B Cells Directly Tolerize CD8+ T Cells

By Sally R.M. Bennett,*‡ Francis R. Carbone,§ Tracey Toy,* Jacques F.A.P. Miller,* and William R. Heath*

From the *Immunology Division, The Walter and Eliza Hall Institute of Medical Research, The Royal Melbourne Hospital, Melbourne, Victoria 3050, Australia; ‡The Cooperative Research Centre for Vaccine Technology at the Queensland Institute of Medical Research, The Royal Brisbane Hospital, Herston, Queensland 4029, Australia; and the §Department of Pathology and Immunology, Monash Medical School, Prahran, Victoria 3181, Australia

Summary

This report investigates the response of CD8+ T cells to antigens presented by B cells. When C57BL/6 mice were injected with syngeneic B cells coated with the Kb-restricted ovalbumin (OVA) determinant OVA257–264, OVA-specific cytotoxic T lymphocyte (CTL) tolerance was observed. To investigate the mechanism of tolerance induction, in vitro–activated CD8+ T cells from the Kb-restricted, OVA-specific T cell receptor transgenic line OT-I (OT-I cells) were cultured for 15 h with antigen-bearing B cells, and their survival was determined. Antigen recognition led to the killing of the B cells and, surprisingly, to the death of a large proportion of the OT-I CTLs. This involved Fas (CD95), since OT-I cells deficient in CD95 molecules showed preferential survival after recognition of antigen on B cells. To investigate the tolerance mechanism in vivo, naive OT-I T cells were adoptively transferred into normal mice, and these mice were coinjected with antigen-bearing B cells. In this case, OT-I cells proliferated transiently and then lost from the secondary lymphoid compartment. These data provide the first demonstration that B cells can directly tolerize CD8+ T cells, and suggest that this occurs via CD95-mediated, activation-induced deletion.

Key words: CD8+ T lymphocytes • cytotoxic T lymphocytes • antigen presentation • B cells • ovalbumin

B cells express relatively high levels of class I MHC molecules and therefore potentially play a role as APCs for CD8+ T cells. In vitro evidence suggests that B cells can stimulate IL-2 production and CTL activity by either primed CD8+ T cell clones or hybridomas (1–3). However, studies in B cell–deficient mice suggest that B cells are not required as APCs during the inductive phase of naive CD8+ T cell responses (4). In fact, it has been reported that B cells fail to induce naive CD8+ T cells to generate primary CTL activity in vitro (5) and can induce secondary in vitro unresponsiveness in CD8+ T cell clones (6). In adult mice, B cells have been shown to induce in vivo CTL tolerance to the minor antigen H-Y (7), but whether this was due to direct tolerance of the CD8+ T cell compartment was not addressed. CTL responses to H-Y are known to be CD4+ T cell dependent (8, 9), and there is a great deal of evidence that B cell presentation of antigen to mature CD4+ T cells is tolerogenic (10–16). Thus, it was unclear whether H-Y-specific CTL tolerance was due to the direct tolerance of CD8+ T cells, or occurred because H-Y-specific CD4+ helper T cells were tolerized. In this latter case, the CTLs themselves might have been completely unaffected by antigen-bearing B cells, but failed to be primed in the absence of CD4+ T cell help. In this report, we demonstrate that CD8+ T cells can be directly tolerized after encounter with antigen on B cells. The basis of this tolerance is examined.

Materials and Methods

Mice. Mice were bred and maintained at the Walter and Eliza Hall Institute for Medical Research. OT-I mice have been described previously (17), and were maintained on the recombination-activating gene (RAG)-1–deficient C57BL/6 (B6) background. Some experiments used OT-I mice expressing the lpr mutation (OT-I.lpr) or back-crossed to bm1; both of these strains were RAG-1 sufficient. For all experiments, mice between 8 and 16 wk of age were used.

B Cell Purification. B cells were purified as described previously (16). In brief, spleen cells were depleted of red blood cells,
and passed over a 30-35 ml Sephadex G-10 column (Amersham Pharmacia Biotech, Uppsala, Sweden) to remove adherent cells. T cells were then removed by treatment with a mixture of anti-Thy1.2 (J11), anti-CD8 (3.168), and anti-CD4 (R L172) antibody supernatants at 4°C for 30 min, followed by two successive treatments with rabbit C (C-Six Diagnostics Inc., M equon, W1) at 37°C for 20 min. After passage over a second Sephadex G-10 column, the cells were analyzed by flow cytometry using anti-B220–FITC and found to be >95% B220+. 

To separate small, resting B cells, the B cell suspension was centrifuged at 3,000 rpm for 30 min over a discontinuous Percoll (Amersham Pharmacia Biotech) density gradient containing ρ = 1.05–1.09 layers as described previously (16). Cells from the ρ = 1.07–1.08 interface were used as resting B cells. B cells were coated at 5 × 10^6/ml with 0, 1.0, or 10 μg/ml OVA 257–264 peptide in Hepes Eagle’s Medium (HEM) containing 2.5% FCS, or left uncoated.

Dendritic Cell Generation. Bone marrow–derived dendritic cells were prepared as described previously (18) with the following modifications. In brief, bone marrow cells from B6 mice were cultured in 94-mm tissue culture dishes (Greiner Labortechnik, Frickenhausen, Germany) at a density of 5 × 10^5/ml in complete DMEM with 10% FCS, 50 μM 2-ME, 2 mM l-glutamine, and 2.5 × 10^6/ml irradiated syngeneic irradiated B6 spleen cells. The LNs and spleen of each mouse were analyzed by flow cytometry on day 3, 7, or 14 after the initial B cell immunization using anti-Thy1.1–FITC (OX-7; Pharmingen, San Diego, CA), anti-CD8-PE (CT-CD8α; Caltag Laboratories, Inc.), and anti-V_2 (B20.1)-biotin followed by Streptavidin–Tri-color (Caltag Laboratories Inc.). The number of OT-I cells was determined for each organ by multiplying the cell number by the percentage of Thy1.1 CD8^V_2^ cells. For individual litters, the background number of Thy1.1 CD8^V_2^ cells of a control littermate that did not receive an injection of OT-I cells was subtracted from the number of CD8^Thy1.1 CD8^V_2^ cells found in the same organ of each test host mouse. To measure the CTL function of adoptively transferred OT-I cells, 5, 2.5, 1.25, 0.63, 0.31, or 0.16 × 10^6 LN cells from the (B6 × B6.Ka thy1F)F1 host mice were cultured in triplicate with 5 × 10^5 irradiated, OVA-loaded spleen cells in 200 μl RPMI containing 10% FCS, 50 μM 2-ME, and 2 mM l-glutamine. 

The number of OT-I responders per well was calculated by multiplying the number of LN cells by the percentage of Thy1.1 CD8^V_2^ cells as determined by flow cytometry. After 5 d, the cytotoxicity of each well was assessed on 10^4 51Cr-labeled EL4 cells alone or EL4 cells coated with 1 μg/ml OVA 257–264 during 51Cr labeling as described (19).

Results

B Cell Presentation of Antigen Causes Direct Tolerization of CD8^+ T Cells. Presentation of antigen by B cells has been shown to cause CTL tolerance for the male-specific anti-H-Y response (7). However, this response is dependent on CD4^+ T cell help (8, 9), raising the possibility that CTL tolerance occurred indirectly by the induction of CD4^+ T cell tolerance. To investigate whether antigen-bearing B cells can induce tolerance by directly affecting the CD8^+ T cell population, B cells coated with the K_b-restricted OVA peptide OVA 257–264 were tested for their ability to tolerize OVA-specific CTL responses generated by intravenous injection of irradiated spleen cells loaded intracytoplasmically with OVA protein (OVA-loaded spleen cells [21]). Injection of B6 mice with 10^7 OVA 257–264-coated B cells reduced the subsequent OVA-specific CTL response upon challenge with OVA-loaded spleen cells (Fig. 1 a). On average, the ability to generate OVA-specific CTLs was diminished 10-fold, but many mice showed >100-fold weaker responses (Fig. 1 d). Tolerance induction by B cells was long-lasting, as thymectomized mice showed no response upon challenge with OVA-loaded spleen cells (data not shown). The level of antigen expression also affected tolerance induction, which was slightly more efficient when B cells were coated with a higher concentration (10 vs. 1 μg/ml) of peptide (Fig. 1 d). Although CD4^+ T cell tolerance induction by B cells has been shown to be more

1978 B Cells Directly Tolerize CD8^+ T Cells
effective using resting B cells (11, 12), we found that for CD8⁺ T cells, small resting B cells were no more tolerogenic than unfractionated B cells (Fig. 1 c). Under similar conditions, peptide-pulsed dendritic cells were not tolerogenic, but instead primed hosts for stronger responses to OVA-loaded spleen cell challenge (data not shown).

We have found that the priming protocol used to measure CD8⁺ T cell cell tolerance induction by B cells, i.e., OVA-loaded spleen cell challenge, is CD4⁺ T cell dependent (19). Therefore, it was possible (though unlikely given that tolerization only involved the class I–restricted determinant) that CTL tolerance in this model may have also occurred indirectly through the induction of CD4⁺ T cell tolerance. To determine whether tolerance resulted from a direct effect on CD8⁺ T cells, we examined cell tolerance induction under conditions where CTL generation was not CD4⁺ T cell dependent, i.e., by priming subcutaneously with OVA257–264 in CFA (19). OVA257–264-coated B cell–primed mice challenged subcutaneously with OVA257–264 in CFA also failed to generate OVA-specific CTLs (Fig. 1 b), confirming that the CD8⁺ T cells were directly tolerated.

Activated CD8⁺ T cells die after recognizing antigen on B cells in vitro. To investigate the mechanism of CTL tolerance induction by antigen-bearing B cells, we examined the in vitro response of activated CD8⁺ T cells to antigen-bearing B cells. For these experiments, in vitro–activated CD8⁺ T cells from the OVA-specific TCR transgenic line OT-I were used. These mice are of a B6 genotype. OT-I cells plus syngeneic B6 B cells (unpulsed or pulsed with OVA257–264) were cultured together for 15 h and then analyzed by flow cytometry to determine the survival of each cell population relative to an internal control population of bm1 B cells. These latter cells bear the Kbm1 MHC molecule and therefore cannot present OVA257–264 to OT-I cells (20). The survival of the OT-I cells (B220⁺, Kb⁺) and B6 B cells (B220⁺, Kb⁻) was then examined relative to the bm1 control B cells (B220⁺, Kb⁻) (Fig. 2, a and b). As expected, the activated OT-I cells killed a large proportion of B6 B cells, as seen by the reduction in B6 B cells relative to bm1 B cells in the presence of antigen. Surprisingly, there was also a dramatic loss of OT-I cells relative to the bm1 B cell control population. Six separate experiments revealed a consistent loss of the CD8⁺ CTLs during their 15-h culture with antigen-bearing B6 B cells. This result was the same whether the bm1 B cells were cocultured as described above, or whether they were simply added at the end of the culture period, just before flow cytometric analysis. In an example of the latter experiment, there were 2.1 × 10⁵ OT-I cells and 1.7 × 10⁵ B6 B cells present after 15 h of
culture in the absence of peptide. When the B6 B cells were first coated with peptide, only $0.31 \times 10^6$ OT-I cells and $0.83 \times 10^6$ B6 B cells remained. This represents an 85% loss of OT-I cells and a 51% loss of B6 B cells.

The antigen-specific loss of OT-I cells was not due to fratricide as a result of re-presentation of OVA peptide by CTLs to each other, since OT-I cells bearing the nonpresenting Kbm1 molecule, instead of K*, were also killed (data not shown). To investigate whether Fas (CD95)-mediated signaling (for a review, see reference 22) was involved in the death of activated CD8$^+$ T cells, the OT-I mice were crossed to lpr mice, which express a genetic defect in CD95 (23). When activated OT-I.lpr cells were cultured for 15 h with antigen-bearing B cells, survival was greatly improved (Fig. 2, c and d). This suggested that activated cells were killed by a CD95-dependent mechanism. Unlike activated OT-I cells, naive OT-I cells did not kill antigen-bearing B cells and were not killed during this 15-h culture period (data not shown).

Expansion and Loss of Naive OT-I T Cells in Response to Antigen-Bearing B Cells In Vivo. As shown earlier, CTL tolerance induction in B6 mice involved recognition of antigen on B cells by naive CD8$^+$ T cells. To further characterize this response in vivo, naive OT-I cells were injected into Thy1 congenic (B6 $\times$ B6.Kathy)F1 mice. After injection of OVA$_{257-264}$-coated B cells, the fate of the adoptively transferred OT-I cells was followed by flow cytometry. The number of OT-I cells recovered from the LNs and spleen of individual mice was then calculated as a percentage of the number of OT-I cells in unprimed control mice (Fig. 3). Exposure to antigen-bearing B cells resulted in a large expansion of OT-I cells by day 3, as seen by an increase in the relative number of OT-I cells in primed versus untreated controls (Fig. 3 a), and, in separate experiments, by the observed proliferation of carboxyfluorescein succinimidyl ester-labeled OT-I cells under similar conditions (data not shown). The extent of OT-I cell expansion was similar to that induced by OVA-loaded spleen cells (Fig. 3 a), which are known to induce strong OVA-specific CTL responses (21). However, by day 7 most of the OT-I cells generated in response to OVA$_{257-264}$-coated B cells had disappeared (Fig. 3 b), and the number of OT-I cells remained similar to or below that of unprimed mice to day 14 (Fig. 3 c). In contrast, mice immunized with OVA-loaded spleen cells showed further expansion of OT-I cells up to day 14. The OT-I cells remaining in B cell-treated mice were not anergic, as they were able to lyse OVA-expressing targets as efficiently as naive OT-I cells, after 5 d restimulation in vitro (Fig. 4). Therefore, although an immunogenic form of OVA (OVA-loaded spleen cells) was able to induce sustained proliferation of OT-I cells, tolerance induction by B cells was characterized by proliferation and then rapid deletion of OVA-specific CD8$^+$ T cells.

To test whether the incomplete deletion of OT-I cells was the result of insufficient antigen exposure, OT-I cell survival was examined after two injections of B cells, 5 d apart. Under these circumstances, deletion of OT-I cells was slightly more efficient (Fig. 3 d).

**Discussion**

Most studies examining tolerance induction by B cells have focussed on the response of CD4$^+$ T cells (10–16). This report represents the first demonstration that naive CD8$^+$ T cells can be directly tolerized by recognition of antigen on B cells. One other study has addressed the role of B cells as tolerogenic APCs in vivo for CD8$^+$ T cell responses by showing that injection of male B cells into female mice resulted in H-Y-specific CTL tolerance (7). However, generation of H-Y-specific CTLs has been reported to require CD4$^+$ T cell help (8, 9). Thus, failure to induce H-Y-specific CTLs might have simply reflected tolerant CD4$^+$ T helper cells. We showed that OVA-spe-
cific CTLs were directly tolerized by OVA 257–264–Coated B cells, since mice primed with OVA 257–264 peptide-coated B cells responded weakly to both OVA–loaded spleen cells (Fig. 1, a and c) and OVA peptide in CFA (Fig. 1 b). This latter response is CD4+ T cell independent, indicating that CTLs exposed to B cells bearing class I–restricted determinants are directly tolerized.

Since CTL tolerance has been reported to be the default response when CD4 help is unavailable (24, 25), it is possible that B cells induced CTL tolerance simply because they failed to provide determinants for stimulation of CD4+ T cell help. Even if class II–restricted determinants had been available, naïve CD4+ T cells are reported to be tolerized by recognition of antigen on B cells, which should also lead to a lack of help and, consequently, CTL tolerance. It remains to be addressed whether provision of primed CD4+ T cell help, which cannot be tolerized by B cells, will allow B cells to stimulate naïve CTL responses.

Our in vitro studies showed that peptide-coated B cells could be lysed by activated OT-I cells, confirming that CD8+ T cells could recognize antigen on B cells (Fig. 2). Moreover, importantly, these experiments revealed that activated, but not naïve, OT-I cells died shortly after interacting with antigen-bearing B cells. The loss of activated OT-I cells after recognition of antigen on B cells suggested that the pathway to B cell–mediated tolerance induction may require the interaction of activated CD8+ T cells with B cells. Protection of activated OT-I.lpr CD8+ T cells from death implied that CD95–mediated signaling played an important role in this death pathway.

A great deal of evidence suggests that CD95–mediated signaling is the predominant mediator of CD4+ T cell death in vitro via a mechanism termed activation-induced cell death (AICD [26–30]). This is mediated through the interaction of CD95 with its ligand (CD95L [31, 32]), expressed on the same (26, 27) or neighboring (33, 34) antigen-activated T cells, and occurs within 24 h for previously activated cells (35). Although there is some evidence that CD95 is involved in the death of activated CD8+ T cells (33, 36, 37), TNFR/TNF-mediated apoptosis has also been suggested to be important (35, 38, 39). This latter mechanism takes 40–48 h to induce apoptosis of activated T cells (35). Our observation that up to 85% of activated CD8+ T cells were killed within 15 h argues against a role for TNFR signaling for B cell–induced deletion of CD8+ T cells. The fact that activated CD8+ T cells expressing the mutant CD95 gene were largely protected from deletion suggests that CD95/CD95L interactions are more important in this case.

It was at first surprising that naïve OT-I cells, in contrast to activated OT-I cells, were not killed when cultured for 15 h in vitro with antigen-bearing B cells. Interestingly, cultures containing naïve OT-I cells showed extensive proliferation on day 2 (data not shown). This is consistent with the idea that to be deleted, OT-I cells must first be activated and, as a consequence, may proliferate before being killed. However, the combined effects of proliferation, nutrient utilization, and activation–induced cell death in vitro made it very difficult to analyze the response of naïve OT-I cells under these conditions; therefore, we concentrated our efforts on examining the response of naïve OT-I cells in vivo.

Deletional tolerance after antigen-specific activation in vivo has been reported for both CD4+ and CD8+ T cells responding to a variety of antigens. These include conventional peptide or protein antigens (40–42), superantigens (43–47), minor antigens (48), viral antigens (49, 50), and tissue-specific antigens (51–53). These studies were characterized by an early, transient period of antigen-specific T cell proliferation followed by rapid deletion of most responding T cells. OT-I cells displayed similar response kinetics upon exposure to peptide-coated B cells in vivo (Fig. 3). At day 3, there was marked proliferation of OT-I cells, but by day 7 the number of OT-I cells had declined to below prestimulation levels, where it remained. Altogether, our data suggest that B cells tolerize CD8+ T cells via activation of naïve cells followed by CD95–mediated deletion of their activated progeny.

Interestingly, not all OT-I cells were deleted in response to OVA-bearing B cells. Unlike other models where those T cells that remain become hyporesponsive to further antigen challenge (40, 41, 44, 48, 52), the OT-I cells that were not deleted showed full CTL function (Fig. 4). Given that deletion is reported to be antigen dose dependent (42, 43), these cells may represent OT-I cells that were inadequately stimulated by the tolerogenic B cells. Perhaps all of the B cells were killed before all activated CD8+ T cells could reencounter antigen for induction of CD95–mediated death. We attempted to address this issue by introducing a second dose of OVA–coated B cells 5 d after the first immunization, but this only slightly enhanced OT-I deletion at day 14. However, in TCR transgenic models, changes to the extent and nature of tolerance induction may require very large variations in antigen dose due to the relatively high number of responsive T cells (52, 54).

It is important to note that we have not addressed whether B cells are unique in their ability to induce deletion of CD8+ T cells. It may be that any cell type lacking the appropriate accessory signals will cause such deletion. The aim of this report was to specifically examine the effect of antigen presentation by B cells to CD8+ T cells. To this end, we have shown that B cells are directly tolerogenic for CD8+ T cells. This tolerance appears to be preceded by activation and proliferation of antigen-specific CD8+ T cells. Once activated, CD8+ T cells appear to be susceptible to CD95–mediated killing by reencounter with antigen on B cells, at least in vitro. Taken together, our data suggest that B cell presentation of antigen to CD8+ T cells leads to activation followed by deletion of the antigen-specific population.
We thank Freda Karamalis, Paula Nathan, Jenny Falso, and Tatiana Banjanin for technical assistance.

This work was supported by the Cooperative Research Centre for Vaccine Technology, the National Institutes of Health (grant AI-29385), and grants from the National Health and Medical Research Council and the Australian Research Council.

Address correspondence to William R. Heath, Immunology Division, The Walter and Eliza Hall Institute, Post Office The Royal Melbourne Hospital, Melbourne, Victoria 3050, Australia. Phone: 61-3-9345-2482; Fax: 61-3-9347-0852; E-mail: heath@wehi.edu.au

Received for publication 26 December 1997 and in revised form 15 September 1998.

References

1. Ke, Y., and J.A. Kapp. 1996. Exogenous antigens gain access to the major histocompatibility complex class I processing pathway in B cells by receptor-mediated uptake. J. Exp. Med. 184:1179–1184.

2. Yefenof, E., R. Zehavi-Feferman, and R. Guy. 1990. Control of primary and secondary antibody responses by cytotoxic T lymphocytes specific for a soluble antigen. Eur. J. Immunol. 20:1849–1853.

3. Barnaba, V., A. Franco, A. Alberti, R. Benvenuto, and F. Balsamo. 1990. Selective killing of hepatitis B envelope antigen-specific B cells by class I-restricted, exogenous antigenspecific T lymphocytes. Nature. 345:258–260.

4. Epstein, M.M., F. Di Rosa, D. Jankovic, A. Sher, and P. Matzinger. 1994. Induction of B cell and T cell tolerance by cross-priming requires cognate CD4 help. J. Exp. Med. 186:65–70.

5. Eynon, E.E., J.D. Nieland, T.N. Schumacher, H.L. Fuchs, E.J., and P. Matzinger. 1992. B cells turn off virgin but not memory T cells. Science. 258:1156–1159.

6. Simpson, E., and R.D. Gordon. 1977. Responsiveness to HY antigen. J. Immunol. 122:3013–3020.

7. Fuchs, E.J., and P. Matzinger. 1992. A fail-safe mechanism of large numbers of dendritic cells from mouse bone marrow cultures supplemented with granulocyte/macrophage colony-stimulating factor. J. Exp. Med. 176:1693–1702.

8. Husmann, L.A., and M.J. Bevan. 1988. Cooperation between helper T cells and cytotoxic T lymphocyte precursors. Ann. N.Y. Acad. Sci. 532:158–169.

9. Eynon, E.E., and D.C. Parker. 1992. Small B cells as antigen-presenting cells in the induction of tolerance to soluble protein antigens. J. Exp. Med. 175:131–138.

10. Yeheskel, E., and D.C. Parker. 1993. Parameters of tolerance induction by antigen targeted to B lymphocytes. J. Immunol. 151:2958–2964.

11. Bennett, S.R., F.R. Carbone, F. Karamalis, J.F.A.P. Miller, and W.R. Heath. 1997. Induction of a CD8 cytotoxic T lymphocyte response by cross-priming requires cognate CD4 help. J. Exp. Med. 186:923–930.

12. Simpson, E., and R.D. Gordon. 1977. Responsiveness to HY antigen. J. Immunol. 122:3013–3020.

13. Morris, S.C., A. Lees, and F.D. Finkelman. 1994. Induction of B cell and T cell tolerance in vivo. J. Exp. Med. 171:377–387.

14. Bennett, S.R., F.R. Carbone, F. Karamalis, J.F.A.P. Miller, and W.R. Heath. 1997. Induction of a CD8 cytotoxic T lymphocyte response by cross-priming requires cognate CD4 help. J. Exp. Med. 186:65–70.

15. Morris, S.C., A. Lees, J.M. Holmes, R.D. Jeffries, and F.D. Finkelman. 1994. Induction of B cell and T cell tolerance in vivo by anti-CD23 mAb. J. Immunol. 152:3768–3776.

16. Webb, S.R., J.H. Li, D.B. Wilson, and J. Sprent. 1985. Capacity of small B cell-enriched populations to stimulate mixed lymphocyte reactions: marked differences between irradiated vs. mitomycin C-treated stimulators. Eur. J. Immunol. 15:92–96.

17. Hogquist, K.A., S.C. Jameson, W.R. Heath, J.L. Howard, M.J. Bevan, and F.R. Carbone. 1994. T cell receptor antagonist peptides induce positive selection. Cell. 76:17–27.

18. Inaba, K., M. Inaba, N. R. Omori, H. Aya, M. Deguchi, S. Ikehara, S. Muramatsu, and R.M. Steinman. 1992. Generation of large numbers of dendritic cells from mouse bone marrow cultures supplemented with granulocyte/macrophage colony-stimulating factor. J. Exp. Med. 176:1693–1702.

19. Bennett, S.R., F.R. Carbone, F. Karamalis, J.F.A.P. Miller, and W.R. Heath. 1997. Induction of a CD8 cytotoxic T lymphocyte response by cross-priming requires cognate CD4 help. J. Exp. Med. 186:65–70.

20. Kurts, C., W.R. Heath, F.R. Carbone, J. Allison, J.F.A.P. Miller, and H. Kosaka. 1996. Constitutive class I-restricted exogenous presentation of self antigens in vivo. J. Exp. Med. 184:923–930.

21. Carbone, F.R., and M.J. Bevan. 1990. Class I-restricted processing and presentation of exogenous cell-associated antigen in vivo. J. Exp. Med. 171:377–387.

22. Nagata, S., and P. Golstein. 1995. The Fas death factor. Science. 267:1449–1456.

23. Fuchs, E.J., and P. Matzinger. 1992. B cells turn off virgin but not memory T cells. Science. 258:1156–1159.

24. Simpson, E., and R.D. Gordon. 1977. Responsiveness to HY antigen Ir gene complementation and target cell specificity. Immunol. Rev. 35:59–75.

25. Husmann, L.A., and M.J. Bevan. 1988. Cooperation between helper T cells and cytotoxic T lymphocyte precursors. An n. Y. Acad. Sci. 532:158–169.

26. Fuchs, E.J., and P. Matzinger. 1992. B cells turn off virgin but not memory T cells. Science. 258:1156–1159.

27. Eynon, E.E., and D.C. Parker. 1992. Small B cells as antigen-presenting cells in the induction of tolerance to soluble protein antigens. J. Exp. Med. 175:131–138.

28. Eynon, E.E., and D.C. Parker. 1993. Parameters of tolerance induction by antigen targeted to B lymphocytes. J. Immunol. 151:2958–2964.

29. Eynon, E.E., and D.C. Parker. 1993. Parameters of tolerance induction by antigen targeted to B lymphocytes. J. Immunol. 151:2958–2964.

30. Morris, S.C., A. Lees, and F.D. Finkelman. 1994. In vivo activation of naïve T cells by antigen-presenting B cells. J. Immunol. 152:3777–3785.

31. Morris, S.C., A. Lees, J.M. Holmes, R.D. Jeffries, and F.D. Finkelman. 1994. Induction of B cell and T cell tolerance in vivo by anti-CD23 mAb. J. Immunol. 152:3768–3776.

32. Morris, S.C., A. Lees, J.M. Holmes, R.D. Jeffries, and F.D. Finkelman. 1994. Induction of B cell and T cell tolerance in vivo by anti-CD23 mAb. J. Immunol. 152:3768–3776.

33. Morris, S.C., A. Lees, J.M. Holmes, R.D. Jeffries, and F.D. Finkelman. 1994. Induction of B cell and T cell tolerance in vivo by anti-CD23 mAb. J. Immunol. 152:3768–3776.

34. Guerder, S., and P. Matzinger. 1989. Activation versus tolerance: a decision made by T helper cells. Cold Spring Harbor Symp. Quant. Biol. 2:799–805.

35. Guerder, S., and P. Matzinger. 1992. A fail-safe mechanism for maintaining self-tolerance. J. Exp. Med. 176:553–564.

36. Dhein, J., H. Walczak, C. Baumler, K.M. Debatin, and P.H. Krammer. 1995. Autoimmune T-cell suicide mediated by APO-1 (Fas/CD95). Nature. 373:438–441.

37. Brunn, R., J. Mogil, D. LaFace, N. J. Yoo, A. Mahboubi, F. Echeverri, S.J. Martin, W.R. Force, D.H. Lynch, C.F. Ware, et al. 1995. Cell-autonomous Fas (CD95)/Fas-ligand interaction mediates activation-induced apoptosis in T-cell hybridomas. Nature. 373:441–444.

38. Ju, S.T., and D.J. Panka, A. Cui, D.H. Lu, C.J. Martin, W.R. Force, D.H. Lynch, C.F. Ware, et al. 1995. Cell-autonomous Fas (CD95)/Fas-ligand interaction mediates activation-induced apoptosis in T-cell hybridomas. Nature. 373:441–444.

39. O wen-Schaub, L.B., S. Yonehara, W.L. Crump III, and E.A. Grimm. 1992. DNA fragmentation and cell death is selec-
tively triggered in activated human lymphocytes by Fas antigen engagement. Cell. Immunol. 140:197–205.
30. Alderson, M. R., T. W. Tough, T. Davis-Smith, S. Braddy, B. Falk, K. A. Schooley, R. G. Goodwin, C. A. Smith, F. R. amsdell, and D. H. Lynch. 1995. Fas ligand mediates activation-induced cell death in human T lymphocytes. J. Exp. Med. 181:71–77.
31. Suda, T., T. Takahashi, P. Golstein, and S. Nagata. 1993. Molecular cloning and expression of the Fas ligand, a novel member of the tumor necrosis factor family. Cell. 75:1169–1178.
32. Suda, T., and S. Nagata. 1994. Purification and characterization of the Fas-ligand that induces apoptosis. J. Exp. Med. 179:873–879.
33. Vignaux, F., and P. Golstein. 1994. Fas-based lymphocyte-mediated cytotoxicity against syngeneic activated lymphocytes: a regulatory pathway? Eur. J. Immunol. 24:1181–1185.
34. Vignaux, F., and P. Golstein. 1994. Fas-based lymphocyte-mediated cytotoxicity against syngeneic activated lymphocytes: a regulatory pathway? Eur. J. Immunol. 24:1181–1185.
35. Zheng, L., G. Fisher, R. E. Miller, J. Peschon, D. H. Lynch, and M. J. Lenardo. 1995. Induction of apoptosis in mature T cells by tumour necrosis factor. Nature. 377:348–351.
36. Russell, J. H., B. Rush, C. Weaver, and R. W. Wang. 1993. Mature T cells of autoimmune lpr/lpr mice have a defect in antigen-stimulated suicide. Proc. Natl. Acad. Sci. USA. 90:4409–4413.
37. Kurts, C., W. R. Heath, H. Kosaka, J. F. A. P. Miller, and F. R. Carbone. 1998. The peripheral deletion of autoreactive CD4+ T cells induced by cross-presentation of self-antigens involves signaling through CD95 (Fas, Apo-1). J. Exp. Med. 188:415–420.
38. Alexander-Miller, M. A., G. R. Leggatt, A. Sarin, and J. A. Berzofsky. 1996. Role of antigen, CD8, and cytokotoxic T lymphocyte (CTL) avidity in high dose antigen induction of apoptosis of effector CTL. J. Exp. Med. 184:485–492.
39. Speiser, D. E., E. Sebzda, T. Ohteki, M. F. Bachmann, K. Pfeiffer, T. W. Mak, and P. S. Ohashi. 1996. Tumor necrosis factor receptor p55 mediates deletion of peripheral cytokotoxic T lymphocytes in vivo. Eur. J. Immunol. 26:3055–3060.
40. Kuburz, D., P. Aichele, D. E. Speiser, H. H engartner, R. M. Zinkernagel, and H. Pircher. 1993. T cell immunity after a viral infection versus T cell tolerance induced by soluble viral peptides. Eur. J. Immunol. 23:1956–1962.
41. Kearney, E. R., K. A. Pape, D. Y. Loh, and M. K. Jenkins. 1994. Visualization of peptide-specific T cell immunity and peripheral tolerance induction in vivo. Immunity. 1:327–339.
42. Liblau, R. S., R. Tisch, K. Shokat, X. Y. Yang, N. Dumont, C. C. Goodnow, and H. O. M. Devitt. 1996. Intravenous injection of soluble antigen induces thymic and peripheral T-cells apoptosis. Proc. Natl. Acad. Sci. USA. 93:3031–3036.
43. Webb, S., C. M.orris, and J. Sprent. 1990. Exthymic tolerance of mature T cells: clonal elimination as a consequence of immunity. Cell. 63:1249–1256.
44. Kawabe, Y., and A. Ochi. 1991. Programmed cell death and extrathymic reduction of Vβ8+ CD4+ T cells in mice tolerant to Staphylococcus aureus enterotoxin B. Nature. 349:245–248.
45. MacDonald, H. R., S. Baschieri, and R. K. Lees. 1991. Clonal expansion precedes energy and death of Vβ8+ peripheral T cells responding to staphylococcal enterotoxin B in vivo. Eur. J. Immunol. 21:1963–1966.
46. McCormack, J. E., J. E. Callahan, J. Kappler, and P. C. Marrack. 1993. Profound deletion of mature T cells in vivo by chronic exposure to exogenous superantigen. J. Immunol. 150:3785–3792.
47. Gonzalo, J. A., I. Moreno de Alboran, J. E. Ales-Martinez, C. Martinez, and G. Kroemer. 1992. Expansion and clonal deletion of peripheral T cells induced by bacterial superantigen is independent of the interleukin-2 pathway. Eur. J. Immunol. 22:1007–1011.
48. Rocha, B., and H. von Boehmer. 1991. Peripheral selection of the T cell repertoire. Science. 251:1225–1228.
49. Moskophidis, D., F. Lechner, H. Pircher, and R. M. Zinkernagel. 1993. Virus persistence in acutely infected immunocompetent mice by exhaustion of antiviral cytotoxic effector T cells. Nature. 362:758–761.
50. Zinkernagel, R. M., D. Moskophidis, T. Kundig, S. Oehen, H. Pircher, and H. Hengartner. 1993. Effector T-cell induction and T-cell memory versus peripheral deletion of T cells. Immuno. Rev. 133:199–223.
51. Kurts, C., H. Kosaka, F. R. Carbone, J. F. A. P. Miller, and W. R. Heath. 1997. Class I-restricted cross-presentation of exogenous self antigens leads to deletion of autoreactive CD8+ T cells. J. Exp. Med. 186:239–245.
52. Lanoue, A., C. Baena, H. von Boehmmer, and A. Sarukhan. 1997. Conditions that induce tolerance in mature CD4+ T cells. J. Exp. Med. 185:405–414.
53. Bertolino, P., W. R. Heath, C. L. Hardy, G. Morahan, and J. F. A. P. Miller. 1995. Peripheral deletion of autoreactive CD8+ T cells in transgenic mice expressing H-2Kb in the liver. Eur. J. Immunol. 25:1932–1942.
54. Rocha, B., A. Grandien, and A. A. Freitas. 1995. Anergy and exhaustion are independent mechanisms of peripheral T cell tolerance. J. Exp. Med. 181:993–1003.