A Fail-Safe Mechanism for Maintaining Self-Tolerance
By Sylvie Guerder and Polly Matzinger

Summary
Using cytotoxic T lymphocyte (CTL) responses to the class I histocompatibility antigen Qa1 and to the minor histocompatibility antigen H-Y, we show that the immune system maintains a peripheral screening process that is able to tolerize a wide variety of potentially autoimmune CTL. The critical factor is the presence or absence of specific T helper cells. If T help is available, CTL precursors that recognize antigen are activated. In the absence of help, they are tolerized. Thus, T helper cells are guardians of peripheral tolerance in CTL.

Although the first step in the induction of T cell tolerance occurs in the thymus, where newly arising autoreactive T cells are inactivated (1–7), four sorts of autoreactive T cells potentially exist in the periphery: rare "escapees" from thymic deletion, newly autoreactive cells arising by mutation (8, 9), T cells developed outside the thymus (10, 11) and T cells recognizing tissue-specific peripheral antigens that may be underrepresented in the thymus. To deal with this last category, which has been seen in mice, chickens, and frogs expressing an antigen only on a peripheral organ (12–21), we had suggested (22) that antigens may be transported to the thymus by circulating APC. However, since acquired antigens are more efficiently presented in association with MHC class II than class I molecules (23), tolerizing to peripheral antigens may be much more effective for CD4+ than for CD8+ T cells. How then are CD8+ CTL prevented from reacting to peripheral antigens?

22 yr ago, Bretscher and Cohn (24) proposed a two-signal model of activation for B cells, designed to maintain peripheral tolerance in the face of somatic hypermutation. They suggested that B cells received one signal from the antigen and a second from a helper cell specific for the same or a linked antigen, and that tolerance would result from the perception of signal one without signal two. Recently, several studies suggested that both B cell and CTL tolerance may be maintained this way. For example, mice primed in the absence of help were subsequently unable to reject Qa1-bearing grafts (19), generate specific CTL to Qa1 (19, 25) or H-Y (25), or produce antibody responses to protein antigens (26, 27).

Although these results were interpreted in the context of the two-signal model, other mechanisms are possible. Here we have analyzed a variety of potential mechanisms and found that the recognition of antigen in the absence of help is indeed a tolerogenic event for CTL, whether they be thymocytes or mature peripheral lymphocytes.

Materials and Methods
Mice. C57BL/6 (H-2b, Qa1b) and BALB/c (H-2d, Qa1b) mice were from Iffa-Credo (L'Arbresle, France), BALB.B (H-2b, Qa1b) and B6.TIa* (H-2b, Qa1b) mice were from Olac (Bicester, England), and A.Tlaa (H-2b, Qa1b) mice were bred in our facility (Kaiser-Augst, Switzerland).

In Vivo Immunization. Mice were immunized by an intraperitoneal injection of 107 splenocytes (prepared in serum-free medium) unless otherwise specified.

T Cell Depletion. Spleen cells were panned on petri dishes that had been coated with a mixture of T cell-specific mAbs: J1J, 100 μg/ml (anti-Thy-1; 28); YTS 169.4, 20 μg/ml (anti-CD8; 27); and GK1.5, 10 μg/ml (anti-CD4; 29), and then further depleted by two-step complement lysis with these mAbs and 10% rabbit complement (Low Tox-M; Cedarlane Laboratories, Hornby, Canada) followed by passage over Ficoll-Paque (Pharmacia Fine Chemicals, Piscataway, NJ).

Anti-CD4 Depletion In Vivo. Mice were injected intraperitoneally twice, 36 and 12 h before priming with 1 mg of GK1.5 and 0.1 mg YTA 3.1.2 (30), two synergistic anti-CD4 mAbs. FACS analysis (Becton Dickinson & Co., Mountain View, CA) revealed that CD4+ cells were completely depleted in the spleen and lymph nodes. Thymocytes, though coated with the mAbs were not depleted. Repopulation with CD4+ cells began after 1 wk and was complete at 8 wk.

Medium and Supplements. Cultures were set up in IMDM containing 10% FCS, 6 × 10−5 M α- thyoglycerol, and 50 μg/ml gentamycin. CAS, used in thymocyte cultures, was a supernatant of rat spleen cells activated for 48 h by 5 μg/ml of Con A.

In Vitro MLR and CTL Cultures. 4 × 106 spleen cells or 9 × 106 thymocytes were stimulated in 2-ml cultures for 5 d with 2 × 106 or for 7 d with 7 × 106 irradiated (3,000 rad) stimulator spleen cells, respectively. 10% Con A supernatant (CAS) (plus 10 mM α-methyl-mannoside) was added to thymocyte cultures as a source of helper factors. Serial 3× dilutions of cultured responders

1 Abbreviation used in this paper: CAS, Con A supernatant; R/T, responder to target.
were tested for lysis of $10^4$ 51Cr-labeled targets (3-d Con A blasts) in a 3.5-h assay. Responder to target (R/T) ratio, shown as responder dilution, is calculated from the number of responders initially cultured. Spontaneous release was done in the absence of killer cells and maximal release was induced with Zaponin (Zap/O/globin; Coulter Electronics, Luton, England). Percent specific lysis equals: 

$$\frac{100 \times (\text{experimental release} - \text{spontaneous release})}{\text{maximal release} - \text{spontaneous release}}.$$

**Summary Figures.** Each point represents the specific lysis generated by spleen cells from an individual mouse at an R/T ratio corresponding to the point, in each experiment, where the responses of the control mice drop off the plateau.

Data are presented according to Tufte (31).

**Results**

**A Hapten-Carrier System for Cytotoxic T Cells**

To study the effects of signal one on CTL activity, we used the response of B6.Tla$^a$ (B6$^a$) mice to the MHC class I antigen Qa1 (32, 33), an experimental system described by Keene and Forman (34) in which the CTL response shares many characteristics with the hapten-carrier systems used to study T-B cell interactions. Unlike other class I molecules, Qa1 does not usually elicit primary CTL responses in vitro (35–37), and, with rare exceptions (37–39), it does not act as a restricting element. In fact, in H-2b mice, Qa1 elicits CTL responses only when coupled with a carrier antigen able to activate T cell help, a defect that may result from its inability to couple well enough with A$^b$ to elicit strong CD4$^+$ helper responses.

This hapten-like character of Qa1 is illustrated in Fig. 1 by the responses of B6$^a$ mice to B6, two congenic strains carrying different Qal alleles (31). Fig. 1 a shows that unprimed B6$^a$ mice (which are Qa-1$^a$ [40]) generate very weak CTL responses to B6 (which are Qa-1$^b$), and mice primed in vivo with B6 cells do no better (Fig. 1 b). However, mice primed with B6 male cells (bearing Qa-1$^b$ plus H-Y) respond well in vitro to B6 female cells (Fig. 1 c), revealing the carrier effect of H-Y. This carrier effect is seen only if Qa1 and H-Y are expressed by the same cell (data not shown, and 34), a form of hapten-carrier linkage (41). Fig. 1 d shows that the minor histocompatibility antigens of BALB.B can also serve as carrier antigens, indicating that Qa1, like typical haptens, can be linked to many different kinds of carriers (41).

Fig. 1, e–h shows that in vivo priming depends on the presence of CD4$^+$ T helper cells. We depleted female B6$^b$ mice of peripheral CD4$^+$ T cells, injected them with male or female spleen cells bearing Qa1$^b$, and 12 wk later, when they had replenished their helper cells, tested them for in vitro CTL responses. The mice that had been primed during the CD4-depleted period were totally unresponsive to Qa1, whether they had been primed with Qa1 alone (Fig. 1 f), or Qa1 plus H-Y (h). Thus, CD4$^+$ T helper cells are essential for in vivo priming to Qa1.

They are also necessary for the in vitro boost. Although primed CTL precursors do not need carrier antigens in vitro (Fig. 1 d), they do need CD4$^+$ T helper cells (42, 43). Our interpretation for this paradox is that B6$^a$ mice may have a few T helpers able to see Qa1$^b$; too few to generate a strong primary CTL response but, either because of expansion or a change of state, enough to help a secondary response. In

**Figure 1.** Cytotoxic T cells specific for Qa1 need T cell help. (Top) The cytotoxic responses of B6$^a$ female mice primed to Qa1, alone or coupled with various carrier antigens. (Bottom) The responses of mice that were primed with or without CD4 depletion. The mice were primed with nothing or with an injection of B6 female (Q), B6 male (Q+ H-Y), or BALB.B female (Q+ m) spleen cells as indicated above each panel. 3 wk (a–d) or 12 wk later (e–h), their spleen cells were stimulated in vitro with B6 female or allogeneic A.Tla$^a$ stimulators and tested for anti-Qa1 lytic activity on B6 female targets, for allo-activity on A.Tla$^a$ targets, and for nonspecific lysis on syngeneic B6.Tla$^a$ female targets. Titration of effectors, shown as responder dilution, begins with a maximum responder to target (R/T) ratio of 100:1 (a–d) and 133:1 (e–h).
any case, since both primary and secondary CTL responses depended on T helper cells, we chose this system to study the effect of delivering antigen to CTL precursors in the absence of help.

Three Tests for Peripheral Tolerance

To look for evidence of tolerance induction, we primed B6 mice with Qa1b under three sets of conditions, each designed to deliver signal one in the absence of signal two. (Set 1) We immunized B6 female mice with cells bearing Qa1b without carrier antigens. (Set 2) We depleted the mice of CD4+ cells before immunizing with Qa1b plus a carrier. (Set 3) We immunized B6 male mice with Qa1b plus H-Y, a carrier to which males are tolerant.

The mice were then rested for various periods of time, reimmunized with cells bearing Qa1b plus a known effective carrier, and tested 2 wk later for their ability to generate CTL in vitro.

Set 1: Priming with Qa1 in the Absence of a Carrier Antigen Leads to Permanent Unresponsiveness

To test the effect of immunizing with cells bearing Qa1 alone, we primed mice with B6 female (Fig. 2, g-h) or male (d-f), or left them unprimed (a-c), boosted them with B6 female (Q), male (Q+H-Y), or BALB.B (Q+m) cells, and tested for their ability to generate CTL responses. As usual, mice that had been injected once responded only if immunized with carrier-linked Qa1 (Fig. 2, b and c), and all the mice primed with Qa1+H-Y produced CTL regardless of the boosting antigen, but mice that had been injected with cells bearing Qa1 in the absence of a carrier (Fig. 2, g and h) were unresponsive to any further challenge with Qa1. The lack of response was antigen specific, since the mice responded well to the carriers used in the boosting immunizations (e.g., H-Y and BALB minis). Thus, the initial injection is crucial. If Qa1 is given with a carrier, the mice are primed. If it is given without a carrier, the mice are rendered tolerant and no amount of help later will rescue the response.

Qa1 Alone Also Tolerizes Thymocytes. Since a proportion of mature thymocytes are fully immunocompetent (45, 46), we asked whether their responses would be the same as those of peripheral cells. Fig. 3 shows that thymic CTL behaved like those from the spleen. They were not primed to Qa1 by cells bearing only a Qa1 difference (Fig. 3 a), they did respond to cells bearing carrier-linked Qa1 (b and c), and they

Figure 2. Mice immunized with Qa1 alone become unresponsive. (Top) The titrated responses (maximal R/T is 100:1) of individual B6 female mice tested in two experiments; and (bottom) a summary of 54 mice, tested in 20 experiments. B6 female mice were left unprimed or were primed with B6 male (Q+H-Y), or female spleen cells (Q). 3 wk later, they were boosted in vivo against B6 female, B6 male, or BALB.B (Q+m) as indicated above each panel. 2 wk later, their spleen cells were stimulated in vitro with BALB.B (Q+m) and tested for lysis on B6 female cells (Q alone) and BALB.B female (Q+m) targets, or stimulated and tested against H-Y (B6 male). Each point in the summaries represents the specific lysis generated by an individual mouse at an R/T ratio of 30–40:1, and the thin lines to the left of each group indicate the mean ± 2 SEM. Differences between the groups were evaluated by student's t test. For Qa1, groups a and g differ from b, and h differs from c at p < 0.001. The BALB.B and H-Y responses do not differ significantly. Lysis of B6 female targets never exceeded 3% of the lysis seen on specific targets. About one third of the mice do not respond to H-Y. This is not unusual as H-Y is a minor antigen to which mice of most haplotypes do not respond and, even in responsive strains, many individuals give weak responses which are easily competed by other antigens (44).
were turned off by prepriming with antigen in the absence of a carrier determinant (g and h).

Because small numbers of peripheral T cells, especially activated T cells, can circulate through the thymus (47, 48), we thought that the thymic responses might be due to recirculating activated peripheral CTL rather than activated thymocytes. To distinguish between these two possibilities, we studied the kinetics of tolerance induction. We first injected mice with B6 female cells (Q), and then, to see how quickly tolerance became entrenched, injected them later with B6 male cells (Q + H-Y). Fig. 4 shows that, in the spleen, tolerance induced by female cells was completely reversed by coinjection of male cells (time 0). By day 2, injection of male cells rescued only 30% of the response, and by day 4 the spleens were >95% unresponsive. Tolerance occurred slightly faster in the thymus, suggesting that both newly arising thymocytes and any recirculating peripheral cells were tolerized there. Had the activity in the thymus been due to recirculating T cells previously activated (or tolerized) in the periphery, tolerance should have appeared later in the thymus than in the spleen. Thus, thymocytes specific for Qa1 follow the same rules as peripheral T cells. They are able to be activated in the presence of a helper determinant and are tolerizable in its absence.

Surprisingly, the tolerant state lasted for 7 mo. Although such a durable state of tolerance in nontoyectomized mice suggested that the injected B6 female cells had established some level of chimerism, we could not detect them in spleen, thymus, or lymph nodes by FACS analysis, by staining of tissue sections with anti-Qa1 sera, or by injecting cells labeled with FITC or 51Cr. Nevertheless, mice injected with mitomycin C-treated cells were only unresponsive to Qa1 for a few days, suggesting that long-lasting tolerance requires the persistence of at least some of the tolerizing cells.

The Induced State of Unresponsiveness Is Not Simply the Result of Thymic Tolerance. Suppose that immunizing with signal one alone were a nonevent, having no effect on peripheral CTL. If virgin CTL had a fairly short lifespan, they would soon die and, by the time we challenged the mice, the peripheral pool would consist of cells that had been immature thymocytes at the time of the first inoculation. The tolerance we measured in the periphery could therefore simply reflect the normal path of thymic tolerance induction, rather than a direct effect on the mature peripheral CTL population.

To test this possibility, we thymectomized a group of adult mice, waited 1 mo for the periphery to stabilize, and immunized them with Qa1. Fig. 5 shows that these CTL can be as effectively immunized as those of normal mice by B6 male and paralyzed by B6 female cells. Thus, this form of tolerance induction operates on mature peripheral CTL precursors. We conclude that CTL in the spleen, any peripheral cells circulating through the thymus, and newly arising thymocytes can all be paralysed in the same way and at about the same time.

Set 2: Removing T Helper Cells Generates Tolerogenic Conditions

This experimental group was designed to ask whether carrier antigens mediated their effect through CD4+ T helper cells or whether they altered presentation of Qa1 to the killers themselves. We tested this by giving carrier-free (tolerogenic) and carrier-associated (immunogenic) Qa1 to mice from which...
the CD4⁺ T helper cells had been temporarily removed. We injected mice twice, 24 h apart, with mAbs to CD4, and primed them 12 h later with B6 female (Qa1) or male (Qa1+H-Y) cells. 12 wk later, when the CD4⁺ cells had recovered, we injected a mix of cells carrying Qa1 plus various carriers. Any remaining functional CTL should now, surrounded by T helper cells and carrier antigen, be easily immunized.

We found that mice immunized during the depleted period became unresponsive, whether they were primed with carrier-free (Fig. 6 d) or carrier-linked (e) Qa1. This was not simply due to temporary removal of CD4⁺ cells, because mice that

---

**Figure 4.** Kinetics of tolerance induction. B6 female mice were injected with spleen cells at time 0 and again on the days indicated. 15 d after the second injection, their spleen cells were cultured with B6 female stimulators and assayed for Qa1-specific lysis as in Fig. 1. R/T is 133:1 for the splenocytes and 300:1 for the thymocytes.

**Figure 5.** Tolerance induction in adult thymectomized mice. 6-wk-old B6 female mice were thymectomized or left untreated (Normal). 1 mo later, some of the thymectomized mice were primed with B6 female cells (Q), and 3 wk later, all the mice were boosted with B6 male (Q+H-Y). 2 wk after boosting, their spleen cells were cultured with B6 female or allogeneic BALB/c stimulators and tested for cytotoxic activity on B6 and BALB/c targets, respectively. The maximum R/T is 133:1.
were not immunized during the depleted period did produce CTL (Fig. 6 c), though slightly less than controls, and all the mice responded to the BALB.B minors (Q + m). Fig. 6 e shows that depleted mice primed with male cells also failed to generate CTL to H-Y, a finding indicating that tolerance induction in the absence of help is not found only for the medial antigen Qal but also extends to more typical, MHC-restricted minor antigens.

In the thymus, CD4+ cells were coated but not removed by anti-CD4 mAbs. Nevertheless, Fig. 7 shows that thymic CTL were also turned off if immunized during the period of treatment (d and e). So, it appears that helper cells need not be deleted; impairing their activation is enough to prevent the delivery of help and induce tolerance.

Thus, an injection of carrier-linked Qal, which is immunogenic in the presence of help from CD4+ cells, becomes tolerogenic in its absence. From this, we concluded that carrier antigens do not directly affect CTLs by altering the structure or tissue distribution of Qal, but act indirectly through CD4+ T helper cells.

Set 3: Tolerance of the Carrier Antigen Neutralizes Its Efficacy

Two possibilities were left. Either carrier-specific helper cells could assist Qal-specific CTL, or the carrier altered the expression or processing of Qal itself so that it was effectively presented by class II molecules to helpers specific for Qal peptides (49). By taking advantage of the natural self-tolerance that male mice have for H-Y, we were able to distinguish between these two. We assumed that, if H-Y exerts its effect through H-Y-specific helper cells, it should be use-

less in males. However, if its effect were to alter Qal presentation to Qal-specific helper cells, then B6 males should respond to B6 male cells (12, 50).

Fig. 8 shows that B6 males behave essentially like their sisters. They do not make strong primary CTL to Qal (b) and can be primed by carrier-linked Qal (c). But, unlike females, males do not respond if the only available carrier is H-Y (d), in fact they are tolerized (e). Since the presence of H-Y does not inhibit the carrier effect of BALB minors (c), the tolerogenic property of male cells is unlikely to be due to dominant suppression, but rather to the lack of H-Y-specific helper T cells. Thus, the carrier effect is due to the independent recognition of the carrier antigens by T helper cells and not to an indirect effect on the presentation of Qal itself.

The Tolerizing Effect Is Not Due to Thy-1+ Veto Cells

Since Qal is a class I antigen, albeit a rather atypical one, expressed by activated T cells; since activated T cells are able to migrate to the thymus (48) and activated CD8+ T VETO cells can turn off CTL responses against their own MHC class I molecules (51); since the tolerizing effect can sometimes be reversed by adding class II differences (helper determinants?) to the VETO cells and reinstated by removing CD4+ T helper cells (52), we considered the possibility that VETO cells (53) were the APC responsible for tolerance induction to Qal.

To test this we first found the minimum tolerizing and immunizing doses of Qal+ spleen cells (Fig. 9). Though as few as 103 male (Q+H-Y) cells were enough to prime,
tolerance induction required 100-fold more female (Q) cells (not surprising since the vast majority of specific CTL must be inactivated). Fig. 10 shows that Thy-1- female cells (Q) induced tolerance as effectively as untreated cells in both spleen and thymus, and Thy-1- male cells (Q+H-Y) were actually more effective at priming. Comparing Figs. 9 and 10, we see that our lowest dose of Thy-1- female cells contained 40-fold fewer T cells than the minimum tolerizing dose of untreated cells. Thus, Thy-1+ cells had little effect on the induction of tolerance or immunity to Qa1. Although some NK precursors in bone marrow are Thy-1+ (54), most splenic VETO cells (T and NK cells) are Thy-1+ (55). From this, we conclude that tolerance to Qa1 after an injection of B6 female cells is unlikely to be due to VETO cells but rather to recognition of antigen in the absence of help.

Recapitulation

From these results, we conclude that: (a) both thymocytes and peripheral CTL specific for Qa1 need help from CD4+

Figure 7. When Th cell activation is prevented, thymic CTL are inactivated by signal one. These are the thymocyte responses of the mice described in Fig. 6. They were stimulated and tested as in Fig. 6, except that the cultures contained 10% CAS. Maximal R/T for the titrations is 225:1 for Qa1 and minor responses, and 300:1 for H-Y. Summaries are shown at an R/T of 75–300:1. For Qa1, a differs from b by p = 0.00002, d and e differ from c by p = 0.008 and p = 0.03, respectively. For H-Y, e differs from c by p = 0.2 and from b by p = 0.01.

Figure 8. H-Y does not generate signal two in male mice. B6+ female or male mice were left unprimed or were immunized with B6 male (Q+H-Y), 3 wk later, some of the mice were boosted with BALB.B male as indicated above each panel. 2 wk later, their spleen cells and thymocytes were stimulated as in Figs. 2 and 3 and tested on B6 (Q) or BALB.B female (Q+m) targets. Maximal R/T is 100:1 for spleen and 300:1 for thymocytes.
T cells in order to be activated. (b) They can receive this help from T cells specific for other determinants (carriers) present on the immunizing cells. (c) In the absence of help, whether caused by lack of a carrier determinant or lack of T helper cells, recognition of Qa1 is a tolerogenic event.

We found that: (a) Qa1 is not unique; H-Y-specific CTL were also tolerized in the absence of help. Though this finding contrasts with a recent claim for the helper independence of H-Y-specific CTL in transgenic mice (4), it agrees with earlier studies showing that help was required (56). (b) The tolerant state was completely antigen specific; CTL to BALB minors were unaffected by injections of Qa1, and responses to H-Y dropped only when male cells were given under tolerogenic conditions, i.e., in the absence of T helper cells. (c) Removing the carrier antigen and removing T helper cells were equally effective ways to induce tolerance. (d) Thymic CTL were tolerized when the CD4+ cells were coated but not depleted by anti-CD4 mAbs. Thus, as Waldmann (57) suggested, it may not be necessary to physically remove the helpers. If they can be prevented from responding, whether or not they themselves become tolerant in the process (58), the lack of help results in tolerance of CTL.

Taken together, our results confirm that the thymus is not the only site of tolerance induction, nor are immature thymocytes the only tolerizable cells. We have shown here that mature peripheral CTL are tolerized in the absence of help. Such
Discussion

These results split CTL activation into three stages. In stage one, the CTL precursor, like a B cell, advances to a critical point and waits for help. If help is not received within about 24 h (Fig. 3, and 59, 60), the CTL is shut down, but if help arrives it moves to the second stage and becomes fully activated, ready for step three, in which it can be triggered to lyse a target in the complete absence of help (61, 62). Although activation (steps one and two) and triggering (step three) are both consequences of the same external event (antigen recognition), the difference in helper requirements points to a fundamental distinction between the two. The distinction is not simply a property of virgin vs. memory CTL, because resting memory CTL are also tolerantized by antigen in the absence of help (25). Nor is it a property of the APC, because the same APC can activate in a helper-dependent fashion and be killed in the absence of help (63). The distinction must be intrinsic to the intracellular signaling pathways themselves such that a second signal is needed to kick a resting CTL into cell division, synthesis of the lytic machinery, etc., but signal one is enough to trigger activated CTL to kill their targets (64). This distinction between activation and triggering may also hold for T helper cells, where some of the second signals necessary to induce IL-2 production from resting T cells are not needed for IL-4 (65, 66), which seems to be released when an activated T cell (67, 68) is reactivated.

How is help delivered? Because resting CTL, being MHC class II negative, cannot be seen by class II-restricted helpers, Keene and Forman (34) suggested that help was delivered via secreted IL-2, and that efficient delivery occurred only if the CTL and the helper were attached to the same APC.

An alternative view is that help for CTL is routed through the APC rather than supplied directly from helper to killer. This is based on Lafferty and Cunningham's (69) proposal that T cells need costimulatory signals from APCs, on the finding that lack of such costimulatory signals leads to tolerance (59), and on the evidence that T helpers can activate APC to deliver such signals (70, 71). We picture a system in which a helper cell binds to an APC and, rather than simply secreting IL-2 into the surrounding medium, it induces the APC to produce costimulatory signals for naïve CTL. By delegating the help function to activated APC, a single T helper would be able to assist many CTL precursors without needing to see the antigen at precisely the same time, and the activity of a small number of antigen specific helpers could be effectively amplified. Help delivered through APC could also explain why CTL responses to some viruses are helper independent (72). If a virus were itself able to activate APC or if it infected dendritic cells, a cell type that may constitutively express costimulation (73, 74), CTL responses would ensue without the need of CD4+ T helper cells.

Last we come to the question of who controls the controllers. If help is the controlling factor in peripheral tolerance, what governs the activation of helpers? More helpers? Cohn (75) suggests that T helpers also need help (75), but this leaves him with the chicken-egg problem of what cell gives signal two to the first helper. There is some evidence that helpers may get their second signals from professional APC (76–78). This would solve the problem of who came first but, since APC present both self and nonself antigens (79–81), it does not deal with the maintenance of tolerance. B cells may have a role here. Since B cells are the largest population of class II+ cells, a newly emerging autoreactive CD4+ T cell's first antigen encounter would most likely be with a B cell. If mammalian B cells like those of chickens (82), cannot deliver costimulatory signals to virgin T cells, this encounter would be tolerogenic. Indeed, resting B cells can present rabbit Ig to tolerize virgin T cells (83), and female mice can be rendered specifically unresponsive to H-Y by an injection of purified male B cells (E. Fuchs and P. Matzinger, manuscript submitted for publication). However, B cell presentation cannot account for tolerance to antigens found only on nonlymphoid tissues such as skin or liver. Do other cell types also have the ability to tolerize virgin T cells? Bowen et al. (84) have shown a slow induction of tolerance in mice transplanted with thyroid or pancreas from which the APCs were removed; Bal et al. (85) found that antigen presentation by APC depleted keratinocytes can turn off antigen-specific T cell clones, and the VETO phenomenon is yet another example of tolerance induction by tissues (CD8+ T cells or NK cells) that are not professional APCs. We wonder, in fact, whether some of the studies that have been classed as VETO phenomena (52) are not actually cases of presentation of signal one without signal two, since tolerance in these cases can be reversed by the addition of a carrier antigen. Immunologists may be dealing here with two quite different mechanisms (tolerance due to VETO cells and tolerance due to antigen presentation in the absence of signal two) that sometimes mimic each other. We presume that any cell capable of delivering signal one (antigen associated with MHC) without signal two will turn off virgin T cells. However, since many tissues do not express class II, there must either be a small set of peripheral antigens to which T helper cells are truly not tolerant or perhaps still more tolerogenic mechanisms to be found.

Finally, on a practical note, if the effector T and B cells of the immune system are consistently rendered unresponsive by signal one in the absence of help, then T helper cells are those on which we should concentrate our efforts to achieve transplantation tolerance and the reversal of autoimmune disease. If helper cells (whether CD4 or CD8) can be reliably rendered unresponsive, the rest of the immune system will follow.
We thank our colleagues Albert Bendelac, Adriana Bonomo, Kurt Brorson, Delanie Cassell, Klaus-Michael Debatin, Ephraim Fuchs, Robert Homer, Charlie Janeway, Steve String fellow, and the Ron's (Germain, Palacios and Schwartz) for their strong, helpful critiques of the work and the paper, and Ernst Wagner for maintaining the truly clean mouse colony, which allowed this work to be possible.

The Basel Institute is wholly supported by Hoffman La Roche, Basel, Switzerland.

Address correspondence to P. Matzinger, Laboratory of Cellular and Molecular Immunology, Building 4, Room 111, National Institute of Allergy and Infectious Disease, National Institutes of Health, Bethesda, MD 20892. S. Guerder's present address is the Section of Immunobiology, School of Medicine and Howard Hughes Medical Institute, Yale University, New Haven, CT 06511.

Received for publication 5 September 1991 and in revised form 7 April 1992.

References


the role of lymphocytes in regulating their development. *Immunological Rev.* 123:115.


