and *$Q7^b$*-plasmid-transfected cells (Fig. 5*b*) and *$Q9^b$*-transfected cells (not shown). With the exception of two cell lines expressing high levels of $Q7^b$, cell lines derived from the *$Q7^b$* transfection of Hepa-1 displayed consistently 2- to 3-fold higher levels of cell-surface expression than did the analogous *$Q7^d$* transfectants. When selected subpopulations of these clones were analyzed for levels of Q7 polypeptide biosynthesis and secretion, similar results were obtained (data not shown). These observations suggest that variation in the levels of *Qa-2* expression in Qa-2^{hi} and Qa-2^{low} strains may, in part, be explained by quantitative variation in expression of individual alleles at the transcriptional or posttranscriptional level.

Taken together with the gene-dosage differences between C57BL and BALB/c strains, our data can account for the 6-fold differences in the *Qa-2* expression between these high and the low strains (16).

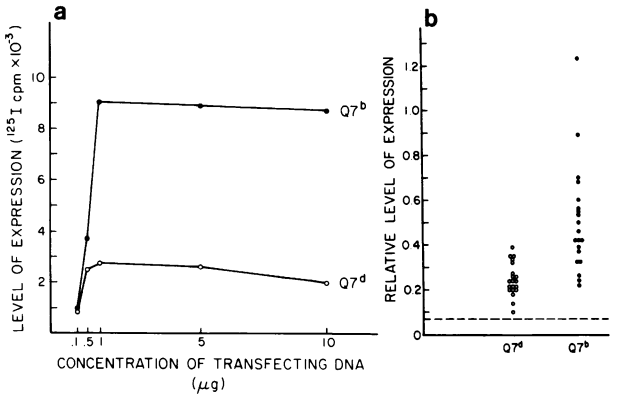

FIG. 5. Comparison of cell-surface expression of $Q7^b$ and $Q7^d$ polypeptides on Hepa-1 transfectants. (*a*) Increasing concentrations of homologous *Eco*RI DNA fragments carrying the *$Q7^b$* or *$Q7^d$* genes were transfected into Hepa-1 cells, and the levels of cell-surface expression were measured by a quantitative RIA using mAb 20-8-4 and ^{125}I-labeled protein A. The experimental errors from duplicate experiments were <10% of the values shown. (*b*) Cell-surface expression of Q7 products was measured as above on individual clones derived from transfection of Hepa cells with 1 μg of *$Q7^d$*- or *$Q7^b$*-containing plasmids. The results were standardized relative to the expression of the internal control H-$2D^b$, estimated with the H-$2D^b$-specific mAb 28-14-8. ○, Relative level of $Q7^d$ expression in individual clones; ● relative level of $Q7^b$ expression in individual clones. The experimental errors from duplicate experiments were <10% of the values shown. The average relative level of expression, estimated for 20 clones, was: for $Q7^d$, 0.26 ± 0.02; and for $Q7^b$, 0.50 ± 0.05. The dashed line indicates the level of detection measured on untransfected Hepa-1 cells.

DISCUSSION

Strains expressing the serologically defined Qa-2 antigens have been reported to display wide variation in the levels of Qa-2 antigen cell-surface expression (5, 6, 15–17, 26, 27). Several factors can control expression levels of cell surface-proteins. These include gene dosage as well as variations in transcriptional levels, mRNA processing/transport time, and post-translational processing steps. To address these possibilities, we have conducted two sets of studies. First, the protein product of another *Q*-region class I gene, *$Q9^b$*, a close homologue of *$Q7^b$* isolated from a Qa-2^{hi} (B10) strain, was biochemically characterized to determine its relationship to the Q7 protein and the Qa-2 protein expressed on lymphoid cells. In a second approach, the allelic *Q*-region class I genes *$Q7^b$* and *$Q7^d$* were individually transfected into a cell line capable of supporting Q7 cell-surface expression (Hepa-1 cells), and the levels of Q7 expression were determined by a quantitative RIA.

Gene-transfection studies have shown that the *$Q7^b$* and the *$Q9^b$* genes both encode a M_r 40,000 cell-surface polypeptide. Two-dimensional gel analysis of the M_r 40,000 $Q7^b$, $Q9^b$, and lymphocyte Qa-2 molecules fails to reveal reproducible differences between these species. However, after removal of the N-linked carbohydrate structures, a clear difference in charge heterogeneity was observed between the M_r 34,000 $Q7^b$ and $Q9^b$ core polypeptides. The complete sequences of the *$Q7^b$* and *$Q9^b$* gene are known (ref. 18, D. Nickerson, I.S., and L.H., unpublished observation). A single base change in exon 3, leading to a glutamine in $Q7^b$ and a glutamic acid in $Q9^b$ at position 173, is observed. This information is consistent with the observed more acidic profile of the $Q9^b$ versus the $Q7^b$ polypeptide. Importantly, the two-dimensional gel pattern observed when a mixture of the $Q7^b$ and $Q9^b$ core polypeptides are analyzed is identical to the core polypeptide pattern obtained from B6 Qa-2 molecules. These observations, together with the findings that the $Q7^b$ and $Q9^b$ proteins display identical serological properties and lipase sensitivities, suggest strongly that the *$Q7^b$* and *$Q9^b$* genes collectively contribute to the population of glycolipid-anchored Qa-2 molecules expressed by lymphoid cells in the Qa-2^{hi} strain B10 (and B6). The finding that *$Q7^b$* and *$Q9^b$* both encode a soluble M_r 39,000 protein product also indicates that both genes contribute to the population of secreted Qa-2 molecules in this strain.

The $Q7^b$ and $Q9^b$ polypeptides display reproducible charge microheterogeneity even after removal of the N-linked carbohydrate. Since the $Q7^b$ and $Q9^b$ polypeptides are anchored to the cell membrane by a PtdIns-bearing glycolipid anchor, variation in the complex glycan component of the anchor structure may result in charge microheterogeneity. However, at this time the participation of other post-translational modifications cannot be ruled out.

Serological and biochemical studies have determined that mouse strains can display up to 15-fold variation in the levels of Qa-2 antigen expression on lymphocytes (5, 6, 15–17, 26, 27). Clearly, gene dosage falls short of explaining this broad range in Qa-2 expression levels. As an alternative, variations in transcriptional levels, mRNA processing/transport time, and post-translational processing rates between allelic forms of *Q7* (or *Q9*) genes may contribute to variations in cell-surface expression. To address this possibility, equal copy numbers of the allelic class I genes *$Q7^b$* and *$Q7^d$* were

individually transfected into Hepa-1 cells, and the levels of Q7 expression on the transfected cells was determined. This analysis revealed that the $Q7^b$-transfected cells display ≈3-fold more Q7 protein on the cell surface than $Q7^d$-transfected cells. In addition, metabolic radiolabeling of $Q7^b$- or $Q7^d$-transfected Hepa-1 cells has determined that the levels of cell-associated and secreted $Q7^b$ biosynthesis are up to 3-fold higher than $Q7^d$ (data not shown). These observations indicate that allele-specific variation in *Q7* expression may contribute to the broad range in *Qa-2* expression seen among strains. It is not clear at this time whether the observed differences in $Q7^b$ versus $Q7^d$ cell-surface protein expression and biosynthesis result from variations in transcriptional activity or protein-processing steps. Similar postulates were put forward to explain the quantitative variation in TL antigen expression among mouse strains (28).

Based on the above observations, we propose a model in which both gene dosage and allelic-specific variation in expression levels contribute, in varying degrees, to the strain-specific Qa-2 expression levels. Thus, the high levels of expression exhibited by B6 and B10 strains are due to the presence of two functional *Qa*-region class I genes ($Q7^{hi}$ and $Q9^{hi}$) encoding high levels of the Qa-2 molecule. Lower levels of expression would be accounted for by the presence of a single gene expressing Qa-2 at a high level, two genes expressing Qa-2 at a low level, or a single gene expressing Qa-2 at a low level. The identification of a single Qa-2-encoding gene ($Q7^d$) in the Qa-2^{low} strain BALB/cJ supports this model (12). Furthermore, in the strain B6.K2, which expresses ≈50% of the Qa-2 antigen levels relative to B6 (16, 17) a $Q9^b$, but not a $Q7^b$, equivalent has been biochemically identified on the lymphocyte cell surface (M.J.S., unpublished observation). In the analysis reported above, we have tested only two allelic counterparts of the *Q7* gene. It is likely that additional *Q7* or *Q9* allelic variants exist that will display further variation in protein expression levels. In addition, it is possible that other, yet unidentified, class I genes may contribute to Qa-2 antigen expression in strains of different genetic backgrounds.

Analysis of Qa-2 antigen expression revealed that the *H-2D* subregion influences Qa-2 levels (15–17). Strains bearing $H\text{-}2D^b$ display high levels of Qa-2 expression, while $H\text{-}2D^d$ strains display lower levels. This observation led to the suggestion that sequences in the *H-2D* region regulate expression levels of *Qa* region class I genes. We would suggest that the association of Qa-2 levels with the *H-2D* region reflects the close linkage of the *Qa*-region and *D*-region chromosomal segments. Thus, strains bearing $H\text{-}2D^b$ would often bear a *Qa*-region segment similar to the B10 (B6) strain and contain two high-level Qa-2-expressing class I genes. Strains bearing $H\text{-}2D^d$ would bear a *Qa*-region chromosomal segment, similar to that described for BALB/c mice, in which a single *Q*-region class I gene contributes to the cell surface M_r 40,000 Qa-2 molecules (12, 33). Interestingly, the strain B6.K3 (K^k,D^k,$Qa\text{-}2^a$) displays levels of lymphocyte Qa-2 expression equivalent to B10.A(2R) (K^k,D^b,$Qa\text{-}2^a$) and B6 (K^b,D^b,$Qa\text{-}2^a$), indicating that, in this case, the high level of expression is independent of the $H\text{-}2D^b$ region (H. Tien and M.J.S., unpublished observation).

The observation that the Qa-2 molecule is attached to the cell surface via a glycolipid anchor has suggested that Qa-2 may serve a role in cell signaling analogous to that suggested for other glycolipid-linked lymphocyte cell surface molecules such as Thy-1 and TAP [Ly-6] (12, 13, 29–32). However, it is puzzling that, if such a central role is served by Qa-2 molecules, the variation in expression levels (high > low > null) would be tolerated. One possible explanation would be that all strains express similar levels of glycolipid-anchored tissue-specific class I molecules. However, in various strains, all (Qa-2^{hi}), some (Qa-2^{low}), or none (Qa-2^-) are detectable by the available anti-Qa-2 serological reagents.

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