THE *QA2* SUBREGION CONTROLS THE EXPRESSION OF TWO ANTIGENS RECOGNIZED BY *H-2*-UNRESTRICTED CYTOTOXIC T CELLS*

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Tla is a region on the 17th chromosome located telomeric to the *D* region of *H-2* (1). This segment contains two subregions, *Qa2* and *Tla*. The *Tla* subregion has two loci, *Tla* and *Qa-1*, that control the expression of TL and Qa-1, respectively. TL is an antigen that is expressed on thymocytes and leukemias of T cell origin, whereas Qa-1 has a more widespread tissue distribution that includes thymocytes, peripheral T and B cells, and T and B cell lymphoblasts (1–3). In functional assays, it has been demonstrated that Qa-1 is expressed on two subpopulations of T lymphocytes involved in feedback suppression (4, 5).

The *Qa2* subregion controls the expression of four antigenic specificities, Qa-2, Qa-3, Qa-4, and Qa-5, each of which is principally detected on the membrane of lymphoid cells and/or their precursors (1, 6, 7). The Qa-2 antigen has a wide tissue distribution, including most normal T lymphocytes, a portion of thymocytes, T and B cell lymphoblasts, multipotential stem cells, progenitors of granulocytes/macrophages, and some natural killer (NK)[1] cells (6, 8–11). This molecule has been analyzed structurally and consists of a 40,000 mol wt heavy chain noncovalently associated with beta 2-microglobulin, making it similar in structure to class I H-2 antigens (12, 13). Further, peptide map analysis of the heavy chain indicates considerable structural homology between Qa-2 and H-2 (14).

Currently, the function of the genes in the *Qa2* subregion is not understood. Further, it is not known whether the *Qa2* subregion contains several nonpolymorphic loci that encode for individual Qa antigens, or alternatively, whether this region contains one gene with several alleles. To approach this question, we generated cytotoxic T lymphocytes (CTL) against antigens controlled by the *Qa2* subregion in an attempt to (*a*) determine whether Qa-2 functions in a manner similar to that of H-2 class I antigens and (*b*) to charactertize the genetic organization of this region.

We provide evidence that CTL can be generated against two different *Qa2* subregion-controlled antigens. One target antigen is (*a*) controlled by the *Qa-2* gene, (*b*) recognized by *H-2*-unrestricted effector cells, (*c*) detected with monoclonal anti-

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[1] *Abbreviations used in this paper:* Con A, concanavalin A; CTL, cytotoxic T lymphocytes; NK, natural killer; NMS, normal mouse serum.

Qa-2 antibody, and (*d*) is not the product of a polymorphic locus. A second target antigen is also described and is associated with the expression of the Qa-5 specificity.

Materials and Methods

Mice. All mice were obtained from our breeding colonies at The University of Texas Health Science Center at Dallas, Dallas, TX, and the New York State Department of Health, or were purchased from The Jackson Laboratory, Bar Harbor, ME. The B10.W (wild) strains of mice were kindly provided by Dr. Jan Klein, Max Planck Institute, Tubingen, Germany. The *H-2* and *Qa/Tla* genotypes of the strains used are listed in Table I.

Generation and Assay for CTL Activity. These methods have been described previously (15). Briefly, animals were primed in vivo with 30×10^6 Qa-2-incompatible spleen cells. After an interval of 3 wk to 6 mo, the spleens from the primed mice were removed, a single cell suspension made, and the cells cultured with irradiated stimulator spleen cells in vitro. After 5 d, the effector cells generated were tested for cytotoxic activity against ^{51}Cr-labeled concanavalin A (Con A) lymphoblast target cells. Net release of isotope represents percent release of isotope from target cells in the presence of immune cells minus the percent release of isotope from target cells in the presence of nonimmune cells (control release). Control release ranged from 15 to 30%, and the standard error from triplicate wells did not exceed 10%.

TABLE I

H-2 and Qa-1/Tla Genotypes, Qa2 Subregion Phenotypes

Strain	*H-2*	*Qa*					*Tla*
		1	2	3	4	5	
B6	*b*	*b*	+	+	+	+	*b*
B6.K1	*b*	*b*	+	+	+	+	*b*
B6.K2	*b*	*b*	+	+	+	+	*b*
B10	*b*	*b*	+	+	+	+	*b*
A.BY	*b*	*b*	+	+	+	+	*b*
BALB/cJ	*d*	*b*	+	+	+′*	−	*c*
BALB/cByJ	*d*	*b*	−	−	−	−	*c*
B10.D2	*d*	*b*	+	+	+′	−	*c*
DBA/2	*d*	*b*	+	+	+′	−	*c*
DBA/1	*q*	*b*	+	−	+′	−	*b*
SWR	*q*	*a*	+	−	+′	−	*a*
A/J	*a*	*a*	+	+	+′	−	*a*
A/Boy	*a*	*a*	+	+	+′	−	*a*
B10.A	*a*	*a*	+	+	+′	−	*a*
B10.M	*f*	*c*	−	−	·‡	·	*d*
B10.BR	*k*	*a*	−	−	−	−	*a*
A.SW	*s*	*b*	+	+	+	·	*b*
A.TL	*t1*	*b*	+	+	+′	−	*c*
B10.S	*s*	*b*	+	+	·	·	*b*
B10.STC77	*w14*	·	−	−	·	·	*c*′§
B10.KPB128	*w19*	·	+	+	·	·	*c*′
B10.KEA5	*w5*	·	+	+	·	·	*a*′
B10.SNA70	*w8*	·	+	+	·	·	*c*′
B10.CAA2	*w11*	·	−	−	·	·	*a*′
B10.STA12	*w13*	·	+	+	·	·	*a*′
B10.GAA37	*w21*	·	+	+	·	·	*b*′

Data taken from references 1, 21, 33, and this publication.

* Indicates weak reaction with serological typing reagent. Cells from all Qa-5^b strains show reduced reactivity with anti-Qa-4 (7).

‡ Not typed.

§ See Table IV for explanation.

CTL Competition Assay. This methodology has been described previously (16). Briefly, 10^6 effector and 10^4 labeled target cells were added to individual wells in microtiter plates together with unlabeled inhibitor cells. The number of unlabeled cells ranged from 10^5 to 1.5×10^6 cells per well.

Blocking of CTL Activity with Antibodies. Alloantisera and monoclonal antibodies or normal mouse sera (NMS) and myeloma proteins at varying concentrations were added to individual wells of microtiter plates together with target cells. After a 45-min incubation at 37°C, effector cells were added, and the cultures were incubated for an additional 4 h before harvesting.

Antisera. Anti-H-2^b and anti-H-2^d sera were produced by multiple inoculations of C57BL/10 spleen cells into B10.D2 recipients and vice-versa. Anti-TL sera were produced by immunization of (B6 × A.CA)F_1 mice with B6.Tla^a cells (anti-TL 5, 6) and (B6 × A.Tla^b)F_1 mice with ASL-1 tumor (H-2^a) cells (anti-TL 1,2,3,5,6). Anti-Qa sera was produced by immunizing B6.K1 mice with B6 lymphoid cells, as described previously (6). This sera has known activity against Qa-2 and Qa-3 and can potentially recognize Qa-4 and Qa-5. Anti-Qa-2 monoclonal antibody was produced by somatic cell hybridization according to the method of Galfre et al. (17). This antibody has a cytotoxic titer of $>10^6$ and was defined as anti-Qa-2 because it killed a higher percentage of peripheral T cells than monoclonal anti-Qa-4. Anti-Qa-5 monoclonal antibody has been described previously (7) and has a cytotoxic titer of >150.

Results

The CTL used in this study were generated against antigens controlled by the *Qa2* subregion. The strain distribution of the antigens controlled by this subregion are listed in Table I. Because there are no strains that dissociate Qa-2 and Qa-4, in this section we have tentatively designated these two specificities as defining the same antigen, Qa-2 (see Discussion). However, it should be kept in mind that these two specificities could each be controlled by a separate gene.

The Ability of Qa Congenic Strains to Generate CTL Activity. We previously demonstrated (15) that BALB/cByJ mice immunized in vivo and boosted in vitro with BALB/cJ spleen cells generate anti-Qa-2 CTL activity. Because these two sublines of BALB/c mice have been separated for >30 yr (18), it is possible that there are extensive genetic differences, in addition to that at the *Qa2* subregion, between these strains. Therefore, we used the recently developed congenic strains, B6.K1 and B6.K2, which differ at the *Qa2* subregion but not *Qa-1* or *Tla*, to generate anti-Qa CTL (6).

When B6.K1 mice were primed in vivo with B6.K2 spleen cells and then boosted in vitro with B6.K2 stimulator cells, no CTL activity was generated (data not shown). Because we have previously shown in the Qa-1 CTL system that B6.Tla^a mice are nonresponders when primed with Qa-1/Tla congenic spleen cells, but can be converted to responders by priming in vivo with Qa-1/Tla disparate noncongenic splenocytes (19), we decided to test the same approach in these experiments. Accordingly, B6.K1 mice were primed with A.BY cells in vivo. A.BY mice share the *H-2* and *Qa-1/Tla* complex with B6.K1 but differ with respect to Qa-2, Qa-3, and Qa-5 (see Table I) as well as at multiple minor H antigens. As demonstrated in Table II, B6.K1 animals primed in vivo and boosted in vitro with A.BY cells generate anti-Qa CTL activity (lines 1–5). It is not necessary to use A.BY stimulator cells for the in vitro cultures, as B6.K2 stimulator cells also generate anti-Qa CTL activity (lines 6–10). Further, B6.K2 stimulator cells generate as much CTL activity from B6.K1 spleen cell CTL precursors as do A.BY stimulator cells when tested against B6.K2 and BALB/cJ but not against A.BY target cells (lines 1, 3, 4, 6, 8, 9). In the case of the latter target cells, the B6.K1 anti-A.BY CTL also recognize minor H antigens on A.BY but not B6.K2 or BALB/cJ target cells (data not shown). Further, an alloan-

TABLE II

Ability of Qa2 Congenic Strains to Generate Anti-Qa CTL Responses

Line	Responder cells	Stimulator cells		Target cells	Net ^{51}Cr release, E:T		
		In vivo	In vitro		100	50	10
1	B6.K1	A.BY*	A.BY	A.BY	30	25	12
2				B6.K1	2	1	1
3				B6.K2	16	11	5
4				BALB/cJ	11	8	3
5				BALB/cByJ	3	2	2
6			B6.K2	A.BY	21	14	4
7				B6.K1	1	0	0
8				B6.K2	20	18	4
9				BALB/cJ	13	9	2
10				BALB/cByJ	4	1	1
11	B6.K1 ♀	B6.K2 ♂‡	B6.K2 ♂	BALB/cJ	23	14	4
12				BALB/cByJ	4	5	6
13				B6.K2	20	14	8

* B6.K1 animals were primed in vivo with sex-matched A.BY spleen cells before in vitro culture.

‡ B6.K1 ♀ animals were primed in vivo with ♂ B6.K2 spleen cells before in vitro culture. Target cells were from ♀ animals.

tigenic difference at H-Y also allows for successful priming of B6.K1 anti-Qa CTL (lines 11–13).

Therefore, immunization of B6 congenic pairs of mice differing at the *Qa2* subregion (above data) or Qa-1 (19) does not produce anti-Qa-2-5 or anti-Qa-1^b CTL, respectively. However, priming such mice in vivo with noncongenic cells followed by in vitro stimulation with Qa congenic cells permits specific cytotoxic activity to be generated. In this study, we refer to anti-Qa2 subregion effector cells as B6.K1 anti-A.BY/B6.K2, to reflect the immunizations received. The role of "helper" determinants on noncongenic cells that allow for in vivo priming of CTL.P has been addressed elsewhere (20).

We previously reported (15) that BALB/cJ mice immunized against BALB/cByJ cells did not produce CTL that recognized a Qa-2^- antigen. Similar results have been obtained using A.BY (Qa-2^+, 3^+, 5^+) animals primed in vivo and boosted in vitro with (non-Qa congenic) B6.K1 (Qa-2^-, 3^-, 5^-) spleen cells. No anti-Qa activity was generated, as determined by testing against BALB/cByJ (Qa-2^-, 3^-, 5^-) target cells (data not shown). Therefore, consistent with current serologic data, strains that are Qa-2^-, 3^-, 5^- do not express a *Qa2* subregion-controlled CTL antigen.

The Qa-3 Specificity Is Not Recognized by Anti-Qa CTL. Our prior data (15) indicated that BALB/cByJ (2^-, 3^-, 5^-) anti-BALB/cJ (2^+, 3^+, 5^-) effector cells were able to lyse DBA/1 (Qa-2^+, 3^-, 5^-) target cells, demonstrating that Qa-2 is a target antigen. However, that data did not exclude the possibility that Qa-3 could also be recognized by anti-Qa CTL. To address this issue, B6.K1 animals were sensitized against Qa antigens by priming with A.BY spleen cells in vivo, followed by boosting with B6.K2 in vitro and tested for cytolytic activity against BALB/cJ targets, which express Qa-2,3. The data presented in Fig. 1 show that these effector cells cause lysis of BALB/cJ targets, as expected (net release at 100:1 ratio is 42%). To determine whether Qa-3 is a target antigen recognized by these effector cells, we asked whether DBA/1 (Qa-2)

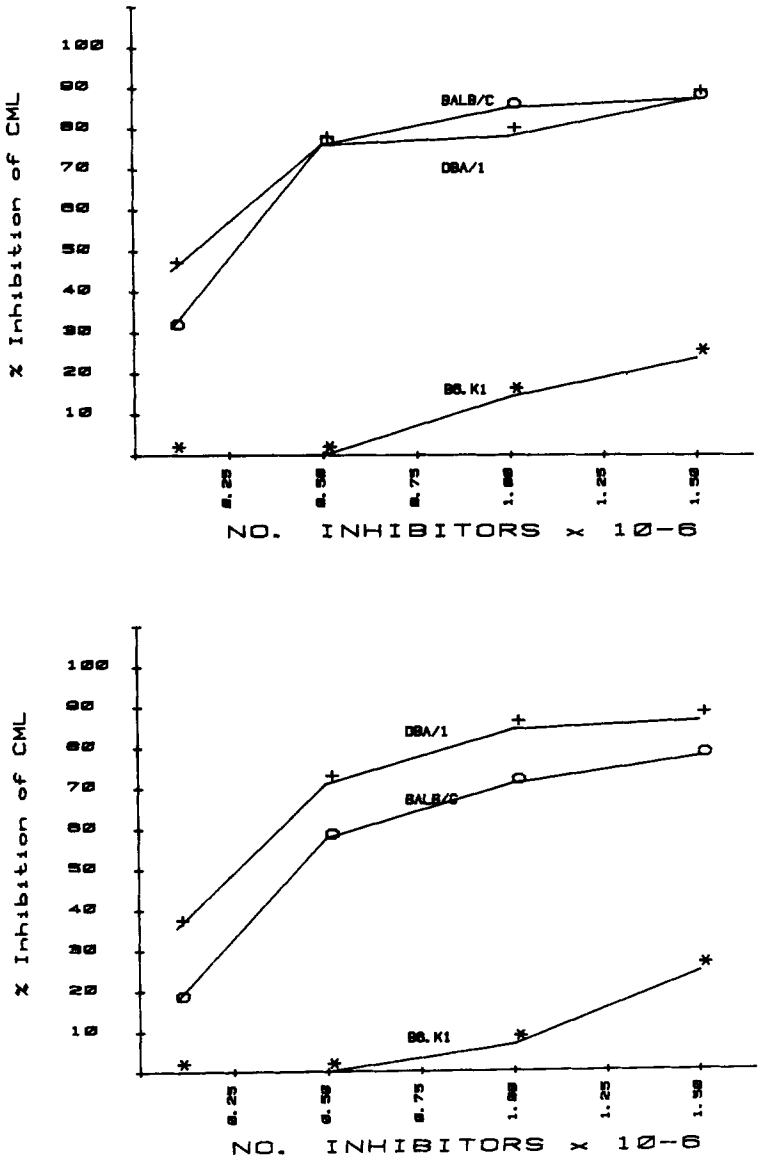

FIG. 1. Qa-3 is not a target antigen for anti-Qa CTL. B6.K1 anti-A.BY/B6.K2 effector cells were tested for their cytotoxic activity against BALB/cJ (top panel) or DBA/1 (bottom panel) target cells in the presence of unlabeled inhibitor cells from B6.K1 (*), BALB/cJ (O), and DBA/1 (+). Net release in the absence of inhibitor cells was 42% for BALB/cJ targets and 45% for DBA/1 targets.

$$\text{percent inhibition of CML} = 1 - \frac{\text{net release in presence of inhibitor cells}}{\text{net release in absence of inhibitor cells}} \times 100.$$

cold target inhibitor cells were less effective than BALB/cJ (Qa-2,3) inhibitors in blocking CTL activity. The data presented in Fig. 1 (top panel) demonstrate that both inhibitor cells block CTL activity to the same extent. As a control, we also show that both DBA/1 and BALB/cJ inhibitor cells equally block cytolytic activity against DBA/1 targets (Fig. 1, bottom panel).

Therefore, these data demonstrate that CTL are not generated against Qa-3; rather, CTL activity detected by B6.K1 anti-A.BY/B6.K2 effector cells is directed against either Qa-2 or both Qa-2 and Qa-5.

Qa-5 Is Associated with a Target Antigen Recognized by Anti-Qa CTL. In testing whether Qa-3 was a target antigen for B6.K1 anti-A.BY/B6.K2 effector cells, we noted when using B6.K2 rather than BALB/cJ cells as targets that inhibitor cells from B10.D2 mice (Qa-2,3) unexpectedly blocked only part of the CTL activity, similar to DBA/1 cells (Fig. 2 A). When DBA/1 cells were used as targets, all three inhibitor cells (DBA/1, B6.K2, and B10.D2) blocked CTL activity to an equivalent extent (data not shown). Further testing using a larger panel of cells (Fig. 2 B) revealed two patterns of blocking: one complete, using inhibitor cells from B6 and B6.K2, and the other partial, using inhibitor cells from B10.D2, A/Boy, A/J, DBA/2, and A.TL. Because the latter cells were only able to partially block CTL activity, this suggested that they may not express a specificity being detected by the effector cells. Examination of the Qa antigen distribution on the strains used in these experiments (Table I) indicates that strains unable to completely block lack the Qa-5 specificity.

Although the above data suggest that one of the specificities that CTL recognize is associated with Qa-5 expression, other explanations are possible. For example, B6.K1 and B6.K2 may differ at a minor H antigen so that CTL activity is generated against this specificity, which is restricted by $H\text{-}2^b$. Because only $H\text{-}2^b$ cells express Qa-5 (see Table I), these would be the only cells expected to completely block in the CTL competition assay. We rule out this possibility because the cytotoxic activity generated is completely blocked by anti-Qa-2 subregion sera and not anti-H-2 sera (see following section). In addition, it is unlikely that this postulated minor H antigen is expressed on cells that have a different background with respect to C57BL/6 mice, viz., A.BY and C3H.SW, because these cells completely block CTL activity in the competition assay (data not shown).

A second possible explanation for the failure of inhibitor cells from certain strains to completely block CTL activity in the competition assay is that these cells express a relatively low amount of Qa-2 on Con A-stimulated lymphoblasts. To address this issue, we generated B6.K1 effector cells sensitized to Qa-2 but not Qa-5 and tested them in the CTL competition assay. These effector cells were produced by priming B6.K1 animals with A.BY cells in vivo, followed by boosting with BALB/cJ ($Qa\text{-}5^-$) cells in vitro. The data in Table III demonstrate that B6.K1 animals primed in vivo with A.BY cells and boosted in vitro with B6.K2 cells generate anti-Qa CTL activity, as expected. Further, the same primed cells boosted with BALB/cJ stimulator cells also display anti-Qa CTL activity, although to a lesser extent, which may be due to the anti-H-2 response elicited concomitantly. If Qa-2 is expressed in greater quantities on $H\text{-}2^b$ ($Qa\text{-}5^+$) Con A lymphoblasts, then boosting the B6.K1 effector cells with $Qa\text{-}5^-$ stimulators (BALB/cJ) should produce the same pattern of inhibition in the CTL competition assay as seen in Fig. 2. However, this was not observed. Accordingly, B6.K1 mice primed in vivo with A.BY cells and boosted with BALB/cJ stimulators have their CTL activity blocked by $Qa\text{-}5^+$ and $Qa\text{-}5^-$ inhibitor cells to an equivalent extent (Fig. 3 A), whereas the same responder cells boosted in vitro with B6.K2 cells have only part of their CTL activity blocked by BALB/cJ ($Qa\text{-}5^-$) inhibitors (Fig. 3 B). Thus, the presence of Qa-5 (or Qa-5 itself) is associated with a specificity recognized by anti-Qa CTL.

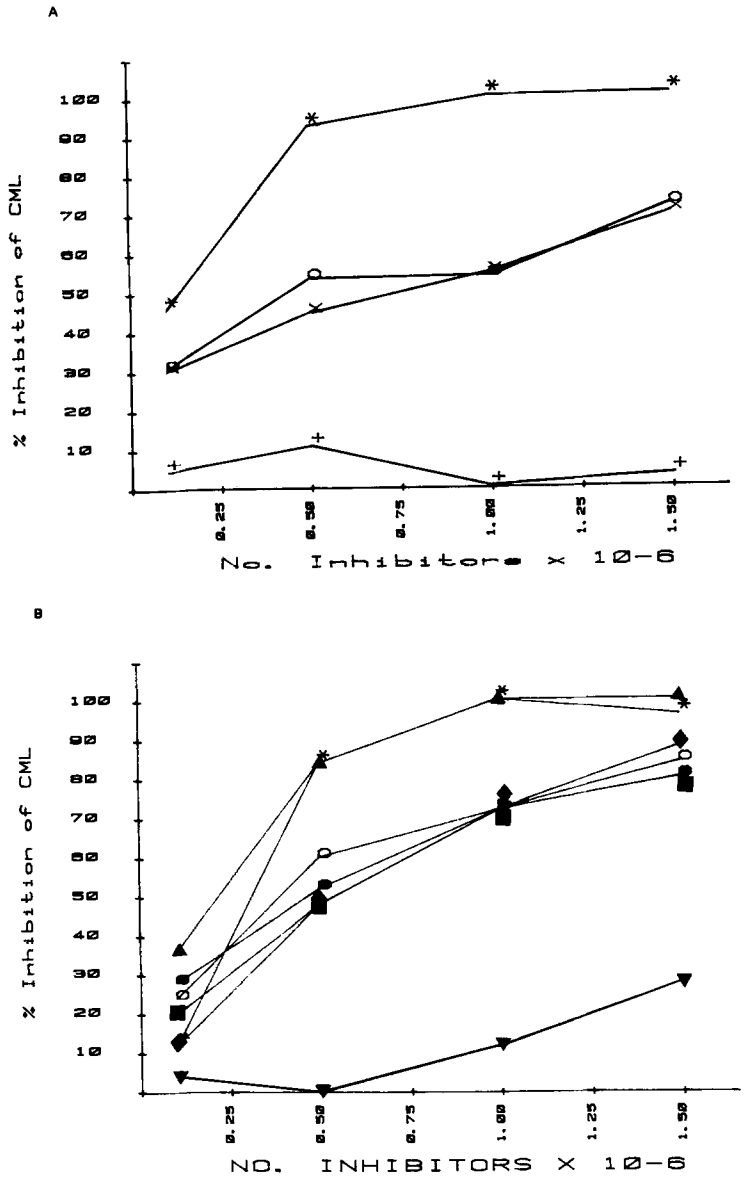

FIG. 2. Qa-5 is associated with a target antigen for anti-Qa CTL. B6.K1 anti-A.BY/B6.K2 effector cells were tested for their cytotoxic activity against B6.K2 target cells in the presence of unlabeled inhibitor cells from B6.K1 (▼), DBA/1 (×), B6.K2 (*), B10.D2 (○), B10.BR (+), B6 (▲), DBA/2 (◆), A/J (●), and A/Boy (■). Net release in the absence of inhibitor cells was 31% for the experiment presented in panel a and 25% for the experiment presented in panel b.

The Qa-2 CTL Antigen Is on the Same Molecule as the Serologically Defined Qa-2 Determinant. The previous data indicate that CTL can be generated by cross-immunizing Qa-incompatible strains and that the specificity of the effector cells is for Qa-2 and Qa-5, as defined by serological typing of target cells. However, the target antigens could either be encoded for by genes that control the serologically defined Qa molecules, or alternatively, they could be controlled by closely linked genes that

TABLE III

Generation of Anti-Qa CTL Activity in B6.K1 Mice Using Qa-5⁻ Stimulator Cells

Cells			Net release, E:T		
Responders	Stimulators	Targets	100	50	10
B6.K1 primed with A.BY	B6.K2	BALB/cJ	47	44	25
		BALB/cByJ	15	11	5
		B6.K2	53	50	32
		B6.K1	7	7	5
	BALB/cJ	BALB/cJ	41	38	18
		B6.K2	22	18	9
		B6.K1	0	3	4

B6.K1 animals were primed in vivo with A.BY spleen cells, followed by boosting in vitro with either B6.K2 (Qa-5⁺) or BALB/cJ (Qa-5⁻) stimulator cells.

express the same positive alleles in the laboratory strains that we have tested. Therefore, to determine whether the same locus controls both Qa-2 CTL and serological determinants, we used two different approaches.

The first was to type seven B10.W (wild) strains for the presence of serologically defined Qa-2, using immune sera, and Qa-2 CTL determinants, using BALB/cByJ anti-BALB/cJ effector cells. B10.W mice have their *H-2* haplotype and *Qa2* subregion derived from individual wild mouse donors (21). Therefore, if there are two separate genes for *Qa-2*, one defined by serology and the other by CTL, then recombination would likely occur in the wild and allow us to detect a discordance between these two antigens. The data in Table IV demonstrate that of the seven B10.W strains tested, five expressed the Qa-2 determinant detected by CTL, whereas two strains were negative. The serological data using anti-Qa2 subregion sera indicates an identical strain distribution for the antigen. In contrast, TL alloantigens show no correlation with Qa-2. Therefore, these data are consistent with the idea that the Qa-2 CTL determinant is identical with serologically defined Qa-2.

A second approach used to determine whether serological and CTL determinants are the same is to use anti-Qa2 subregion antibodies in an attempt to block anti-Qa CTL activity. The data in Table V demonstrate that B6.K1 anti-A.BY/B6.K2 effector cells have their CTL activity completely blocked when the B6.K2 target cells are exposed to a polyvalent anti-Qa2 subregion sera before their addition (lines 3 and 4, columns A and B). The same antisera has no effect in blocking the anti-H-2 CTL response (line 10, column A). Monoclonal anti-Qa-2 only blocks ~50% of CTL activty directed against B6.K2 target cells (lines 5 and 6, column B). This partial inhibition is not because of incomplete masking of the Qa-2 CTL determinant because this antibody efficiently blocks CTL activity directed against Qa-5⁻ target cells (see below). Monoclonal anti-Qa-2 had no activity in blocking anti-H-2^b-specific CTL (data not shown).

Because Qa-5 defines a second CTL target antigen (see previous section), the failure of monoclonal anti-Qa-2 to completely block CTL activity is expected. However, monoclonal anti-Qa-5 either alone or together with monoclonal anti-Qa-2 has no blocking activity (lines 7 and 8, column B). This result could either be the result of an inability of monoclonal anti-Qa-5 (which has a relatively low titer) to properly mask

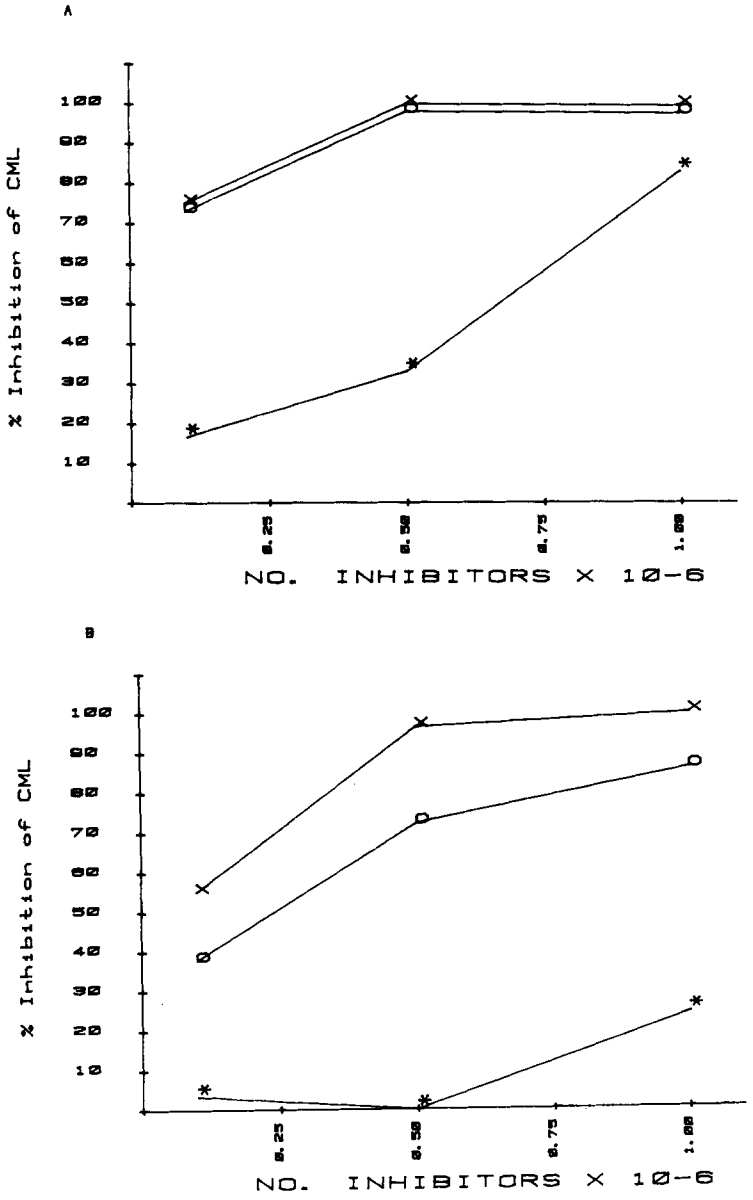

FIG. 3. Qa-5 is associated with a target antigen for anti-Qa CTL. Spleen cells from B6.K1 mice primed in vivo with A.BY cells were boosted in vitro with stimulator cells from BALB/cJ (panel A) or B6.K2 (panel B) and tested for their cytotoxic activity against B6.K2 target cells in the presence of unlabeled inhibitor cells from B6.K1 (*), B6.K2 (×), and BALB/cJ (○). Net release in the absence of inhibitor cells was 12% for the target cells used in panel A and 29% for the target cells used in panel B.

the Qa-5 CTL determinant or possibly that a molecule other than Qa-5 contains the target specificity. This issue is currently under investigation.

The same kind of testing was done on BALB/cJ (Qa-5$^-$) target cells to eliminate anti-Qa-5-associated CTL activity. In this case, both polyvalent K1 anti-B6 and the

TABLE IV

Distribution of Serological and CTL Qa-2 Determinants in B10.W (wild) Mice

Strain	Presence of CTL determinant	Qa-2 serology	*Tla* genotype
BALB/cJ	+*	+‡	*c*§
BALB/cByJ	−	−	*c*
B10.STC77	−	−	*c'*
B10.KPB128	+	+	*c'*
B10.KEA5	+	+	*a'*
B10.SNA70	+	+	*c'*
B10.CAA2	−	−	*a'*
B10.STA12	+	+	*a'*
B10.GAA37	+	+	*b'*

Effector cells were BALB/cByJ spleen cells obtained from mice primed in vivo with B10.D2 spleen cells. The primed cells were cultured in vitro with BALB/cJ splenocytes for 5 d before testing.

* Plus sign represents net release >10% at E:T of 50:1.

‡ Tested using B6.K1 anti-B6 sera.

§ Tested using (B6 × A.CA)F_1 anti-B6.Tla^a sera (anti-Tla 5,6) and (B6 × A.Tla^b)F_1 anti-ASL-1 sera (anti-Tla 1,2,3,5,6). Preliminary work in L. Flaherty laboratory indicates that these mice probably possess unique TL phenotypes. We have therefore denoted them *a'* (strongly positive with anti-TL 1, 2, 3, 5, 6 and anti-TL 5, 6), *c'* (reactive with anti-TL 1, 2, 3, 5, 6 only), or *b'* (unreactive with either antisera).

monoclonal anti-Qa-2 reagent block CTL activity to an equivalent extent (lines 14–17, columns A and B). Although most of the CTL activity (75–86%) in the experiment presented in column A is inhibitable by both antisera (lines 14 and 16), only ~50% of the activity is blocked in the experiment presented in column B (lines 14 and 16). We interpret this inability to completely block lysis in the following manner. B6.K1 anti-A.BY/B6.K2 effector cells exert, in most experiments, a low level of lysis against non-Qa2 subregion antigens on BALB/c target cells. Examples of this reactivity are presented in the experiments shown in Table III and Table V (line 11, column B), where significant lysis was detectable on BALB/cByJ targets. Because this lysis is not Qa2 subregion directed, killing against BALB/cJ targets should not be completely inhibitable with anti-Qa sera and accounts for a result of only partial inhibition, viz., Table V (lines 14–17, column B).

Therefore, these antibody-blocking experiments define two determinants recognized by anti-Qa2 subregion effector cells. One determinant is expressed on the Qa-2 molecule and can be masked by monoclonal anti-Qa-2 antibody when the target cells are from BALB/cJ mice. The same monoclonal anti-Qa-2 antibody does not completely block lysis on B6.K2 target cells, although a polyvalent anti-Qa-2 reagent does. This indicates that there is another CTL target antigen and is in agreement with the results presented in the previous section, which also show that Qa-5 (or a molecule coordinately expressed with Qa-5) is a target specificity.

Even though anti-Qa-2 CTL are *H-2* unrestricted, it is still possible that H-2K or D play a role in controlling the specificity of the effector cells. For example, Qa-2 may associate on the cell membrane with H-2 in such a way that a determinant is formed that is cross-reactive with all Qa-2$^+$ strains. Alternatively, the H-2 molecule may be

TABLE V

Ability of Anti-Qa and Anti-H-2 Sera to Block CTL Activity

Line	Cells		Sera	Net release	
	Effector	Target		A*	B
1	B6.K1 anti-B6.K2‡	B6.K2	NMS§ (1/5) ‖	20	33
2			MP (1/5)		39
3			K1 a-B6 (1/5)	1 (95)¶	0 (100)
4			(1/10)	2 (93)	0 (100)
5			M a-2 (1/5)		23 (41)
6			(1/10)		19 (51)
7			M a-5 (1/5)		38 (3)
8			+ {M a-2 (1/10) M a-5 (1/10)}		22 (44)
9	B10.S anti-B6.K2		NMS (1/5)	22	
10			K1 a-B6 (1/5)	22 (0)	
11	B6.K1 anti-B6.K2	BALB/cBy		0	16
12		BALB/cJ	NMS (1/5)	16	42
13			MP (1/5)	14	44
14			K1 a-B6 (1/5)	4 (75)	19 (55)
15			(1/10)	7 (56)	21 (50)
16			M a-2 (1/5)	2 (86)	25 (43)
17			(1/10)	7 (50)	22 (50)
18			M a-5 (1/5)		40 (9)

* Data in this Table are taken from three different experiments. Experiment 1 is listed under the column marked A, lines 1–10, E:T = 100; experiment 2 is listed under A, lines 11–18, E:T = 25; experiment 3 is listed under B, lines 1–18, E:T = 50.

‡ See Table II.

§ NMS, and is control for K1 a-B6; MP = TEPC183 (uk) 1 mg/ml and is a control for monoclonal antibody; K1 a-B6 is a polyvalent anti-Qa2 subregion antiserum; M a-2 is monoclonal anti-Qa-2; M a-5 is monoclonal anti-Qa-5.

‖ Represents final concentration of serum or antibody in microtiter wells during CTL assay.

¶ The percent inhibition is $1 - \frac{\text{(net release in presence of antisera)}}{\text{(net release in presence of control serum)}} \times 100$.

requisite for target cell lysis; e.g., after effector cell interaction with Qa-2$^+$ target cells, a secondary interaction may be induced involving H-2 molecules that permits target cell lysis. Accordingly, we attempted to block anti-Qa-2 CTL activity with anti-H-2 sera. The data in Table VI demonstrate that anti-H-2 sera block the activity of anti-H-2 CTL. However, the same sera are unable to block the cytotoxic potential of anti-Qa2 subregion effector cells when tested against either B6.K2 or BALB/cJ targets. Therefore, this data further confirms that anti-Qa CTL are *H-2* unrestricted and that H-2K or D molecules do not play an obligatory role in target cell lysis mediated by CTL.

Qa-2 Is Not Polymorphic. The serological data suggest that there are only two *Qa-2* alleles, *a* and *b* (see Table VIII, alternative A). To further test if the *Qa-2* CTL gene is polymorphic, we generated anti-Qa-2 CTL using BALB/cByJ anti-BALB/cJ effector cells and tested against BALB/cJ targets in the presence of inhibitor cells from Qa-2$^-$ (B6.K1) or Qa-2$^+$ strains. The latter included B6.K2 and 3 B10.W strains. If *Qa-2* is polymorphic, then it is likely that B10.W strains will express this polymorphism such that some of the wild strains would be unable to block all the anti-Qa-2 CTL activity in the competition assay. However, the data in Fig. 4 (panel A)

TABLE VI

Inability of Anti-H-2 Sera to Block Anti-Qa CTL Activity

Cells		Sera	Net release
Effector	Target		
B6.K1 anti-B6.K2*	B6.K2	NMS‡ (1/5)§	26.9
		(1/10)	24.4
		Anti-H-2^b (1/5)	21.6 (20)
		(1/10)	24.1 (1)
B10.D2 anti-B10 ‖			37.8
		Anti-H-2^b (1/5)	3.5 (91)
		Anti-H-2^b (1/10)	14.1 (63)
B6.K1 anti-B6.K2	BALB/cJ	NMS (1/4)	25
		(1/8)	30
		Anti-H-2^d (1/4)	32 (−28)¶
		(1/8)	34 (−13)
B6.K1 anti-BALB/cJ		NMS (1/4)	14
		(1/8)	13
		Anti-H-2^d (1/4)	3 (79)
		(1/8)	5 (62)

* ‡ § ¶ See Table V for explanation.

‖ These effector cells were generated from primary cultures and are specific for H-2 antigens. Anti-H-2 sera are described in Materials and Methods.

demonstrate that all four Qa-2^+ strains are able to block CTL activity when tested against B6.K2 targets. Further, when the same effector cells were tested against the three B10.W target cells, all four inhibitors showed similar activity in blocking anti-Qa-2 CTL responsiveness. Although the extent of inhibition varied somewhat against different targets (see panels A and C), this was not a consistent finding in two other experiments. Therefore, the *Qa-2* locus controlling the CTL determinant is not polymorphic.

The Qa2 Subregion Does Not Restrict the Specificity of Antigen-specific CTL. Because the Qa-2 molecule is structurally similar to H-2 class I antigens and serves as a target antigen for *H-2*-unrestricted CTL, it is possible that this molecule has a function similar to H-2 in that it restricts antigen-specific CTL responses. However, the data in Fig. 5 indicate that anti-minor H antigen CTL do not have their specificity restricted by the *Qa-2* subregion. Accordingly, B10.A (*H-2*^{a}) anti-A/J (*H-2*^{a}) anti-minor H antigen CTL lyse A/J and A.TL targets, as expected, because they share all or the *D-end* of the *H-2*^{a} haplotype with *H-2*^{a}, whereas A.BY (*H-2*^{b}) and A.SW (*H-2*^{s}) target cells are not lysed (Fig. 5, top panel). More importantly, although all four of these target cells are Qa-2^+,3^+, it is H-2 and not Qa that determines whether a target cell is sensitive to lysis. In the reverse direction, A.BY (*H-2*^{b}) anti-B10 (*H-2*^{b}) CTL lyse B10 but not B10.A (*H-2*^{a}) target cells, even though they both express Qa-2,3 in common (Fig. 5, bottom panel). Therefore, the *Qa2* subregion does not restrict CTL generated against minor H antigens.

Discussion

The data in this report provide functional evidence to indicate that Qa-2 is similar to class I H-2 antigens. Accordingly, cytotoxic T cells can be generated against Qa-2 that are *H-2* unrestricted in their specificity. This was demonstrated not only by the

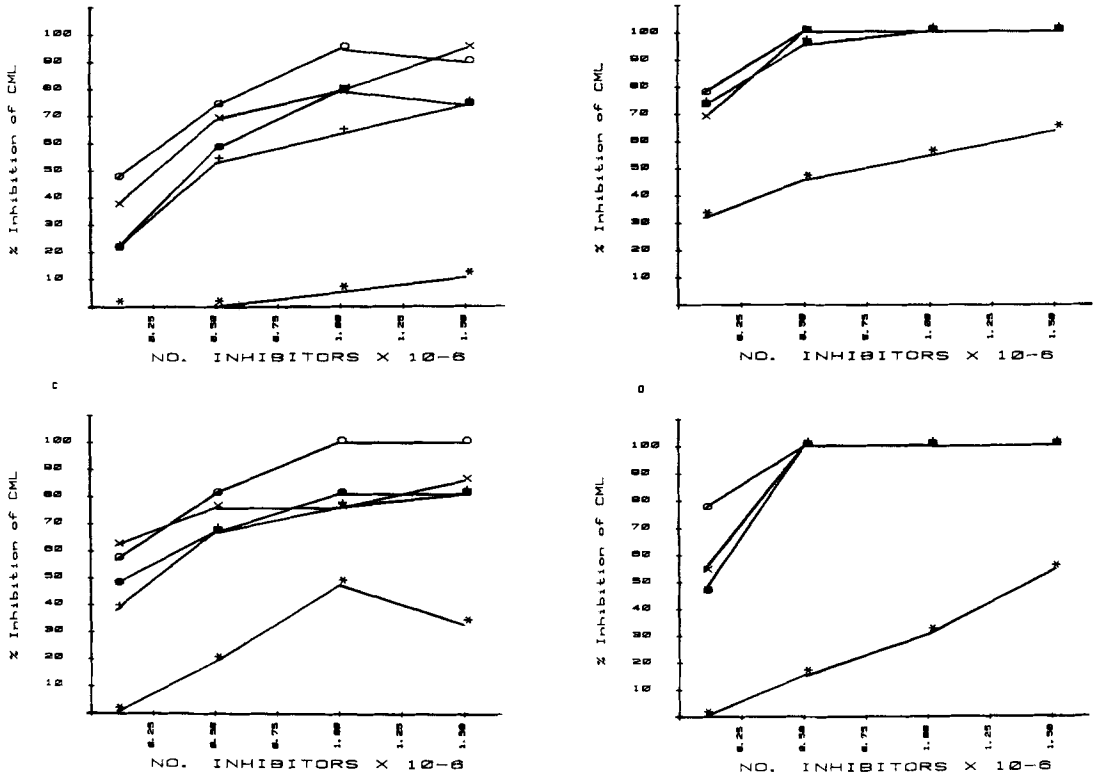

FIG. 4. *Qa-2* is not polymorphic. BALB/cByJ mice were primed with B10.D2 cells in vivo, followed by boosting with BALB/cJ cells in vitro. The resultant effector cells were tested against targets in the presence of unlabeled inhibitor cells from B6.K1 (*), B6.K2 (×), B10.KPB128 (○), B10.KEA5 (■), and B10.GAA37 (+). Target cells are B6.K2 (panel A), B10.GAA37, (panel B) B10.KPB128 (panel C), and B10.KEA5 (panel D). Net release in the absence of inhibitor cells was 19% in A, 22% in B, 21% in C, and 13% in D.

ability of anti-Qa-2 effector cells to lyse Qa-2^+ target cells with differing *H-2* haplotypes, including five B10.W strains, but also by the fact that anti-Qa-2 CTL activity can be blocked by pretreating the target cells with a monoclonal anti-Qa-2 antibody but not anti-H-2 sera. Therefore, in addition to H-2 class I and II antigens, Qa-2 serves as a target antigen for CTL, without the imposition of a specificity restriction by another locus. Similar results have been reported for the Qa-1 antigen (19, 22, 23).

In addition to functional data, there are remarkable similarities at the structural level between H-2 and Qa-2. For example, Qa-2 and H-2K/D molecules consist of a 40,000 to 44,000 mol wt heavy chain glycoprotein noncovalently associated with beta 2-microglobulin (12, 13). Soloski et al. (14) compared tryptic peptides between Qa-2 and H-2 heavy chains and noted that 20–40% were homologous. This level of homology is found when H-2K and H-2D alloantigens are compared and has been interpreted to indicate a sequence homology of between 75 and 90% (24, 25). Therefore, these studies re-enforce the idea that H-2 and Qa-2 have similar functions.

However, in spite of the above-mentioned similarities, several differences exist

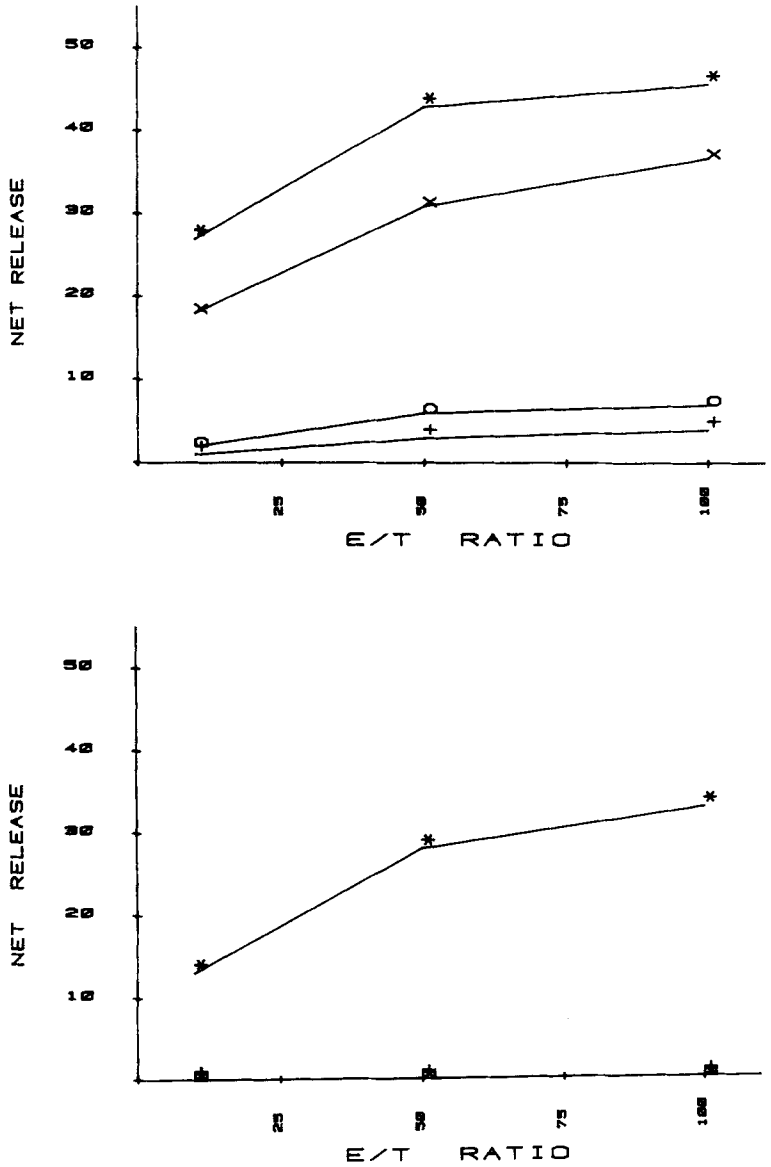

FIG. 5. *Qa2* does not restrict anti-minor H-antigen CTL. B10.A animals were sensitized against A/J spleen cells and tested against target cells from A/J (*), A.TL (×), A.BY (○), and A.SW (+), top panel. A.BY animals were sensitized against B10 spleen cells and tested against target cells from B10 (*), B10.BR (+), B10.A (○), and B10.M (×), bottom panel.

between Qa-2 and H-2, which are summarized in Table VII. For example, H-2K, D, and L (class I antigens) serve as restricting elements for antigen-specific CTL, whereas Qa-2 does not. In this report, we demonstrated that CTL generated against minor H antigens are not *Qa-2*-subregion restricted. Examination of previous studies designed to determine the specificity of anti-minor H, -viral, -tumor, and -hapten sensitized CTL, also reveal that restriction is at *H-2* rather than *Qa2* (16, 26–28). However, data

TABLE VII
Similarities between Qa-2 and H-2K/D

	Qa-2	H-2K/D
Structure	40,000 mol wt H-chain associated with beta-2 microglobulin, peptide map similar to H-2K/D	44,000 mol wt H-chain associated with beta-2 microglobulin, peptide map similar to Qa-2
Primary in vitro CTL	−	+
Unrestricted CTL target antigen	+	+
Restricting locus for antigen-specific CTL	− (?)	+
Polymorphism	−	+
Tissue distribution	Peripheral T cells, T and B cell lymphoblasts, some thymocytes, multipotential stem cells, progenitors of granulocytes/macrophages, some NK cells	All cells

indicating a lack of *Qa2* restriction needs to be interpreted with caution because it is possible that there is an antigen that has not yet been tested that will be *Qa2* restricted. Further, *H-2* restriction may predominate over *Qa2* restriction, so that even if the latter potential exists, it might not be detected.

A second difference between H-2 and Qa-2 is that primary anti-H-2 CTL responses can be generated, whereas anti-Qa-2 responses cannot. The simplest interpretation of this data is that there is a low number of anti-Qa-2 CTL precursors that need in vivo priming for expansion so that they can then be detected in the in vitro assay. This issue is addressed in detail by Keene and Forman (20).

Based on serological data, it has been determined that *Qa-2*, unlike most *H-2* genes, expresses only a positive and null allele. Therefore, we used a different approach, namely, an analysis of Qa-2 CTL determinants, to further investigate whether this gene is polymorphic. This was approached by generating anti-Qa-2 CTL and testing for antigenic polymorphism on Qa-2^+ B10.W (wild) strains of mice. The data from CTL competition assays revealed no detectable differences between Qa-2 CTL determinants on three different B10.W strains and one standard laboratory strain. In addition, comparative peptide maps of Qa-2 taken from several laboratory and wild strains of mice reveal no structural differences (M. Soloski, J. W. Uhr, and E. S. Vitetta, personal communication). Taken together, these data demonstrate that *Qa-2* is a nonpolymorphic locus.

It is likely that class I *H-2* genes function by allowing for T lymphocyte recognition of pathogenic microorganisms that express antigenic determinants on cell membranes. The polymorphism of these genes is thought to allow individual members of a species to mount an immune response against antigenically different pathogens. This interpretation of *H-2* makes it unlikely that *Qa-2* functions in a similar manner because *Qa-2* has not been demonstrated as a restricting locus for antigen-specific CTL, nor is it polymorphic. We favor the possibility that *Qa-2* and *H-2* arose from a common ancestral gene and that *Qa-2* diverged through evolution to acquire a different and yet unknown function.

There is a parallel between the IE_a gene in the *H-2* complex and *Qa-2* in that both

genes display relatively limited or no polymorphism, and each has a null allele (29). Strains with a null IE_a allele presumably use the product of a duplicated gene, *IA*, for immune function (30). In a similar manner, *Qa-2*^{b} strains may have a duplicated gene that functions in its stead. It should also be noted that a null *Qa-2* allele does not rule out the possible presence of a Qa backbone molecule that is invariant in all mouse strains, including those that type as Qa-2$^-$.

In addition to the Qa-2 antigen, the *Qa2* subregion controls the expression of three other cell surface antigenic specificities, Qa-3, Qa-4, and Qa-5. The strain distribution of Qa-2 and Qa-4 is identical, and both antigens have a similar tissue distribution, although Qa-4 seems to be present in lesser amounts on the cell surface than Qa-2 (7, and unpublished data). However, this difference could be accounted for by the anti-Qa-4 monoclonal antibody having a lower affinity for antigen relative to anti-Qa-2. Therefore, because there is insufficient evidence to consider these determinants as different, we propose that these two specificities define the same antigen, Qa-2. With this interpretation, the genetic organization of this subregion can be explained by postulating that there are three genes. Each gene has two alleles, one encodes for a Qa antigen and is designated *a*, and the second is a null allele, *b*. Alternatively, there may be only one gene in this subregion with four alleles. Three of the alleles, *a*, *c*, and *d*, encode for Qa-2 alloantigens, whereas the fourth allele, *b*, is null (see Table VIII). We favor the former possibility because anti-Qa-2,3 CTL did not detect any antigenic differences between Qa-2$^+$3$^-$ and Qa-2$^+$3$^+$ inhibitor cells in a cold target competition assay, and no structural differences, as determined by comparative peptide maps, have been detected between Qa-2 molecules isolated from Qa-2$^+$3$^+$ and Qa-2$^+$3$^-$ strains (M. Soloski, E. S. Vitetta, and J. W. Uhr, personal communication).

In addition to Qa-2, we found a second *Qa2* subregion-controlled target antigen

TABLE VIII

Interpretations of the Qa-2 Subregion

A				
	Region		*Qa2*	
	Loci	*Qa-2*	*Qa-3*	*Qa-5*
	Alleles	*a*	*a*	*a*
		b	*b*	*b*
	Antigens	Qa-2[4]*	Qa-3	Qa-5
B				
	Region		*Qa2*	
	Loci	*Qa-2*		
	Alleles	*a*		
		b		
		c		
		d		
	Antigens	Qa-2^a (2[4]^a)		
		Null		
		Qa-2^c (2[4], 3)		
		Qa-2^d (2[4], 3, 5)		

* Qa-4 has the same strain distribution as Qa-2 and a similar tissue distribution. Because it is likely that Qa-2 and Qa-4 are the same antigen, we have defined the *Qa2* subregion with this assumption. Two alternative interpretations of the genetic organization of the *Qa2* subregion are presented. The data presented in this paper favors possibility A. See text.

that is associated with Qa-5 expression. This was demonstrated in a cold target competition assay in which anti-Qa-2,5 CTL effector activity could not be completely blocked with Qa-2^+5^- inhibitor cells when tested against Qa-2^+5^+ targets. We further demonstrated that this result was not because of a greater level of Qa-2 expression on Qa-5^+ cells because effector cells generated only against the Qa-2 antigen and tested against Qa-2^+5^+ targets could have their activity completely blocked with Qa-2^+5^- inhibitor cells. On the other hand, monoclonal anti-Qa-5 antibody did not block any anti-Qa-2,5 effector activity. Therefore, at present we cannot determine whether the failure of this antibody to block is the result of an inability to mask CTL determinants on the Qa-5 molecule or possibly that a molecule other than Qa-5 expresses the target antigen. All Qa-5^+ strains express both Qa-2 and H-$2D^b$. This suggests that anti-Qa-5 CTL are both H-2 and Qa-2 restricted. However, this possibility is unlikely because neither anti-H-2 nor anti-Qa-2 sera inhibits anti-Qa-5-associated CTL activity.

We (16) have previously suggested that H-2 may possess an enzymatic function on target cells that permits their destruction by CTL. Langman (31), and Cohn and Epstein (32) have suggested that H-2K or D play a role in the formation of membrane channels or sites that cause cell lysis after interaction with effector cells. Accordingly, these postulates require an active role for H-2K/D on target cells for lysis to occur, irrespective of effector cell specificity. However, the data in this report indicate that H-2 does not play a necessary role on target cells for their CTL-mediated lysis. This was demonstrated not only by the fact that anti-Qa2 CTL are *H-2* unrestricted in their specificity, but also by the observation that anti-H-2 sera is unable to block anti-Qa-2 CTL activity. On the other hand, if Qa-2 has a function similar to class I H-2 antigens, then the above suggested functional role for H-2 may be substituted for by Qa-2.

Summary

B6.K1 mice were immunized with spleen cells from B6.K2, a *Qa2*-subregion congenic strain. Cytotoxic T cells were generated that recognize two target antigens controlled by this region.

One of the target antigens is Qa-2. This was demonstrated by the findings that pretreatment of target cells with monoclonal anti-Qa-2 antibody blocked lysis of target cells, and Qa-2 target antigens and serological determinants had a concordant distribution on a panel of B10.W (wild) mice. The gene controlling the Qa-2 target antigen is not polymorphic because B6.K2 and three strains of Qa-2^+ B10.W mice express the same antigens, as determined by a CTL cold target competition assay. Anti-Qa-2 CTL were *H-2* unrestricted because effector cells lysed Qa-2^+ targets irrespective of their *H-2* haplotype, including five B10.W strains, and lysis was not inhibited by pretreating target cells with anti-H-2 sera. The *Qa2* subregion does not act as a restricting locus for anti-minor-H antigen CTL.

A second target antigen was detected that was associated with the expression of the Qa-5 determinant. However, CTL activity could not be blocked by pretreating target cells with monoclonal anti-Qa-5. Therefore, the CTL target antigen may be expressed on a Qa-5^- molecule. Although the Qa-5 associated CTL specificity is only detected on H-$2D^b$ strains, it is unlikely that CTL recognition is *H-2* restricted because anti-H-2^b sera has no effect in blocking this reactivity.

Qa-2 and H-2 class I antigens share a similar structure and serve as target antigens

for unrestricted CTL. However, unlike class I *H-2* genes, *Qa-2* neither restricts antigen-specific CTL nor is polymorphic. Therefore, it is likely that *Qa-2* and *H-2* are derived from a common ancestral gene and have evolved to serve different functions.

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References

1. Flaherty, L. 1981. The "Tla" region antigens. *In* The Role of the Major Histocompatibility Complex in Immunobiology. M. Dorf, editor. 33–57.
2. Stanton, T. H., and E. A. Boyse. 1976. A new serologically defined locus, *Qa-1* in the *Tla* region of the mouse. *Immunogenetics*. 3:525.
3. Nell, L. J., D. L. Kastner, and R. R. Rich. 1980. *Qa-1*-associated antigens. III. Distribution of Qa-1 region antigens on lymphoid subpopulations. *J. Immunol.* **125:**2597.
4. Eardley, D. D., J. Hugenberger, L. McVay-Boudreau, F. W. Shen, R. K. Gershon, and H. Cantor. 1978. Immunoregulatory circuits among T-cell sets. I. T-helper cells induce other T-cell sets to exert feedback inhibition. *J. Exp. Med.* **147:**1106.
5. Cantor, H., J. Hubenberger, L. McVay-Boudreau, D. D. Eardley, J. Kemp, F. W. Shen, and R. K. Gershon. 1978. Immunoregulatory circuits among T-cell sets. Identification of a subpopulation of T-helper cells that induces feedback inhibition. *J. Exp. Med.* **148:**871.
6. Flaherty, L. 1976. The *Tla* region of the mouse: identification of a new serologically defined locus, *Qa-2*. *Immunogenetics*. **3:**533.
7. Hämmerling, G., U. Hämmerling, and L. Flaherty. 1979. Qat-4 and Qat-5, new murine T-cell antigens governed by the *Tla* region and identified by monoclonal antibodies. *J. Exp. Med.* **150:**108.
8. Koo, G. C., J. Jacobson, and G. Hammerling. 1980. Antigenic profile of murine natural killer cells. *J. Immunol.* **125:**1003.
9. Kincade, P. W., L. Flaherty, G. Lee, J. Watanabe, and J. Michaelson. 1980. Qa antigen expression on functional lymphoid, myeloid, and stem cells in adult mice. *J. Immunol.* **124:** 2879.
10. Sullivan, K. A., and L. Flaherty. 1979. The Qa-2 antigen on lymphocyte subpopulations: mixed lymphocyte culture and cell-mediated lympholysis. *J. Immunol.* **123:**2920.
11. Flaherty, L., D. Zimmerman, and K. A. Sullivan. 1978. Qa-2 and Qa-3 antigens on lymphocyte subpopulations. I. Mitogen responsiveness. *J. Immunol* **121:**1640.
12. Michaelson, J., L. Flaherty, E. Vitetta, and M. D. Poulik. 1977. Molecular similarities between the Qa-2 alloantigen and other gene products of the 17th chromosome of the mouse *J. Exp. Med.* **145:**1066.
13. Michaelson, J., L. Flaherty, Y. Bushkin, and H. Yudkowitz. 1981. Further biochemical data on Qa-2. *Immunogenetics.* **14:**129.
14. Soloski, M. J., J. W. Uhr, L. Flaherty, and E. S. Vitetta. 1981. Qa-2 H-2K and H-2 alloantigens evolved from a common ancestral gene. *J. Exp. Med.* **153:**1080.
15. Forman, J., and L. Flaherty. 1978. Identification of a new CML target antigen controlled by a gene associated with the *Qa-2* locus. *Immunogenetics*. **6:**227.
16. Forman, J. 1975. On the role of the *H-2* histocompatibility complex in determining the specificity of cytotoxic effector cells sensitized against syngeneic trinitrophenyl-modified targets. *J. Exp. Med.* **142:**403.
17. Galfre, G., S. Howe, C. Milstein, G. W. Butcher, and J. C. Howard. 1977. Antibodies to the major histocompatibility antigens produced by hybrid cell lines. *Nature (Lond.)*. **266:** 550.

18. Altman, P. J., and D. D. Katz, editors. 1979. *In* Inbred and Genetically Defined Strains of Laboratory Animals. Part 1. Mouse and Rat. *Fed. Am. Soc. Exp. Biol.* **3**:16.
19. Forman, J. 1979. *H-2* unrestricted cytotoxic T cell activity against antigens controlled by genes in the Qa/Tla region. *J. Immunol.* **123**:2451.
20. Keene, J., and J. Forman. 1982. Helper activity is required for the in vivo generation of cytotoxic T lymphocytes. *J. Exp. Med.* In press.
21. Zaleska-Rutezynska, Z., and J. Klein. 1977. Histocompatibility-2 system in wild mice. V. Serological analysis of sixteen B10.W congenic lines. *J. Immunol.* **119**:1903.
22. Klein, J., and C. Chaing. 1978. A new locus (H-2T) at the D end of the H-2 complex. *Immunogenetics.* **6**:235.
23. Kastner, D. L., and R. R. Rich. 1979. H-2 nonrestricted cytotoxic response to an antigen encoded tetomeric to H-2D. *J. Immunol.* **122**:196.
24. Brown, J. L., K. Kato, J. Silver, and S. G. Nathenson. 1974. Notable diversity in peptide composition of murine H-2K and H-2D alloantigens. *Biochemistry.* **13**:3174.
25. Coligan, J. E., T. J. Kindt, B. Ewenstein, H. Uchara, T. Nisizawa, and S. G. Nathenson. 1978. Primary structure of murine major histocompatibility complex alloantigens: amino acid sequence studies of the cyanogen bromide fragments of the H-2K^b glycoprotein. *Proc. Natl. Acad. Sci. U. S. A.* **75**:3390.
26. Zinkernagel, R., and P. C. Doherty. 1979. MHC-restricted cytotoxic T cells: studies in the biological role of polymorphic major transplantation antigens determining T cell restriction-specificity function and responsiveness. *Adv. Immunol.* **27**:51.
27. Bevan, M. J. 1975. The major histocompatibility complex determines susceptibility to cytotoxic T cells directed against minor histocompatibility antigens. *J. Exp. Med.* **142**:1349.
28. Gomard, E., V. Duprez, T. Reme, M. J. Colombani, and J. P. Levy. 1977. Exclusive involvement of H-2D^b or H-2K^d product in the interaction between T-killer lymphocytes and syngeneic H-2^b or H-2^d viral lymphomas. *J. Exp. Med.* **146**:909.
29. Jones, P. P., D. B. Murphy, and H. O. McDevitt. 1978. Two-gene control of the expression of a murine Ia antigen. *J. Exp. Med.* **148**:925.
30. Klein, J. 1979. The major histocompatibility complex of the mouse. *Science* (*Wash. D. C.*). **203**:516.
31. Langman, R. E. 1978. Cell-mediated immunity and the major histocompatibility complex. *Rev. Physiol. Biochem. Pharmacol.* **81**:1.
32. Cohn, M., and R. Epstein. 1978. T-cell inhibition of humoral responsiveness. II. Theory on the role of restrictive recognition in immune regulation. *Cell. Immunol.* **39**:125.
33. Klein, J., L. Flaherty, J. L. VardeBerg, and D. C. Schreffler. 1978. H2 haplotypes, genes, regions, and antigens: first listing. *Immunogenetics.* **6**:489.