Brief Definitive Report

Self Major Histocompatibility Complex Class I Antigens Expressed Solely in Lymphoid Cells Do Not Induce Tolerance in the CD4$^+$ T Cell Compartment

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Summary

Transgenic mice expressing self major histocompatibility complex (MHC) class I (H-2K^b) antigen solely in lymphoid cell lineages do not acquire tolerance to H-2K^b expressed on skin grafts. H-2K^b–specific cytotoxic T cell responses were completely abrogated in these mice, even after they had rejected skin grafts. Moreover, thymocytes expressing T cell receptors that confer H-2K^b reactivity on cytotoxic CD8$^+$ T cells were eliminated. The ability to reject grafts correlated with the presence of a novel population of H-2K^b–reactive CD4$^+$ T cells. At least some of these CD4$^+$ T cells recognize peptides derived from H-2K^b by processing. We conclude that self MHC I antigens induce tolerance in the CD8 T cell compartment via negative selection when expressed exclusively by lymphoid cells. In contrast, tolerance to MHC class II–restricted self peptides derived by processing of such MHC I antigens is not induced in the CD4 T cell compartment. This suggests that effective transfer of self antigens from lymphoid cells to MHC II–positive cells that can process and present them as self peptides to thymocytes or CD4$^+$ T cells does not take place in vivo. Thus, sequestration of self antigens and MHC II molecules in distinct cell types in the thymic microenvironment allows potentially autoreactive and functionally competent CD4$^+$ T cells that recognize cryptic MHC II–restricted self peptides to mature into the peripheral T cell repertoire under normal physiological circumstances.

Self tolerance is induced in the T cell compartment by a number of mechanisms (1–4). The most effective tolerogenic mechanism is clonal deletion of self-reactive thymocytes, but the effectiveness of this mechanism depends on whether thymocytes are exposed to self antigens. Self antigens must be expressed in thymus or transferred to thymic APC from "producer" cells in peripheral tissues. However, different APC may present distinct peptides derived from the same protein because (*a*) the route of antigen delivery; (*b*) the mechanism of antigen uptake (for extracellular proteins), and (*c*) differential biochemical processing pathways may all influence peptide presentation. These issues are particularly important when considering how tolerance is acquired in the CD4 T cell compartment because few cell types express MHC II constitutively in vivo. In essence, self antigens not expressed by MHC II–positive thymic cells are sequestered because peptides derived from them cannot be presented directly to thymocytes in the context of MHC II. Self antigens in this category must be transferred to MHC II–positive cells and processed into peptides that associate with MHC II. Failure to deliver self antigens from MHC II–negative producer cells to MHC II–positive APC would render self peptides invisible, or cryptic, to the CD4 T cell compartment (5, 6).

Intercellular transfer of some self antigens occurs via passive mechanisms. For example, complement factor C5, a blood-borne protein, is internalized, by thymic MHC II–positive dendritic cells (DC) (7). Presumably, cell-associated proteins cannot be transferred unless there is intimate cell–cell cocntact or cell destruction allowing neighboring MHC II–positive APC to internalize cell contents. This is an attractive scenario for murine thymocytes (MHC II negative) because large numbers of thymocytes are engulfed by thymic macrophages after, or before, their death by apoptosis (8).

To investigate this issue, we used a set of transgenic (Tg) mice that express the murine H-2K^b MHC I gene exclusively in lymphoid cells (9, 10). This provides a unique opportunity to investigate whether tolerance is induced in the CD4 T cell compartment by a cell-associated self antigen expressed only by MHC II–negative cells in the thymus. In this report, we describe the tolerance status of the CD4 and CD8 T cell compartments in such mice.

Materials and Methods

Mice. Mice were bred in a specific pathogen–free facility (National Institute for Medical Research [NIMR]). CD2K^b-3– (9,

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10) and CBK- (11) Tg mice have been described previously. TCR-Tg mice expressing two different clonotypic receptors that confer H-2K^b recognition on CD8$^+$ T cells (BM3 and DES) have been described previously (10, 12).

Skin Grafts. Skin grafts from CBK donor mice were applied to CD2K^b-Tg or CBA/Ca recipients using standard procedures (13). Plaster casts protecting graft beds were removed 10 d after grafting, and grafts were inspected daily for signs of rejection.

Cytofluorimetric Analyses. 10^6 thymocytes were stained with biotinylated MV3 (anti-H-2K^b) antibody (14) and developed with streptavidin-PE (Biogenesis, Poole, UK). Thymocytes were gated, and selected events were displayed as histograms using FACSplot software (NIMR Computing Division) using a FACScan® (Becton Dickinson & Co., Mountain View, CA) cytofluorimeter. Thymocytes from DES TCR-Tg mice or [DES × CD2K^b-4] double-Tg mice were stained with anti-CD8-FITC and anti-CD4-PE as described previously (10).

Generation of DC. DC-enriched cell suspensions from CBK and CBA mice were prepared by culturing bone marrow in the presence of GM-CSF (10, 12, 15).

Bioassay for H-2K^b Expression on DC. 4×10^6 LN cells from BM3 TCR-Tg mice (12) were incubated with 4×10^6 bone marrow–derived DC for 5 d. IFN-γ release was estimated by ELISA assay (12).

Cytotoxicity Assays. CD2K^b-Tg mice were injected subcutaneously with 10^7 irradiated splenocytes from CBK mice. After 2 wk, splenocytes from primed mice were cultured with irradiated CBK splenocytes for 5 d in vitro (9). Responder cells were harvested and incubated, in triplicate, with EL4 (H-2^b) target cells in the ratios shown (see Fig. 3) for 3 h. 25 μl of supernatant was harvested from each well, and specific release of ^{51}Cr (Amersham, Amersham, UK) was determined (16).

T Cell Proliferation Assays. Proliferation assays (9) were set up and harvested after 4 d with the following modifications. LN responder cells were obtained from naive CD2K^b-Tg mice or from CD2K^b-Tg mice after they had rejected CBK skin grafts (primed). 2.5×10^5 responder cells were stimulated with 10^5 bone marrow–derived DC in flat-bottomed 96-well plates and cultured in medium containing 1% mouse serum. Cells were pulsed with 1 μCi [^{3}H]thymidine (Amersham) for 8 h before harvesting. In some cases, anti-CD4 antibody (GK1.5, TIB 207, a gift from Dr. R. Zamoyska, NIMR) was added. Synthetic peptide Kb[80-92] (YWERETQKAKGNE; based on the 61–69 kb peptide described in reference 17) was made by the NIMR Peptide Synthesis Service. Peptide was added to standard proliferation assays in the amounts indicated. All assays were carried out in triplicate.

Results

Expression of H-2K^b in CD2K^b-Tg Mice. CD2K^b-Tg mice carry a transgene in which transcriptional promoter elements from the human CD2 gene are joined to the H-2K^b structural gene (9). Mice from three sublines (CD2K^b-3, CD2K^b-4, CD2K^b-11) differ only in transgene copy number and chromosomal integration sites because oocytes from an inbred strain (CBA/Ca) were used to make CD2K^b-Tg mice by DNA microinjection. The CD2K^b transgene drives H-2K^b expression in lymphoid cell lineages in all CD2K^b-Tg mice. Mature B and T lymphocytes (data not shown) and thymocytes (Fig. 1 *A*) express H-2K^b. CD2K^b-Tg thymocytes express ~10 times more H-2K^b than thymocytes from non-Tg C57BL/10 (H-2^b) mice. Myeloid cells (DC and macrophages) from CD2K^b-4 and CD2K^b-11 mice do not express H-2K^b as determined by cytofluorimetric analyses (data not shown), whereas myeloid cells from CD2K^b-3 mice express H-2K^b (10). Results from a more sensitive, T cell–dependent bioassay for H-2K^b expression (12) confirm that bone marrow–derived DC from CD2K^b-4 or CD2K^b-11 mice express no detectable H-2K^b (Fig. 1 *B*). Similar results were obtained when stimulators used in this bioassay were cultured DC or macrophages originating from spleen or thymus (18). These results show that H-2K^b is expressed at high level in lymphoid cell lineages in all sublines of CD2K^b-Tg mice but that expression in myeloid cell lineages occurs only in mice of the CD2K^b-3 subline.

Tolerance Status of CD2K^b Tg Mice. Tolerance to H-2K^b was assessed by grafting CD2K^b-Tg mice with tail skin from CBK-Tg mice (11). CBK mice express H-2K^b in all cells because they carry a transgene that is a subclone of the

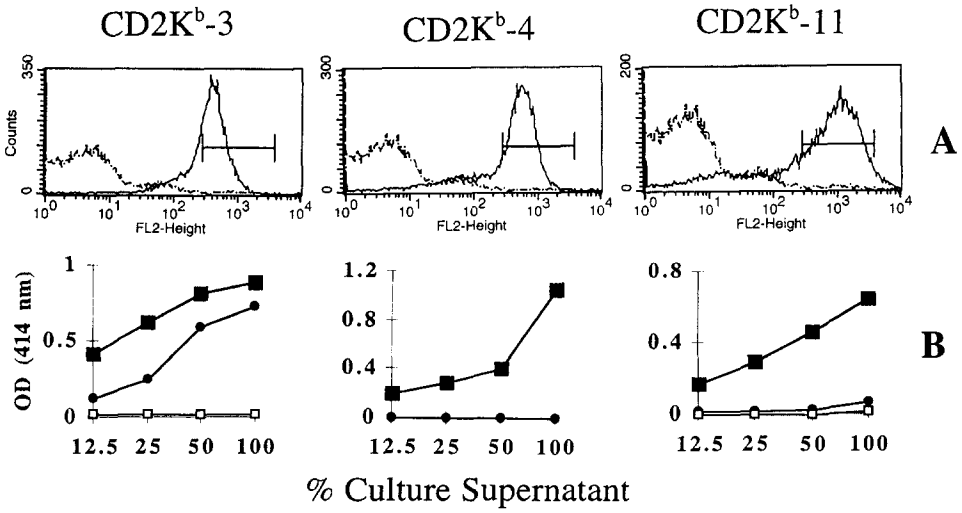

Figure 1. H-2K^b expression by thymocytes and DC from CD2K^b mice. (*A*) Histograms show H-2K^b–specific antibody staining profiles on thymocytes from CD2K^b (*solid lines*) or CBA mice (*dotted lines*). Markers show the relative level of H-2K^b expression on PBL from C57BL/10 mice. (*B*) IFN-γ release when H-2K^b–specific, CD8$^+$ T cells from BM3 TCR-Tg mice (10) after coculture with DC from CD2K^b (●), CBA (□), or C57BL/10 (■) mice.

Table 1. *Rejection of Skin Grafts by CD2K^b-Tg Mice*

Recipient	Donor	Rejected/total	Mean survival time	Range
			d	
CBA/Ca	CBK	4/4	16	14–20
CD2K^b-3	CBK	0/8	>100	–
CD2K^b-4	CBK	6/7	43	23–84
CD2K^b-11	CBK	7/11	27	13–55

entire H-2K^b gene including the H-2 promoter. Consequently, the only difference between donor CBK and recipient CD2K^b-Tg mice is the pattern of H-2K^b expression. As was found previously (9), CD2K^b-3 mice accept CBK grafts (Table 1). Even though H-2K^b is a self antigen expressed at high level by lymphoid cells from CD2K^b-4 and CD2K^b-11 mice, most of these mice reject CBK skin grafts. Thus, most CD2K^b-4 and CD2K^b-11 mice are not tolerized to H-2K^b, and we conclude that expression of H-2K^b solely in lymphoid cells is not sufficient to generate tolerance to H-2K^b.

CBK skin grafts survived much longer on CD2K^b-4 and CD2K^b-11 recipients than on CBA recipients (Table 1). Grafts on CD2K^b-4 and CD2K^b-11 recipients had unusual appearances before rejection in that they became dehydrated, shrank slowly in size, and were not shed. In contrast, grafts rejected by CBA recipients quickly became necrotic and were shed, as is normally observed when MHC-mismatched grafts are rejected. Qualitative differences in effector cells and/or their relative frequencies in recipient mice could explain these discrepancies.

H-2K^b–specific CD8$^+$ T Cells Are Eliminated in CD2K^b Tg Mice. The fate of H-2K^b–reactive thymocytes in CD2K^b mice was examined by intercrossing CD2K^b-Tg mice with TCR-Tg mice (10). The DES TCR clonotype confers H-2K^b reactivity because the DES TCR-α and TCR-β transgenes were derived from an allo-(H-2K^b)–reactive, CD8$^+$ T cell clone (Désiré; see reference 19). Thymocytes from [DES × CD2K^b-4] double-Tg mice were eliminated efficiently and at an early stage in development, because the total number of thymocytes was substantially reduced (~90%), and virtually all immature CD4$^+$CD8$^+$ thymocytes were eliminated (Fig. 2). Almost all (87%) remaining thymocytes were immature CD4$^-$CD8$^-$ thymocytes. The relatively high proportion (but low number) of mature CD4$^+$CD8$^-$ thymocytes probably escape deletion because of rearrangements of endogenous TCR genes. Almost identical results were obtained when CD2K^b-3 mice were intercrossed with BM3 or DES TCR-Tg mice (10). Thus, thymocytes that recognize native cell surface–associated H-2K^b molecules are eliminated in the thymus of CD2K^b-Tg mice.

More general tests for T cells that respond to H-2K^b were carried out by measuring cytotoxicity generated in vitro after coculturing T cells from CD2K^b-Tg mice with irradiated stimulator cells from CBK mice. Splenocytes

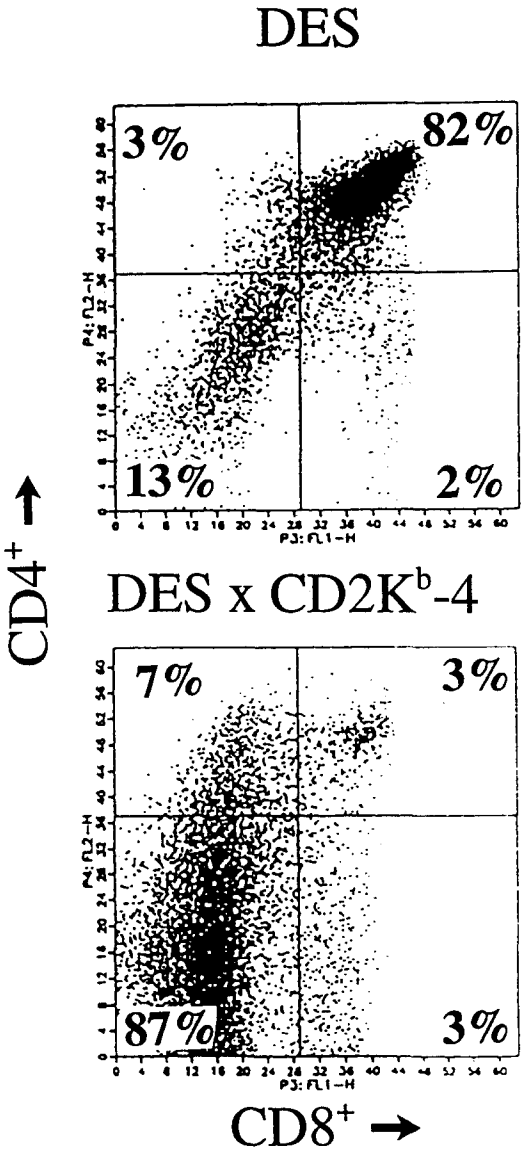

Figure 2. Elimination of H-2K^b–reactive thymocytes in CD2K^b-4 mice. Thymocytes from DES TCR-Tg mice (10) or [DES × CD2K^b-4] double Tg mice were stained with anti-CD4 (*vertical axis*) and anti-CD8 (*horizontal axis*) antibodies. 60–80 × 10^6 (DES) and 9–10 × 10^6 (DES × CD2K^b-4). thymocytes were obtained. Results shown are representative of several experiments.

from CD2K^b-4 or CD2K^b-11 mice that had been immunized with CBK splenocytes before setting up MLC did not lyse target cells expressing H-2K^b at levels above background (Fig. 3), whereas T cells from immunized CBA mice lysed these targets efficiently. H-2K^b–specific cytotoxicity was not detected when responder T cells from naive CD2K^b-Tg mice or CD2K^b-Tg mice that had rejected CBK skin grafts were tested (data not shown). Thus, cytotoxic T cell responses were completely abrogated in CD2K^b-4 and CD2K^b-11 mice, even after in vivo immunization against H-2K^b, and we conclude that allo-(H-2K^b)–reactive T cells (mostly CD8$^+$) are absent in CD2K^b-

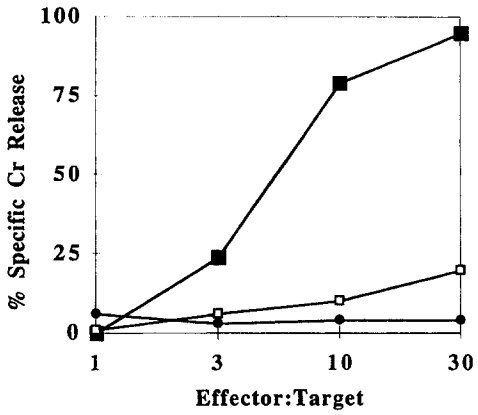

Figure 3. LN cells from CBA (■), CD2K^b-4 (□) or CD2K^b-11 (●) mice that had been immunized subcutaneously with CBK splenocytes were cocultured with CBK spleen cells and tested for cytolytic activity. Graphs show the percentage of specific release of ^{51}Cr at four E/Target ratios. Results shown are representative of several experiments.

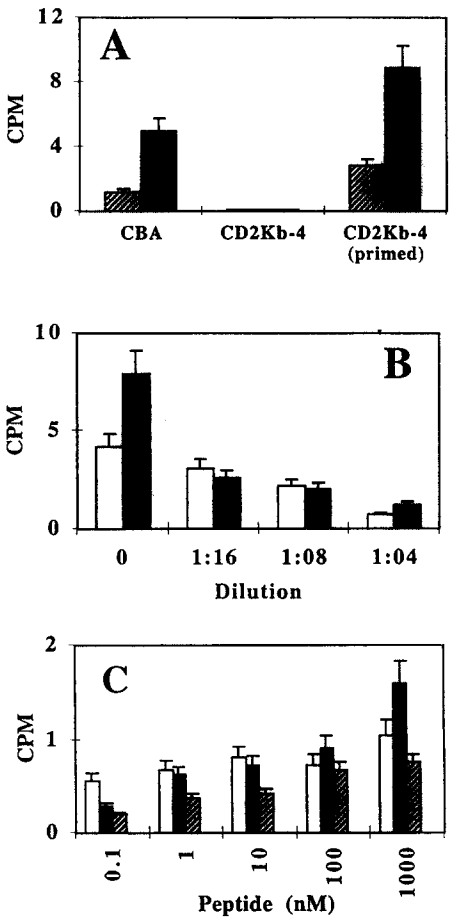

Figure 4. Assays for H-2K^b–specific T cell proliferation. (*A*) LN T cells from CD2K^b-4 mice that had rejected skin grafts from CBK (CD2K^b-4–primed), naive CD2K^b-4, and CBA mice were stimulated with DC from CBK (*solid bars*) or CBA (*striped bars*) mice. (*B*) LN T cells from CBA (*open bars*) or immunized CD2K^b-4 (*solid bars*) mice were stimulated with DC from CBK mice in the presence of different amounts of hybridoma culture supernatant containing anti-CD4 mAb. (*C*) LN T cells from CBA (*open bars*), CD2K^b-4 (*solid bars*), or CD2K^b-11 (*striped bars*) mice were stimulated with CBA DC pulsed with Kb[80-92] peptide. All cultures were carried out in triplicate, and the results shown are representative of three separate experiments. Error bars show the standard deviation. Vertical axes show CPM × 10^{-4}.

Tg mice. This may occur because thymocytes can present native (i.e., cell associated) H-2K^b to other thymocytes in CD2K^b-Tg mice (10, 20).

H-2K^b Peptide–specific CD4$^+$ T Cells Are Present in CD2K^b Tg Mice. H-2K^b–specific T cell proliferation was detected when LN cells from CD2K^b-4 (Fig. 4) or CD2K^b-11 (data not shown) mice were stimulated with cultured DC derived from CBK bone marrow. However, this response was detected only if LN cells were obtained from mice that had previously rejected a CBK skin graft (Fig. 4 *A*) or had been immunized by injection of CBK splenocytes (data not shown). Proliferative responses were abrogated completely by including anti-CD4 mAb in the culture medium (Fig. 4 *B*). This shows that H-2K^b–reactive CD4$^+$ T cells are present in CD2K^b-4 and CD2K^b-11 mice.

Reasoning that H-2K^b–reactive CD4$^+$ T cells might recognize processed H-2K^b peptides presented on self MHC II molecules (H-2A^k or H-2E^k), we synthesized an H-2K^b peptide (Kb[80-92]) that binds to H-2A^k molecules and stimulates proliferation of CD4$^+$ T cells in CBA mice (17). Furthermore, this peptide is produced naturally from native H-2K^b protein in CBA mice since peptide-specific T cell responses were induced after immunization of CBA mice with splenocytes from B10 mice (17). T cells from naive (unprimed) CD2K^b-4 and CD2K^b-11 mice proliferated in a dose-dependent manner when cocultured with CBA DC pulsed with Kb[80-92] peptide (Fig. 4 *C*). These responses were comparable with responses made by control CBA mice to the same peptide. Thus, at least some of the H-2K^b–specific CD4$^+$ T cells present in CD2K^b-4 and CD2K^b-11 mice recognize H-2K^b peptides that bind to self MHC II.

Discussion

One explanation for the failure of lymphoid cells to induce tolerance in the CD4 T cell compartment of CD2K^b-4 and CD2K^b-11 mice is that thymocytes cannot present MHC II–restricted peptides because they do not express MHC II. However, tolerance to serum proteins, such as complement factor C5, is induced in the CD4 T cell compartment after C5 uptake and processing before peptide presentation on thymic MHC II–positive cells, which induces clonal deletion of C5-reactive thymocytes (7). Transfer of proteins between cells may provide a general mechanism by which thymocytes are exposed to MHC II–restricted peptides that originate from self antigens expressed only by MHC II–negative cells. Protein transfers between cells might occur by passive uptake of antigen (as for C5) or after cell destruction and consequent release of self antigens into local microenvironments from which they are inter-

nalized, processed, and presented to $CD4^+$ thymocytes or T cells by MHC II–positive cells. This scenario may occur during necrosis, when dying cells release their contents directly into the surrounding microenvironment (21). However, most thymocytes die by apoptosis, during which cells remain intact until scavenger macrophages engulf and destroy them (8).

We conclude that there is no effective mechanism for transferring H-$2K^b$ molecules between thymocytes and MHC II–positive cells to present MHC II–restricted H-$2K^b$ peptides because H-$2K^b$ specific $CD4^+$ T cells are present in $CD2K^b$-4 and $CD2K^b$-11 mice. This provides unequivocal evidence that a cell-associated self antigen expressed solely by lymphoid cells does not tolerize the CD4 T cell compartment under normal physiological conditions in vivo. This finding should be generally applicable to other self proteins that are expressed only by cells that do not express MHC II constitutively in vivo unless special factors prevent transfers of cell surface–associated proteins from thymocytes to thymic MHC II–positive cells. Under normal physiological conditions, proteins sequestered inside cells that produce them would be even less likely to be transferred to other cells than proteins such as H-$2K^b$, which is located on the external surface of the cell membrane. If our findings are generally applicable, we predict that large numbers of self peptides that could be generated by processing of self antigens and that could bind to self MHC II are not available for tolerance induction in the CD4 T cell compartment because of (*a*) segregated (i.e., tissue-specific) expression of self antigens and MHC II; (*b*) intracellular compartmentalization of processed peptides and newly expressed MHC II, or (*c*) a combination of these alternatives. Exposure of such "cryptic" self peptides could lead to functional activation of mature $CD4^+$ T cells, as was observed in $CD2K^b$-4 and $CD2K^b$-11 mice grafted with CBK skin. Altered physiological conditions that lead to changes in normal patterns of self protein or self MHC II expression may provide novel opportunities for transferring proteins between cells or presenting previously cryptic self peptides to T cells (5, 22, 23). In effect, rejection of CBK skin grafts by $CD2K^b$-4 and $CD2K^b$-11 mice mimics such circumstances because there is no genetic (i.e., structural) difference between recipient and donor tissues. Cryptic H-$2K^b$ peptides are either displayed naturally by cells in the CBK graft and/or are generated as a result of inflammation after surgery.

We assume that expression of H-$2K^b$ by myeloid cells, as well as lymphoid cells in $CD2K^b$-3 mice, explains why these mice do not reject CBK skin grafts. Presumably, coexpression of H-$2K^b$ and MHC II molecules by thymic myeloid cells allows these cells to display processed H-$2K^b$ peptides that remain sequestered in $CD2K^b$-4 and $CD2K^b$-11 mice. There is substantial evidence that peptides derived from intracellular proteins can associate with MHC II molecules synthesized in the same cells (24–27).

The role and status of B cells in $CD2K^b$-4 and $CD2K^b$-11 mice is of interest because they coexpress H-$2K^b$ and MHC II in $CD2K^b$-Tg mice. B cells tolerize naive, but not antigen-experienced, $CD4^+$ T cells in vivo creating an unresponsive state (anergy) in T cells (28). Nevertheless, naive B cells and activated B cell blasts are relatively inefficient APCs for T cell activation because they do not express adhesion and costimulatory molecules such as CD54 and CD80 (26). Thus, even if B cells display H-$2K^b$–peptide/MHC II complexes, they may not influence the tolerance status of $CD4^+$ T cells that recognize these complexes. However, it will be interesting to examine whether the relationship between H-$2K^b$ peptide–specific $CD4^+$ T cells and B cells in $CD2K^b$-4 and $CD2K^b$-11 mice is altered after graft rejection.

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References

1. Kisielow, P., W. Swat, B. Rocha, and H. von Boehmer. 1991. Induction of immunological unresponsiveness in vivo and in vitro by conventional and superantigens in developing and mature T cells. *Immunol. Rev.* 122:69–85.
2. Hämmerling, G.J., G. Schönrich, I. Ferber, and B. Arnold. 1993. Peripheral tolerance as a multi-step mechanism. *Immunol. Rev.* 133:93–104.
3. Marrack, P., P. Hugo, J. McCormack, and J. Kappler. 1993.

Death and T cells. *Immunol. Rev.* 133:119–130.
4. Miller, J.F.A.P., and W.R. Heath. 1993. Self-ignorance in the peripheral T-cell pool. *Immunol. Rev.* 133:131–150.
5. Lanzavecchia, A. 1995. How can cryptic epitopes trigger autoimmunity? *J. Exp. Med.* 181:1945–1948.
6. Sercarz, E.E., P.V. Lehmann, A. Ametani, G. Benichou, A. Miller, and K. Moudgil. 1993. Dominance and crypticity of T cell antigenic determinants. *Annu. Rev. of Immunol.* 11: 729–766.
7. Zal, T., A. Volkmann, and B. Stockinger. 1994. Mechanisms of tolerance induction in major histocompatibility complex class II–restricted T cells specific for a blood-borne self-antigen. *J. Exp. Med.* 180:2089–2099.
8. Surh, C.D., and J. Sprent. 1994. T cell apoptosis detected *in situ* during positive and negative selection in the thymus. *Nature (Lond.).* 372:100–103.
9. Simpson, S.J., P. Tomlinson, and A.L. Mellor. 1993. Immunological consequences of T cell specific expression of H-2K^b molecules in transgenic mice. *Int. Immunology.* 5:189–198.
10. Sponaas, A.-M., P.D. Tomlinson, R. Schulz, J. Antoniou, P. Ize-Iyamu, A.-M. Schmitt-Verhulst, and A.L. Mellor. 1994. Tolerance induction by elimination of subsets of self-reactive thymocytes. *Int. Immunology.* 6:1593–1604.
11. Yeoman, H., and A.L. Mellor. 1992. Tolerance and MHC restriction in transgenic mice expressing an MHC class I gene in erythroid cells. *Int. Immunology.* 4:59–65.
12. Sponaas, A.-M., P.D. Tomlinson, J. Antoniou, N. Auphan, C. Langlet, B. Malissen, A.-M. Schmitt-Verhulst, and A.L. Mellor. 1994. Induction of tolerance to self MHC class I molecules expressed under the control of milk protein or β globin gene promoters. *Int. Immunol.* 2:277–287.
13. Billingham, R.E., and P.B. Medawar. 1951. The technique of free skin grafting in mammals. *J. Exp. Biol.* 28:385–401.
14. Eccles, S.J., M.A. Vidal, C.J. Wrighton, F.G. Grosveld, and J.F. Burke. 1989. Rapid detection of ultraviolet-induced reversion of an amber mutation in mouse L cells. *Proc. Natl. Acad. Sci USA.* 86:1297–1299.
15. Inaba, K., M. Inaba, N. Romani, 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.
16. Simpson, E., and P. Chandler. 1986. Analysis of cytotoxic T cell responses. *In* Handbook of Experimental Immunology. Vol. 2. D.M. Weir, L.A. Herzenberg, and L.A. Herzenberg, editors. Blackwell Scientific, Oxford, UK. 68–92.
17. Singh, B., C.A. Waters, and D. Swanlund. 1986. Alloantigenic sites on class I major histocompatibility complex antigen: 61–69 region in the first domain of the H-2K^b molecule induces specific antibody and T cell responses. *J. Immunology.* 137:2311–2318.
18. Schulz, R. 1995. Tolerance status of transgenic mice expressing a major histocompatibility complex class I molecule under the control of human CD2 regulatory elements. Ph.D. thesis. University College, University of London.
19. Hua, C., M. Buferne, and A.-M. Schmitt-Verhulst. 1985. Lysis of hybridoma cells bearing anticlonotypic surface immunoglobulin by clonotype-expressing alloreactive cytotoxic T cells. *Eur. J. Immunol.* 15:1029–1032.
20. Schonrich, G., G. Strauss, K.P. Muller, L. Dustin, D.Y. Loh, N. Auphan, A.-M. Schmitt-Verhulst, B. Arnold, and G. Hammerling. 1993. Distinct requirements of positive and negative selection for selecting cell type and CD8 interaction. *J. Immunol.* 151:4098–4105.
21. Kychlinsky, A., L.M. Zheng, C.-C. Liu, and J.D.-E. Young. 1991. Cytolytic lymphocytes induce both apoptosis and necrosis in target cells. *J. Immunol.* 146:393–400.
22. Quarantino S., M. Feldman, C.M. Dayan, O. Acuto, and M. Londei. 1996. Human self-reactive T cell clones expressing identical T cell receptor β chains differ in their ability to recognize a cryptic self-epitope. *J. Exp. Med.* 183:1–10.
23. Salemi S., A.P. Caporossi, L. Boffa, M.G. Longobardi, and V. Barnaba. 1995. HIVgp120 activates autoreactive CD4-specific T cell responses by unveiling of hidden CD4 epitopes during processing. *J. Exp. Med.* 181:2253–2257.
24. Lin, R.H., and B. Stockinger. 1989. T cell immunity or tolerance as a consequence of self antigen presentation. *Eur. J. Immunol.* 19:105–112.
25. McKisic, M.D., A.J. Sant, and F.W. Fitch. 1991. Some cloned murine CD4$^+$ T cells recognize H-2L^d class I MHC determinants directly. Other cloned CD4$^+$ T cells recognize H-2L^d class I MHC determinants in the context of class II MHC molecules. *J. Immunol.* 147:2868–2874.
26. Guéry, J.-C., and L. Adorini. 1995. Dendritic cells are the most efficient in presenting endogenous naturally processed self-epitopes to class II-restricted T cells. *J. Immunol.* 154: 536–544.
27. Guéry, J.-C., A. Sette, E. Appelle, and L. Adorini. 1995. Constitutive presentation of dominant epitopes from naturally processed self-β2-microglobulin to class II-restricted T cells leads to self-tolerance. *J. Immunol.* 154:545–554.
28. Fuchs, E.J., and P. Matzinger. 1992. B cells turn off virgin but not memory T cells. *Science (Wash. DC).* 258:1156–1159.