CD4+ T Cell Division in Irradiated Mice Requires Peptides Distinct from Those Responsible for Thymic Selection

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Summary

We investigated the mechanism by which α/β T cells expand upon transfer to T cell–deficient host mice by injecting carboxyfluorescein diacetate succinimidyl ester–labeled T cells into mice depleted of T cells by sublethal irradiation. We found that CD4+ T cells divided when transferred to irradiated hosts and that the division of more than half of these cells required class II expression. However, division of transferred CD4+ T cells did not occur in irradiated hosts that expressed class II molecules occupied solely by the peptide responsible for thymic selection, indicating that peptides distinct from those involved in thymic selection cause the division of CD4+ T cells in irradiated mice. These data establish that class II–bound peptides control the expansion of CD4+ T cells transferred to T cell–deficient hosts and suggest that the same peptides contribute to the maintenance of T cell numbers in normal mice.

Key words: T cell homeostasis • peptides • peripheral selection • T cell receptor–major histocompatibility complex interaction • T cell–deficiency

The number of mature T cells in an adult mouse is controlled by the rates of T cell production and division, and by the factors that influence T cell survival. Each of these processes involves interactions between TCR and MHC molecules. The production of new T cells occurs in the thymus (1), where contact between newly expressed α/β TCRs and self-peptides presented by MHC molecules selects thymocytes to mature (2–7). TCR–MHC interactions also affect mature T cell survival. Studies in which T cells were transferred to mice that lack MHC expression have shown that a normal CD4+ or CD8+ T cell life span depends on peripheral expression of the selecting MHC molecule (8–12). Whether the peptides bound to MHC proteins also affect T cell survival has not been addressed, but at least one study suggests that the selecting peptide–MHC ligand may be sufficient to promote a normal T cell life span (9).

The size of the T cell pool is also affected by the division of mature T cells (13–16). In normal mice, most T cells divide infrequently, such that approximately half the T cells in a mouse divide within a period of 3–8 wk (17, 18). However, if a T cell deficit exists, the rate of T cell division increases for unknown reasons. For example, when T cells are transferred to nude, irradiated, SCID or TCR-α knockout (KO)[1] mice, the transferred cells expand rapidly (19–23). Whether such expansion requires antigen presence and/or MHC expression has been the subject of several reports. Studies have shown that naive transgenic CD8+ T cells specific for the male HY antigen expand in normal mice only in the presence of antigen (12, 24). A different study demonstrated that 2B4 transgenic CD4+ T cells specific for pigeon cytochrome *c* in the context of E^k expand in irradiated, bone marrow–reconstituted mice only in the presence of antigen (23). This study also established that expansion of naturally occurring CD4+ T cells after transfer to irradiated mice is diminished when class II blocking antibody is administered to the host. The finding that CD4+ T cell expansion in T cell–deficient hosts requires class II expression has been confirmed by a recent report showing that CD4+ T cells could not expand in TCR-αKO mice lacking class II expression (22). Whether different peptides presented by class II molecules influence this process is unknown. Because TCR transgenic T cells require antigen to expand in T cell–deficient mice, peptides presented by class II molecules are presumably required for naturally occurring CD4+ T cells to divide when transferred to T cell–deficient mice. However, the involvement of class II–bound peptides has not been confirmed for normal T cells.

The purpose of our study was to determine whether class II–bound peptides influence the division of CD4+ T cells transferred to T cell–deficient mice. Because we sought a

[1]*Abbreviations used in this paper:* CFSE, carboxyfluorescein diacetate succinimidyl ester; CyC, cychrome; Ii, invariant chain; KO, knockout; SEB, staphylococcal enterotoxin B.

direct measure of the extent of division of individual T cells after transfer to each host, T cells were labeled with the dye carboxyfluorescein diacetate succinimidyl ester (CFSE) before transfer to various sublethally irradiated hosts. Due to the approximately twofold decrease in CFSE fluorescence after each cell division, CFSE allowed us to assess the extent of division of individual transferred T cells (25, 26). We found that a significant fraction of $CD4^+$ T cells divided when placed into irradiated normal mice. Most of the $CD4^+$ T cell division that occurred in irradiated normal mice failed to occur in irradiated MHC class II KO (MHCIIKO) mice, confirming that $CD4^+$ T cell division in T cell–deficient mice requires MHC class II expression. When the peptide presented by class II in the irradiated recipient was restricted to the peptide responsible for positive and negative selection of the T cells, division of the transferred $CD4^+$ T cells did not occur. These experiments establish that peptides distinct from those involved in thymic selection control $CD4^+$ T cell expansion in T cell–deficient mice and suggest that these same peptides may influence the T cell pool in normal mice by causing mature $CD4^+$ T cells to divide.

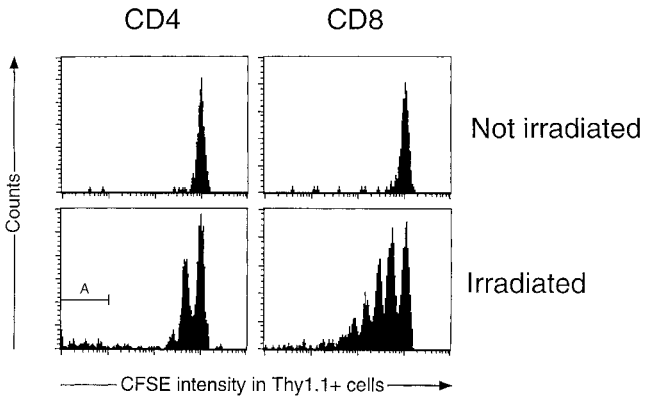

Figure 1. $CD4^+$ T cells divide after transfer to irradiated hosts. Purified, CFSE-labeled B6.PL (Thy1.1) T cells were transferred to normal or 450-rad irradiated B6 (Thy1.2) mice. Each host received 1.8×10^6 B6.PL $CD4^+$ T cells. 7 d later lymph node and spleen cells were stained with PE anti-Th1.1, CyC anti-CD8, and allophycoerythrin anti-CD4. Histograms show the intensity of CFSE fluorescence of $CD4^+Thy1.1^+$ or $CD8^+Thy1.1^+$ cells. Total $CD4^+Thy1.1^+$ cells recovered from the spleen and lymph nodes of each nonirradiated or irradiated host were 2.4×10^5 and 2.7×10^5, respectively.

Materials and Methods

Reagents and Mice. B6.PL-Thy1a/Cy (B6.PL) and BALB/cByJ (BALB/c) mice were purchased from The Jackson Laboratory. C57BL/6NTacfBR (B6) and C57BL/6TacfBR-[KO]$A^b\beta$ (MHCIIKO) animals were purchased from Taconic Farms. A^b EpTKO (β2-microglobulin KO, wtA^bKO, IiKO, A^bEp^+), TKO (β2-microglobulin KO, wtA^bKO, IiKO), wtA^{b+}DKO (β2-microglobulin KO, wtA^{b+}, IiKO), and DO TCR^+ RAG2KO BALB/c mice were bred under specific pathogen-free conditions in the Biological Resource Center (BRC) at National Jewish Medical and Research Center. CFSE was purchased from Molecular Probes Inc. PE-labeled mAbs against Thy1.1 and CD4, cy-chrome (CyC)-labeled anti-CD8, allophycoerythrin-labeled anti-CD4, and CyC-labeled streptavidin were purchased from PharMingen. FITC-labeled and biotinylated anti–TCR-β (H597) and anti-DO 11.10 idiotype (KJ1) were prepared in our laboratory. Cells were stained and analyzed as previously described (5, 27).

T Cell Transfer. T cells were purified to >95% using Cellect Mouse T columns (Biotex Labs.). They were stained with CFSE by incubation in 1.5 μM CFSE in balanced salt solution for 15 min at 37°C at 10^7 cells/ml, washed once, and transferred intravenously. Mice were irradiated immediately before transfer with 450 rads (^{137}Cs source; AEC). Similar results were obtained if transfer was delayed until 36 h after irradiation.

Results

T Cells Divide When Transferred to T Cell–deficient Mice. The effect of sublethal irradiation of host mice on the rate of division of T cells transferred to the host was determined. T cells were purified from the lymph nodes of B6.PL ($Thy1.1^+$) mice, labeled with CFSE, and injected into B6 ($Thy1.2^+$) recipients that either had or had not been sublethally irradiated. $CD4^+$ and $CD8^+$ T cells that were transferred to normal, nonirradiated B6 mice showed little evidence of division 7 d after transfer (Fig. 1). However, $CD4^+$ and $CD8^+$ T cells that were transferred to irradiated B6 mice divided substantially during the same period. In irradiated B6 hosts, $CD8^+$ T cells divided more quickly than did $CD4^+$ T cells, although a small subpopulation of $CD4^+$ T cells divided so many times that it had almost lost its CFSE fluorescence (Fig. 1, histogram region A). These data showed, as expected from previous studies, that transferred T cells divided more quickly in irradiated host mice than in nonirradiated host mice.

Because whole-body irradiation affects many tissues, it is possible that the T cell division observed in irradiated mice did not result from T cell deficiency, but instead from additional effects of irradiation. To be certain that a T cell deficiency results in the increased division of transferred T cells, CFSE-labeled T cells were transferred to mice that lacked T cells due to induced genetic mutations. CFSE-labeled T cells from BALB/c mice were transferred to RAG2KO (α/β-TCR T $cell^-$, γ/δ-TCR T $cell^-$, B $cell^-$) mice, and CFSE-labeled T cells from B6.PL mice were transferred to TCR-βKO (α/β-TCR T $cell^-$, γ/δ-TCR T $cell^+$, B $cell^+$) mice. Both RAG2KO and TCR-βKO mice lack endogenous α/β T cells. 7 d after transfer, $CD4^+$ and $CD8^+$ T cells had divided in both RAG2KO and TCR-βKO recipient mice (Fig. 2). However, the rate of $CD4^+$ T cell division was much faster in the RAG2KO and TCR-βKO hosts than it was in irradiated hosts. Because the rates of T cell division in irradiated and genetically T cell–deficient hosts differed, it is possible that the mechanism responsible for division in each host also differed. However, together these experiments indicate that the rate of α/β-TCR^+ $CD4^+$ and $CD8^+$ T cell division increased in response to an α/β T cell deficit.

Most $CD4^+$ T Cell Division in Irradiated Mice Requires MHC Class II Expression. To determine whether division of T cells that were transferred to irradiated mice required

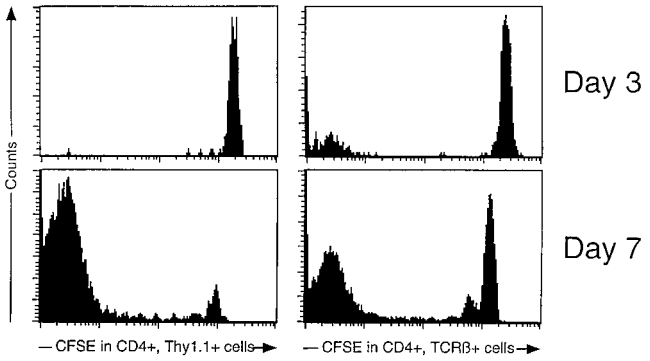

Figure 2. $CD4^+$ T cells divide rapidly after transfer to untreated TCR-βKO (left) or RAG2KO (right) mice. Sublethally irradiated TCR-βKO or RAG2KO mice were injected with 3×10^6 CFSE-labeled B6.PL (left) or BALB/c (right) T cells. 7 d later lymph node and spleen cells were removed for analysis. Cells from TCR-βKO recipients were stained with PE anti-Th1.1, CyC anti-CD8, and allophycoerythrin anti-CD4, and cells from RAG2KO recipients were stained with PE anti-CD4 and CyC anti–TCR-β. The data shown represent $CD4^+Thy1.1^+$ lymph node cells from TCR-βKO recipients and $CD4^+TCR\text{-}\beta^+$ lymph node cells from RAG2KO recipients.

TCR–MHC interactions, we tested whether $CD4^+$ T cells divided in hosts that did not express MHC class II molecules. Purified B6.PL lymph node T cells were transferred to nonirradiated or irradiated B6 or MHCIIKO mice. Analyses 7 d after transfer showed that transferred $CD4^+$ T cells did not divide in nonirradiated B6 or MHCIIKO hosts (Table I). However, transferred $CD4^+$ T cells did divide in both irradiated B6 and irradiated MHCIIKO hosts, although more division occurred in irradiated B6 hosts than in irradiated MHCIIKO hosts (Fig. 3, summarized in Table I). 55% of transferred $CD4^+$ T cells divided in irradiated B6 hosts, whereas only 22% of transferred $CD4^+$ T cells divided in irradiated MHCIIKO hosts. As shown in Table I, the difference in the percentage of transferred $CD4^+$ T cells that divided in irradiated B6 and irradiated MHCIIKO mice was consistent over many experiments. These data indicate that most of the division that occurred among $CD4^+$ T cells transferred to irradiated B6 mice required class II expression, which confirmed previous studies of $CD4^+$ T cell expansion in T cell–deficient mice (22, 23).

Although most of the $CD4^+$ T cells that were transferred to irradiated hosts required class II expression to divide, some $CD4^+$ T cells divided in the absence of class II expression. Among the transferred $CD4^+$ T cells that divided in irradiated MHCIIKO mice were two groups of cells. The first group of $CD4^+$ T cells that divided in irradiated MHCIIKO mice went through a single round of cell division. This single round of division may represent a response by the T cells to the cytokine environment generated in the host by irradiation (28–30). The second group of $CD4^+$ T cells divided many times in MHCIIKO mice (see Fig. 1, histogram region A). During and after division these cells expressed high levels of CD44 (data not shown), which suggests that antigen, possibly presented by class I or nonclassical MHC molecules, caused these cells to divide. The rapidly dividing cells were excluded from the calculations presented here because they appeared to represent a distinct phenomenon.

After the transfer of T cells to irradiated mice, the total number of transferred T cells recovered from the lymph nodes and spleen of an irradiated recipient was typically 10–20% of the total number of cells that were initially

Table I. *Unfamiliar Class II–bound Peptides Are Involved in $CD4^+$ T Cell Division in Irradiated Hosts*

Source of donor T cells	Recipient	Recipient irradiated	No. of independent experiments	Percentage of transferred $CD4^+$ T cells that have undergone a given number of divisions		
				0 divisions	1 division	2 divisions
					average ± SEM	
B6.PL	B6	no	5	97.0 ± 0.5	2.8 ± 0.4	0.3 ± 0.5
	A^bKO	no	4	96.8 ± 0.5	3.3 ± 0.3	0.0 ± 0.0
	B6	yes	5	57.7 ± 2.9	38.9 ± 2.4	4.5 ± 0.9
	A^bKO	yes	4	79.2 ± 2.3	19.3 ± 2.2	1.3 ± 0.4
A^bEpTKO	A^bEpTKO	yes	4	91.8 ± 4.6	7.5 ± 4.0	1.1 ± 0.8
	TKO	yes	4	85.7 ± 9.8	13.3 ± 9.7	1.7 ± 0.9
A^bEpTKO‡	A^bEpTKO	yes	1	96.6 ± 2.7*	3.4 ± 2.6*	0.8 ± 0.7*
	TKO	yes	1	90.2 ± 2.2*	7.9 ± 2.1*	3.8 ± 3.6*
DO TCR + RAG2KO	BALB/c	yes	3	89.6 ± 2.1	6.5 ± 1.0	2.6 ± 0.3

Donor T cells were purified and labeled with CFSE before transfer to untreated or irradiated recipients. 7 d after transfer, recipients were analyzed and the percentage of transferred $CD4^+$ T cells that had not divided, divided once, or divided twice was determined using histogram markers as indicated in Fig. 3. In the experiment in which A^bEpTKO donor mice were injected with SEB and LPS before transfer, the data shown indicate the percentage of $V\beta8^+CD4^+$ ($V\beta8^+$ T cells are activated by SEB) cells that went through a given number of divisions.

*Data from $CD4^+V\beta8^+$ cells.

‡Injected 1 wk before transfer with 150 μg SEB and 7 μg LPS.

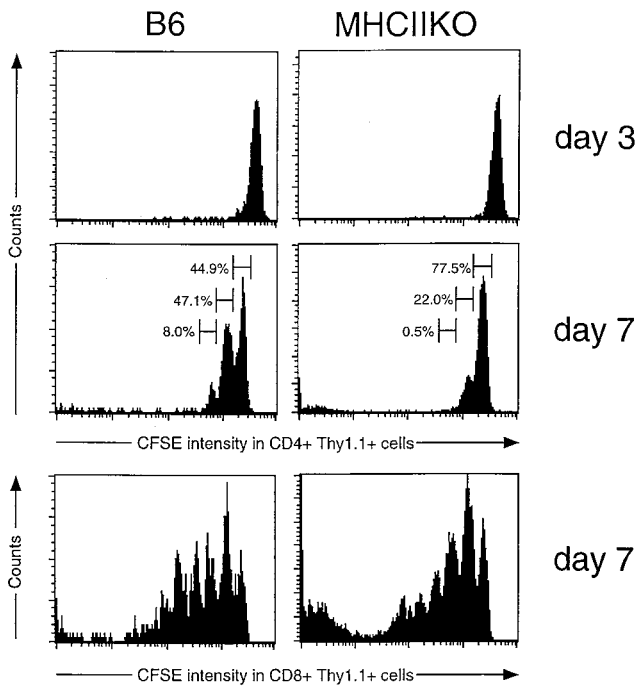

Figure 3. Much of the proliferation of $CD4^+$ T cells in irradiated hosts requires MHC class II expression. 5×10^6 B6.PL CFSE-labeled T cells (2.1×10^6 $CD4^+$ T cells) were transferred to 450-rad sublethally irradiated B6 or MHCIIKO mice. 7 d later lymph node and spleen cells were stained and analyzed as described in Fig. 1. Numbers indicate the percentages of cells that have not divided, divided once, or divided twice, with the percentage calculation excluding cells that have gone through more than two divisions (see Fig. 1). Total $CD4^+$Thy1.1 cells recovered from each B6 or MHCIIKO mouse were 2.9×10^5 and 5.4×10^5, respectively.

transferred. To exclude the possibility that transferred T cells trafficked to and divided within tissues other than spleen and lymph node, lymphocytes were prepared from the liver, lung, and small intestine of irradiated B6 or irradiated MHCIIKO mice that had been injected with CFSE-labeled B6.PL T cells 7 d earlier. The total number of transferred T cells obtained from these tissues represented ~4% of the number initially transferred, but the T cells obtained from these tissues had divided less than had the T cells in the lymph nodes and spleen from the same mice (data not shown). Thus, trafficking of transferred $CD4^+$ T cells to tissues other than the spleen and lymph node did not influence the division of $CD4^+$ T cells in irradiated B6 or MHCIIKO mice. This finding provided additional support for the conclusion that most $CD4^+$ T cells required class II expression to divide in irradiated hosts.

The Peptide–MHC Ligand Responsible for Thymic Selection Does Not Cause Division of $CD4^+$ T Cells in Irradiated Mice. To assess whether peptides influenced MHC class II–dependent $CD4^+$ T cell division, we exploited mice that express a single class II–peptide combination, A^b covalently linked to a peptide from Eα (A^bEp), and no other class II or class I proteins, and no invariant chain (Ii) (A^bEpTKO). A recent report involving mice constructed to express a single peptide bound to class II molecules by a different method suggested that such mice contained many class II–bound peptides (31). Experiments similar to those performed in this report indicate that this is not the case in A^bEpTKO animals (Marrack, P., unpublished data). All tests of A^b EpTKO mice indicate that positive and negative selection of all $CD4^+$ T cells in A^bEpTKO mice occurs only on the A^bEp molecule, and that the APCs from these mice are unable to present any other self- or foreign peptide in association with class II molecules due to 100% occupancy of A^b by Eα (5, 32). Because a normal array of self-peptides is not presented by class II during negative selection of $CD4^+$ T cells in A^bEpTKO mice, mature $CD4^+$ T cells in these mice are not tolerant to the self-peptides presented by class II in a wild-type A^{b+} mouse. Consequently, a large percentage of A^bEpTKO $CD4^+$ T cells react strongly to the normal self-peptides presented by A^b (5). As discussed below, we used this reactivity as a control to show that A^bEpTKO T cells were capable of division.

To determine whether interactions between TCR and the class II–peptide ligand responsible for thymic selection could induce division of $CD4^+$ T cells in irradiated mice, A^bEpTKO T cells were labeled with CFSE and transferred to irradiated hosts of three genotypes: (i) mice that expressed no MHC class I, class II, or Ii (TKO); (ii) mice that expressed only the selecting ligand (A^bEpTKO mice); and (iii) mice that expressed wild-type A^b, but no class I or Ii (wtA^{b+}DKO). 7 d after transfer, host lymph node and spleen cells were stained for TCR and CD4 and analyzed. In each of these hosts a population of cells stained positively for TCR and CD4 but were CFSE negative. These cells were derived from the host, not the donor, and they appear in Fig. 4 because there was no surface marker that could be used to distinguish between host and donor $CD4^+$ T cells.

As expected, few $CD4^+$ A^bEpTKO T cells divided when transferred to irradiated TKO hosts, which do not express class II molecules (Fig. 4, summarized in Table I). $CD4^+$ A^bEpTKO T cells also failed to divide when transferred to irradiated A^bEpTKO hosts, which express the class II–peptide ligand responsible for thymic selection. In fact, on average, more of the A^bEpTKO T cells divided in irradiated TKO recipients than in irradiated A^bEpTKO recipients (Table I). Thus, the selecting ligand had no effect on the rate of division of A^bEpTKO T cells transferred to irradiated recipients. The lack of division by the transferred A^bEpTKO T cells was not due to a defect in the A^bEpTKO T cells themselves; A^bEpTKO T cells divided extensively in irradiated wtA^{b+}DKO hosts, which express wild-type A^b bound to peptides to which A^bEpTKO T cells are not tolerant (Fig. 4). These data showed that interactions between TCR and the specific peptide–MHC ligand responsible for thymic selection did not cause division of T cells in irradiated hosts.

A recent study found that although naive transgenic $CD8^+$ T cells required antigen to divide in irradiated hosts, memory transgenic $CD8^+$ T cells divided in irradiated mice in the absence of specific antigen (12). To address whether the peptide–MHC ligand responsible for thymic selection is capable of causing the division of memory $CD4^+$ T cells, $V\beta8^+$ T cells from A^bEpTKO mice were activated before transfer by injecting the mice with 150 μg

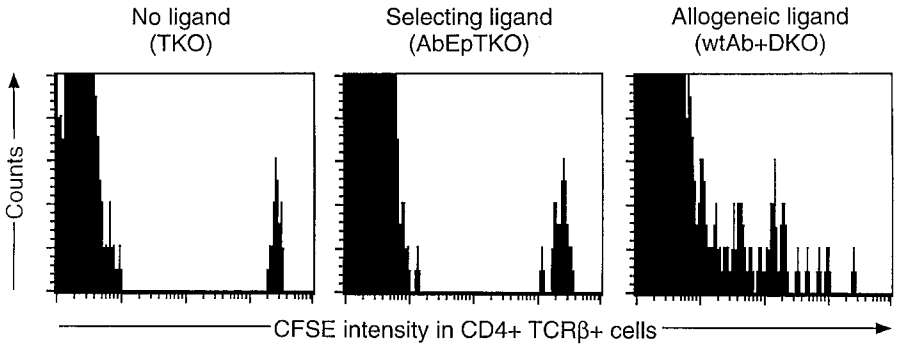

Figure 4. The selecting ligand does not drive $CD4^+$ T cell division in irradiated hosts. 3.8×10^5 CFSE-labeled $CD4^+$ A^bEpTKO T cells were transferred to 450-rad sublethally irradiated hosts. Hosts were: TKO (β2M KO, wtA^bKO, IiKO); A^bEpTKO (β2M KO, wtA^bKO, IiKO, A^bEp^+); $wtA^{b+}DKO$ (β2M KO, $wtA^{b+}+$, IiKO). 7 d after transfer cells were stained with PE anti-CD4 and CyC anti–TCR-β and analyzed. Histograms represent the data from live $CD4^+TCR^+$ lymph node cells.

staphylococcal enterotoxin B (SEB) and 7 μg LPS. Injection of SEB and LPS resulted in the activation of $V\beta8^+$ T cells, as assessed by the increased number of $V\beta8^+$ cells observed 2 d after SEB injection (data not shown). 1 wk after the SEB injection, T cells from these A^bEpTKO mice were purified, labeled with CFSE, and transferred to irradiated A^bEpTKO, TKO, and $wtA^{b+}DKO$ recipients. 1 wk later, the amount of division that had occurred among transferred $V\beta8^+$ $CD4^+$ cells was determined (Table I). The data indicate that little division of previously activated $V\beta8^+$ $CD4^+$ T cells occurred in irradiated mice that lacked MHC expression or expressed only the selecting MHC–peptide ligand. As expected, the transferred $V\beta8^+$ $CD4^+$ cells divided rapidly in $wtA^{b+}DKO$ mice, indicating that the lack of division of these cells in A^bEpTKO and TKO mice was not due to anergy induced by SEB priming (data not shown). Thus, unlike memory $CD8^+$ T cells, memory $CD4^+$ T cells do not appear to divide in irradiated mice that express only the selecting MHC molecule.

Transgenic DO T Cells Do Not Divide in Irradiated Hosts That Support Positive Selection. To provide additional evidence that the peptide–MHC ligand responsible for thymic selection could not induce division of $CD4^+$ T cells in irradiated mice, CFSE-labeled transgenic $CD4^+$ DO T cells, which bear TCRs specific for OVA peptide in the context of A^d and are positively selected in BALB/c mice (33), were transferred to irradiated BALB/c mice. 7 d after transfer, analysis showed that few DO T cells divided in irradiated BALB/c hosts (Fig. 5, summarized in Table I). Because the peptides found in the periphery of BALB/c mice are similar to the peptides that support selection of the DO TCR in the thymus (34), this experiment indicated that ligands involved in thymic selection could not induce division of DO T cells in an irradiated recipient.

To demonstrate that DO T cells were able to divide in response to antigen, irradiated BALB/c mice that had previously received CFSE-labeled DO T cells were injected with OVA peptide, for which DO T cells are specific. 7 d after the injection of 0.1, 1.0, or 10 μg of OVA peptide, the extent of division among DO T cells was determined (Fig. 5). Analysis showed that the amount of division of DO T cells reflected the amount of antigen administered to the irradiated BALB/c recipient. Although injection of 0.1 μg OVA peptide into an irradiated BALB/c host induced little division, injection of 1.0 or 10 μg OVA peptide caused a progressive increase in the extent of division of DO T cells. This result confirmed the previous finding that transgenic $CD4^+$ T cells required antigen to divide when transferred to T cell–depleted mice (23) and indicated that the extent of division among DO T cells in irradiated BALB/c mice reflected the amount of peptide antigen administered. These findings suggest that the one or two rounds of division seen among normal B6.PL $CD4^+$ T cells that were transferred to irradiated B6 hosts may have

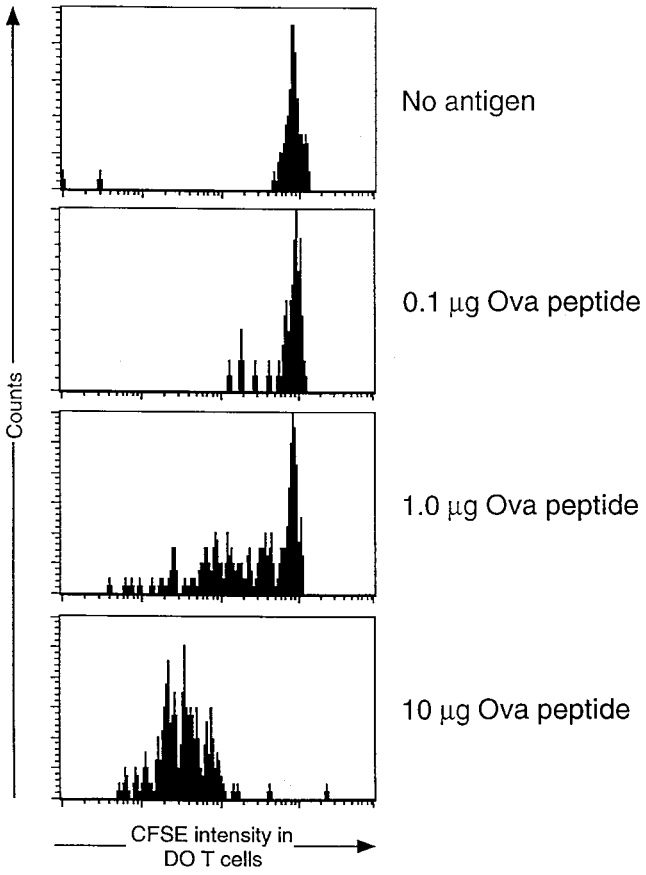

Figure 5. Transgenic DO T cells do not divide in irradiated mice that support thymic selection of the DO TCR. DO TCR^+RAGKO BALB/c, CFSE-labeled cells were transferred to sublethally irradiated BALB/c mice. 24 h later, mice were injected intravenously with soluble OVA peptide as indicated on the figure. 7 d later, cells were stained with PE anti-CD4 and CyC KJ1 (anti-DO idiotype) and analyzed. Data shown are $CD4^+KJ1^+$ lymph node cells.

occurred in response to TCR stimulation by relatively low levels of peptides.

Discussion

Previous reports have demonstrated that depletion of T cells in mice creates an environment in which T cells divide when transferred into these mice (19–23). Past experiments have also shown that the T cell division in such mice depends on TCR contact with MHC. For example, division of $CD4^+$ T cells transferred to T cell–deficient mice depends on the expression of class II molecules (22, 23). The findings presented here confirm that $CD4^+$ T cell division in T cell–deficient mice requires class II expression and establish that class II–bound peptides control this division.

As expected, a smaller percentage of $CD4^+$ T cells from normal mice divided when transferred to irradiated MHCIIKO mice than divided when transferred to irradiated B6 mice. To determine whether the peptides presented by class II were involved in this class II–dependent division, $CD4^+$ T cells from mice expressing only one class II–peptide combination were transferred to irradiated hosts. A^bEpTKO $CD4^+$ T cells, which were selected in the thymus exclusively by Ep bound to A^b, did not divide when transferred to irradiated A^bEpTKO hosts, in which all A^b molecules were occupied by the selecting Ep peptide. Because A^bEpTKO hosts expressed class II molecules, the lack of division among transferred A^bEpTKO T cells indicated that division of $CD4^+$ T cells in irradiated mice depends on peptide presentation by class II molecules and not on other effects class II expression may have on irradiated hosts. Therefore, the class II–dependent division of naturally occurring B6.PL $CD4^+$ T cells after transfer to irradiated B6 hosts was caused by peptides presented by class II molecules.

The lack of A^bEpTKO T cell division in irradiated A^bEpTKO hosts also established that the peptides that controlled division of $CD4^+$ T cells in irradiated mice were distinct from those responsible for thymic selection. These peptides have not been specifically identified, but we refer to them as unfamiliar because they are distinct from those involved in thymic selection. These unfamiliar peptides were expressed extrathymically after irradiation and may be derived either from intestinal flora or from the mouse itself.

The requirement for unfamiliar peptides suggests that TCR specificity determined whether a particular $CD4^+$ T cell divided when transferred to an irradiated host. This suggestion is supported by findings described here and in a previous report (23) that transgenic $CD4^+$ T cells did not divide in T cell–deficient hosts in the absence of antigen. Thus, $CD4^+$ T cells divided when transferred to irradiated mice in response to specific interactions between TCRs and unfamiliar peptides presented by class II molecules. The reason these specific interactions occurred in irradiated mice but did not occur in nonirradiated mice is unknown. There are at least two explanations for the observation that $CD4^+$ T cells experienced greater exposure to unfamiliar peptides when transferred to irradiated mice than when transferred to normal mice. First, irradiation may have changed the distribution of peptides presented by class II molecules by altering the representation of specific self- or foreign peptides. This could have resulted from the phagocytosis of large numbers of apoptotic mouse cells by APCs or from transient infection of irradiated mice by normal flora due to the breakdown of mucosal barriers. Second, by causing apoptosis of lymphocytes, irradiation could have decreased the competition among T cells for interaction with peptides normally presented by class II molecules. The explanation that decreased competition led to increased exposure of $CD4^+$ T cells to peptides is particularly appealing because it could also explain the rapid rate of division of T cells that were transferred to TCR-βKO and RAG2KO hosts, both of which lack endogenous T cells to compete with transferred $CD4^+$ T cells for interactions with APCs. Further work will be required to determine whether increased contact between transferred $CD4^+$ T cells and unfamiliar peptides in irradiated mice occurred because of decreased competition among T cells or due to a change in the distribution of peptides presented by class II molecules in the host.

The data presented here demonstrate that $CD4^+$ T cells from normal mice divide in response to interactions with unfamiliar class II–bound peptides when transferred to irradiated hosts. Because the unfamiliar peptides that induce division in irradiated mice may also be presented, perhaps at lower levels, by class II molecules in normal mice, it is possible that the same peptides that cause division of $CD4^+$ T cells in irradiated mice are also responsible for causing extrathymic expansion (13) of T cells in normal mice. Thus, the 20% of the $CD4^+$ T cell repertoire that required class II expression to divide in irradiated hosts may have been created in donor mice by extrathymic expansion in response to the same peptides that induced division in irradiated mice. If so, then the peptides presented by class II molecules in a normal, healthy mouse may significantly influence the T cell repertoire, as has been suggested in an earlier study of peripheral T cell selection (24). Additional work will have to be done to determine whether unfamiliar peptides influence the T cell repertoire in normal mice by causing $CD4^+$ T cell division and, if so, whether this division occurs only in the context of an active immune response.

The authors thank Ella Kushnir for breeding the A^bEpTKO and TKO mice and Terry Potter for evaluating the manuscript.

This work was supported by United States Public Health Service grants AI17134, AI18785, and AI22295.

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Submitted: 25 February 1999 Revised: 19 May 1999 Accepted: 15 June 1999

References

1. Miller, J.F., and D. Osoba. 1967. Current concepts of the immunological function of the thymus. *Physiol. Rev.* 47:437–520.
2. Nikolic-Zugic, J., and M.J. Bevan. 1990. Role of self-peptides in positively selecting the T-cell repertoire. *Nature.* 344:65–67.
3. Miyazaki, T., P. Wolf, S. Tourne, C. Waltzinger, A. Dierich, N. Barois, H. Ploegh, C. Benoist, and D. Mathis. 1996. Mice lacking H2-M complexes, enigmatic elements of the MHC class II peptide-loading pathway. *Cell.* 84:531–541.
4. Martin, W.D., G.G. Hicks, S.K. Mendiratta, H.I. Leva, H.E. Ruley, and L. Van Kaer. 1996. H2-M mutant mice are defective in the peptide loading of class II molecules, antigen presentation, and T cell repertoire selection. *Cell.* 84:543–550.
5. Ignatowicz, L., J. Kappler, and P. Marrack. 1996. The repertoire of T cells shaped by a single MHC/peptide ligand. *Cell.* 84:521–529.
6. Hsu, B.L., B.D. Evavold, and P.M. Allen. 1995. Modulation of T cell development by an endogenous altered peptide ligand. *J. Exp. Med.* 181:805–810.
7. Fung-Leung, W.P., C.D. Surh, M. Liljedahl, J. Pang, D. Leturcq, P.A. Peterson, S.R. Webb, and L. Karlsson. 1996. Antigen presentation and T cell development in H2-M-deficient mice. *Science.* 271:1278–1281.
8. Brocker, T. 1997. Survival of mature CD4 T lymphocytes is dependent on major histocompatibility complex class II–expressing dendritic cells. *J. Exp. Med.* 186:1223–1232.
9. Kirberg, J., A. Berns, and H. von Boehmer. 1997. Peripheral T cell survival requires continual ligation of the T cell receptor to major histocompatibility complex–encoded molecules. *J. Exp. Med.* 186:1269–1275.
10. Rooke, R., C. Waltzinger, C. Benoist, and D. Mathis. 1997. Targeted complementation of MHC class II deficiency by intrathymic delivery of recombinant adenoviruses. *Immunity.* 7:123–134.
11. Takeda, S., H.R. Rodewald, H. Arakawa, H. Bluethmann, and T. Shimizu. 1996. MHC class II molecules are not required for survival of newly generated $CD4^+$ T cells, but affect their long-term life span. *Immunity.* 5:217–228.
12. Tanchot, C., F.A. Lemonnier, B. Perarnau, A.A. Freitas, and B. Rocha. 1997. Differential requirements for survival and proliferation of CD8 naive or memory T cells. *Science.* 276: 2057–2062.
13. Stutman, O. 1986. Postthymic T-cell development. *Immunol. Rev.* 91:159–194.
14. Rocha, B., A.A. Freitas, and A.A. Coutinho. 1983. Population dynamics of T lymphocytes. Renewal rate and expansion in the peripheral lymphoid organs. *J. Immunol.* 131:2158–2164.
15. Bell, E.B., and S.M. Sparshott. 1997. The peripheral T-cell pool: regulation by non-antigen induced proliferation? *Semin. Immunol.* 9:347–353.
16. Bruno, L., H. von Boehmer, and J. Kirberg. 1996. Cell division in the compartment of naive and memory T lymphocytes. *Eur. J. Immunol.* 26:3179–3184.
17. Tough, D.F., and J. Sprent. 1994. Turnover of naive- and memory-phenotype T cells. *J. Exp. Med.* 179:1127–1135.
18. Sprent, J., and D.F. Tough. 1994. Lymphocyte life-span and memory. *Science.* 265:1395–1400.
19. Bell, E.B., S.M. Sparshott, M.T. Drayson, and W.L. Ford. 1987. The stable and permanent expansion of functional T lymphocytes in athymic nude rats after a single injection of mature T cells. *J. Immunol.* 139:1379–1384.
20. Miller, R.A., and O. Stutman. 1984. T cell repopulation from functionally restricted splenic progenitors: 10,000-fold expansion documented by using limiting dilution analyses. *J. Immunol.* 133:2925–2932.
21. Mackall, C.L., F.T. Hakim, and R.E. Gress. 1997. Restoration of T-cell homeostasis after T-cell depletion. *Semin. Immunol.* 9:339–346.
22. Beutner, U., and H.R. MacDonald. 1998. TCR-MHC class II interaction is required for peripheral expansion of CD4 cells in a T cell–deficient host. *Int. Immunol.* 10:305–310.
23. Mackall, C.L., C.V. Bare, L.A. Granger, S.O. Sharrow, J.A. Titus, and R.E. Gress. 1996. Thymic-independent T cell regeneration occurs via antigen-driven expansion of peripheral T cells resulting in a repertoire that is limited in diversity and prone to skewing. *J. Immunol.* 156:4609–4616.
24. Rocha, B., and H. von Boehmer. 1991. Peripheral selection of the T cell repertoire. *Science.* 251:1225–1228.
25. Weston, S.A., and C.R. Parish. 1990. New fluorescent dyes for lymphocyte migration studies. Analysis by flow cytometry and fluorescence microscopy. *J. Immunol. Methods.* 133:87–97.
26. Lyons, A.B., and C.R. Parish. 1994. Determination of lymphocyte division by flow cytometry. *J. Immunol. Methods.* 171:131–137.
27. Teague, T.K., P. Marrack, J.W. Kappler, and A.T. Vella. 1997. IL-6 rescues resting mouse T cells from apoptosis. *J. Immunol.* 158:5791–5796.
28. Marrack, P., T. Mitchell, J. Bender, D. Hildeman, R. Kedl, K. Teague, and J. Kappler. 1998. T-cell survival. *Immunol. Rev.* 165:279–285.
29. Chang, C.M., A. Limanni, W.H. Baker, M.E. Dobson, J.F. Kalinich, and M.L. Patchen. 1997. Sublethal gamma irradiation increases IL-1alpha, IL-6, and TNF-alpha mRNA levels in murine hematopoietic tissues. *J. Interferon Cytokine Res.* 17:567–572.
30. Ul'ianova, L.P., and R.S. Budagov. 1997. [Changes in the hematopoietic system and in the concentrations of blood-regulatory cytokines after acute irradiation and a combined radiation-thermal lesion] (in Russian). *Radiats. Biol. Radioecol.* 37:182–188.
31. Barton, G.M., and A.Y. Rudensky. 1999. Requirement for diverse, low-abundance peptides in positive selection of T cells. *Science.* 283:67–70.
32. Wilson, N.A., P. Wolf, H. Ploegh, L. Ignatowicz, J. Kappler, and P. Marrack. 1998. Invariant chain can bind MHC class II at a site other than the peptide binding groove. *J. Immunol.* 161:4777–4784.
33. Murphy, K.M., A.B. Heimberger, and D.Y. Loh. 1990. Induction by antigen of intrathymic apoptosis of $CD4^+CD8^+$ TCR^{lo} thymocytes in vivo. *Science.* 250:1720–1723.
34. Marrack, P., L. Ignatowicz, J.W. Kappler, J. Boymel, and J.H. Freed. 1993. Comparison of peptides bound to spleen and thymus class II. *J. Exp. Med.* 178:2173–2183.