Qa-2, H-2K, AND H-2D ALLOANTIGENS EVOLVED FROM A COMMON ANCESTRAL GENE*

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The murine 17th chromosome contains two regions of particular interest to biologists, the T/t complex and the major histocompatibility complex (MHC).[1] The T/t complex controls a number of loci that have important functions in embryonic development (1); the MHC encodes gene products that are involved in cellular interactions critical for immune function (2). A third region, Tla, located telomeric to H-$2D$ (3), has recently been described. The Tla region controls the expression of several serologically defined lymphoid cell surface alloantigens termed TL and Qa antigens (3). At present, five Qa antigens (Qa-1–5) have been described and the expression of these antigens appears to be confined primarily to hematopoietic cells (4–7). Earlier studies (3–12) indicated that Qa antigens were found predominantly on T cells but that Qa-1, Qa-2, Qa-3, and Qa-4 are expressed on mitogen-stimulated B cells as well. More recent studies have demonstrated that Qa-2 is expressed on a variety of hematopoietic cells, including subsets of B cells, myeloid progenitor cells, and multipotential stem cells (13). In addition, Qa-4 and Qa-5 antigens are expressed on natural killer cells (14).

Antisera recognizing Qa-1 or Qa-2 antigens define cell surface glycoproteins of 44,000 mol wt that are noncovalently associated with β-2 microglobulin (15, 16). Thus, Qa-1 and Qa-2 may be related to H-2 alloantigens at the primary structural level.

In the present report, we describe studies of the Qa-2 antigen at the primary structural level. Isotopically labeled Qa-2, H-2K, and H-2D antigens from lysates of mitogen-stimulated C57BL/6 spleen cells were immunoprecipitated with congenic alloantisera. Comparative mapping of the tryptic peptides obtained from Qa-2 and H-2 molecules indicate that these two molecules have different primary structures, but that there is considerable structural homology between them. The data suggest, therefore, that the genes encoding the Qa-2 and H-2 molecules have arisen from a common primordial gene.

[1] Abbreviations used in this paper: Con A, concanavalin A; FCS, fetal calf serum; GAMIg, goat anti-mouse immunoglobulin; HBSS, Hanks' balanced salt solution; HGG, human gammaglobulin; LPS, lipopolysaccharide; MHC, major histocompatibility complex; NMS, normal mouse serum; NP-40, nonidet P-40; PA, pyridine acetate; PHA, phytohemagglutinin; RAMIg, rabbit anti-mouse immunoglobulin; SDS-PAGE, sodium dodecyl sulfate-polyacrylamide gel electrophoresis; TBS, Tris-buffered saline; 2-ME, 2-mercaptoethanol.

Materials and Methods

Mice. Adult mice, 6–10 wk of age, were used in these experiments. C57BL/6 mice (B6) were purchased from The Jackson Laboratory, Bar Harbor, Maine. The B10.A and B6·K1 strains were inbred strains obtained from the colony maintained at The University of Texas Health Science Center at Dallas.

Antisera. Antisera reactive against Qa-2 were prepared by immunizing B6·K1 mice with B6 spleen and lymph node cells as described previously (6). The [B10.A (5R) × R III]F₁ α B10 antiserum (αD^b) was obtained from the Research Resources Branch of the National Institutes of Health, Bethesda, Md. All other anti H-2 alloantisera were prepared by immunizing the appropriate recipients with donor spleen and lymph node cells as described in Table I. Anti-H-2D vs. anti-H-2K activity was determined by immunoprecipitation analysis of lysates from spleens of appropriate recombinant mice. Rabbit anti-mouse immunoglobulin (RAMIg) and goat anti-mouse immunoglobulin (GAMIg) were prepared as described previously (17, 18). Normal mouse serum (NMS; Pel-Freeze Biologicals Inc., Rogers, Ariz.) was obtained from outbred mice.

Preparation and Labeling of Mitogen-activated Spleen Cells. Mouse spleens were teased into Hanks' balanced salt solution (HBSS) and single cell suspensions were prepared as described previously (19). Cell viability was assessed using trypan blue exclusion. Concanavalin A (Con A)-activated spleen cells were routinely prepared by resuspending 7.2 × 10^8 viable cells in 200 ml RPMI-1640 containing 5% fetal calf serum (FCS), 5 μg/ml Con A (ICN Nutritional Biochemicals, Cleveland, Ohio), 5 × 10^{-5} M 2-mercaptoethanol (2-ME), glutamine, and antibiotics. To obtain lipopolysaccharide (LPS)-activated cells, 3 × 10^8 viable spleen cells were resuspended in 150 ml RMPI-1640 containing 20% FCS, 5 × 10^{-5} M 2-ME, 2× nonessential amino acids and glutamine, 1 mM sodium pyruvate, 50 μg/ml LPS (*Salmonella typhosa* 0901; Difco Laboratories, Detroit, Mich.), and antibiotics. The cultures were then gassed with 10% CO_2 and incubated for 48 h at 37°C.

The mitogen-activated cells were harvested and labeled at 1 × 10^7 cells/ml for 8 h with ^{3}H (150 μCi/ml) or ^{14}C (15 μCi/ml) amino acids (New England Nuclear, Boston, Mass. or Amersham-Searle Corp., Arlington Heights, Ill.) as detailed elsewhere (20). Labeled cells were harvested by centrifugation, washed twice in Tris-buffered saline (TBS), pH 7.4, and lysed for 15 min at 4°C in 5–6 ml TBS containing 0.5% nonidet P-40 (NP-40; Gallard, Schlesinger, New York). Nuclei and debris were subsequently removed by centrifugation for 15 min at 2,000 *g*.

TABLE I

Description of H-2 Alloantisera

Antiserum designation	Recipient/donor combination	H-2 haplotypes*		Strains used for immunoprecipitation	H-2 alloantigen recognized
		I		I	
		K ABJEC SD		(K ABJEC SD)	
αD^b	Recipient: [B10.A(5R) × RIII]F₁	b bbkkd dd		B6	D^b
		r rrrrr rr		(b bbbbb bb)	
	Donor: B10	b bbbb<u>b bb</u>			
αK^b	Recipient: [B10.D2 × A/J]F₁	d ddddd dd		B6	K^b
		k kkkkd dd		(b bbbbb bb)	
	Donor: B10.A(5R)	<u>b bb</u>kkd dd			
αD^d	Recipient: B10.BR	k kkkkk kk		B10.A	D^d
				(k kkkkd dd)	
	Donor: B10.D2	<u>d ddddd dd</u>			
αK^k	Recipient: [BALB/cKh × B10.D2]F₁	d ddddd dd		B10.A	K^k
		d ddddd dd		(k kkkkd dd)	
	Donor: B10.BR	<u>k kkkkk kk</u>			

* Regions of potential reactivity are underlined.

Immunoprecipitation and Isolation of H-2K, H-2D, and Qa-2 Molecules.   Glycoproteins were isolated from NP-40 lysates of labeled cells by fractionation on Lentil-lectin Sepharose (20). Lentil-lectin was purified from *Lens culinaris* and coupled to Sepharose 4B as described previously (21). Lysates were applied to the lectin column in TBS containing 0.25% NP-40 and the nonadherent proteins were eluted in the same buffer. The adherent material was eluted with 0.3 M α-methylmannoside in TBS with 0.25% NP-40, and concentrated to ~5 ml by negative-pressure dialysis.

The concentrated glycoprotein pools were centrifuged at 10,000 g for 15 min before immunoprecipitation. Immunoglobulin was immunoprecipitated from the concentrated glycoprotein pool by incubation with RAMIg (300 μl/3 × 10^8 cells) for 15 min at 37°C and 30 min at 4°C. Immune complexes were removed by the addition of protein A-bearing Cowan I strain of *Staphylococcus aureus*, followed by incubation for 15 min at 37°C and 30 min at 4°C (22). Immunoglobulin-depleted glycoprotein pools were then depleted of "nonspecific" material by adding NMS (250 μl/3 × 10^8 cells) for 15 min at 37°C and 30 min at 4°C, followed by two treatments with *S. aureus.* "Precleared" glycoprotein pools were then reacted with the appropriate alloantisera at 37°C for 15 min and 4°C for 2–12 h. All alloantisera were titered so as to remove all reactive molecules. Immune complexes were removed by either immunoprecipitating them with GAMIg or by absorbing them to *S. aureus*. Immunoprecipitates or *S. aureus*-bound complexes were washed three times with TBS containing 0.1% sodium dodecyl sulfate (SDS), 0.25% NP-40, and 0.2% deoxycholate, followed by one wash with TBS. Immune complexes were eluted from the *S. aureus* or immune precipitates were dissolved by boiling for 2 min in electrophoresis sample buffer with 5% 2-ME. All insoluble material was removed by centrifugation at 10,000 g for 5 min. Samples were electrophoresed for 4–5 h on 12.5% polyacrylamide gels using the Laemmli discontinuous system as described previously (23). All gels were fractionated using a Savant gel crusher (Savant Instruments Inc., Hicksville, N. Y.). In some experiments, gels were fractionated directly into scintillation vials and then counted in Beckman EP cocktail (Beckman Instruments Inc., Fullerton, Calif.). Alternatively, the fractionated gels were incubated overnight in 0.01% SDS to allow the radiolabeled protein to elute from the gel matrix. Aliquots of each fraction were then removed and counted in scintillation cocktail. The appropriate fractions were pooled, gel pieces were removed by filtration through a 0.45-μ Millipore filter (Millipore Corp., Bedford, Mass.), and the samples were then lyophilized.

Preparation and Use of Sepharose-GAMIg.   GAMIg serum (10) was affinity-purified on Sepharose-mouse euglobulin and coupled at 1 mg/ml to cyanogen-activated Sepharose 4B (24).

Trypsin Digestion and Comparative Peptide Mapping.   The techniques described below have been detailed elsewhere (25, 26). Lyophilized H-2D, H-2K, or Qa-2 molecules were redissolved in 2–3 ml dH$_2$O containing 0.5–1.0 mg human gammaglobulin (HGG) as carrier and dialyzed against several changes of 0.1 M NH$_4$HCO$_3$, pH 8.0. The molecules to be compared (e.g., ^{3}H-Qa-2 and ^{14}C-H-2K^b) were then mixed at a ^{3}H:^{14}C ratio of 2–3:1 and relyophilized. The lyophilized samples were dissolved in 1.0 ml 0.1 M NH$_4$HCO$_3$, pH 8.0, and 20 μg TPCK-trypsin was added. After a 1-h incubation at 37°C, additional trypsin (100–200 μg) was added and the incubation was continued for 12–16 h at 37°C. The pH was then lowered by the addition of 2–3 drops of glacial acetic acid and the sample was lyophilized. Before chromatography, the peptides were solubilized in 2.0 ml 0.05 M pyridine acetate (PA), pH 3.13. Insoluble material was removed by centrifugation at 10,000 g for 5 min. Routinely 75–95% of the input cpm were recovered in the acid soluble fraction. Tryptic peptides were analyzed by cation exchange chromatography on Technicon chromobeads type P (Technicon T & T Corp., McFarland, Wis.), using a 3- × 150-mm microbore column maintained at 54°C by a water jacket. The solubilized peptides were applied to the column in 0.05 M PA, pH 3.13. After washing the column with 5–6 ml of starting buffer, a pH-ionic strength gradient was applied using a Varigard gradient maker (Phoenix Precision Instruments, Gardiner, N. Y.). 30 ml of each of the following PA buffers was used: 0.05 M, pH 3.13; 0.1 M, pH 3.54; 0.2 M, pH 4.02; 0.5 M, pH 4.50; 2.0 M, pH 5.0. After completion of the gradient, 2–3 ml of 2 N NaOH was applied to remove all bound material. 12 drop fractions were collected into minivials and allowed to evaporate. Water (0.35 ml) and scintillation fluid were added and the samples were counted. The ^{3}H and ^{14}C cpm were normalized and corrected for channel spillover.

Results

Isolation of Qa-2 and H-2 Molecules. The Qa-2 antigen is expressed on subpopulations of lymphocytes and the surface density is several-fold lower than H-2D or H-2K antigens, as determined by immunoprecipitation of radiolabeled molecules from cell lysates (15). Hence, it was important to obtain a population of lymphocytes that was enriched for Qa-2 antigens. Studies by Flaherty et al. (11) have demonstrated that 75–90% of the cells in populations of mitogen (Con A, phytohemagglutinin [PHA], LPS)-activated lymphocytes express serologically detectable Qa-2. Therefore, Con A-stimulated spleen cells were used for preparation of Qa-2 molecules. An SDS gel profile of a typical immunoprecipitate using extracts prepared from B6 Con A blasts is shown in Fig. 1A. As demonstrated earlier (15), B6·K1αB6 (αQa-2) serum recognizes a polypeptide of ~44,000 mol wt. β-2 microglobulin, which is known to be associated with Qa-2 molecules, was allowed to migrate off the gel under the electrophoretic conditions employed. When glycoproteins pools from B6·K1 (Qa-2⁻) Con A blasts were treated with αQa-2, no peak corresponding to 44,000 mol wt is precipitated, thus establishing the specificity of our antisera (Fig. 1B). When glycoproteins from B6 Con A blasts were precipitated with NMS, a significant amount of material migrating at 44,000 mol wt could also be detected. Thus radioactive material, presumably non-Qa-2 in nature, could contaminate our Qa-2 preparations and thereby interfere with subsequent structural analysis. Additional experiments demonstrated that the material that was nonspecifically precipitated could be significantly reduced by preincubating glycoprotein pools with NMS and *S. aureus*. Under the preclearing conditions, a maximum of 5–10% of the 44,000-mol wt peak could represent nonspecific contamination. Sears et al. (27) reported a similar problem in their structural studies of the H-2L alloantigen, and observed that nonspecific precipitation could be reduced by preincubating the lysate with NMS coupled to Sepharose.

H-2K or H-2D alloantigens were immunoprecipitated from mitogen-stimulated B10.A or B6 spleen cells. Preliminary experiments revealed that the preclearing regimen was applicable to the isolation of *H-2* gene products. The anti-H-2 sera used are complex and contain reactivities toward H-2K, D, and I region determinants (Table I). However, by selecting the appropriate recombinant congenic strain, specific
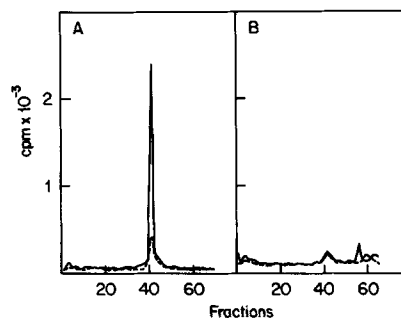

Fig. 1. SDS-PAGE analysis of an αQa-2 immunoprecipitate. Glycoprotein pools obtained from ³H-arginine labelled B6(A) or B6·K1(B) Con A-activated spleen cells were precleared with RAMIg and NMS as described in Materials and Methods. Aliquots (approximately 2 × 10⁷ cell equivalents) of the precleared glycoprotein pool were removed and incubated with 30 µl αQa-2 (———) or NMS (-----). Immunoprecipitates were formed by the addition of GAMIg and subsequently were washed, solubilized, reduced, and analyzed on 12.5% polyacrylamide gels.

for the K or D end antigens of the H-2 complex, specific immunoprecipitations can be obtained. For example, B10·D2αB10·BR will contain reactivity toward determinants carried by K^k, I-A^k, I-E/C^k, and D^k (Table I). By using B10.A (K^k, D^d) cells, reactivity is confined to the K end. Any I region-controlled molecules that are coprecipitated can be readily separated from H-2 by SDS-polyacrylamide gel electrophoresis (PAGE) (see Fig. 2A and B).

Do Qa-2, H2-K, and H2-D Alloantigens Exist as Separate Molecules in NP-40 Lysates? The next series of experiments addressed the question of whether the alloantigens detected by anti-Qa-2 or anti-H-2 sera in detergent lysates of Con A-treated B6 splenocytes exist as separate molecular species. The experimental design was to remove all Qa-2 molecules from the lysate and then determine if H-2K^b or H-2D^b molecules had been removed. Thus, glycoprotein pools from [^{3}H]arginine-labeled B6-Con A blasts were precleared as described in Materials and Methods. The precleared extracts (~10^7 cell equivalents) were then incubated with sufficient αQa-2 (controls received equivalent amounts of NMS) to ensure removal of all Qa-2 molecules. The immune complexes thus formed were removed by the addition of affinity-purified GAMIg coupled to Sepharose. This immunoabsorbant was used because αQa-2 antisera do not react with *S. aureus*. The Sepharose was removed by filtration and the sample was treated with appropriate alloantisera.

The results of these experiments are shown in Fig. 2. Under conditions in which 95-100% of the Qa-2 was removed (Fig. 2E and F), there was no removal of immunoprecipitable H-2D^b (Fig. 2C and D) or H-2K^b (Fig. 2A and B). This result
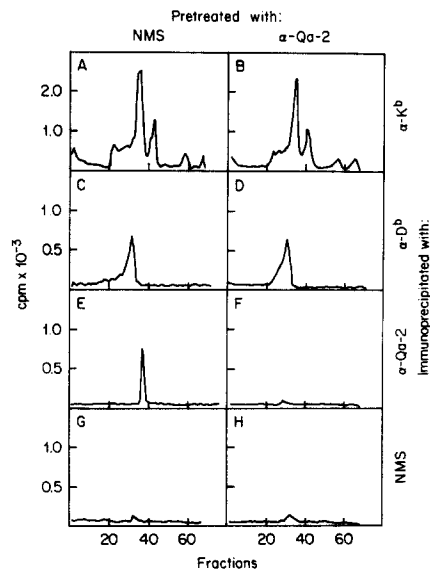

Fig. 2.   Effect of the removal of Qa-2 alloantigen on the subsequent isolation of H-2K^b or H-2D^b alloantigen. Precleared glycoprotein pools were obtained from a NP-40 lysate of [^{3}H]arginine-labeled B6 Con A blasts. Aliquots (~10^7 cell equivalents) were removed and incubated with 30 μl of αQa-2 or NMS. Affinity-purified GAMIg coupled to Sepharose was then added to remove any immune complexes that had formed. The Sepharose was then removed by filtration and the remaining sample was reacted with 30 μl αK^b (A and B), αD^b (C and D), αQa-2 (E and F), or NMS (G and H). Each sample was then precipitated with GAMIg and the immunoprecipitate was washed, solubilized, reduced, and analyzed by SDS-PAGE.

confirms earlier studies by Michaelson et al. (15) and indicates that the H-2K, H-2D, and Qa-2 alloantigens reside on distinct molecular species.

Tryptic Peptides of Isolated [³H]arginine-labeled Qa-2 Molecules. Qa-2 molecules labeled with [³H]- or [¹⁴C]arginine were isolated from B6-Con A blasts and digested with trypsin as described in Materials and Methods. Each digest was analyzed by cation exchange chromatography employing a PA pH-ionic strength gradient. Fig. 3 (top) shows a peptide map in which [³H]arginine- and [¹⁴C]arginine-labeled Qa-2 were co-analyzed. 11–13 arginine peptides can be reproducibly resolved when Qa-2 molecules are digested and analyzed. The last peak in each map represents material eluted from the column by 2 N NaOH. The amount of radioactivity recovered in this peak was variable and is not scored as a peptide in this analysis. Routinely 85–95% of the input radioactivity is soluble in the acidic (pH 3.13) starting buffer, and 85–95% of this acid soluble radioactivity is recovered from the column. Thus, the vast majority of arginine-labeled peptides are being detected by this analysis. It should be noted that the maps generated from [³H]arginine- or [¹⁴C]arginine-labeled Qa-2 are completely superimposable. This finding demonstrates the reproducibility of the "double-label" mapping technique. The advantage of performing a double-label analysis versus aligning pH gradients or internal markers in single-label experiments has been discussed previously (25).

The amount of radioactivity under the majority of the peaks is approximately equal throughout the map (Fig. 3, top). The simplest interpretation of this observation is that these peptides are derived from a single major molecular species. However, several minor peaks are observed. The αQa-2 serum used was produced by immunizing B6·K1 mice with B6 spleen and lymph node cells (6). Therefore, this serum is potentially polyvalent with reactivities toward Qa-2, Qa-3, Qa-4, and Qa-5 (13). Because reactivity toward Qa-4 and Qa-5 has only been observed using monoclonal
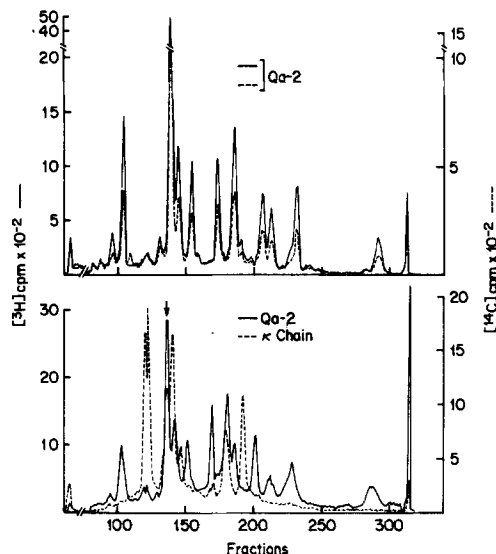

Fig. 3. Ion exchange chromatography of tryptic digests of Qa-2 alloantigen. Qa-2 alloantigen labeled with [³H]arginine was compared with [¹⁴C]arginine-labeled Qa-2 (top) or with a monoclonal κ chain labeled with [¹⁴C]arginine (bottom). The bold arrow in the bottom panel indicates where free arginine and the major Qa-2 peak co-elute.

antibodies (7), the minor peaks observed might be derived from a 44,000-mol wt polypeptide bearing the Qa-3 alloantigen. Alternatively, because the majority of these minor species are not always observed, some may be either incomplete trypsin digestion products or the result of occasional trypsin-mediated cleavages at tyrosine residues (28). Nevertheless, these minor species did not detract from our studies because only major peaks were scored in the comparisons. In addition, one should note the large peak eluting at fractions 135–140. We explored the possibility that this peak represents free arginine by mixing [^{3}H]arginine-labeled Qa-2 with free [^{14}C]-arginine, digesting with trypsin and analyzing the products by cation exchange chromatography. Free arginine co-chromatographed with the major Qa-2 peak (data not shown). This free arginine could have arisen from background contamination carried over during the purification procedure, or alternatively could be generated by the trypsin cleavage of adjacent arg-arg or lys-arg residues. The latter possibility seems most likely because amino acid sequence analysis of H-2K, H-2D, and H-2L alloantigens indicate several sites in which such cleavages could occur (29–32).

It is well documented that the technique of cation exchange chromatography used in our experiments is very sensitive for detecting structural differences (33–36). A single amino acid substitution in a given peptide can markedly alter its elution position from the column (34–36). Hence, structurally distinct polypeptides should show little peptide homology. Fig. 3 (bottom) shows a peptide map comparison of Qa-2 with a mouse Ig κ-chain isolated from the IgG2A$_κ$ secreted by P3 myeloma cells (37). None of the 12 Qa-2 peptides co-eluted with peptides derived from this κ-chain. The free arginine peptide is indicated by the arrow. This result confirms that structurally unrelated molecules show little or no homology at the level of comparative peptide mapping.

Comparison of the Arginine Tryptic Peptides of Qa-2 and H-2 Alloantigens. Fig. 4 shows a comparative peptide map analysis of Qa-2 and H-2K^b and H-2D^b molecules. Clearly, Qa-2 is structurally distinct from H-2K^b and D^b because numerous peptide differences are observed. However, there are several Qa-2 peptides that co-elute with peptides derived from H-2K^b or H-2D^b. Thus, 5/12 (42%) and 2/12 (17%) of the Qa-2 peptides co-eluted with peptides derived from H-2K^b and H-2D^b, respectively. This level of peptide homology is similar to that reported for various H-2K, H-2D, and H-2L alloantigens when their tryptic peptides were compared (26, 27, 38).

Because the Qa-2 and H-2 molecules compared above were isolated from the same mouse strain, it was of interest to determine whether Qa-2 molecules have demonstrable peptide homology with H-2 molecules from another haplotype. Fig. 5 shows the comparative peptide maps of Qa-2 and H-2K^k or H-2D^d. Again, considerable structural homology is observed with 4/12 (33%) and 5/12 (42%) Qa-2 peptides co-eluting with H-2K^k and H-2D^d peptides, respectively. A summary of the peptide map comparisons of Qa-2 vs. H-2 alloantigens is shown in Table II. It should be noted that the major Qa-2 peak, which we have demonstrated to be free arginine, is not included in the peptide homology comparison of this study.

The majority of the coincident peaks have similar ratios of ^{3}H:^{14}C. This is the expected observation if the peaks are structurally identical (33, 38). However a few coincident peaks differ markedly in their ^{3}H:^{14}C ratios (for example, Fig. 4, top fractions 210–215, and Fig. 5, top fractions 170–175). We are including these peaks in the comparisons because we cannot exclude the possibility that a given peak may
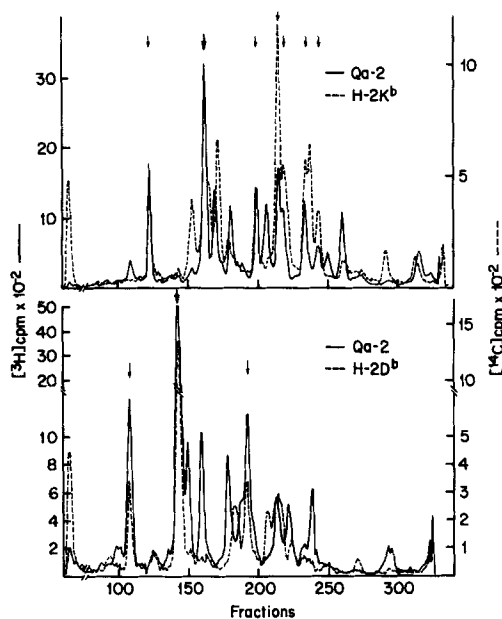
Fig. 4. Ion exchange chromatography of the arginine-labeled tryptic peptides from Qa-2 and H-2^b alloantigens. Qa-2 labeled with [^{3}H]arginine was compared to [^{14}C]arginine-labeled H-2K^b (top), or H-2D^b (bottom) isolated from B6-Con A blasts. The arrows indicate peptides that co-elute. The bold arrow marks the position of the major Qa-2 peak, which was identified as free arginine.

contain two or more peptides, one that is homologous, the other not. Indeed, the difference in the ^{3}H:^{14}C ratio suggests that this is the case.

A third *H-2* locus, *H-2L*, which shares public but not private specificities with H-2D alloantigens, has recently been described (39, 40). Hence, antisera used to immunoprecipitate H-2D molecules could also detect H-2L alloantigens. We believe that it is unlikely that this complexity affects comparisons between Qa-2 and H-2D antigens for the following reasons: (*a*) no H-2L^b alloantigen has yet been detected by immunoprecipitation (40); (*b*) the tryptic peptide map of H-2D^d (Fig. 5, bottom) is very similar to the maps reported by Sears et al. (27; Figs. 3 and 4) in which H-2D^d was immunoprecipitated using an antiserum directed against an H-2D^d private specificity; and (*c*) previous tryptic peptide maps of samples containing both H-2D^d and H-2L^d showed that the H-2L^d molecule represented only a very small fraction of the total radioactivity (38). We conclude therefore that our H-2D antisera are largely recognizing private H-2D^d or H-2D^b determinants. Any minor peaks that could represent H-2L alloantigens were not included in the comparison.

The tryptic peptide comparisons indicate that Qa-2 is structurally related to H-2K and H-2D alloantigens and that the degree of relatedness is equivalent to that observed among H-2 alloantigens (26, 27, 38).

Peptide Map Comparison of Qa-2 and H-2K^k Lysine-containing Peptides.   Fig. 6 shows a peptide map comparison of the lysine-labeled tryptic peptides derived from Qa-2 and H-2K^k. As observed with the arginine maps, Qa-2 is structurally distinct from H-2K^k. However, homologous peptides are again observed with 3/7 (43%) Qa-2 peptides co-eluting with H-2K^k-derived peptides.
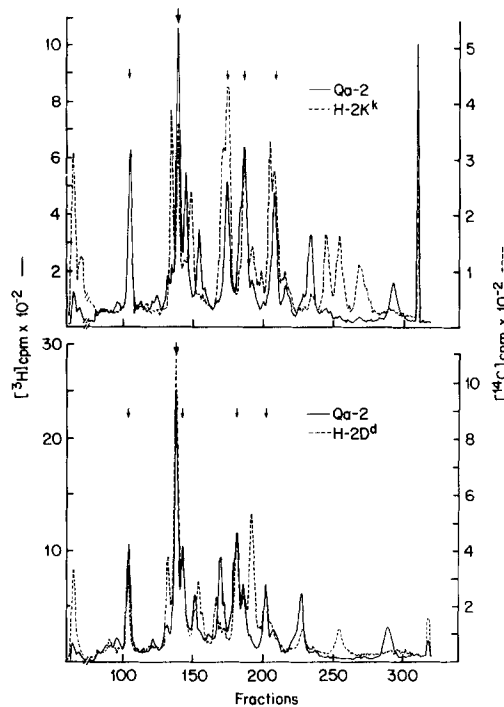
FIG. 5.   Ion exchange chromatography of the arginine-labeled tryptic peptides from Qa-2 and H-2^a alloantigens. Qa-2 labeled with [^{3}H]arginine was compared with [^{14}C]arginine-labeled H-2K^k (top) or H-2D^d (bottom) isolated from B10.A LPS blasts. The arrows indicate peptides that co-elute. The bold arrow marks the position of the major Qa-2 peak, which was identified as free arginine.

TABLE II

Average Peptide Homology between Qa-2 and H-2 Alloantigens

	H-2K^b	H-2D^b	H-2K^k	H-2D^d
Qa-2	$\frac{8}{24}$ (33)	$\frac{4}{19}$ (21)	$\frac{8}{27}$ (30)	$\frac{10}{23}$ (43)

* Percentage of homology (numbers in parentheses)

$$= \frac{2 \times \text{number of peptides in common}}{\text{number of Qa-2 peptides} + \text{number of H-2 peptides}}.$$

Discussion

The present results at the primary structural level indicate that the Qa-2, H-2K^b, and H-2D^b alloantigens isolated from C57BL6/J mice are sufficiently distinct to show that the Qa-2 alloantigen is encoded by a separate locus. In addition, there is sufficient structural homology between Qa-2 and H-2 alloantigens to prove that they evolved from a common primordial gene. Previous serological studies demonstrated that the strain and tissue distribution of Qa-2 antigens are different from any known H-2 antigens, thereby suggesting that Qa-2 and H-2 alloantigens are encoded by different loci. Genetic evidence has mapped the *Qa-2* locus to the *Tla* region, telomeric to *H-2D* (5). In addition, biochemical studies have shown that the determinants recognized by αQa-2 or αH-2 alloantisera reside on separate molecular species (15; Fig. 2). However,
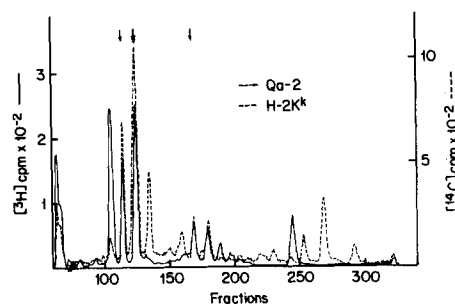
FIG. 6.   Ion exchange chromatography of the lysine-labeled tryptic peptides from Qa-2 and H-2K^k. Qa-2 labeled with [^{3}H]lysine was isolated from B6 Con A blasts and compared with [^{14}C]-lysine-labeled H-2K^k isolated from B10.A LPS blasts. The arrows indicate peptides that co-elute.

all of the above information cannot formally exclude the possibility that the *Qa-2* locus encodes for an enzyme that modifies an H-2 alloantigen post-translationally. Thus, when the tryptic peptides from Qa-2 and H-2 alloantigens are compared, considerable structural variation is detected. Such extensive differences are not due to differences in glycosylation, because glycopeptides are not resolved in our analysis (34). The possibility that other modifications, such as methylation or acetylation of amino acids, could account for the structural variation observed is highly unlikely because such extensive modifications are unprecedented (41, 42). We therefore conclude that the observed structural differences are due to variations in the amino acid sequence. This implies that the 44,000-mol wt Qa-2 polypeptide is encoded by a locus distinct from that encoding H-2K and H-2D.

The comparative peptide mapping of Qa-2 antigen with several H-2 alloantigens has shown that 21–43% of their arginine-labeled tryptic peptides are homologous (Table II). This degree of structural homology is also observed when various H-2K, H-2D, and H-2L alloantigens are compared (26, 27, 38). Because we have no information on the size of these homologous peptides, it is possible that this homology represents only a very restricted region(s) of the molecules. However, molecules showing such levels of peptide homology have been repeatedly demonstrated to exhibit 75–90% amino acid sequence homology (29–32). It is therefore possible that Qa-2 and H-2 alloantigens will show similar degrees of amino acid sequence homology. This is the first observation that there exist genes in the *Tla* region that show primary structural homology to H-2K and H-2D gene products.

Two general hypotheses have been developed to explain the presence of numerous structurally related loci on the 17th chromosome (43). The first postulates that these loci arose from duplications and subsequent divergence of a primordial gene. The alternative hypothesis states that these loci were always separate but the structural homologies are the result of convergent evolution due to common functional constraints. The finding that there are at least four loci encoding structurally related gene products (H-2D, -K, -L, and Qa-2) and additional loci encoding other potentially homologous proteins (TL, Qa-1, etc.) argues against convergent evolution and thereby makes the duplication hypothesis more attractive. The observation that there are cell surface structures resembling Qa and TL antigens on guinea pig and human lymphocytes suggests that the duplication event occurred before speciation (44–46).

The Qa-2 and H-2 alloantigens share several biochemical properties. For example,

all bind β-2 microglobulin, all are glycosylated, and all are membrane proteins (47). In this regard, our comparisons have detected two Qa-2 peptides that appear to have an H-2-derived homologue in all alleles and loci tested. Hence, some of the homologies observed may represent regions of the molecule involved in glycosylation, membrane attachment, or interactions with β-2 microglobulin. One would predict, therefore, that the Qa-1 and TL antigens would also share primary structural homology with H-2 because these proteins possess similar biochemical features (16, 18, 47).

There is abundant evidence that indicates that the *T/t* and *H-2* complexes contain a variety of loci that are implicated in cell-cell recognition. Our findings regarding Qa-2 alloantigen suggest that there is a cluster of genes in the *Tla* region that encodes gene products structurally resembling H-2 alloantigens. It is therefore tempting to speculate that Qa antigens may function on hematopoietic cells as immune recognition units similar to H-2K, H-2D, and H-2L. The recent finding that cytotoxic effector T cells can be generated against Qa-1 or Qa-2 alloantigens (48, 49) supports this idea.

Summary

The *Tla* region located on the murine 17th chromosome controls several serologically defined cell surface antigens. These antigens, referred to as Qa-1–5 and TL, are expressed on a variety of hematopoeitic cell populations. In the present studies we have immunoprecipitated isotopically labeled Qa-2 and H-2 molecules from mitogen-stimulated B6 spleen cells. Sequential immunoprecipitation experiments have shown that the determinants recognized by αQa-2, αH-2K^b, and αH-2D^b alloantisera reside on separate molecular species. Comparative mapping of the arginine-labeled tryptic peptides from Qa-2, H-2K^b, and H-2D^b molecules indicate that Qa-2 is structurally distinct but that there is considerable structural homology; 21–43% of the Qa-2 peptides co-chromatograph with peptides derived from H-2D^b and H-2K^b, respectively. Similar levels of homology are observed when Qa-2 is compared with H-2K^k or H-2D^d. The results show that the Qa-2 alloantigen is encoded by a locus separate from the loci encoding H-2K or H-2D alloantigens, but that the Qa-2, H-2K, and H-2D alloantigens are sufficiently related at the primary structural level to indicate that they evolved from a common primordial gene.

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