The Class I-b Molecule Qa-1 Forms Heterodimers with H-2Ld and a Novel 50-kD Glycoprotein Encoded Centromeric to I-Eβ

By Paula R. Wolf and Richard G. Cook

From the Department of Microbiology and Immunology, Baylor College of Medicine, Houston, Texas 77030

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-2b and H-2d mouse strains. We now demonstrate that the 50-kD protein coprecipitated with Qa-1 is the class I-a antigen Ld in all H-2Ld-positive mouse strains examined. Further analyses of a panel of recombinants revealed that the 50-kD protein coprecipitated with Qa-1 in H-2b haplotype mouse strains is encoded or controlled by a gene centromeric to major histocompatibility complex class II I-Eβ. We have designated this gene and corresponding protein product as Qsm, Qa-1 structure modifier. Both Ld and Qsm can interact with Qa-1 to form cell surface–expressed heterodimers in vivo. These Qa-1 heterodimers are not expressed in H-2k haplotype cells. The Qa-1/Ld 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 Ld as well, and that the Ld molecules participating in these interactions with Qa-1 and Qsm may be devoid of β2-microglobulin and/or peptide. These data represent the first demonstration that class I molecules can be expressed as heterodimers (Qa-1/Ld) 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 Ld. 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 Ld molecules.

Stable cell surface expression of a class I molecule is dependent on association of the H chain with both β2-microglobulin (β2m) and peptide. Abnormally low levels of class I molecules reach the surface of β2m-deficient cells, indicating that β2m 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/β2m 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 β2m varies among class I allomorphs, as does the rate of β2m association. Both H-2Ld and H-2Dd are capable of being transported to the cell surface without β2m, whereas other class I molecules generally are not detected in β2m-deficient cells (10-12). Both properly folded (conformed) as well as unfolded (nonconformed) Dβ H chains are expressed on the cell surface of β2m- murine cell lines, such as RIE-Dβ (10). In the same manner, two distinct antigenic forms of Ld distinguishable by mAbs can be found both intracellularly and at the surface of normal cells. Confirmed Ld (30-5-7s reactive) is both β2m and peptide bound, whereas alternative Ld (Ldalt; 64-3-7s reactive) is only weakly β2m associated and not bound to peptide (12-14). Thus, although some class I molecules are expressed at the cell surface in the absence of β2m 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 β2m (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).

Abbreviations used in this paper: anti-T23c, antipeptide serum directed against the cytoplasmic tail of T23; β2m, β2-microglobulin; B6, C57BL/6; CAB, Con A-activated T cell blasts; 1-D, 2-D, one and two dimensional, respectively; ER, endoplasmic reticulum; Ldalt, alternative form of Ld; 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 β2m 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-2Dk (Qdm) mouse strains could not be recognized by Qdm-dependent CTL, whereas target cells expressing Qa-1 from all other haplotype mouse strains (non-H-2Dk, Qdm*) were recognized. Qdm-independent CTL were able to lyse Qa-1-expressing cells from all haplotypes (20). Data now suggest that the Qdm+ epitope results from Qa-1 presenting a peptide derived from H-2Lδ or H-2Dkδ but not H-2Dk (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 γ/δ T cells (23) and α/β (24) T cells 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-1δ mouse strains as T23 revealed an additional molecular mass species (50 kD) that coprecipitated with the 48-kD Qa-1δ molecule with an antipeptide serum directed toward the cytoplasmic tail of T23 (anti-T23c) (18). This 50-kD protein, present in both H-2A and H-2D 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-2b) 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.Tla and B6.K1 mouse strains were bred in our mouse colony at Baylor College of Medicine. C3H.L1 transgenics, B6.AK1, BALB/c-H2mice, 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 spleen 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 (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-2Lδ mAb 28-14-8s was obtained from American Type Culture Collection (Rockville, MD). The H-2Lδ mAb 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, 5 x 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 μCi of [35S]methionine (Amersham Corp., Arlington Heights, IL). At the end of the labeling period, cold methionine-containing medium (supplemented with 10% FCS) was added to stop the labeling reaction. Cells were then 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 x 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 x 10^6 cells were labeled in PBS with 2.5 μCi [125I] (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 μg/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 (12 x 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 H2O. 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 radiography or autoradiography.

Peptide Mapping. Anti-T23c and anti-Qa-1.2 immunoprecipitates from [35S]methionine-labeled B6 lysates were separated on 1-D SDS-PAGE gels under reducing conditions. The gels were dried and analyzed by fluorography or autoradiography.
SDS Treatment of Cell Lysates. Iodinated B6 and BALB/c CAB were lysed in 0.2% NP-40 (50 × 10⁶ 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 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-2kd 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 [35S]methionine; the 50-kD species could be detected only after a 1-h chase in B6 (H-2b). 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 (≈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 oligosaccharides (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-2b). Therefore, we compared the expression of the alternative molecular mass anti-T23c-reactive proteins in H-2k, H-2d, and H-2k mouse strains by both 1-D and 2-D gels. Immunoprecipitation with the anti-T23c serum from lysates of 129i-surface labeled H-2b (B6, A.BY) and H-2d (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-2d (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-2d, H-2d, and H-2d haplotype cells after a 30-min pulse with [35S]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-2d and H-2d haplotype strains but not in any of the three H-2d strains analyzed (data not shown).

Although a 50-kD protein was precipitated with the anti-T23c serum from both H-2d and H-2d 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-2d haplotype mouse strains with those from two H-2d haplotype strains. The 50-kD species from b haplotype strains exhibited 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-2d strains and the difference in pl 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.

Ld Physically Associates with Qa-1 on the Surface of H-2d Haplotype Cells, Resulting in the Coprecipitation of Qa-1 and Ld with the Anti-T23c Serum. H-2d strains have been reported to express lower levels of Qa-1b when analyzed with alloantisera and CTL; this effect was localized to the H-2d 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 mCTL (Qdm-dependent) failed to lyse targets from H-2d/Qa-1b strains but did lyse targets from all other Qa-1b strains regardless of H-2 genotype. H-2d/Qa-1b strains were designated Qdm+ and all other Qa-1b strains as Qdm− (20). Aldrich et al. (21) now have data to suggest that the Qdm+ epitope results from presentation of an H-2Ld-derived peptide by T23-encoded Qa-1. The C3H strain is Qdm+ and, therefore, cannot be lysed by Qdm-dependent mCTL. However, targets from C3H mice expressing an H-2Ld transgene (designated as C3HLd transgenic) are recognized and lysed by Qdm-dependent mCTL (21). Thus, the Qdm+ epitope can be restored to the Qdm− strain C3H by H-2Ld.

Because Qa-1b/H-2d (Qdm+) 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/Ld was involved in the expression of the higher molecular mass species. CAB from C3H and C3HLd 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 C3HLd transgenic mouse cells (Fig. 3 A). Analysis of the 50-kD protein from C3HLd on 2-D gels revealed the same IEF pattern as seen for the previously examined H-2d mouse strains (Fig. 3 B). Surprisingly, the 50-kD species precipitated with anti-T23c also was biochemically indistinguishable from Ld-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 Ld antigen coprecipitated with Qa-1. Analysis of a panel of recombinant mouse strains positive for Ld but differing in haplotype at other MHC loci yielded consistent results. The C3HLd-transgenic mice expressed more Ld associated with Qa-1 than any mouse strain examined, which is consistent with the higher level of total Ld 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<sup>d</sup> 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>lem</sup> (H-2<sup>d</sup>), which does not express L<sup>d</sup> due to a mutation in the H-2L<sup>d</sup> gene (Fig. 3A) (35). The presence of L<sup>d</sup> in anti-T23 immunoprecipitates is not due to crossreactivity of the antiserum with L<sup>d</sup>, as demonstrated by the failure to precipitate L<sup>d</sup> from T23<sup>b</sup>, L<sup>d</sup> spleen cells (see Fig. 8) and from L<sup>d</sup>-transfected fibroblasts (data not shown). Thus, these data indicate that L<sup>d</sup> associates with Qa-1 to form a heterodimer expressed on the cell surface.

Not all Qa-1 and L<sup>d</sup> 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<sup>d</sup> 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<sup>d</sup> 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<sup>d</sup> panels). For this reason, Qa-1 is not visible in the anti-L<sup>d</sup> precipitates. Overexposures were too complex to define the presence of Qa-1. To determine if the anti-L<sup>d</sup> mAb were capable of reacting with the Qa-1/L<sup>d</sup> heterodimer and to characterize the form of L<sup>d</sup> involved in this interaction, lysates were precleared with each of the L<sup>d</sup> mAbs and then immunoprecipitated with anti-T23c. Based on these immunodepletion experiments, the 28-14-8s and 64-3-7s anti-L<sup>d</sup> mAb reacted with the Qa-1/L<sup>d</sup> heterodimer, removing L<sup>d</sup> 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-2b versus H-2k haplotype mouse strains. B6, AKR, C3H, and CBA CAB 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 La in expression of the 50-kD protein coprecipitated with Qa-1. CAB from C3H, C3H.La transgenics, and BALB/c-H-2dm2 mice were iodinated and lysates immunoprecipitated with anti-T23c, 28-14-8s (La and Laalt), 30-5-7s (La), or 64-3-7s (Laalt), 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-2dm2. (B) The 2-D gel comparison of the anti-T23c and 28-14-8s precipitates from C3H.La-transgenic mice. The downward arrowhead in both panels denotes La, 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>2</sup> | Qa-1/Qsm |
| C57BL6/J, A.BY  | b | b | 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>a</sup> | k | k | k | k | k | k | k | k | k | - | k | k | k | - | - | - | - | - |
| A.Tla<sup>b</sup> | k | k | k | k | k | k | k | k | k | - | k | k | - | - | - | - | - | - |
| A.TL<sup>c</sup> | k | k | k | k | k | k | k | k | k | - | k | k | - | - | - | - | - | - |
| C3H.L<sup>d</sup> | k | k | k | k | k | k | k | k | k | - | k | k | - | - | - | - | - | - |
| B6.K1           | b | b | b | b | b | b | b | b | b | b | - | + | k | k | - | - | - | - |
| C3H.OH          | d | d | d | d | d | d | d | d | d | d | - | k | k | - | - | - | - | - |
| B6.AK1          | b | b | b | b | b | b | b | b | b | b | - | + | b | b | - | - | - | - |
| B10.A(2R)       | k | k | k | k | k | k | k | k | d | + | k | k | + | - | - | - | - | - |
| 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 | d | a | a | - | - | - | - | - |
| B10.D2(R107)    | b | b | b | b | b | b | b | b | b | b | ? | ? | ? | d | d | ? | d | d | + |

MHC allele designations adapted from references 32-34.

28-14-8s is conformation independent (α3-domain reactive), mAb 64-3-7s recognizes L<sup>alt</sup> molecules (does not bind peptide and is only weakly associated with β<sub>m</sub>), and mAb 30-5-7s recognizes conformed L<sup>d</sup> molecules (both peptide and β<sub>m</sub> associated). Thus, the L<sup>alt</sup> 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/β<sub>m</sub>/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 [35S]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 pl 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 when 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^d CAB were pulsed with [3S]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^d 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^h haplotype. The downward arrowhead in C3H.L^d, 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^a and H-2^b haplotype mouse strains. B6 and BALB/c CAB were iodinated, and the resulting lysates boiled in the presence (+) 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.
The digested samples were then analyzed on 1-D gels. Lanes 1, 4, and 6 represent the anti-T23c-precipitated 50-kD protein. Lanes 2, 5, and 8 represent the anti-T23c-precipitated 48-kD Qa-1 molecule. Lanes I, 4, and 2 #g/ml of V8 protease or Lys C at 37°C for 45 min or left untreated. The eluted proteins were digested with 2 μg/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.

The recombinant B10.D2(R107) is positive for both Ld and Qsm (b haplotype to the left of I-Eδ) and, therefore, has the potential to express Qa-1 associated with either Ld, 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 Ld, 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, Ld 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 Ld precipitates from the Qsm−transgenic mice C3H.Ld (Fig. 7 B). Consistent results were obtained with an additional recombinant, B10.A(3R), which is similar to R107 but is T23 − (Table 1). Both 28-14-8s and 64-3-7s mAbs coprecipitated Qsm with Ld from iodinated 3R lysates, whereas only Ld alone was detected in the Qsm−mouse strain BALB/c (Fig. 7 C); thus, the interaction of Qsm with Ld can be independent of Qa-1 expression. Therefore, an unidentified gene designated Qsm and located to the left of I-Eβ in H-2b haplotype mice controls expression of a protein that can form a heterodimer with Qa-1 or Ld 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/Ld and Qa-1/Qsm heterodimers.

As discussed earlier, the Ldalt but not conformed Ld molecule formed heterodimers with Qa-1. To determine the antigenic conformation of the Ld 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 Ld precipitated with 30-5-7s differed from that of the Qsm-associated Ld molecule detected with either 64-3-7s or 28-14-8s, as well as from that of the Qa-1-associated Ld molecule. These data indicate that Ldalt rather than conformed Ld participates in heterodimer formation with Qsm and Qa-1.

Heterodimer Formation Between Qa-1 and Ld or Qa-1 and Qsm Occurs In Vivo and Is Not Due to Cross-Reactivity. To determine whether the Qa-1/Ld/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, Ld was not coprecipitated with Qa-1 when AKR (T23+Ld+) and A/J (T23−Ld+) cells were mixed. Similarly, Qsm was not detected when lysates of B10.A(4R) (T23−Qsm−) and B10.A(3R) (T23−Qsm−) were analyzed (Fig. 8). In addition, these data document that the presence of Ld and Qsm in anti-T23c precipitates from T23−Ld− or T23−Qsm− strains is not due to cross-reactivities of the antisera for Ld or Qsm. Ld molecules were not immunoprecipitated from the A/J plus AKR lysate mixture (or A/J alone, not shown), which contains Ld. Also, Qsm was not immunoprecipitated from the 4R plus 3R lysate mixture (or 3R alone, not shown) which has the Qsm−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 Ld in all H-2Ld+ mouse strains examined. In addition, an unidentified protein encoded centromeric to the MHC class II region in H-2b haplotype mice also formed a cell surface–expressed hetero-

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. 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 μg/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.
Figure 7. Formation of heterodimers between Qa-1, Qsm, and Ld 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.Ld 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 Ld; the upward arrowhead denotes Qa-1. (C) 2-D gel analysis of anti-Ld (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+, Ld+) and AKR mixed with A/J (T23+, Ld+). Shown in the righthand panels are B10.A(4R) (T23+, Qsm-) and 4R mixed with B10.A(3R) (T23-, Qsm+).
dimer with either Qa-1 or Ld or even potentially a heterotrimer with both.

The 50-kD proteins coprecipitated with Qa-1 from all H-2Ld+ strains, including the C3H.Ld+ transgenic mice, were biochemically indistinguishable from Ld when compared on 2-D gels. Furthermore, a 50-kD molecule was not detected in the mutant mouse BALB/c-H-2k, which does not express Ld (35). The ability to dissociate Qa-1 from Ld 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-2b 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 Ld. The gene responsible for this protein was mapped to the left of the MHC class I-Eβ 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 was also found to heterodimerize with Ld 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-2Kb and H-2Kd loci are the only class I genes known to exist centromeric to class II. Our previous experiments, comparing Qa-1 with Kb on 2-D gels, demonstrated that Kb is not the protein associated with Qa-1 (18). The H-2Kb gene is reportedly a pseudogene (36). The complete sequence is not available for H-2Kd, and it remains possible that, unlike Kd, the Kd 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-2Ld, H-2Db, and H-2Da H chains can form disulfide-linked homodimers in vivo via a conserved cytoplasmic domain cysteine (position 340) (38). These homodimers were not associated with βm and, therefore, were unreactive with certain α1 and α2 domain-specific antibodies. In βm− 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 βm− cells examined were homodimers, the rest being free H chains. In contrast, homodimer formation in βm+ 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 βm. The authors suggested that formation of homodimers between free, un assembled 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/Ld, Qa-1/Qsm, and Ld/Qsm heterodimers may be composed of H chains that fail to assemble properly with βm 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/Ld and Qa-1/Qsm heterodimers (18). Therefore, one would expect to see more βm in the anti-T23c precipitates than in the anti-Qa-1.2 precipitates. However, the amount of βm 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 Ld and Qsm may not be βm associated. The same may be true for the Ld molecules participating in heterodimer formation. The ability to precipitate the Ld/Qa-1 and Ld/Qsm heterodimers with 64-3-7s that only detects unfolded Ld molecules weakly associated with βm, coupled with an inability to precipitate the heterodimers with 30-5-7s that only recognizes Ld molecules associated with βm and peptide, implies that the Ld molecules involved may not be βm 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 un assembled Qa-1 and Ld 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 Ld 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 expression of L\(d\) has been attributed to the failure to be saturated with the 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-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-2\(^L\) or H-2\(^D\) but not H-2\(^D\) (21). This observation coupled with our data document two mechanisms by which H-2\(^L\) 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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Address correspondence to Dr. Richard G. Cook, Department of Microbiology and Immunology, Baylor College of Medicine, One Baylor Plaza, Houston, TX 77030. Dr. P. R. Wolf's present address is Center for Cancer Research, Massachusetts Institute of Technology, Cambridge, MA 02139.

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