

# The Class I-b Molecule Qa-1 Forms Heterodimers with H-2L<sup>d</sup> and a Novel 50-kD Glycoprotein Encoded Centromeric to *I-E $\beta$*

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## Summary

Recent biochemical characterization of the *T23*-encoded Qa-1 molecule revealed an additional higher molecular mass species of 50 kD coprecipitated with the 48-kD Qa-1 molecule in *H-2<sup>b</sup>* and *H-2<sup>d</sup>* mouse strains. We now demonstrate that the 50-kD protein coprecipitated with Qa-1 is the class I-a antigen L<sup>d</sup> in all *H-2L<sup>d</sup>*-positive mouse strains examined. Further analyses of a panel of recombinants revealed that the 50-kD protein coprecipitated with Qa-1 in *H-2<sup>b</sup>* haplotype mouse strains is encoded or controlled by a gene centromeric to major histocompatibility complex class II *I-E $\beta$* . We have designated this gene and corresponding protein product as *Qsm*, Qa-1 structure modifier. Both L<sup>d</sup> and *Qsm* can interact with Qa-1 to form cell surface-expressed heterodimers in vivo. These Qa-1 heterodimers are not expressed in *H-2<sup>k</sup>* haplotype cells. The Qa-1/L<sup>d</sup> and Qa-1/*Qsm* heterodimers are associated by noncovalent interactions and occur only between fully processed proteins. In addition, we show that the *Qsm*-encoded protein can form heterodimers with L<sup>d</sup> as well, and that the L<sup>d</sup> molecules participating in these interactions with Qa-1 and *Qsm* may be devoid of  $\beta_2$ -microglobulin and/or peptide. These data represent the first demonstration that class I molecules can be expressed as heterodimers (Qa-1/L<sup>d</sup>) on the cell surface, and map a gene (*Qsm*) that may potentially encode a novel class I molecule, or another protein, that associates with both Qa-1 and L<sup>d</sup>. These interactions may enable increased levels of Qa-1 to reach the cell surface and may subsequently influence T cell recognition of Qa-1 and/or L<sup>d</sup> molecules.

Stable cell surface expression of a class I molecule is dependent on association of the H chain with both  $\beta_2$ -microglobulin ( $\beta_2m$ )<sup>1</sup> and peptide. Abnormally low levels of class I molecules reach the surface of  $\beta_2m$ -deficient cells, indicating that  $\beta_2m$  association is crucial for efficient intracellular transport of the H chain to the cell surface (1–3). Likewise, the requirement of peptide for the proper assembly and stable expression of class I molecules at the cell surface was demonstrated with the use of mutant cell lines deficient in peptide transporter subunits associated with antigen processing (TAP) (4–7). Cell surface expression of the unstable “empty” class I H chain/ $\beta_2m$  complexes in these cells can be increased by addition of exogenous peptides or incubation at reduced temperatures (8, 9).

The ability to be expressed in the absence of  $\beta_2m$  varies among class I allomorphs, as does the rate of  $\beta_2m$  association. Both H-2L<sup>d</sup> and H-2D<sup>b</sup> are capable of being transported to the cell surface without  $\beta_2m$ , whereas other class I molecules generally are not detected in  $\beta_2m$ -deficient cells (10–12). Both properly folded (conformed) as well as unfolded (nonconformed) D<sup>b</sup> H chains are expressed on the cell surface of  $\beta_2m^-$  murine cell lines, such as RIE-D<sup>b</sup> (10). In the same manner, two distinct antigenic forms of L<sup>d</sup> distinguishable by mAbs can be found both intracellularly and at the surface of normal cells. Confirmed L<sup>d</sup> (30-5-7s reactive) is both  $\beta_2m$  and peptide bound, whereas alternative L<sup>d</sup> (L<sup>d</sup>alt; 64-3-7s reactive) is only weakly  $\beta_2m$  associated and not bound to peptide (12–14). Thus, although some class I molecules are expressed at the cell surface in the absence of  $\beta_2m$  and peptide, they are expressed at lower levels and in a less stable form than fully assembled molecules.

The *T23*-encoded class I-b molecule Qa-1, characterized by a short half-life, low cell surface levels, and seemingly inefficient association with  $\beta_2m$  (15, 16), demonstrates additional requirements for stable expression on the cell surface. Stable expression of Qa-1 on the cell surface is extremely dependent on asparagine (N)-linked glycosylation (17, 18).

<sup>1</sup> Abbreviations used in this paper: anti-T23c, antipeptide serum directed against the cytoplasmic tail of T23;  $\beta_2m$ ,  $\beta_2$ -microglobulin; B6, C57BL/6; CAB, Con A-activated T cell blasts; 1-D, 2-D, one and two dimensional, respectively; ER, endoplasmic reticulum; L<sup>d</sup>alt, alternative form of L<sup>d</sup>; mCTL, monoclonal CTL; N, asparagine; Qdm, Qa-1 determinant modifier; *Qsm*, Qa-1 structure modifier; sMDM, supplemented Mishell-Dutton medium; TAP, transporters associated with antigen processing.

Other laboratories (19) have reported reduced expression of certain HLA molecules on the surface of cells treated with glycosylation inhibitors. The dependence on N-linked glycosylation for Qa-1 expression may indicate that carbohydrates are necessary for stable association of the H chain with  $\beta_2m$  and/or peptide. In addition to carbohydrate requirements, an *H-2D*-linked locus, *Qdm* (Qa-1 determinant modifier), was shown to control the expression of certain monoclonal CTL (mCTL) defined Qa-1 determinants (20). Qa-1 expressing target cells from *H-2D<sup>k</sup>* (*Qdm<sup>k</sup>*) mouse strains could not be recognized by *Qdm*-dependent CTL, whereas targets expressing Qa-1 from all other haplotype mouse strains (non-*H-2D<sup>k</sup>*, *Qdm<sup>+</sup>*) were recognized. *Qdm*-independent CTL were able to lyse Qa-1-expressing cells from all haplotypes (20). Data now suggest that the *Qdm<sup>+</sup>* epitope results from Qa-1 presenting a peptide derived from *H-2L<sup>d</sup>* or *H-2D<sup>b,d</sup>* but not *H-2D<sup>k</sup>* (21).

Despite these atypical requirements for intracellular transport and cell surface expression, Qa-1 exhibits widespread tissue distribution and possible functions analogous to the class I-a antigens (20). A recent report has implied a role for Qa-1 in the presentation of stress-related peptides (22). Both  $\gamma/\delta$  (23) and  $\alpha/\beta$  (24) T cells can recognize peptides presented by Qa-1. Aldrich et al. (24) have reported peptide loading of Qa-1 by TAP-dependent and -independent pathways.

Our previous identification of the gene encoding Qa-1 of *Qa-1<sup>b</sup>* mouse strains as *T23* revealed an additional higher molecular mass species (50 kD) that coprecipitated with the 48-kD *Qa-1<sup>b</sup>* molecule with an antipeptide serum directed toward the cytoplasmic tail of *T23* (anti-*T23c*) (18). This 50-kD protein, present in both *H-2<sup>b</sup>* and *H-2<sup>d</sup>* haplotype mouse strains, had not been detected in previous biochemical analyses with alloantisera specific for Qa-1. The 48- (*Qa-1*) and 50-kD proteins precipitated from C57BL/6 (B6) (*H-2<sup>b</sup>*) were not distinguished upon digestion with *N*-glycanase, suggesting that the higher molecular mass species resulted from a post-translational modification of Qa-1 involving N-linked oligosaccharides (18). The possibility of alternatively glycosylated forms of Qa-1 expressed on the cell surface was intriguing, since the stable expression of Qa-1 was shown to be extremely dependent on N-linked oligosaccharides. This report describes further biochemical analyses of Qa-1 expression in various recombinant mouse strains, focusing on the nature of and mechanisms responsible for generating the Qa-1-associated 50-kD protein. The culmination of these studies represents the first demonstration of heterodimer formation between class I molecules in intact cells.

## Materials and Methods

**Mice and Cell Culture.** A.T1a<sup>b</sup> and B6.K1 mouse strains were bred in our mouse colony at Baylor College of Medicine. C3H.L<sup>d</sup> transgenics, B6.AK1, BALB/c-*H-2<sup>dmm2</sup>*, and B10.A(3R) mice were generous gifts from Carla Aldrich and James Forman (University of Texas Southwestern Medical School, Dallas, TX). B6 and all other mice were purchased from The Jackson Laboratory (Bar Harbor, ME). Con A-activated T cell blasts (CAB) were prepared from mouse spleens as described (17).

**Serological Reagents.** The anti-*T23c* peptide serum (207-1) directed against the cytoplasmic tail of the *T23* gene product (amino acids 326–337) has been previously described (25) and was a gift from John E. Coligan (National Institutes of Health, Bethesda, MD). The majority of experiments used anti-*T23c* serum obtained from rabbits injected with the corresponding peptide (synthesized in our laboratory) coupled to KLH. Antiserum to the *Qa-1<sup>b</sup>* (anti-*Qa-1.2*) gene product was prepared as previously described (26). The anti-*Qa-1.2* reagent used for immunoprecipitations was an IgG fraction isolated on Affi-gel protein A (Bio Rad Laboratories, Richmond, CA). The *H-2L<sup>d</sup>* mAb 28-14-8s was obtained from American Type Culture Collection (Rockville, MD). The *H-2L<sup>d</sup>* mAbs 64-3-7s and 30-5-7s were gifts from Ted Hansen (Washington University, St. Louis, MO) and Carla Aldrich, respectively (12, 13).

**Radiolabeling of Cells.** For internal labeling,  $50 \times 10^6$  CAB were washed in methionine-free medium and then labeled for 30 min at 37°C in 1 ml HBSS containing 10% FCS and 1 mCi of [<sup>35</sup>S]methionine (Amersham Corp., Arlington Heights, IL). At the end of the labeling period, cold methionine-containing medium (supplemented Mishell–Dutton medium [sMDM]; GIBCO BRL, Gaithersburg, MD) was added to stop the labeling reaction. Cells were then either washed three times with PBS and immediately lysed with 0.5–1% NP-40 in PBS at 4°C or recultured at 37°C in sMDM at  $2 \times 10^6$ /ml and chased for varying lengths of time. After each chase, cells were washed and lysed as above (18). For cell surface iodination,  $50 \times 10^6$  cells were labeled in PBS with 2.5 mCi <sup>125</sup>I (Amersham Corp.) by the lactoperoxidase catalyzed procedure (27). At the end of the labeling period, the cells were washed and lysed as above. All cells were lysed in the presence of 1 mM PMSF and 33  $\mu$ g/ml of aprotinin (Boehringer Mannheim Biochemicals, Indianapolis, IN) to inhibit endogenous protease activity.

**Immunoprecipitation, SDS-PAGE, and Two-dimensional Gel Analysis.** Cell lysates were depleted of Ig and “nonspecific” material by preclearing with BSA-Sepharose, goat anti-mouse Ig-Sepharose, and protein A-agarose (Pierce Chemical Co., Rockford, IL). Aliquots of the lysates ( $\sim 12 \times 10^6$  cell equivalents) were incubated with specific or control antibodies at 4°C overnight. The immune complexes were recovered on protein A-agarose and washed four times with PBS containing 0.5% NP-40, 0.25% sodium deoxycholate, 0.1% SDS, and twice with H<sub>2</sub>O. For one-dimensional (1-D) gels, immunoprecipitates were eluted from protein A-agarose with solubilizing buffer and analyzed by SDS-PAGE on 12.5% gels under reducing conditions as described by Laemmli (28). For 2-D gels, immunoprecipitates were eluted from protein A-agarose in IEF solubilizing buffer and analyzed on a pH 5–7 IEF tube gel followed by SDS-PAGE on a 12.5% gel (17). BSA (68 kD), ovalbumin (45 kD), carbonic anhydrase (30 kD), chymotrypsinogen (24 kD), and cytochrome C (12.5 kD) were used as molecular mass markers. Gels were dried and analyzed by fluorography or autoradiography.

**Peptide Mapping.** Anti-*T23c* and anti-*Qa-1.2* immunoprecipitates from <sup>125</sup>I-labeled B6 lysates were separated on 1-D SDS-PAGE gels under reducing conditions. The gels were dried on 3-mm Whatman paper, and the anti-*Qa-1.2*, anti-*T23c* 48-kD (*Qa-1*), and anti-*T23c* 50-kD bands were excised and rehydrated in 1 ml of H<sub>2</sub>O. The 48- and 50-kD proteins were eluted out of the corresponding gel slices in 0.03 M Tris (pH 6.8), 2.5% glycerol, and 0.0125% SDS overnight at 37°C. The eluted samples were lyophilized and resuspended in H<sub>2</sub>O. 40% of each sample was digested in a 100- $\mu$ l volume with 2  $\mu$ g/ml V8 proteases (Boehringer Mannheim Biochemicals) or Lys C at 37°C for 45 min, and 20% of each was left undigested. The digested samples were then analyzed by 1-D SDS-PAGE under reducing conditions.

**SDS Treatment of Cell Lysates.** Iodinated B6 and BALB/c CAB were lysed in 0.2% NP-40 ( $50 \times 10^6$  cells/0.5 ml), and then boiled in the presence or absence of 2% SDS for 5 min. The resulting lysates were diluted to a final concentration of 0.2% SDS in PBS containing 0.2% NP-40, precleared as described above, divided, and immunoprecipitated with either preimmune or anti-T23c serum. Immunoprecipitates from the nontreated and SDS-treated lysates were compared on 1-D and 2-D gels under reducing conditions.

## Results

We previously reported that *T23* encodes the class I-b antigen Qa-1 of *Qa-1<sup>b</sup>* mouse strains (18). By use of an antiserum directed toward a peptide in the cytoplasmic tail of T23, the predicted 48-kD Qa-1 protein and a higher molecular mass species of 50 kD were precipitated from the cell surface of iodinated *H-2<sup>b,d</sup>* Con A-activated T cells. The 50-kD species was not precipitated with anti-Qa-1 alloantisera. Only a single protein was detected with the anti-T23c serum after a 30-min pulse with [<sup>35</sup>S]methionine; the 50-kD species could be detected only after a 1-h chase in B6 (*H-2<sup>b</sup>*). The fully processed 48- and 50-kD proteins seen in B6 displayed very similar IEF patterns on 2-D gels. Furthermore, both anti-T23c-specific proteins were equally dependent on N-linked glycosylation for cell surface expression based on studies with tunicamycin, and each displayed the same deglycosylated molecular mass ( $\sim 39$  kD) after treatment with N-glycanase. These data suggested that the 50-kD species was a structural variant of the *T23*-encoded Qa-1 protein, resulting from a posttranslational modification involving N-linked carbohydrates (18).

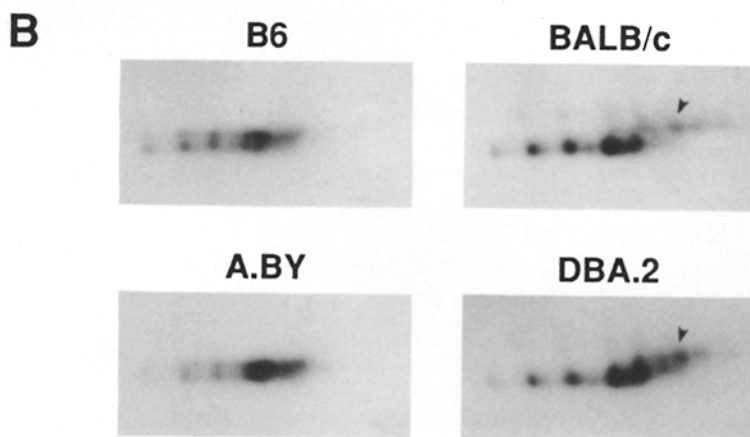
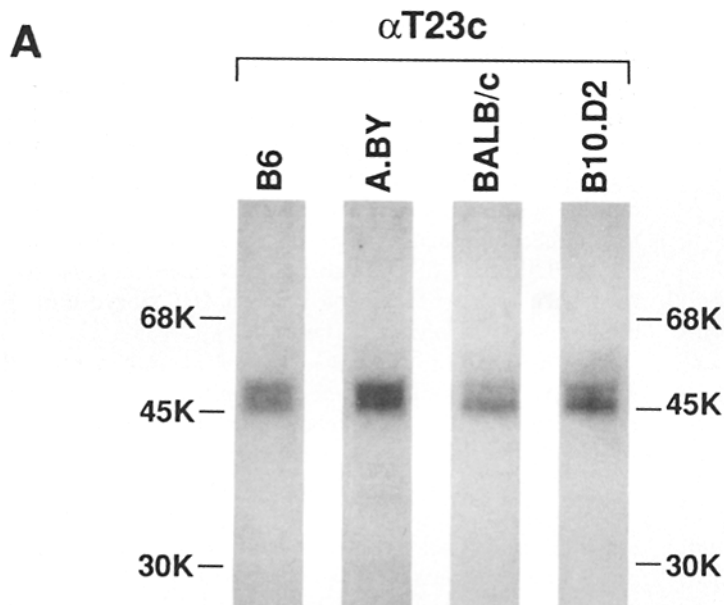
**Biochemical Characterization of the Anti-T23c-specific 50-kD Protein from Various Haplotypes.** In previous studies (18), thorough biochemical examination of the proteins precipitated with anti-T23c sera was performed only on immunoprecipitates from B6 (*H-2<sup>b</sup>*). Therefore, we compared the expression of the alternative molecular mass anti-T23c-reactive proteins in *H-2<sup>b</sup>*, *H-2<sup>d</sup>*, and *H-2<sup>k</sup>* mouse strains by both 1-D and 2-D gels. Immunoprecipitation with the anti-T23c serum from lysates of <sup>125</sup>I-surface labeled *H-2<sup>b</sup>* (B6, A.BY) and *H-2<sup>d</sup>* (BALB/c, DBA/2) CAB resulted in the isolation of both 48- and 50-kD proteins (Fig. 1 A). In contrast, only the 48-kD Qa-1 molecule was precipitated from iodinated *H-2<sup>k</sup>* (AKR, C3H, CBA) CAB; the 50-kD protein was absent (Fig. 2). In additional experiments (not shown), a single protein species representing the high-mannose form of Qa-1 was present in *H-2<sup>b</sup>*, *H-2<sup>d</sup>*, and *H-2<sup>k</sup>* haplotype cells after a 30-min pulse with [<sup>35</sup>S]methionine. The mature complex-sialylated 48-kD form of Qa-1 was seen after a 1.5-h chase in all three haplotypes. Again, the additional higher molecular mass species was detected in the *H-2<sup>b</sup>* and *H-2<sup>d</sup>* haplotype strains but not in any of the three *H-2<sup>k</sup>* strains analyzed (data not shown).

Although a 50-kD protein was precipitated with the anti-T23c serum from both *H-2<sup>b</sup>* and *H-2<sup>d</sup>* haplotype cells (Fig. 1 A), the IEF pattern of this species differed between haplotypes. Fig. 1 B compares 2-D gel profiles from two *H-2<sup>b</sup>* haplotype mouse strains with those from two *H-2<sup>d</sup>* haplotype strains. The 50-kD species from *b* haplotype strains ex-

hibited identical IEF patterns, and the 50-kD proteins from *d* haplotype strains were identical; however, the 50-kD protein differed between *b* and *d* haplotypes. The higher molecular mass species of the *d* haplotype was more acidic and displayed a different pattern of spots. The lack of the 50-kD protein in *H-2<sup>k</sup>* strains and the difference in pI demonstrated for this species between *b* and *d* haplotypes imply that, if this higher molecular mass protein is derived from a structural modification of Qa-1 involving N-linked oligosaccharides, then the modifying gene may be MHC linked and exhibit functional polymorphism.

**L<sup>d</sup> Physically Associates with Qa-1 on the Surface of H-2<sup>d</sup> Haplotype Cells, Resulting in the Coprecipitation of Qa-1 and L<sup>d</sup> with the Anti-T23c Serum.** *H-2<sup>k</sup>* strains have been reported to express lower levels of Qa-1<sup>b</sup> when analyzed with alloantisera and CTL; this effect was localized to the *H-2D<sup>k</sup>* region (29–31). An *H-2D*-linked gene, *Qdm*, was shown subsequently to control the expression of certain Qa-1-specific mCTL determinants (20). Some anti-Qa-1<sup>b</sup> mCTL (*Qdm* dependent) failed to lyse targets from *H-2<sup>k</sup>/Qa-1<sup>b</sup>* strains but did lyse targets from all other *Qa-1<sup>b</sup>* strains regardless of *H-2* genotype. *H-2<sup>k</sup>/Qa-1<sup>b</sup>* strains were designated *Qdm<sup>k</sup>* and all other *Qa-1<sup>b</sup>* strains as *Qdm<sup>+</sup>* (20). Aldrich et al. (21) now have data to suggest that the *Qdm<sup>+</sup>* epitope results from presentation of an *H-2L<sup>d</sup>*-derived peptide by *T23*-encoded Qa-1. The C3H strain is *Qdm<sup>k</sup>* and, therefore, cannot be lysed by *Qdm*-dependent mCTL. However, targets from C3H mice expressing an *H-2L<sup>d</sup>* transgene (designated as C3H.L<sup>d</sup> transgenics) are recognized and lysed by *Qdm*-dependent mCTL (21). Thus, the *Qdm<sup>+</sup>* epitope can be restored to the *Qdm<sup>k</sup>* strain C3H by *H-2L<sup>d</sup>*.

Because *Qa-1<sup>b</sup>/H-2<sup>k</sup>* (*Qdm<sup>k</sup>*) target cells do not express all of the appropriate determinants needed for the recognition and lysis by *Qdm*-dependent mCTL, nor do they express an anti-T23c-specific 50-kD protein (Fig. 2), we asked if *Qdm/L<sup>d</sup>* was involved in the expression of the higher molecular mass species. CAB from C3H and C3H.L<sup>d</sup>-transgenic mice were iodinated and immunoprecipitated with the anti-T23c serum. In contrast to the parental C3H mouse strain, in which only the 48-kD molecule was expressed, both the 48- and 50-kD proteins were precipitated from the C3H.L<sup>d</sup>-transgenic mouse cells (Fig. 3 A). Analysis of the 50-kD protein from C3H.L<sup>d</sup> on 2-D gels revealed the same IEF pattern as seen for the previously examined *H-2<sup>d</sup>* mouse strains (Fig. 3 B). Surprisingly, the 50-kD species precipitated with anti-T23c also was biochemically indistinguishable from L<sup>d</sup> immunoprecipitated with the mAb 28-14-8s, which recognizes a conformation-independent epitope (Fig. 3 B). This implies that the 50-kD protein is not a structurally modified form of Qa-1 under the control of *Qdm* but rather represents the L<sup>d</sup> antigen coprecipitated with Qa-1. Analysis of a panel of recombinant mouse strains positive for L<sup>d</sup> but differing in haplotype at other MHC loci yielded consistent results. The C3H.L<sup>d</sup>-transgenic mice expressed more L<sup>d</sup> associated with Qa-1 than any mouse strain examined, which is consistent with the higher level of total L<sup>d</sup> expression in these mice. Regardless of the MHC haplotype at any of the *H-2K*

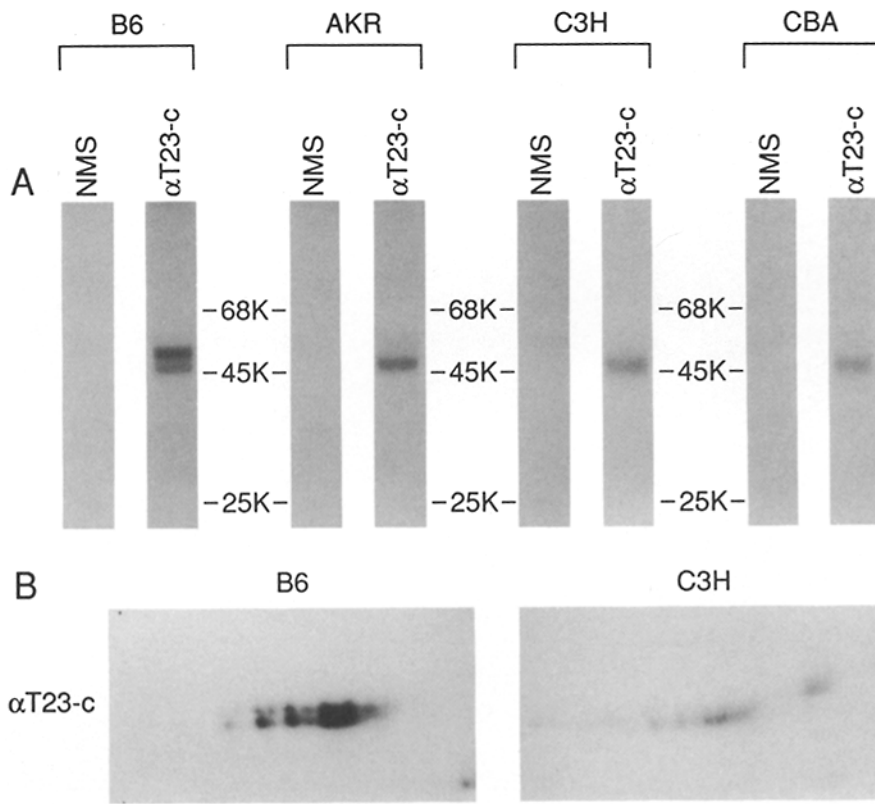


**Figure 1.** Comparison of the 50-kD protein coprecipitated with Qa-1 from *H-2<sup>b</sup>* and *H-2<sup>d</sup>* haplotype mouse strains. CAB from the *H-2<sup>b</sup>* haplotype strains B6 and A.BY and from the *H-2<sup>d</sup>* haplotype strains BALB/c, DBA-2, and B10.D2 were iodinated and lysates immunoprecipitated with the anti-T23c serum or normal rabbit serum for a control (not shown). Precipitates were analyzed on both 1-D (A; DBA-2 not shown) and 2-D (B; B10.D2 not shown) gels. The arrowhead denotes the 50-kD protein in the *d* haplotype strains that is distinct from that in the *b* haplotype strains.

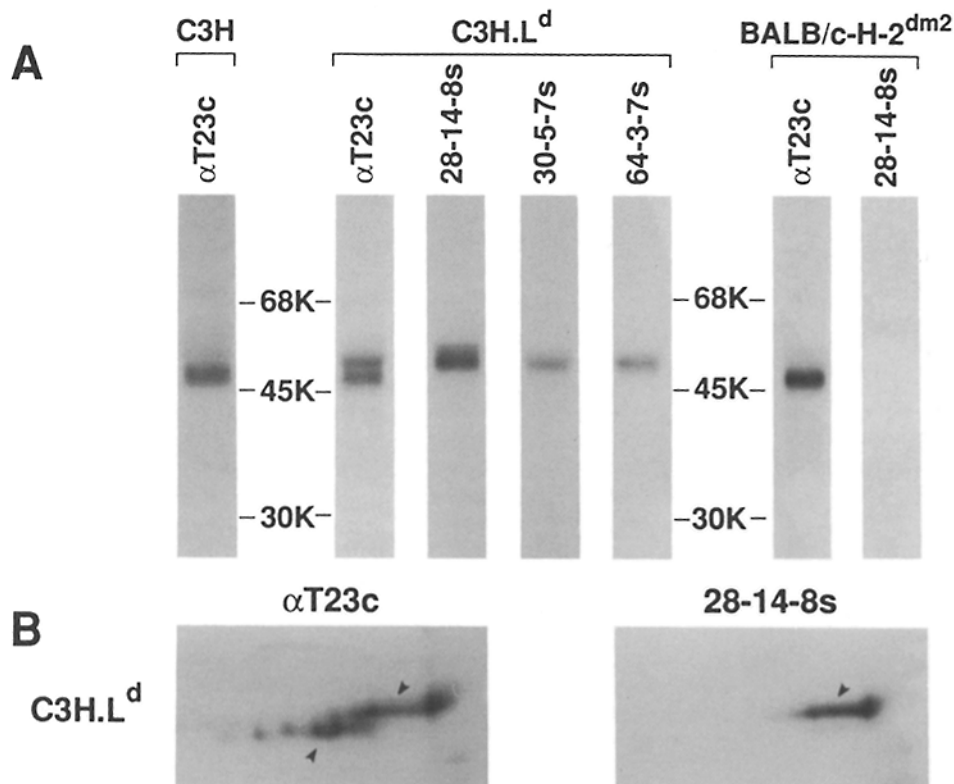
or *H-2D* loci, the anti-T23c serum coprecipitated with the 48-kD Qa-1 molecule, a 50-kD protein indistinguishable on 2-D gels from  $L^d$  in all strains positive for the *H-2L<sup>d</sup>* gene (Table 1). Furthermore, the 50-kD protein was not coprecipitated with Qa-1 from cells of the mouse strain BALB/c-*H-2<sup>dm2</sup>* (*H-2<sup>d</sup>*), which does not express  $L^d$  due to a mutation in the *H-2L<sup>d</sup>* gene (Fig. 3 A) (35). The presence of  $L^d$  in anti-T23 immunoprecipitates is not due to crossreactivity of the antiserum with  $L^d$ , as demonstrated by the failure to precipitate  $L^d$  from T23<sup>-</sup>,  $L^d$ <sup>+</sup> spleen cells (see Fig. 8) and from  $L^d$ -transfected fibroblasts (data not shown). Thus, these data indicate that  $L^d$  associates with Qa-1 to form a heterodimer expressed on the cell surface.

Not all Qa-1 and  $L^d$  molecules expressed on the cell surface participate in heterodimer formation. We previously demonstrated that free, nondimerized Qa-1 is precipitated with Qa-1 alloantisera (18, 26). The inability to coprecipitate  $L^d$  with anti-Qa-1.2 serum likely results from steric

blockage of determinants or an altered conformation. The anti-T23c serum is directed toward the cytoplasmic tail of Qa-1 and would not be affected by this interaction. There is ~10 times more  $L^d$  than Qa-1 expressed on the cell surface based on flow cytometric and immunoprecipitation data (the anti-T23c panels in Fig. 3 are 5–10 times longer exposures than the anti- $L^d$  panels). For this reason, Qa-1 is not visible in the anti- $L^d$  precipitates. Overexposures were too complex to define the presence of Qa-1. To determine if the anti- $L^d$  mAb were capable of reacting with the Qa-1/ $L^d$  heterodimer and to characterize the form of  $L^d$  involved in this interaction, lysates were precleared with each of the  $L^d$  mAbs and then immunoprecipitated with anti-T23c. Based on these immunodepletion experiments, the 28-14-8s and 64-3-7s anti- $L^d$  mAb reacted with the Qa-1/ $L^d$  heterodimer, removing  $L^d$  from the subsequent anti-T23c immunoprecipitation, while the 30-5-7s mAb did not (data not shown). Lie et al. (12) and Smith and coworkers (13, 14) have shown that mAb



**Figure 2.** Comparative 1-D and 2-D gel analysis of anti-T23c precipitates from *H-2<sup>b</sup>* versus *H-2<sup>k</sup>* haplotype mouse strains. B6, AKR, C3H, and CBA mice were iodinated and immunoprecipitated with anti-T23c or normal mouse serum and analyzed by 1-D (A) and 2-D (B) gels. Only anti-T23c immunoprecipitates for B6 and C3H are shown in B.



**Figure 3.** Examination of the role of *L<sup>d</sup>* in expression of the 50-kD protein coprecipitated with Qa-1. CAB from C3H, C3H.*L<sup>d</sup>* transgenics, and BALB/*c-H-2<sup>dm2</sup>* mice were iodinated and lysates immunoprecipitated with anti-T23c, 28-14-8s (*L<sup>d</sup>* and *L<sup>d</sup>alt*), 30-5-7s (*L<sup>d</sup>*), or 64-3-7s (*L<sup>d</sup>alt*), and analyzed on 1-D (A) gels. Only the anti-T23c precipitate is shown for C3H, and only the anti-T23c and 28-14-8s precipitates are shown for BALB/*c-H-2<sup>dm2</sup>*. (B) The 2-D gel comparison of the anti-T23c and 28-14-8s precipitates from C3H.*L<sup>d</sup>*-transgenic mice. The downward arrowhead in both panels denotes *L<sup>d</sup>*, and the upward arrowhead in the anti-T23c panels denotes Qa-1.

**Table 1. Mouse Strains**

Strain	MHC allele													Heterodimers	
	K	TAP	I-A	I-E	C4	Hsp70	Bat-5	TNF	D	L	Qdm	Q	T23	Qa-1/L <sup>d</sup>	Qa-1/Qsm
C57BL6/J, A.BY	b	b	b	b	b	b	b	b	b	-	+	b	b	-	+
BALB/c, DBA/2, B10.D2	d	d	d	d	d	d	d	d	d	d	+	d	d	+	-
C3H/HeJ, CBA/J, AKR, B6.H-2 <sup>k</sup>	k	k	k	k	k	k	k	k	k	-	k	k	k	-	-
A.Tla <sup>b</sup>	k	k	k	k	/	d	d	d	d	d	+	d	d	+	-
A.TL	s	/	k	k	k	k	k	/	d	d	+	d	d	+	-
C3H.L <sup>d</sup>	k	k	k	k	k	k	k	k	k	d	+	k	k	+	-
B6.K1	b	b	b	b	b	b	b	b	b	-	+	/	k	k	+
C3H.OH	d	d	d	d	d	?	k	k	k	-	k	k	k	-	-
B6.AK1	b	b	b	b	b	b	b	/	k	k	-	k	k	-	+
B10.A(2R)	k	k	k	k	/	d	d	/	b	b	-	+	b	b	-
B10.A(4R)	k	k	k	k/b	b	b	b	b	b	-	+	b	b	-	-
B10.A(3R)	b	b	b	b/k	d	d	d	d	d	?	a	a	-	-	
B10.D2(R107)	b	b	b	b	b	?	?	?	d	d	?	d	d	+	+

MHC allele designations adapted from references 32-34.

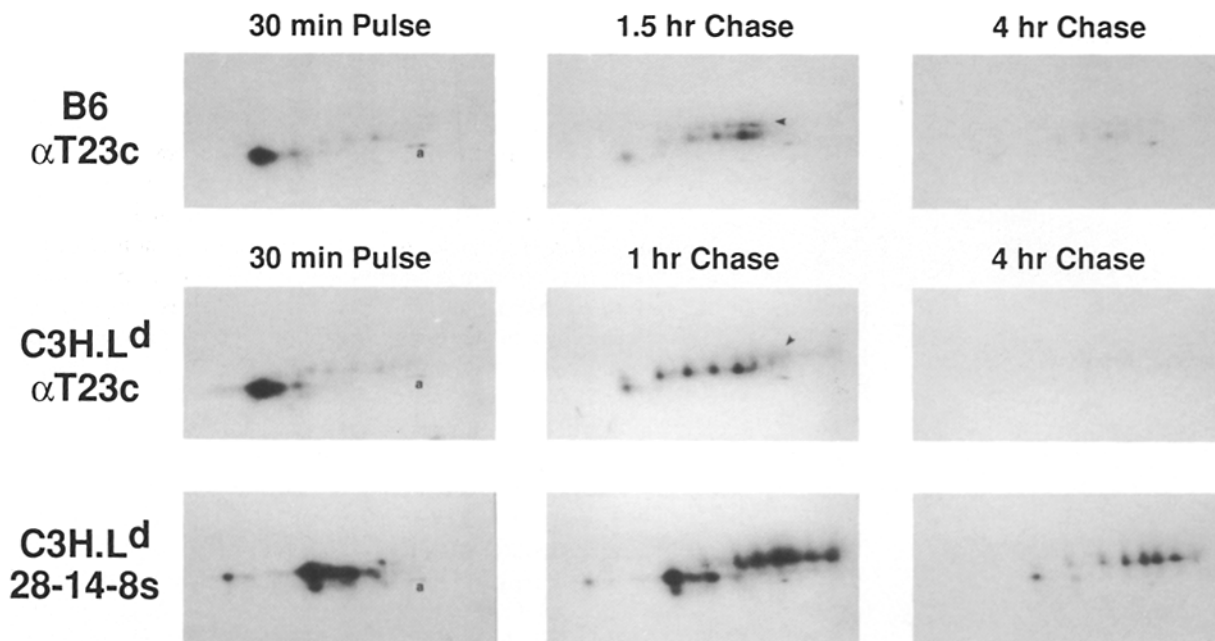
28-14-8s is conformation independent ( $\alpha$ 3-domain reactive), mAb 64-3-7s recognizes L<sup>d</sup>alt molecules (does not bind peptide and is only weakly associated with  $\beta$ 2m), and mAb 30-5-7s recognizes conformed L<sup>d</sup> molecules (both peptide and  $\beta$ 2m associated). Thus, the L<sup>d</sup>alt form only is capable of heterodimerizing with Qa-1.

*Nature of the Qa-1/L<sup>d</sup> Interaction and Its Effect on the Rate of Processing and Stability of the Qa-1 Antigen.* Biosynthetic labeling was used to determine if heterodimer formation between Qa-1 and L<sup>d</sup> occurred in a pre-Golgi compartment, such as during assembly of the H chain/ $\beta$ 2m/peptide complex in the endoplasmic reticulum (ER), or post-Golgi compartment, after the addition of N-linked oligosaccharides. B6 and C3H.L<sup>d</sup> CAB were labeled with [<sup>35</sup>S]methionine for 30 min or labeled for 30 min and then chased for various lengths of time. The resulting lysates were immunoprecipitated with anti-T23c or 28-14-8s and analyzed by 2-D gel electrophoresis. The predominant forms of both Qa-1 and L<sup>d</sup> precipitated from cells pulsed for 30 min were the unprocessed, high-mannose forms (Fig. 4). The high-mannose, immature Qa-1 protein differed in pI from the immature L<sup>d</sup> molecule precipitated with mAb 28-14-8s. Most importantly, the anti-T23c serum did not coprecipitate the immature L<sup>d</sup> proteins and, instead, detected only free Qa-1 molecules. Thus, the high-mannose forms of Qa-1 and L<sup>d</sup> do not associate to form a heterodimer recognized by the anti-T23c serum. Heterodimer formation was detected only between fully processed (mature) Qa-1 and L<sup>d</sup> proteins, as seen most clearly after a 1.5-h chase (Fig. 4, arrow). Therefore, heterodimer formation between these two class I molecules appears to occur only after glycosylation of the proteins is complete, in a post-Golgi compartment or once the molecules are expressed on the cell surface.

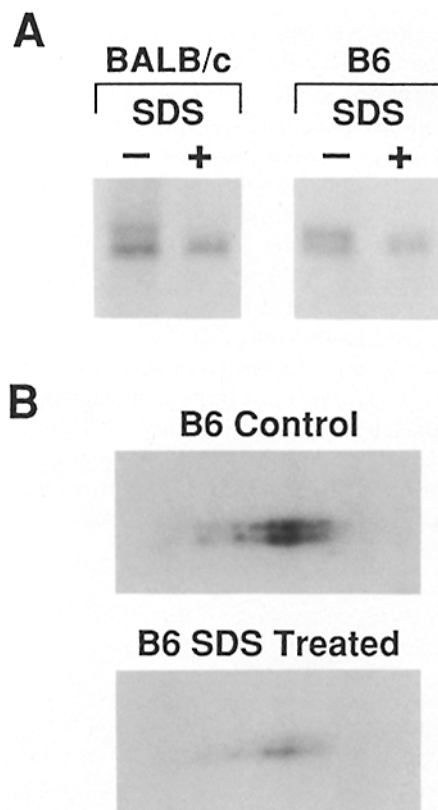
We reported previously (18) that Qa-1 exhibits a rapid turnover rate compared with class I-a molecules. There is little Qa-1 detectable by immunoprecipitation with either the Qa-1.2 alloantiserum or the anti-T23c serum after a 4-h chase (Fig. 4; 18). The class I-a L<sup>d</sup> molecule demonstrated a longer half-life than did Qa-1 and could be detected at significantly high levels even after a 4-h chase (Fig. 4); the same is true for H-2K<sup>b</sup> (18). However, the turnover rate of the heterodimer appeared similar to unassociated Qa-1, suggesting that interaction with L<sup>d</sup> does not increase the stability of Qa-1 on the cell surface.

We next examined the nature of the interaction of Qa-1 with L<sup>d</sup>. Lysates of iodinated BALB/c CAB were boiled in the presence of 2% SDS, immunoprecipitated with the anti-T23c serum, and compared with precipitates from non-SDS-treated labeled lysates on 1-D gels (Fig. 5 A). Treatment of cell lysates with SDS completely dissociated the Qa-1/L<sup>d</sup> heterodimer, resulting in the immunoprecipitation of only the 48-kD Qa-1 molecule by the anti-T23c serum. This indicates that Qa-1 and L<sup>d</sup> are not interacting with each other via interchain disulfide bonds but rather are noncovalently associated.

*An Unidentified H-2<sup>b</sup> Haplotype Gene Located Centromeric to Class II Encodes a Protein that Can Physically Interact with Qa-1 and L<sup>d</sup>.* Interestingly, the majority of the 50-kD protein precipitated with anti-T23c serum from B6 CAB also was not detected after SDS treatment of cell lysates, as shown in Fig. 5 B. This suggests that, in H-2<sup>b</sup> haplotype mouse strains, this species does not represent a structurally modified form of Qa-1 but rather is another protein noncovalently associated with Qa-1. To identify potential differences in protein sequence between the 48- and 50-kD species, peptide mapping was performed. The anti-T23c-reactive 48-kD pro-



**Figure 4.** Analysis of Qa-1 association with  $L^d$  by pulse-chase experiments. B6 and C3H.L<sup>d</sup> CAB were pulsed with [<sup>35</sup>S]methionine for 30 min and/or labeled for 30 min and then chased for 1–1.5 or 4 h. B6 lysates were immunoprecipitated with anti-T23c, and C3H.L<sup>d</sup> lysates were immunoprecipitated with anti-T23c, 28-14-8s, 30-5-7s (not shown), or 64-3-7s (not shown), and electrophoresed on 2-D gels. The arrowhead in B6, 1.5-h chase panel represents the anti-T23c-specific 50-kD protein of  $H-2^b$  haplotype. The downward arrowhead in C3H.L<sup>d</sup>, 1-h chase panel represents  $L^d$  physically associated with Qa-1. *a*, actin.

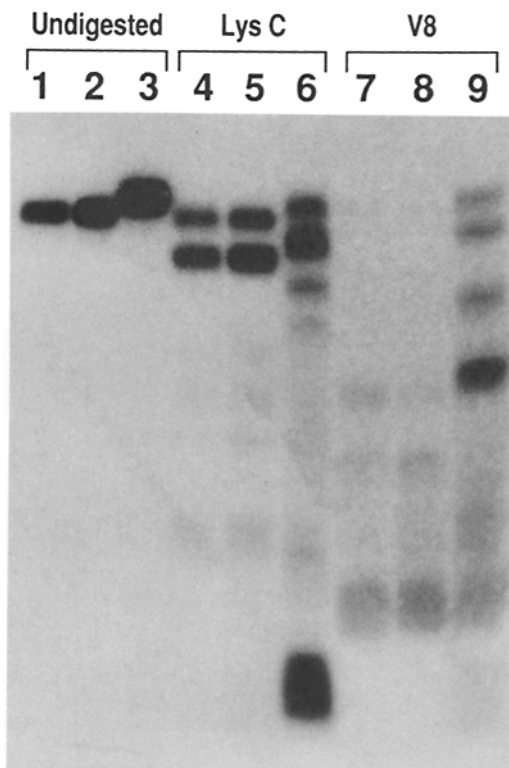


**Figure 5.** Effect of SDS treatment on the coprecipitation of the 50-kD protein with Qa-1 from  $H-2^d$  and  $H-2^b$  haplotype mouse strains. B6 and BALB/c CAB were iodinated, and the resulting lysates boiled in the pres-

tein and associated 50-kD protein and the anti-Qa-1.2-specific 48-kD protein were immunoprecipitated from iodinated lysates of B6 CAB, separated on 1-D gels, eluted, and treated with V8 protease or Lys C protease. Fig. 6 shows the resulting digests analyzed on 1-D gels. A different pattern was obtained with both enzymes for the 50-kD protein compared with the 48-kD Qa-1 molecule. In contrast, the peptide maps were identical for both the anti-T23c- and anti-Qa-1.2-precipitated 48-kD proteins. This suggests that the 50-kD species immunoprecipitated with the anti-T23c serum from B6 is not Qa-1 but a distinct protein. We have designated the protein, and the putative gene or genes controlling expression of this protein, as *Qsm*, since noncovalent association of the *Qsm*-encoded protein with Qa-1 alters the structure of Qa-1 expressed on the cell surface in such a way as to prevent recognition by the Qa-1.2 alloantiserum.

In an effort to identify the protein coprecipitated with Qa-1 in  $H-2^b$  haplotype mouse strains, a panel of recombinants was analyzed for the presence or absence of the anti-T23c-specific 50-kD species. The data obtained are summarized in Table 1. The presence of the 50-kD species in recombinant B6.K1 maps the gene encoding this protein to the left of Q, and expression of this protein in B6.AK1 maps it further to the left of *TNF- $\alpha$*  and thus eliminates D<sup>b</sup> (Table 1). However, the anti-T23c peptide serum did not co-

ence (+) or absence (-) of 2% SDS. Treated and untreated lysates were immunoprecipitated with anti-T23c and analyzed on 1-D (A) and 2-D (B, B6 shown only) gels.



**Figure 6.** Peptide mapping of the anti-T23c-precipitated 48-kD Qa-1 molecule and 50-kD protein. B6 CAB were iodinated and resulting lysates immunoprecipitated with anti-T23c or anti-Qa-1.2. Immunoprecipitates were separated in 1-D gels, the 48- and 50-kD bands excised from the dried gel, and proteins eluted. The eluted proteins were digested with 2  $\mu$ g/ml of V8 protease or Lys C at 37°C for 45 min or left untreated. The digested samples were then analyzed on 1-D gels. Lanes 1, 4, and 7 represent the anti-Qa-1.2-precipitated 48-kD Qa-1 molecule. Lanes 2, 5, and 8 represent the anti-T23c-precipitated 48-kD Qa-1 molecule. Lanes 3, 6, and 9 represent the anti-T23c-precipitated 50-kD protein.

precipitate this protein in either the B10.A(2R) or B10.A(4R) strains (Fig. 7 A). B10.A(2R) maps the controlling gene to the left of *C4*; the site of recombination in B10.A(4R) is further to the left within *I-E $\beta$*  (Table 1). The inability to precipitate the 50-kD species from these strains with the anti-T23c serum indicates the gene (*Qsm*) encoding this protein is located to the left of *I-E $\beta$* .

The recombinant B10.D2(R107) is positive for both *L<sup>d</sup>* and *Qsm* (*b* haplotype to the left of *I-E $\beta$* ) and, therefore, has the potential to express Qa-1 associated with either *L<sup>d</sup>*, the *Qsm*-encoded product, or both (Table 1). The anti-T23c serum coprecipitated a 50-kD species with Qa-1 as predicted; however, 1-D gel analysis could not distinguish whether this molecule represented *L<sup>d</sup>*, *Qsm*, or both (Fig. 7 A). Further analysis on 2-D gels indicated both Qa-1-associated proteins to be present (Fig. 7 B, arrows). In addition, 2-D gel analysis of the 28-14-8s precipitate from R107 demonstrated that, by itself, *L<sup>d</sup>* also interacts with the *Qsm* protein (Fig. 7 B, arrows). Both 28-14-8s and 64-3-7s (not shown) precipitates from R107 revealed additional spots analogous to *Qsm*, compared with *L<sup>d</sup>* precipitates from the *Qsm<sup>-</sup>*-transgenic mice C3H.*L<sup>d</sup>* (Fig. 7 B). Consistent results were obtained with

an additional recombinant, B10.A(3R), which is similar to R107 but is *T23<sup>-</sup>* (Table 1). Both 28-14-8s and 64-3-7s mAbs coprecipitated *Qsm* with *L<sup>d</sup>* from iodinated 3R lysates, whereas only *L<sup>d</sup>* alone was detected in the *Qsm<sup>-</sup>* mouse strain BALB/c (Fig. 7 C); thus, the interaction of *Qsm* with *L<sup>d</sup>* can be independent of Qa-1 expression. Therefore, an unidentified gene designated *Qsm* and located to the left of *I-E $\beta$*  in *H-2<sup>b</sup>* haplotype mice controls expression of a protein that can form a heterodimer with Qa-1 or *L<sup>d</sup>* via non-covalent interactions. Whether or not all three proteins can interact to form a heterotrimer could not be determined, since the coprecipitation of these three proteins from R107 lysates with the anti-T23c serum could either represent expression of a trimeric complex or expression of both Qa-1/*L<sup>d</sup>* and Qa-1/*Qsm* heterodimers.

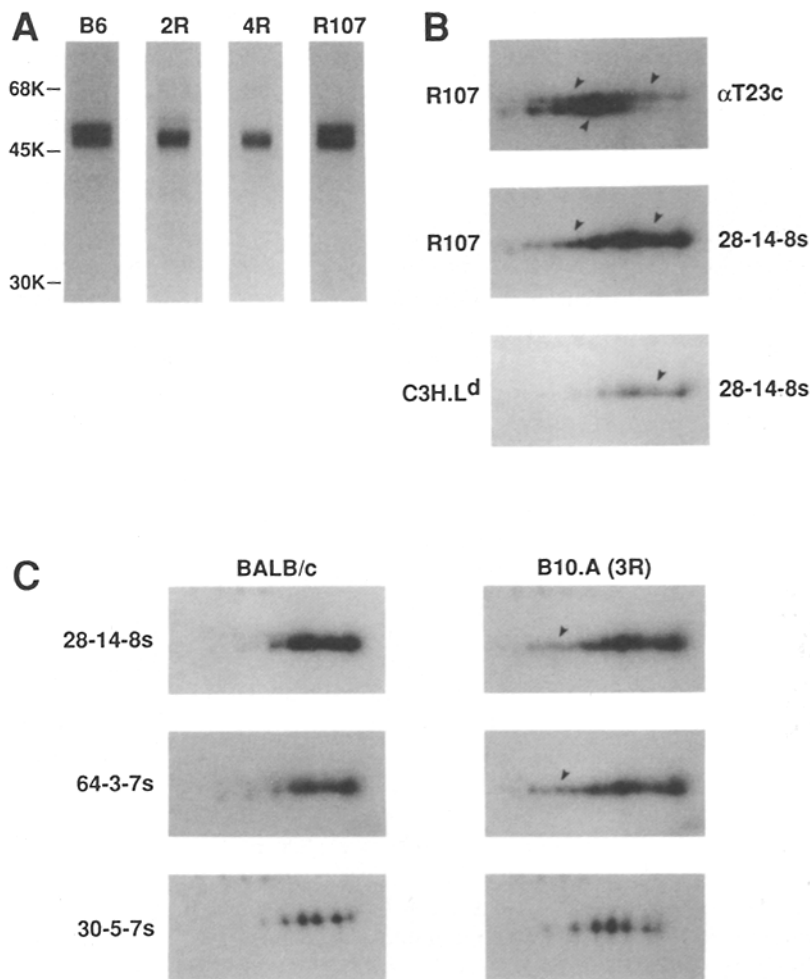
As discussed earlier, the *L<sup>d</sup>alt* but not conformed *L<sup>d</sup>* molecule formed heterodimers with Qa-1. To determine the antigenic conformation of the *L<sup>d</sup>* molecule involved in heterodimer formation with *Qsm*, 64-3-7s precipitates from iodinated 3R CAB were compared with 30-5-7s precipitates on 2-D gels. As shown in Fig. 7 C, the 2-D profile observed for *L<sup>d</sup>* precipitated with 30-5-7s differed from that of the *Qsm*-associated *L<sup>d</sup>* molecule detected with either 64-3-7s or 28-14-8s, as well as from that of the Qa-1-associated *L<sup>d</sup>* molecule. These data indicate that *L<sup>d</sup>alt* rather than conformed *L<sup>d</sup>* participates in heterodimer formation with *Qsm* and Qa-1.

*Heterodimer Formation Between Qa-1 and L<sup>d</sup> or Qa-1 and Qsm Occurs In Vivo and Is Not Due to Cross-Reactivity.* To determine whether the Qa-1/*L<sup>d</sup>*/*Qsm* interactions occurred in vivo or were the result of cell lysis, cells from appropriate strains were mixed, iodinated, lysed, and immunoprecipitated with the anti-T23c serum. As shown by the 2-D gels in Fig. 8, *L<sup>d</sup>* was not coprecipitated with Qa-1 when AKR (*T23<sup>+</sup>L<sup>d</sup>-*) and A/J (*T23<sup>-</sup>L<sup>d</sup>+*) cells were mixed. Similarly, *Qsm* was not detected when lysates of B10.A(4R) (*T23<sup>+</sup>Qsm<sup>-</sup>*) and B10.A(3R) (*T23<sup>-</sup>Qsm<sup>+</sup>*) were analyzed (Fig. 8). In addition, these data document that the presence of *L<sup>d</sup>* and *Qsm* in anti-T23c precipitates from *T23<sup>+</sup>L<sup>d</sup>+* or *T23<sup>+</sup>Qsm<sup>+</sup>* strains is not due to cross-reactivities of the antiserum for *L<sup>d</sup>* or *Qsm*. *L<sup>d</sup>* molecules were not immunoprecipitated from the A/J plus AKR lysate mixture (or A/J alone, not shown), which contains *L<sup>d</sup>*. Also, *Qsm* was not immunoprecipitated from the 4R plus 3R lysate mixture (or 3R alone, not shown) which has the *Qsm<sup>+</sup>* genotype and should express *Qsm*.

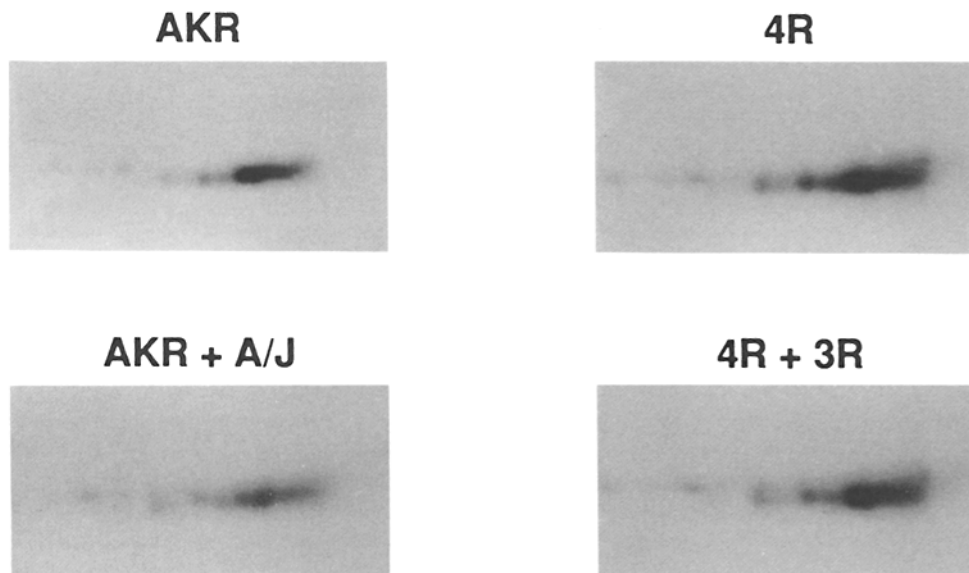
## Discussion

These data describe for the first time the in vivo cell surface expression of heterodimers formed between two different class I molecules. A significant proportion of the *T23*-encoded class I-b antigen Qa-1 was found on the cell surface heterodimerized with the class I-a molecule *L<sup>d</sup>* in all *H-2L<sup>d</sup>+* mouse strains examined. In addition, an unidentified protein encoded centromeric to the MHC class II region in *H-2<sup>b</sup>* haplotype mice also formed a cell surface-expressed hetero-





**Figure 7.** Formation of heterodimers between Qa-1, Qsm, and L<sup>d</sup> in various recombinants. (A) B6, B10.A(2R), B10.A(4R), and B10.D2(R107) CAB were iodinated, immunoprecipitated with anti-T23c, and analyzed on 1-D gels. (B) B10.D2(R107) and C3H.L<sup>d</sup> were iodinated, immunoprecipitated with anti-T23c or 28-14-8s, and analyzed on 2-D gels. Downward arrowheads on the left of each autoradiogram designate Qsm, while downward arrowheads on the right are pointing to L<sup>d</sup>; the upward arrowhead denotes Qa-1. (C) 2-D gel analysis of anti-L<sup>d</sup> (28-14-8s, 64-3-7s, and 30-5-7s) immunoprecipitates from iodinated lysates of BALB/c and B10.A(3R) CAB. Arrowheads denote the Qsm protein.



**Figure 8.** Examination of Qa-1 expression in mixed lysates to determine if heterodimer formation occurs in vivo or results from cell lysis. CAB from AKR, AKR mixed with A/J, B10.A(4R), and B10.A(4R) mixed with B10.A(3R) were iodinated and the resulting lysates immunoprecipitated with anti-T23c and analyzed on 2-D gels. Shown in the lefthand panels are AKR ( $T23^+$ ,  $L^d^-$ ) and AKR mixed with A/J ( $T23^-$ ,  $L^d^+$ ). Shown in the right hand panels are B10.A(4R) ( $T23^+$ ,  $Qsm^-$ ) and 4R mixed with B10.A(3R) ( $T23^-$ ,  $Qsm^+$ ).

dimer with either Qa-1 or L<sup>d</sup> or even potentially a heterotrimer with both.

The 50-kD proteins coprecipitated with Qa-1 from all *H-2L<sup>d+</sup>* strains, including the C3H.L<sup>d</sup>-transgenic mice, were biochemically indistinguishable from L<sup>d</sup> when compared on 2-D gels. Furthermore, a 50-kD molecule was not detected in the mutant mouse BALB/c-*H-2<sup>dm2</sup>*, which does not express L<sup>d</sup> (35). The ability to dissociate Qa-1 from L<sup>d</sup> by SDS treatment of cell lysates demonstrated that heterodimer formation occurred primarily through noncovalent interactions rather than disulfide bond formation.

Our previous analyses of the 50-kD species in the *H-2<sup>b</sup>* strain B6 showed that this protein could not be distinguished from the 48-kD Qa-1 protein after deglycosylation, suggesting that it resulted from a posttranslational modification involving N-linked oligosaccharides. This protein was not detected with the Qa-1 alloantiserum. Since the anti-T23c serum is directed toward the cytoplasmic tail, recognition should not be affected by the additional modification of oligosaccharides, whereas determinants recognized by the alloantiserum could be compromised. Therefore, it was unexpected when the majority of the 50-kD species no longer coprecipitated with Qa-1 after SDS treatment of B6 lysates. Further investigations revealed that this protein had a peptide map different from that of Qa-1 and thus was not a modified form of Qa-1 but rather a distinct protein noncovalently associated with Qa-1 to form a heterodimer in a fashion similar to that of L<sup>d</sup>. The gene responsible for this protein was mapped to the left of the MHC class II *I-E $\beta$*  gene and designated as *Qsm*, since heterodimer formation with *Qsm* modified the structure of Qa-1, rendering it unrecognizable by the Qa-1 alloantiserum. In additional experiments, *Qsm* also was found to heterodimerize with L<sup>d</sup> molecules.

The results presented here raise several important questions. The first concerns the identity of *Qsm*. Whether or not this gene is linked tightly to the MHC has not been determined; however, it is located on chromosome 17. Although we cannot conclude from the results whether *Qsm* encodes or controls expression of a class I molecule, *Qsm* does resemble a class I molecule biochemically. The *H-2K<sup>b</sup>* and *H-2K2<sup>b</sup>* loci are the only class I genes known to exist centromeric to class II. Our previous experiments, comparing Qa-1 with K<sup>b</sup> on 2-D gels, demonstrated that K<sup>b</sup> is not the protein associated with Qa-1 (18). The *H-2K2<sup>k</sup>* gene is reportedly a pseudogene (36). The complete sequence is not available for *H-2K2<sup>b</sup>*, and it remains possible that, unlike *K2<sup>k</sup>*, the *K2<sup>b</sup>* allele may encode a protein capable of forming a heterodimer with Qa-1. It is not uncommon for a class I gene to be a pseudogene in one haplotype but not in another (i.e., *T22*). On the other hand, *Qsm* may not be a novel class I molecule. Investigations (37) have demonstrated that class I molecules are capable of interacting with different hormone receptors, perhaps influencing the function of these proteins. Alternatively, *Qsm* may encode a chaperone involved in Qa-1 assembly, which in some instances is transported to the cell surface with Qa-1 instead of dissociating before exiting the ER.

A second question addresses the biological significance or

benefit of heterodimers formed between distinct class I molecules. A recent investigation by Capps et al. (38) demonstrated that H-2L<sup>d</sup>, H-2D<sup>b</sup>, and H-2D<sup>d</sup> H chains can form disulfide-linked homodimers in vivo via a conserved cytoplasmic domain cysteine (position 340) (38). These homodimers were not associated with  $\beta_2m$  and, therefore, were unreactive with certain  $\alpha 1$  and  $\alpha 2$  domain-specific antibodies. In  $\beta_2m^-$  cells, these homodimers formed immediately after biosynthesis of the class I H chain and were Endo H sensitive. Approximately 40–60% of class I molecules expressed on the surface of the  $\beta_2m^-$  cells examined were homodimers, the rest being free H chains. In contrast, homodimer formation in  $\beta_2m^+$  cells did not occur until after a 1-h chase, at which time the class I molecules were Endo H resistant. Homodimer formation could be prevented with addition of exogenous  $\beta_2m$ . The authors suggested that formation of homodimers between free, unassembled class I H chains may inhibit these molecules from binding and presenting extracellular peptides to T cells, thereby preventing inappropriate cell lysis and subsequent autoimmune-type reactions (38). It is possible that, like the class I homodimers described above, the Qa-1/L<sup>d</sup>, Qa-1/Qsm, and L<sup>d</sup>/Qsm heterodimers may be composed of H chains that fail to assemble properly with  $\beta_2m$  and peptide. The anti-T23c serum precipitates a greater amount of Qa-1 on the cell surface compared with the Qa-1.2 alloantiserum due to its ability to recognize Qa-1/L<sup>d</sup> and Qa-1/Qsm heterodimers (18). Therefore, one would expect to see more  $\beta_2m$  in the anti-T23c precipitates than in the anti-Qa-1.2 precipitates. However, the amount of  $\beta_2m$  coprecipitated with Qa-1 was the same for each antibody (Fig. 1 of ref. 18 and our unpublished data). This implies that Qa-1 molecules associated with L<sup>d</sup> and Qsm may not be  $\beta_2m$  associated. The same may be true for the L<sup>d</sup> molecules participating in heterodimer formation. The ability to precipitate the L<sup>d</sup>/Qa-1 and L<sup>d</sup>/Qsm heterodimers with 64-3-7s that only detects unfolded L<sup>d</sup> molecules weakly associated with  $\beta_2m$ , coupled with an inability to precipitate the heterodimers with 30-5-7s that only recognizes L<sup>d</sup> molecules associated with  $\beta_2m$  and peptide, implies that the L<sup>d</sup> molecules involved may not be  $\beta_2m$  associated. If this is true, then these heterodimers also would be predicted to be incapable of presenting endogenous peptide to T cells (39–42).

Heterodimer formation may be a means to keep unassembled Qa-1 and L<sup>d</sup> H chains (and possibly *Qsm*) that escape the retention mechanisms of chaperones from binding exogenous peptides and thereby initiating inappropriate lysis by T cells, as was suggested for class I homodimer formation (38). An alternative conclusion can be drawn by taking into consideration the apparent specificity of Qa-1 heterodimer formation. If nonspecific dimerization of class I molecules is merely a means to eliminate free H chains on the cell surface, then it is curious why Qa-1 preferentially dimerizes with only L<sup>d</sup> or *Qsm* and does not homodimerize or associate with any other known protein. Qa-1 lacks the conserved cysteine at position 340 shown to be important for class I homodimerization. However, there are two other cysteines unique to Qa-1, one of which is located in the cytoplasmic

domain (position 319) and might be available to form disulfide-bonded homodimers (15). Qa-1 does not heterodimerize detectably with any protein in mouse strains that are negative for either  $L^d$  or  $Qsm$ , such as in the  $H-2^k$  haplotype. There is less Qa-1 detectable by immunoprecipitation on the cell surface of  $k$  haplotype mouse strains (our unpublished observations), implying that either unassembled Qa-1 H chains do not make it out to the cell surface and, therefore, are dealt with in another way, or that free Qa-1 H chains are expressed but are so unstable that they are rapidly degraded during the 15-min labeling period. The apparent specificity of Qa-1 heterodimer formation may be due to striking similarities to  $L^d$ . Both exhibit weakened abilities to associate with  $\beta_2m$  and are expressed on the cell surface at low levels compared with most class I molecules (11, 16). The low cell surface expression of  $L^d$  has been attributed to the failure to be saturated with endogenous peptide ligands (43). Similarly, a limited peptide pool available for Qa-1 binding may explain the relatively low surface expression and instability of this class I-b antigen. If  $Qsm$  encodes a novel class I molecule, then one might expect it to be similar to Qa-1 and  $L^d$  in its peptide- and  $\beta_2m$ -binding properties. Therefore, an alternative explanation for class I heterodimerization may be that class I molecules that inefficiently associate with  $\beta_2m$  may physically associate with one another instead. This physical interaction

may circumvent the necessity for at least one or both of the molecules to be bound to  $\beta_2m$  and/or associated with peptide and allow their cell surface expression. Once on the cell surface, these class I molecules may be free to interact with effector molecules on other cells, or their expression could be stabilized by exogenous peptide. The latter circumstance could suggest that Qa-1 and  $L^d$  may both be involved in the immune response against particular bacterial pathogens. Class I molecules can bind and present exogenous peptides, and it is possible that extracellular peptides of appropriate length derived from bacteria are capable of loading class I molecules exogenously.

The ability of heterodimerization to influence T cell recognition of Qa-1 and/or  $L^d$  molecules on the cell surface remains to be determined. Differential T cell recognition of Qa-1 has been described and shown to be under the control of  $Qdm$  (20). Evidence now exists suggesting the  $Qdm^+$  epitope results from Qa-1 presenting a peptide derived from  $H-2L^d$  or  $H-2D^{b,d}$  but not  $H-2D^k$  (21). This observation coupled with our data document two mechanisms by which  $H-2L^d$  influences the expression of Qa-1. Delineation of the nature of the  $L^d$ /Qa-1 dimer association and the  $L^d$  peptide bound to Qa-1 will provide clues to the biological function of Qa-1.

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We thank Drs. C. Aldrich, J. Forman, and T. Hansen for mice and reagents. We are grateful to Drs. H.-G. Ljunggren, R. Rich, J. Rodgers, and J. Vyas for critical reviews of the manuscript. We also thank E. Chapman for assistance in preparation of the manuscript.

This work was supported in part by National Institutes of Health grant AI-17897.

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Received for publication 8 July 1994 and in revised form 9 September 1994.

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