*Proc. Natl. Acad. Sci. USA*
Vol. 87, pp. 6604–6608, September 1990
Immunology

# Peripheral tolerance in mice expressing a liver-specific class I molecule: Inactivation/deletion of a T-cell subpopulation

KIM WIETIES*, ROBERT E. HAMMER†, SHARON JONES-YOUNGBLOOD†, AND JAMES FORMAN*§

*Department of Microbiology and †Department of Biochemistry, University of Texas Southwestern Medical Center, and Howard Hughes Medical Institute, Dallas, TX 75235-9048

*Communicated by Jonathan W. Uhr, June 12, 1990*

**ABSTRACT** **We previously demonstrated that C3H/HeJ transgenic (TG) mice that express a laboratory-engineered class I molecule, Q10/L, exclusively on liver parenchymal cells show no evidence of hepatic disease even after deliberate immunization. Nevertheless, these animals demonstrate cytotoxic T-lymphocyte (CTL) activity specific for Q10/L, although it is less than that obtained from non-TG littermates. We now show that this decrease in CTL activity is not a reflection of a decrease in precursors, since both TG and normal animals have similar numbers. When non-TG C3H mice are primed with H-2L$^d$ and H-2K$^{bm1}$ antigens, which extensively crossreact with Q10/L, their specific *in vitro* CTL activity directed against H-2L$^d$, H-2K$^{bm1}$, and Q10/L is increased 10- to 20-fold, as expected. Although primed TG mice show similar increases in *in vitro* CTL activity directed against H-2L$^d$ and H-2K$^{bm1}$, they display no increase in anti-Q10/L activity. Whereas anti-H-2L$^d$ spleen cells from non-TG mice readily generate CTL lines and clones specific for H-2L$^d$ and Q10/L, TG cells give rise to anti-H-2L$^d$ lines or clones only. These data indicate that the tolerance in TG mice is accounted for by the inactivation or deletion of an important CTL subpopulation having the capability of recognizing the peripheral antigen *in situ*. To determine whether tolerance would persist in the absence of Q10/L, TG cells were transferred into non-TG recipients. Three weeks later Q10/L-specific lytic activity generated in *in vitro* bulk cultures remained reduced compared to non-TG cells, indicating that the tolerant phenotype was stable during this interval.**

Immunologic tolerance is a process whereby lymphocyte clones capable of self reactivity are deleted or rendered inactive (anergic). Although tolerance to self antigens has been demonstrated to occur in the thymus, tolerance mechanisms must also function in the periphery to eliminate clones capable of recognizing antigens expressed solely on extrathymic tissues. Many laboratories including our own have approached the issue of peripheral tolerance utilizing transgenic (TG) animals that express foreign major histocompatibility complex (MHC) molecules in an organ-specific manner. These studies have demonstrated that animals expressing MHC antigens exclusively on organ parenchymal cells are not autoimmune since no evidence of cellular-induced organ damage is evident *in situ*. However, the basis for this lack of disease varies. In some cases, there is little or no demonstrable antigen-reactive T-cell activity (1–4), whereas in other models, antigen-specific T cells can be detected *in vitro* (5–9). In instances where *in vitro* T-cell activity is detected, deliberate immunization of TG mice does not induce autoimmune disease (6, 8, 9), although in one system *in vitro* cultured cells cause cellular infiltration *in vivo* in the organ expressing the TG antigen (7).

We are studying a model of tolerance directed against the class I molecule Q10. Unlike other class I molecules, Q10 is only produced in the liver and is secreted rather than membrane bound (10). The presence of Q10 in serum does not tolerize antigen-specific cytotoxic T lymphocytes (CTLs) since mice have T cells that recognize Q10/L, a laboratory-engineered form of Q10 that is membrane bound (11). By using the Q10/L gene, we constructed TG mice that express Q10/L exclusively on liver parenchymal cells (5). Although these animals are functionally tolerant based on a lack of hepatic pathology *in vivo*, spleen cells from these mice cultured *in vitro* are capable of recognizing the TG class I molecule. Since deliberate immunization of these TG mice with antigen does not induce disease, these *in vitro*-detectable CTLs presumably lack a subpopulation capable of recognizing the antigen on hepatocytes *in vivo*. In this study, we characterize the CTLs in normal vs. TG mice and describe differences that likely account for the tolerance in the latter animals.

## MATERIALS AND METHODS

**Mice.** All animals were bred and maintained in our colonies at the University of Texas Southwestern Medical Center at Dallas. TG mice were derived by injecting an exon-shuffled gene, Q10$^d$/L$^d$, into C3H/HeJ animals (5). In these mice, Q10$^d$/L$^d$ ($\alpha$1 and $\alpha$2 domains were derived from Q10$^d$; and $\alpha$3, transmembrane, and cytoplasmic domains were from H-2L$^d$) is expressed exclusively on liver parenchymal cells. H-2L$^d$ TG mice express H-2L$^d$ on peripheral lymphoid tissues of C3H mice. They will be described elsewhere in detail.

**CTL Assay.** Approximately $5 \times 10^6$ responder spleen cells were cocultured with $5 \times 10^6$ x-irradiated stimulator splenocytes for 5 days and then tested in a standard $^{51}$CR release assay using L-cell targets expressing H-2L$^d$, Q10/L$^d$, H-2K$^{bm1}$, or H-2D$^d$.

**Generation of Long-Term CTL Lines and Clones.** CTL lines and clones were generated using two methods.

*Method I.* C3H responder cells were cocultured with stimulator cells that alternated weekly between H-2K$^{bm1}$ and BALB/c (H-2L$^d$) to favor the growth of CTLs likely to crossreact on Q10/L (12). Briefly, TG or C3H spleen cells were cocultured with H-2K$^{bm1}$ feeder cells. After 5 days, these cultures were restimulated with fresh irradiated spleen cells in medium containing 20% (vol/vol) rat concanavalin A supernatant (CAS; ref. 12) as a source of interleukin 2. These bulk cultures were placed in limiting dilution (LD) in 96-well flat-bottom plates (96 replicates per dilution) containing $1 \times 10^6$ BALB/c irradiated stimulator cells and CAS in a total volume of 200 $\mu$l. After 10–13 days, wells were visually scored for the presence of cell growth. Wells chosen as positive from dilutions that should contain <1 precursor per well were expanded and tested for CTL activity.

The publication costs of this article were defrayed in part by page charge payment. This article must therefore be hereby marked "*advertisement*" in accordance with 18 U.S.C. §1734 solely to indicate this fact.

Abbreviations: CTL, cytotoxic T lymphocyte; CTL.P, CTL precursor; CTL.P$_f$, CTL.P frequency; TG, transgenic; CAS, concanavalin A supernatant; MHC, major histocompatibility complex; LD, limiting dilution.

§To whom reprint requests should be addressed.

*Method II.* The second method for generating long-term clones utilizes primary CTL cultures containing C3H or TG splenocytes mixed with TG C3H–H-2L$^d$ stimulator cells placed in LD on day 5 (96 replicates per dilution) with BALB/c feeder cells and CAS (13). On days 12 and 19, the cultures are refed with stimulator cells and CAS. On day 25 each well was split and tested for CTL activity on Q10/L and H-2L$^d$ targets.

**CTL Precursor Frequency (CTL.P$_f$) Analysis.** CTL.P$_f$ was measured as described (14). Briefly, wells of the desired number (usually between $5 \times 10^2$ and $5 \times 10^4$) of input lymph node cells (24 replicates per dilution) were cocultured with $1 \times 10^6$ irradiated BALB/c or C3H (control) spleen cells in medium containing CAS. After 6 days, positive and negative wells were identified by testing $^{51}$Cr-labeled targets.

**Adoptive Transfer Protocol.** C3H animals 6–8 weeks of age were anesthetized with Avertin and their thymii were removed. One week after surgery, they were sublethally irradiated (750 cGy) and then received one spleen equivalent of either C3H or TG spleen cells i.v. After 3 weeks, their spleen cells were tested in the CTL assay.

## RESULTS

**General Method for Evaluating Anti-Q10/L CTLs.** The Q10/L antigen represents the product of an exon-shuffled gene expressed on either transfected L cells (15) or hepatocytes of TG mice (5). Since neither of these cell types efficiently activate alloreactive CTL precursors (CTL.Ps), we have taken advantage of the crossreactivity of Q10/L, H-2L$^d$, and H-2K$^{bm1}$ to generate anti-Q10/L CTLs (12). Thus, sensitization of C3H animals with either H-2L$^d$ or H-2K$^{bm1}$ spleen cells allowed for the generation of not only H-2L$^d$- or H-2K$^{bm1}$-specific CTLs, respectively, but also Q10/L-specific CTLs. This crossreactivity can be seen where C3H anti-BALB/c (H-2L$^d$) cultures generated CTLs that lysed not only H-2L$^d$-expressing L cells (Fig. 1*B*) but also Q10/L L cells (Fig. 1*A*). This recognition is specific as no lysis was noted on control L cells (Fig. 1*E*). Further, this *in vitro* crossreaction was physiologically significant *in vivo* since adoptive transfer of primed C3H anti-BALB/c (H-2L$^d$) or H-2K$^{bm1}$ splenocytes caused hepatic pathology in Q10/L TG recipients (5).

**CTL.P$_f$ in Normal and TG Animals.** We have shown (5) that spleen cells taken from C3H-TG mice that express the class I antigen Q10/L exclusively on hepatocytes contained CTLs that specifically recognize Q10/L. However, in bulk CTL cultures, we consistently noted that the amount of lytic activity generated from TG splenocytes was considerably less (≈5 times less) than that observed in splenocytes obtained from their non-TG littermates (ref. 5, also see Fig. 1*A*). To further quantitate the extent of anti-Q10/L-specific reactivity in TG mice, we compared the anti-Q10/L CTL.P$_f$ in lymph nodes of normal and TG animals.

C3H or C3H-TG lymph node cells were cultured at LD with BALB/c (*H-2K$^d$, -D$^d$, -L$^d$*) stimulator cells and 6 days later cytotoxicity was measured against Q10/L, H-2L$^d$, or H-2D$^d$. A representative experiment is shown in Fig. 2 and the data are summarized in Table 1. The CTL.P$_f$ against H-2L$^d$ in control vs. TG mice was similar, as expected [1/21,198 vs. 1/15,977 (Fig. 2); 1/20,223 vs. 1/10,442 (Table 1)]. Although bulk culture CTL activity against Q10/L was reduced in TG animals, the anti-Q10/L CTL.P$_f$ was similar in both normal and TG mice [1/18,002 vs. 1/21,139 (Fig. 2)]. The mean value from four individual TG vs. control animals was 1/10,666 vs. 1/15,200, respectively (Table 1). The CTL.P$_f$ directed against H-2D$^d$ between control and TG mice was also similar, as expected (Table 1). Thus, the relatively low amount of Q10-specific CTL activity detected *in vitro* in TG mice is not reflected by the number of CTL.Ps.

**Anti-Q10/L CTLs in TG Mice Cannot Be Primed *in Vivo*.** We have demonstrated that *in vivo* priming of TG animals with either splenocytes expressing antigens that crossreact with Q10/L- or Q10/L-expressing L cells did not produce any signs of hepatic pathology. Since TG mice contained CTL.Ps that recognize Q10/L, we questioned whether this priming activated anti-Q10/L CTL.Ps *in vivo*. Preliminary data indicated that TG animals injected with Q10/L-crossreacting antigens were not primed *in vivo* to Q10 (5). We have now extended these findings to measure priming of CTLs to Q10-crossreacting antigens, H-2L$^d$ and H-2K$^{bm1}$, as well as to the Q10-noncrossreactive antigen H-2D$^d$.

Either TG or normal C3H mice were primed *in vivo* with BALB/c or H-2K$^{bm1}$ spleen cells on alternate weeks for a 6-week period. Spleen cells from these mice were tested for their lytic activity against target cells and compared with nonprimed splenocytes. CTLs generated from non-TG C3H animals demonstrated a 17.5-fold increase (range 5–33) in lysis of Q10-transfected L-cell targets (Fig. 3). A similar increase in cytolytic activity was also noted on H-2L$^d$, H-2K$^{bm1}$, as well as H-2D$^d$ targets, as expected. Whereas TG splenocytes also demonstrated increases in CTL lytic activity

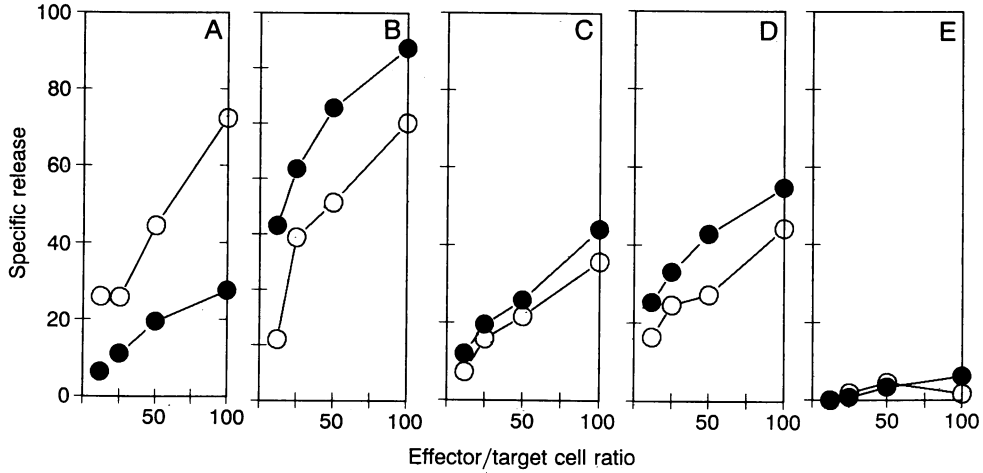


FIG. 1. Transfer of TG T cells into non-TG mice does not alter their phenotype. Spleen cells from C3H and TG mice were transferred into thymectomized and irradiated (750 cGy) C3H recipients. Three weeks later their spleen cells were cocultured with BALB/c stimulator cells and tested in a standard CTL assay against Q10/L (*A*), H-2L$^d$ (*B*), H-2D$^d$ (*C*), H-2K$^{bm1}$ (*D*), or control thymidine kinase gene (*E*) L cells. ○, C3H animals; ●, TG animals. A representative experiment of three experiments is shown.

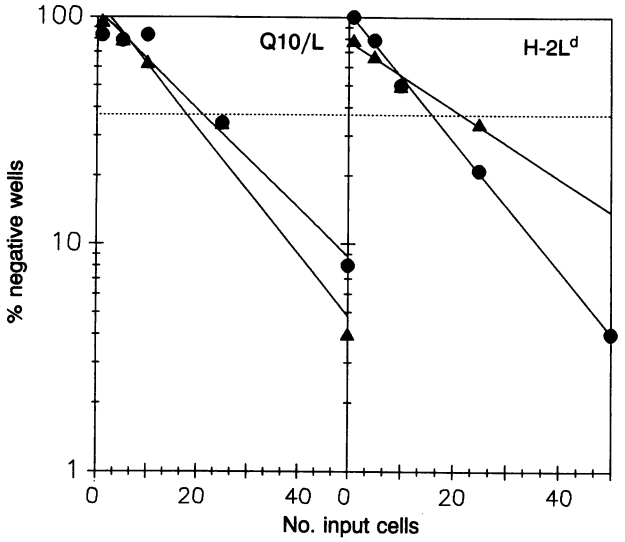


FIG. 2. Anti-Q10/L and H-2L$^d$ CTL.$P_f$ in lymph nodes of C3H (▲) and TG (●) mice. (*Left*) CTL.$P_f$ vs. Q10/L. (*Right*) CTL.$P_f$ vs. H-2L$^d$. Data are from experiment 2 in Table 1.

against H-2L$^d$, H-2K$^{bm1}$, and H-2D$^d$, no increased activity was observed on Q10/L targets ($X_{\text{priming factor}}$ = 0.9). This result indicates that although TG animals have *in vitro* anti-Q10 CTLs, their reactivity is altered *in vivo* such that they are not expanded after *in vivo* priming.

**Ability to Generate Long-Term Anti-Q10 CTL Clones or Lines.** Although the above data indicate that the anti-Q10/L CTL.$P_f$ between TG and C3H animals is similar, other results indicate differences between the CTLs derived from these animals, namely, reduced anti-Q10/L CTL activity of TG splenocytes in bulk culture and inability to *in vivo* prime TG CTLs. To further characterize differences between anti-Q10/L CTLs from normal and TG mice, we set out to generate stable anti-Q10 CTL clones or lines using two methods. Method I analyzes cultures at LD; CTLs obtained using method II were not clonal and thus consisted of both clones and polyclonal lines. The data in Table 2 demonstrate that long-term CTL clones or lines specific for Q10 and H-2L$^d$ were readily obtained from C3H animals, as previously demonstrated (12). Thus, by using culture method I, three C3H mice generated between 10 and 13 anti-H-2L$^d$ clones, all of which crossreact on Q10/L. With culture method II, we noted that between 6 and 30% of positive wells showed anti-H-2L$^d$ activity and between 8 and 16% had anti-Q10/L activity. In contrast, splenocytes from TG mice did not generate Q10/L-reactive CTL using either culture method. This result does not necessarily indicate an absolute lack of clonable anti-Q10/L CTLs in TG mice, since clones may be detected if higher numbers of cells are cultured. Nevertheless, this difference between TG and normal mice demonstrates another alteration in Q10-specific CTLs in TG animals.

**Transfer of TG T Cells to Normal C3H Animals Does Not Reverse Their Reduced Ability to Generate Anti-Q10 CTLs.** The above data demonstrate that the presence of Q10/L on hepatocytes in TG mice alters the activity of Q10/L-specific CTLs. Similar studies using class II molecules as toleragens have suggested that the tolerance observed is not due to clonal deletion but rather T-cell anergy (15–17). Since the altered anti-Q10 activity in TG mice may also be the result of T-cell anergy, we determined whether putative anergic T cells would retain the tolerant phenotype when transferred to non-TG hosts where the toleragen is not expressed. Accordingly, spleen cells from TG mice were transferred into sublethally irradiated thymectomized C3H recipients. After 3 weeks, the animals were sacrificed and their spleen cells were assayed for Q10/L-specific CTLs.

Transfer of normal C3H spleen cells into irradiated recipients followed by culture *in vitro* with BALB/c splenocytes generated strong CTL activity against Q10/L, as expected (Fig. 1*A*). In contrast, CTL activity from spleens for irradiated C3H mice repopulated with TG T cells showed a much reduced anti-Q10/L CTL response, similar to the difference noted when testing splenocytes from TG vs. normal C3H mice directly (5). As expected, CTL activity from TG spleen cells directed against H-2L$^d$, H-2K$^{bm1}$, and H-2D$^d$ was equivalent to that of non-TG spleen cells (Fig. 1 *B–D*). Thus, removal of the toleragen for a 3-week interval did not rescue the altered phenotype.

## DISCUSSION

Q10/L TG mice are functionally tolerant to an antigen expressed exclusively on their liver parenchymal cells. Although animals tolerant to this self molecule might be expected to lack specific antigen-reactive T cells, we have demonstrated (5) this is not the case. In several other TG model systems where animals express antigens restricted to peripheral organs, T-cell reactivity has been found to be either (*i*) absent or reduced (1–4) or (*ii*) apparently normal (5–9). In one example, antigen-specific CTLs are revealed by the addition of exogenous interleukin 2 (8).

In this report, we demonstrate that Q10/L-TG mice have a similar number of antigen-specific CTL.Ps compared to their non-TG littermates. However, in addition to the finding that Q10-specific lytic activity generated in bulk cultures *in vitro* is ≈5 times less than that of non-TG mice, we show that priming of TG mice *in vivo* does not boost the lytic activity of Q10/L-specific CTLs, as detected *in vitro*. Further, these *in vitro*-cultured T cells do not give rise to long-term Q10/L specific CTL or clones. This latter result is consistent with the findings of Wood *et al.* (18) who noted that antigen-specific T cells from neonatally tolerant animals have a diminished growth pattern *in vitro* when grown on feeder cells expressing the toleragen.

Since the anti-Q10/L CTL.$P_f$ in normal and TG mice is approximately equivalent, the difference in CTL activity in TG mice must represent the deletion or inactivation of a small

Table 1. CTL.$P_f$ of C3H and TG mice

| | Reciprocal of CTL.$P_f$ | | | | | |
|---|---|---|---|---|---|---|
| | Q10 | | H-2L$^d$ | | H-2D$^d$ | |
| Exp. | C3H | TG | C3H | TG | C3H | TG |
| 1 | 12,068 (0.93) | 21,019 (0.87) | 17,634 (0.94) | 8,345 (0.98) | 17,634 (0.94) | 20,673 (0.99) |
| 2 | 18,002 (0.97) | 21,139 (0.97) | 21,198 (0.97) | 15,997 (0.99) | | |
| 3 | 5,640 (0.86) | 11,795 (0.92) | | 10,942 (0.98) | | |
| 4 | 6,955 (0.88) | 6,847 (0.83) | 9,167 (0.99) | 4,270 (0.99) | | 4,835 (0.87) |
| 5 | | | 32,891 (0.99) | 12,677 (0.99) | 13,745 (0.89) | 29,120 (0.97) |
| Mean | 10,666 ± 2811 | 15,200 ± 3541 | 20,223 ± 4919 | 10,442 ± 1979 | 15,690 | 18,209 |

$R^2$ value of regression line is in parentheses. Some mean values are expressed ± SEM.

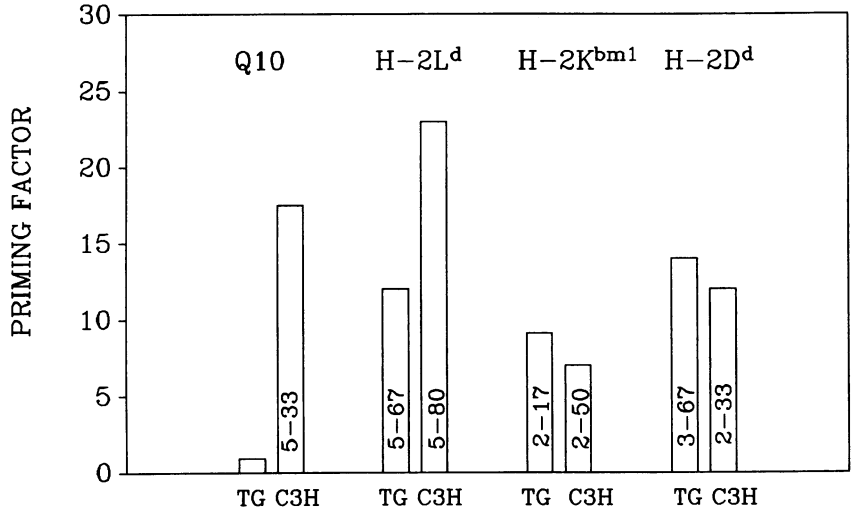


FIG. 3. CTL activity in C3H mice primed against H-2L$^d$ and H-2K$^{bm1}$ antigens. C3H and TG mice were primed alternately *in vivo*, with BALB/c and H-2K$^{bm1}$ spleen cells, six times at weekly intervals. Spleens from unprimed and primed control and TG mice were cocultured *in vitro* with BALB/c splenocytes and tested 5 days later for CTL activity against L cells expressing Q10/L, H-2L$^d$, H-2K$^{bm1}$, and H-2D$^d$. No lysis was detected against L cells transfected with herpes simplex virus thymidine kinase gene only (data not shown). Priming factor = (no. unprimed effectors required for 30% lysis of 5000 targets/no. primed effectors required for 30% lysis of 5000 targets)× 100. Numbers within bars represent range of values for eight comparisons against Q10/L, H-2L$^d$, and H-2K$^{bm1}$ and for six comparisons against H-2D$^d$. Range of priming factor for TG mice vs. Q10/L was 0.6–1.2.

but important subpopulation of the total Q10/L-specific T-cell pool. We suggest that a similar missing subpopulation accounts for the findings in other studies of peripheral tolerance where antigen-specific T-cell reactivity is observed *in vitro* despite a lack of autoimmune reactivity *in vivo*, even after deliberate immunization (6, 8, 9).

Although the basis for these specific alterations in Q10/L TG mice is not known, it is possible that the activity missing in these animals is mediated by T cells with receptors bearing a relatively high affinity for the antigen. This would be consistent with the fact that the expression of Q10/L on hepatocytes is low, ≈5 times lower in density than transfected L cells. This low density of antigen may only be able to tolerize T cells with high-affinity receptors, leaving T cells with lower-affinity receptors unaffected and detectable in our *in vitro* assays. However, it is also possible that other properties could account for this missing subset including the expression of accessory molecules such as CD8 or members of the integrin family.

Table 2. Inability to generate long-term CTL clones and lines from TG splenocytes

| | Method I (no. positive wells) | | Method II (% positive wells) | |
|---|---|---|---|---|
| Animal | Q10/L | H-2L$^d$ | Q10/L | H-2L$^d$ |
| C3H | | | | |
| 1 | 10 | 10 | 16 | 30 |
| 2 | 13 | 13 | 14 | 20 |
| 3 | 13 | 13 | 8 | 6 |
| 4 | ND | ND | 15 | 20 |
| TG | | | | |
| 1 | 0 | 6 | 0 | 40 |
| 2 | 0 | 4 | 0 | 18 |
| 3 | 0 | 8 | 0 | 8 |
| 4 | ND | ND | 0 | 22 |

For method I, C3H or TG anti-H-2K$^{bm1}$ splenocytes were cultured for 5 days and then placed at LD in 96-well plates on BALB/c feeder cells. Wells containing growing cells from cultures expected to contain <1 CTL.P per well expanded and tested for lytic activity against Q10/L and H-2L$^d$. Results are expressed as no. of positive wells from plates containing 96 replicates. For method II, C3H or TG spleen cells were cocultured with TG (C3H-H-2L$^d$) splenocytes for 5 days and placed at LD using BALB/c feeder cells. After 25 days, cultures in wells were split and tested for CTL activity against Q10/L and H-2L$^d$. Results are expressed as percent positive wells (96 total) in cultures containing 50 cells per well. Because of the large number of positive wells, these cultures are not considered clonal. ND, not determined.

The concept of specific tolerance involving only a subset of antigen-specific cells has readily been demonstrated for B cells whereby higher-affinity B-cell clones are tolerized in proportion to the amount of self antigen expressed (19). In TG mice expressing a T-cell receptor specific for both Mls$^a$ and lymphocytic choriomeningitis virus (LCMV), Pircher *et al.* (20) postulated that the partial deletion of V$\beta$8.1-bearing T cells may reflect the differential affinity of T cells for the Mls$^a$ vs. LCMV antigens.

It is possible that peripheral tolerance only need involve deletion/inactivation of those cells that recognize organ-specific peptides in the context of self MHC. Thus, the Q10/L-specific CTLs that we detect in TG mice may represent T cells specific for nonhepatic self peptides presented by Q10/L. These CTLs should not pose a threat to TG mice since these cells would not recognize endogenously produced hepatocyte-specific Q10/L-associated self peptides. If this were true, then inoculation of TG mice with spleen cells expressing Q10/L-crossreactive antigens should prime those anti-Q10/L CTLs reactive against nonhepatic peptides associated with Q10/L. However, we observed that Q10/L-specific T cells from TG mice could not be primed *in vivo* with spleen cells expressing Q10/L-crossreacting antigens (H-2L$^d$ and H-2K$^{bm1}$). Thus, these results rule out the possibility that the alteration in CTL activity in TG mice is accounted for by organ-specific peptides presented by this class I molecule.

The failure to prime TG mice against Q10/L *in vivo*, even though the *in vitro* CTL.P$_f$ was similar in TG and normal mice, might resolve an unexplained paradox regarding *in vivo*-priming against MHC antigens in general. Whereas priming animals against non-MHC antigens usually results in a 10- to 1000-fold increase in T-cell precursors, priming against MHC antigens normally results in a modest increase, usually in the range of 2- to 5-fold (21, 22). If most of the alloreactive CTLs detected in primary *in vitro* cultures are not activatible *in vivo*, as our data show, then this finding would resolve the paradox. Thus, even though *in vivo* priming would expand a relatively small subset of T cells capable of responding to antigen *in vivo*, this increase would be masked by a large background of non-*in vivo*-activatible CTLs in the *in vitro* assay.

Studies examining peripheral tolerance to class II antigens have taken advantage of the V$\beta$ gene usage to follow the fate of tolerized T cells. These studies indicate that clonal deletion is not the mechanism; rather, tolerant animals retain specific T cells in an anergic state (16, 23). In other models of tolerance, clonal deletion has been demonstrated, especially when the toleragen is expressed on bone marrow-derived cells in the thymus (refs. 24–26; for review, see ref. 27).

Whether the lack of this T-cell subpopulation in Q10/L-TG mice reflects anergy or deletion is not known. However, if Q10/L tolerance is similar to other studies of peripheral tolerance where specific T cells are anergic, then the data presented here indicate that transfer of such T cells to a non-TG environment for 3 weeks does not reverse this phenotype.

The possible role of the CTL detected *in vitro* in mediating potential Q10/L reactivity against hepatocytes is not known. We have not been able to clone such cells from TG mice to test this issue directly (Table 2). However, it remains possible that with appropriate cytokines or other signals that some of these cells could be activated *in vivo* and contribute to an anti-self response. If these cells have low-affinity receptors for antigen, then this reactivity may result in a low-grade response, similar to that seen in a variety of autoimmune diseases.

This work is supported by National Institutes of Health Grants RO1-AI28332 and AI13111. K.W. was supported by National Cancer Institute Training Grant CA09082.

1. Lo, D., Burkly, L. C., Widera, G., Cowing, C., Flavell, R. A., Palmiter, R. D. & Brinster, R. L. (1988) *Cell* **53,** 159–168.
2. Allison, J., Campbell, I. L., Morahan, G., Mandel, T. E., Harrison, L. C. & Miller, J. F. A. P. (1988) *Nature (London)* **333,** 529–533.
3. Sarvetnick, N., Liggitt, D., Pitts, S. L., Hansen, S. E. & Stewart, T. A. (1988) *Cell* **52,** 773–782.
4. Lo, D., Burkly, L. C., Falvell, R. A., Palmiter, R. D. & Brinster, R. L. (1989) *J. Exp. Med.* **170,** 87–104.
5. Jones-Youngblood, S. L., Wieties, K., Forman, J. & Hammer, R. E. (1990) *J. Immunol.* **144,** 1187–1195.
6. Boehme, J., Haskins, K., Stecha, P., VanEwijk, W., LeMeur, M., Gerlinger, P., Benoist, C. & Mathis, D. (1989) *Science* **244,** 1175–1183.
7. Murphy, K. M., Weaver, C. T., Elish, M., Allen, P. M. & Loh, D. Y. (1989) *Proc. Natl. Acad. Sci. USA* **86,** 10034–10038.
8. Morahan, G., Allison, J. & Miller, J. F. A. P. (1989) *Nature (London)* **339,** 622–625.
9. Miller, J., Daitch, L., Rath, S. & Selsing, E. (1990) *J. Immunol.* **144,** 334–341.
10. Kress, M., Cosman, D., Khoury, G. & Jay, G. (1983) *Cell* **34,** 189–199.
11. Mann, D. W., Stroynowski, I., Hood, L. & Forman, J. (1987) *J. Immunol.* **138,** 240–245.
12. Mann, D. W., McLaughlin-Taylor, E., Wallace, R. B. & Forman, J. (1988) *J. Exp. Med.* **168,** 307–324.
13. Jenkins, R. N. & Rich, R. R. (1983) *J. Immunol.* **131,** 2147–2153.
14. Pimsler, M. & Forman, J. (1978) *J. Immunol.* **121,** 1302–1305.
15. Straus, D. S., Stroynowski, I., Schiffer, S. G. & Hood, L. (1985) *Proc. Natl. Acad. Sci. USA* **82,** 6245–6249.
16. Rammensee, H.-G., Kroschewski, R. & Frangoulis, B. (1989) *Nature (London)* **339,** 541–544.
17. Qin, S., Cobbold, S., Benjamin, R. & Waldmann, H. (1989) *J. Exp. Med.* **169,** 779–794.
18. Wood, P. J., Socarras, S. & Streilein, J. W. (1987) *J. Immunol.* **139,** 3236–3244.
19. Pickard, A. R. & Havas, H. F. (1972) *J. Immunol.* **109,** 1360–1370.
20. Pircher, H., Burki, K., Lang, R., Hengartner, H. & Zingernagel, R. M. (1989) *Nature (London)* **342,** 559–561.
21. Teh, H. S., Bennink, J. & von-Boehmer, H. (1982) *Eur. J. Immunol.* **12,** 887–892.
22. Ryser, J.-E. & MacDonald, H. R. (1979) *J. Immunol.* **123,** 128–132.
23. Burkly, L. C., Lo, D., Kanagawa, O., Brinster, R. L. & Flavell, R. A. (1989) *Nature (London)* **342,** 562–566.
24. Kappler, J. W., Roehm, N. & Marrack, P. (1987) *Cell* **49,** 273–280.
25. Kisielow, P., Bluthmann, H., Staerz, U. D., Steinmetz, M. & von Boehmer, H. (1988) *Nature (London)* **333,** 742–746.
26. Sha, W. C., Nelson, C. A., Newberry, R. D., Kranz, D. M., Russell, J. H. & Loh, D. Y. (1988) *Nature (London)* **335,** 271–274.
27. Schwartz, R. H. (1989) *Cell* **57,** 1073–1081.