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Age-Dependent TCR Revision Mediated by Interaction between $\alpha\beta$ TCR and Self-Antigens

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Interactions between TCR and self-peptide/MHC complex play an important role in homeostasis and Ag reactivity of mature peripheral T cells. In this report, we demonstrate that the interactions between mature peripheral T cells and endogenous Ags have a negative impact on the maintenance of foreign Ag-specific T cells in an age-dependent manner. This is mediated by RAG-dependent secondary rearrangement of the TCR α-chain (receptor revision). The TCR revision in mature T cells is readily observed in mouse expressing transgenic TCR α-chain inserted into the physiological locus (knockin mouse) but not in conventional transgenic mouse with an identical TCR α-chain. Thus, our results suggest that under physiological conditions in which all TCR α-chains are susceptible to deletion by secondary rearrangement, TCR revision in mature peripheral T cells is an ongoing process in adult animals and contributes to age-dependent changes in T cell function and repertoire. ***The Journal of Immunology,*** **2007, 179: 2163–2169.**

Antigen specificity of the T cell is determined by its surface receptor, which is composed of α- and β-chains (1, 2). During development in the thymus, the interaction between surface receptor and self-MHC molecule bound to peptides derived from a variety of self-proteins determines the fate of immature thymocytes (3–6). Thymocytes expressing TCR having strong affinity to the self-peptide/MHC complex die by apoptosis (negative selection). T cells that fail to interact with the self-peptide/MHC complex via surface receptors also die by apoptosis (death by neglect). In contrast, immature thymocytes expressing surface receptors that show weak affinity to the self-peptide/MHC complex are selected to mature into functional T cells (positive selection) (7–9). This selection process ensures the MHC-restricted, foreign Ag-specific response of T cells and maintains tolerance to self-Ags. Another type of endogenous Ag,"endogenous superantigen," encoded by retrovirus related genes interacts with T cells in a TCR β-chain-dependent and MHC-independent fashion. This interaction in the thymus leads to the deletion and/or induction of anergy of the T cells (10, 11).

In peripheral lymphoid organs, self-peptide/MHC complexes are necessary for the survival, expansion, and homeostasis of naive T cells (12, 13). In mice lacking T cells, introduced naive T cells proliferate rapidly in vivo in a self-peptide/MHC-dependent manner (homeotic expansion) (14). Furthermore, the capacity of T cells to respond to foreign Ag is also dependent on the continuous interaction between TCR and self-peptide/MHC complexes (15). These results demonstrate that the interaction between mature T cells and self-peptide/MHC complexes via TCR plays an important role in maintaining the size and function of T cells in peripheral lymphoid organs. However, no Ag responsible for the interaction in the periphery has been identified and, at this point, it is not possible to study the nature of the interaction.

T cells expressing known self-Ag-specific transgenic (TG) TCR escape thymic-negative selection and emigrate to peripheral lymphoid organs. Subsequent interaction between self-reactive T cells and Ags results in a decrease in the number of self-reactive T cells in the periphery. This decrease is not due to the death of self-reactive T cells; rather, it is due to the change in surface TCR expression in mature T cells. The induction of RAG I and RAG II genes in mature peripheral T cells leads to the secondary rearrangement of the *TCR* locus, eventually replacing existing self-reactive TCR with new TCR (receptor revision) (16–19). Although the biological role of the receptor revision in mature T cells is not well understood, it seems that the interaction between mature T cells and self-Ags is a continuous event and plays an important role in shaping the postthymic T cell repertoire.

We and others have established a new type of TCR-TG mouse in which a functionally rearranged V-J α segment was introduced into the endogenous TCR α-chain locus using homologous recombination in the embryonic stem (ES)[2] cell (20, 21). Using these TCR knockin (KI) mice, we have demonstrated that multiple rearrangements of the TCR α-chain play a critical role in the positive and negative selection of T cells in the thymus. In this report, using the same TCR α KI mouse lines, we demonstrate that the interaction between mature peripheral T cells and endogenous genetic elements decreases the number of T cells expressing KI α TCR in an age-dependent manner. These T cells are specific for well-characterized foreign Ags and do not respond to self-Ags in a conventional T cell activation assay. Thus, mature, foreign Ag-specific T cells are capable of interacting with yet unknown self-Ag(s) in the periphery, similar to the interaction for positive selection in the thymus. This interaction induces the *RAG* gene and the secondary rearrangement of the TCR α-chain, leading to the deletion of the KI TCR α-chain gene. Our findings suggest that receptor revision in mature T cells occurs more frequently than previously anticipated. This would decrease the number of T cells expressing foreign Ag-specific TCRs and induce the generation of nonselected

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[2] Abbreviations used in this paper: ES, embryonic stem; KI, knockin; TG, transgenic.

Table I. *Changes in KI TCR α-chain expression in F_1 mice*[a]

Mouse	KI TcR α-Chain Expression	
	4 wk	8 wk
KI α/Vβ3 TG B10.BR	42.8 ± 2.5[b]	39.2 ± 3.1
KI α/Vβ3 TG B10.BR × CBA/Ca		
1	42.1	10.7
2	46.3	6.7
3	47.0	15.6
4	43.1	11.5
5	30.0	9.9
6	29.3	6.9
7	32.2	8.5
8	45.1	9.1
KI α/Vβ3 TG B10.BR × AKR/J		
1	37.6[c]	16.5
2	33.5	16.4
3	50.9	29.8
4	51.6	24.8
5	30.7	14.8
6	30.1	16.8

[a] KI α/Vβ3 TG male mouse with high KI TCR α-chain expression was mated with B10.BR, CBA/Ca, and AKR/J female mice. Offspring were tested for the expression of KI TCR α-chain and Vβ3 TG and the expression of KI TCR α-chain in CD4 T cell population was monitored at 4 and 8 wk of age.

[b] KI TCR α-chain expression (percent-positive expression in CD4 T cells: mean of five mice analyzed ± SD).

[c] KI TCR α-chain expression (percent-positive expression in CD4 T cells).

peripheral T cells. These mechanisms may play an important role in the age-dependent changes of T cell function (22–24), namely, the loss of T cell response to pathogens, as well as the appearance of the autoimmune phenomenon.

Materials and Methods

Mice

Cytochrome *c*-specific I-E^k-restricted 2B4 TCR α-chain KI mice (20), 2B4 TCR α-chain TG mice (25), and 2B4 TCR β-chain TG mice (26) were described previously. Mouse lines used in this study were generated as follows,

The E14 ES cell line (129 origin) was used to establish the KI ES cells (20). ES cells were injected into the B6 blastocyst. The resulting chimeric mouse was mated with B10.BR mice, and F_1 mice (B10.BR × 129) were screened for the presence of the *KI* gene. KI-positive F_1 mice were mated to 2B4 TCR β-chain (Vβ3) TG B10.BR mice. Offspring were screened for the homozygosity of H-2^k, presence of KI, and TG β-chain TCR and for the absence of mammary tumor virus 3, 11, and 13 that delete Vβ3-positive T cells in the thymus. These KI-positive Vβ3 TG and H-2^k homozygous mice were cross-mated and maintained as KI heterozygous and Vβ3 TG-positive mice (for Figs. 1 and 2). This KI-positive Vβ3 TG (two generations backcrossed to B10.BR) mouse was used to backcross to B10.BR. For each backcross, one male mouse that exhibited a decrease of KI TCR expression was chosen to mate to B10.BR female and offspring were screened for the presence of KI and Vβ TG at 4 wk of age and for the decrease of KI TCR-positive CD4 T cells. After five generations of backcross (male mouse used for experiment in Fig. 3), the offspring exhibit clear segregation: some exhibited a decrease of KI-positive CD4 T cells and others maintained a high percentage of KI-positive CD4 T cells. The single KI^{high} male mouse was used to mate with CBA/ca and AKR/J in the experiment shown in Table I.

The male Ag (H-Y)-specific CD8 T cell-derived TCR αβ-chain TG mouse (27), the TCR β-chain TG mouse (28), and the TCR α-chain KI mouse (21) were described previously. RAG-GFP KI mouse (29) was a gift from Dr. N. Sakaguchi (Kumamoto University, Kumamoto, Japan). B10.BR, CBA/Ca and AKR/J mice were purchased from The Jackson Laboratory. All procedures were approved by the RIKEN Animal Care and Use Committee.

Chimeric mice

Bone marrow chimeric mice were established according to the method described previously (30). Briefly, T cell-depleted bone marrow cells from KI α/Vβ3 TG mice and from Vβ3 TG mice were mixed (10:1 ratio) and injected into lethally irradiated (950 rad) recipients (1×10^7 cells/mouse). Chimeric mice were screened for the expression of 2B4 TCR α-chain before use in the experiments.

Flow cytometric analysis

Preparation of PBMCs, thymocytes, and lymph node cells, and staining of the cells were performed as described previously (20, 30). Abs used were anti-CD4, -CD8, -Vβ3, and -Vβ8 (BD Biosciences); anti-2B4 TCR α-chain Id, a2B4 (a gift from Dr. L. Samelson, National Institutes of Health, Bethesda, MD) (31); and anti H-Y TCR Id, MR14-1 (32). Data were analyzed with CellQuest software (BD Biosciences).

Irradiation

Mice were irradiated with Gammacell 40 Exactor (Nordin).

Cell transfer

T cells (2×10^6) from KI α/Vβ3 TG and RAG-GFP homozygous mice were transferred i.v. into transgene-negative littermate mice. Fifteen days after transfer, lymph node and spleen cells were pooled and analyzed as described above.

Results

Age and genetic background-dependent changes in peripheral T cell repertoire in mice

T cells bearing I-E^k-restricted and cytochrome *c*-specific 2B4 TCR can be efficiently selected in the thymus of H-2K mice to emigrate to peripheral lymphoid organs as CD4 single-positive T cells (20). In H-2K KI α/Vβ3 TG mice (129 and B10.BR mixed genetic background with no Vβ3-specific endogenous superantigens) at 4 wk of age, ~40% of CD4 T cells in lymph node and peripheral blood expressed 2B4 αβ TCR (20). When these KI α/Vβ3 TG mice were tested for the expression of the KI TCR α-chain at 25 wk of age, significant heterogeneity of the expression, ranging from 48.9% to as low as 8.9%, was found in their peripheral blood CD4 T cells (Fig. 1*A*). As reported previously, all KI TCR-negative T cells did not contain the KI gene with no change in the β TCR expression (data not shown), demonstrating change in the level of TCR α-chain deletion among these mice. It should be noted that this heterogeneity was also observed in lymph node T cells in 20-wk-old mice.

The heterogeneity of expression of the KI TCR α-chain in old mice prompted us to examine whether this heterogeneity is dependent on genetic differences among mice with the 129 and B10.BR (all mice have H-2K MHC homozygosity) mixed background. At 4 wk of age, ~40% of peripheral CD4 T cells expressed the KI TCR α-chain in all the mice analyzed, similar to our previous report. However, at 8 wk of age, the CD4 T cells in these mice exhibited significant heterogeneity of expression of the KI TCR α-chain (two examples are shown in Fig. 1*B*). These results indicate that changes in the number of KI TCR α-chain-positive CD4 T cells occur in an age and genetic background-dependent manner.

We then tested whether this decrease in the number of KI TCR α-chain-positive CD4 T cells in KIα/Vβ3 TG mice is due to the decreased output of KI TCR α-chain-positive T cells from the thymus, or is mediated by the change occurring in the periphery. KI α/Vβ3 TG mice with mixed background (20 wk of age) were initially screened for KI TCR α-chain expression. Thymus and lymph node CD4 T cells were analyzed for the expression of KI TCR α-chain (Fig. 2*A*). Although the total number of thymocytes (35×10^6 cells) was significantly smaller than that in young mice, the majority of newly generated CD4 T cells in the thymus expressed the KI TCR α-chain. In contrast, only 18% of CD4 T cells in the lymph nodes were positive for the same KI TCR α-chain. Mice with small numbers of KI TCR α-chain-positive CD4 T cells were also sublethally irradiated (600 rad) to induce death of peripheral lymphocytes and regeneration of new T lymphocytes from

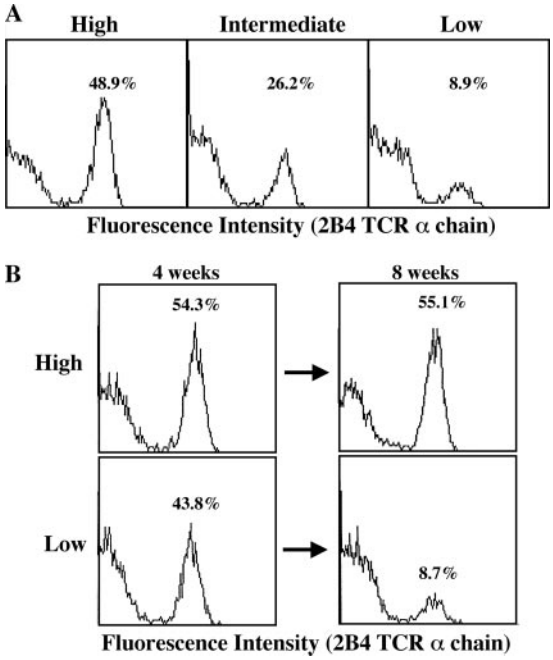

FIGURE 1. Age-dependent change in KI TCR α-chain expression in a TCR α KI/β TG mouse. *A*, PBMCs from 25-wk-old KIα/Vβ3 TG mice with a 129 and B10.BR mixed genetic background were stained with anti-CD4 and -a2B4 Abs. Stained cells were gated for CD4-positive populations and the fluorescence intensity of a2B4 Ab staining is shown. Three representative results (mice with high, intermediate, and low KI TCR α-chain positive CD4 T cell populations) are shown. This heterogeneity was found among 35, 129-B10.BR mixed background mice analyzed including eight mice from the same parents. *B*, PBMCs from KIα/Vβ3 TG mice with 129 and B10.BR mixed background were stained by the same methods at 4 and 8 wk of age. Two examples are shown. Four mice from the same parents were analyzed ranging from 55.1, 38.7, 10.2, and 8.7% KI-positive T cells in the CD4 T cell population at 8 wk of age.

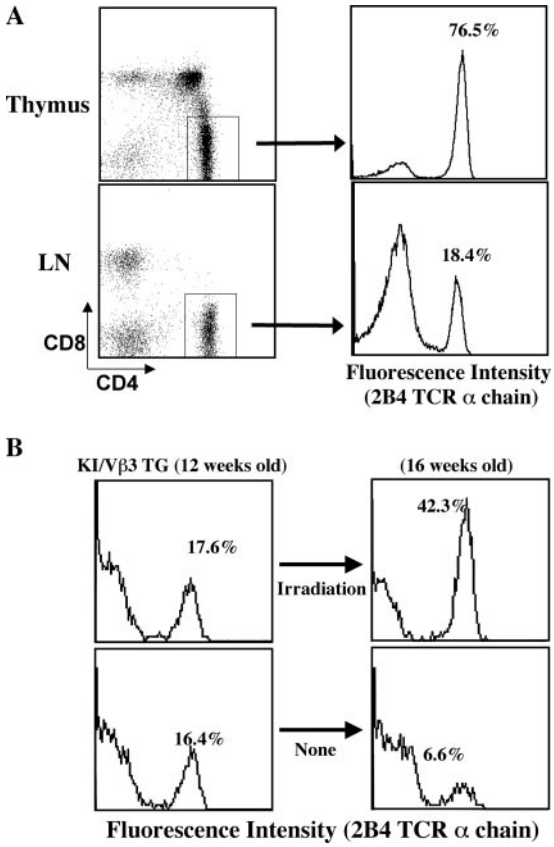

FIGURE 2. Decrease in number of KI TCR α-chain-positive CD4 T cells in peripheral lymphoid organ. *A*, Thymocytes and lymph node T cells from KI α/Vβ3 TG mice (20 wk old) were stained with anti-CD4, -CD8, and -a2B4 Abs and analyzed for KI TCR α-chain-positive CD4 single-positive T cell populations. *B*, Mice were screened for the expression of a2B4-positive peripheral blood CD4 T cells at 12 wk of age. Mice with small numbers of a2B4-positive CD4 T cells were either irradiated (600 rad) or not treated and then tested for a2B4-positive peripheral blood CD4 T cells 4 wk after treatment. Four irradiated mice showed an increase of KI-positive CD4 T cells from 17.1 $\pm$ 4% to 40.3 $\pm$ 5.6 and four nonirradiated mice showed a decrease from 16.8 $\pm$ 2.8 to 7.2 $\pm$ 2.4%.

the thymus. Four weeks after irradiation, the mice were tested for the presence of KI TCR α-chain-positive CD4 T cells. As shown in Fig. 2*B*, mice that recovered from the irradiation had significantly large numbers of KI TCR α-chain-positive CD4 T cells. In contrast, in mice that received no irradiation, the number of KI TCR α-chain-positive CD4 T cells decreased further (we examined four mice per group and found similar results). These results suggest that the thymus of adult mice is still capable of producing KI TCR α-chain-positive CD4 T cells and that the decrease in the number of KI TCR α-chain-positive CD4 T cells occurs in the periphery. This conclusion is also supported by our thymectomy experiments. The mice selected by the same phenotype as those used in Fig. 2*B* were thymectomized and tested for the change of KI-positive CD4 T cells. In control nonthymectomized mice, KI-positive CD4 T cells decreased from 15.8 $\pm$ 2.7% to 7.0 $\pm$ 1.3% (three mice). Change in thymectomized mice was from 16.6 $\pm$ 1.8% to 4.8 $\pm$ 1.7% in thymectomized group (three mice), indicating very little, if any, contribution of the recent thymic emigrant in the decrease of the KI-positive CD4 T cells in the periphery.

Characterization of genetic elements controlling peripheral T cell repertoire

KI α/Vβ3 TG mice (129 and B10.BR mixed genetic background) were backcrossed to B10.BR mice and offspring were screened for the presence of KI TCR-positive CD4 T cells at 12–22 wk of age. The offspring of KI^{low} $\times$ B10.BR mice remained heterogeneous but after five generations of backcrossing to B10.BR mice, these mice started to segregate into KI^{high} and KI^{low} phenotypes (Fig. 3). In contrast, the offspring of KI^{high} $\times$ B10.BR mice were homogeneous and the expression of KI TCR-positive CD4 T cells remained high (50–60% positive at 16 wk of age, similar to F_1 KI^{high} mouse shown in Fig. 3). The results of these backcrossing experiments suggest that T cells expressing KI TCR α-chain and TG TCR β-chain are positively selected in the thymus of the B10.BR and 129 mixed background mouse but the genetic elements from the 129 mouse negatively influence the maintenance of T cells expressing the same TCR in the periphery.

Then, we attempted to examine the 129 mouse genetic elements responsible for the decrease in expression of KI TCR α-chain-positive CD4 T cells. However, the 129 mouse contains three endogenous MTV superantigens, MTV 3, 11, and 13, which interact with Vβ3 TCR (11). The presence of these superantigens that delete all T cells expressing Vβ3 TCR made it difficult to carry out segregation analysis using 129 mouse. We therefore chose two mouse lines, CBA/Ca and AKR/J, which are of H-2K genotype and have no Vβ3-specific endogenous MTV superantigens (11). One KI α/Vβ3 TG male mouse with KI^{high} phenotype was mated with B10.BR, CBA/Ca, and AKR/J females, and offspring were tested for the expression of KI TCR α-chain in the CD4 T cell

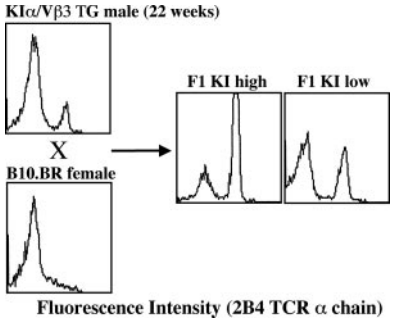

FIGURE 3. Segregation of a2B4-positive CD4 T cell high and low mice after five generations of backcrossing to B10.BR mice. KI α/Vβ3 TG mice were backcrossed to B10.BR mice. Mice with low expression of a2B4-positive CD4 T cells at 22 wk of age were selected for mating with B10.BR mice. After five generations of backcrossing, offspring were tested for the number of a2B4-positive CD4 T cells at 4 wk of age. For this experiment, eight mice from the same parents had both KIα and Vβ3 TG. Four mice had a KI^{high} phenotype (60.1 $\pm$ 7.1% a2B4 positive) and four had a KI^{low} phenotype (30.4 $\pm$ 4.0%).

population. As expected, ~25% of offspring contained a significant number (30–50%) of KI TCR α-chain-positive CD4 T cells at 4 wk of age. It should be noted that all mice with high expression of KI TCR α-chain-positive CD4 T cells also had Vβ3 TG. This finding is compatible with our previous findings (20) that the KI TCR α-chain can be protected from deletion by secondary rearrangement of the TCR α-chain through positive selection in the presence of Vβ3 TG and I-E^k. When the same mice were analyzed at 8 wk of age, there was no significant decrease in the expression of KI TCR α-chain-positive CD4 T cells in KI α/Vβ3 TG B10.BR mice. However, all mice from the CBA/Ca and AKR/J mating had significantly lower expression of KI TCR α-chain-positive CD4 T cells than the mice at 4 wk of age (Table I). It should be noted that the decrease in expression of KI TCR α-chain-positive CD4 T cells in B10.BR $\times$ AKR/J F_1 mice is significantly less than that in B10.BR $\times$ CBA/Ca F_1 mice (an average of 19.9 and 9.8% of CD4 T cells express KI TCR α-chain, respectively, at 8 wk of age). At this point, it is difficult to determine whether this difference is due to the presence of different genetic elements or to the difference in the number of genetic elements in these two mouse strains.

To determine the complexity of the genetic elements in CBA/Ca mice, KI α/Vβ3 TG B10.BR $\times$ CBA/Ca F_1 mice were backcrossed to B10.BR mice, and offspring were screened for the presence of KI TCR α-chain and Vβ TG. The number of CD4-positive T cells expressing the KI TCR α-chain was monitored at 4 and 8 wk of age. As shown in Table II, all KI TCR α-chain and TG β-chain double-positive mice (10 of 50 backcrossed mice analyzed) contained similar numbers of KI TCR α-chain-positive CD4 T cells at 4 wk of age. However, at 8 wk of age, significant heterogeneity of expression of KI TCR α-chain-positive CD4 T cells was observed among these backcrossed mice. Two mice showed no decrease in the percentage of KI TCR α-chain-positive CD4 T cells, while four mice showed >50% decrease. There were also mice that showed moderate decrease (two mice with <25% decrease and two others with <10% decrease). It should be noted that the second series of backcrossed mice also exhibited similar heterogeneity (data not shown). These results clearly suggest an involvement of more than one genetic element in the decrease in expression of KI TCR α-chain-positive CD4 T cells in CBA/Ca mouse.

Table II. *Changes in TCR α-chain expression in backcrossed mice*[a]

Mouse	KI TCR α-Chain Expression		Percentage Decrease
	4 wk	8 wk	
A			
1	44.5[b]	17.1	>50
2	40.1	20.0	>50
3	48.2	17.2	>50
4	36.1	16.3	>50
B			
5	39.8	27.0	>25
6	38.0	26.6	>25
C			
7	42.0	36.0	>10
8	45.0	39.0	>10
D			
9	33.9	33.3	None
10	47.3	48.5	None

[a] KI α/Vβ3 TG B10.BR $\times$ CBA/Ca F_1 male mouse was mated with B10.BR female mice. Offspring were tested for the expression of KI TCR α-chain and Vβ3 TG and the expression of KI TCR α-chain in CD4 T cell population was monitored at 4 and 8 wk of age. Mice were categorized based on the percentage of KI TCR α-chain-positive CD4 T cells.

[b] KI TCR α-chain expression (percent-positive expression in CD4 T cells).

Requirement of $\alpha\beta$ TCR for interaction with genetic elements

In the next set of experiments, we tested whether the age-dependent decrease in expression of the KI TCR α-chain occurs independent of Vβ3 TG. B10.BR mice carrying the KI TCR α-chain alone were mated with CBA/Ca mice and offspring were monitored for the expression of the KI TCR α-chain in the CD4 T cell population. We also examined 2B4 Vβ3 TCR TG F_1 mice with the same genetic background for the decrease in expression of the TG β-chain in the CD4 T cell population. As shown in Table III, only a small percentage of CD4 T cells were positive for the expression of the KI TCR α-chain in KI TCR mice without Vβ3 TG. However, there was no decrease in the percentage of KI TCR α-chain-positive CD4 T cells in the mice with B10.BR $\times$ CBA/Ca F_1 background at 8 wk. There was also no decrease in the percentage of T cells expressing Vβ3 TG in the mice with the same genetic background. Thus, it is clear that the expression of neither KI TCR α nor Vβ3 TG TCR alone on CD4 T cells is responsible for the T cell repertoire change in the periphery in mice with the B10.BR $\times$ CBA/Ca mixed genetic background.

Table III. *Changes in TCR α-chain expression in F_1 mice*[a]

Mouse	TCR α-Chain Expression	
	4 wk	8 wk
KIα.B10BR $\times$ CBA/Ca		
1	3.1[b]	2.9
2	4.3	4.2
3	3.8	3.7
Vβ3 TG.B10.BR $\times$ CBA/Ca		
1	97.3[c]	92.1
2	96.5	90.8
3	97.0	90.6

[a] KI α and Vβ3 TG mice with B10.BR background were mated with CBA/Ca mice and offspring were tested for the expression of KI TCR α-chain and Vα3 TG, respectively. TCR expression in CD4 T cell population was monitored at 4 and 8 wk of age.

[b] KI TCR α-chain expression (percent-positive expression in CD4 T cells).

[c] TG β-chain expression (percent-positive expression in CD4 T cells).

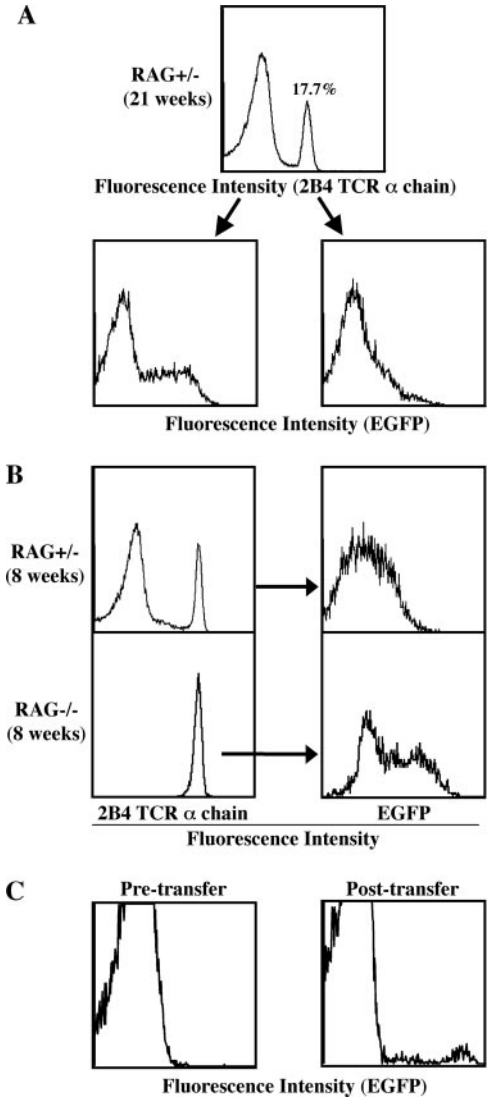

FIGURE 4. Monitoring RAG expression using a RAG I GFP KI mouse. *A*, Lymph node CD4 T cells from KI α/Vβ3 TG and RAG I GFP/RAG wild-type heterozygous mice (20 wk old) were separated based on a2B4 staining and GFP expression was monitored for each fraction. *B*, Lymph node CD4 T cells from KI α/Vβ3 TG and either RAG I GFP heterozygous or homozygous mice were stained with a2B4 Ab±. GFP expression in a2B4-positive CD4 T cells was compared. *C*, GFP-negative KI TCR-positive CD4 T cells (2×10^6) from KI α/Vβ3 TG with RAG I GFP homozygous mice were transferred to mice (littermate lacking both KI and RAG genes). Fifteen days after cell transfer, mice were sacrificed and T cells expressing KI TCR were analyzed for the expression of GFP.

Role of TCR α-chain secondary rearrangement in the change in peripheral T cell repertoire

We initially attempted to detect RAG gene re-expression in peripheral CD4 T cells in KI α/Vβ3 TG mice but failed to detect RAG I and II mRNAs reproducibly even with a high-sensitivity PCR assay. To determine the possible involvement of RAG-mediated secondary rearrangement in the decrease of KI TCR-positive T cells, we mated 2B4 KI α/Vβ3 TG B10.BR mice with RAG GFP mice. In this GFP mouse line, the expression of the introduced *GFP* gene is regulated by RAG I promoter. However, due to the long half-life of GFP protein in nondividing cells, GFP protein can be detected for a significantly long time after the termination of gene transcription. The presence of *GFP* gene in heterozygous RAG I-GFP mice did not affect the decrease in expression of KI TCR-positive CD4 T cells. However, in old mice, the KI TCR-negative CD4 T cell population contained GFP^{high} cells, indicating recent RAG expression (Fig. 4*A*). In contrast, KI TCR-positive CD4 T cell population contained very few GFP^{high} cells.

We also compared KI TCR-positive CD4 T cells from KI α/Vβ3 TG mice with RAG I GFP heterozygous (one copy of the functional *RAG I* gene and *GFP* gene) and RAG I GFP homozygous (both alleles of the *RAG I* gene are replaced with GFP gene) genetic backgrounds for the expression of GFP. In RAG I GFP homozygous mice, all CD4 T cells expressed KI TCR and a significant number of these T cells expressed high levels of GFP protein. In contrast, the number of GFP^{high} cells is small in KI TCR-positive T cells from RAG I GFP heterozygous mice (Fig. 4*B*).

To further analyze whether peripheral mature T cells can re-express the *RAG* gene, GFP-negative KI TCR-positive CD4 T cells (Fig. 4*C*, pretransfer) from KI α/Vβ3 TG with RAG I GFP homozygous mice were transferred to mice (littermate lacking both *KI* and *RAG* genes). Fifteen days after cell transfer, mice were sacrificed and T cells expressing KI TCR were analyzed for the expression of the *RAG* gene. As shown in Fig. 4*C* (posttransfer), significant number (5.3%) of cells were positive for the RAG expression. These results suggest that the interaction between 2B4 $\alpha\beta$ TCR and endogenous gene products induces *RAG* gene re-expression and subsequent secondary rearrangement of the TCR α-chain. In RAG I GFP heterozygous mice, the KI TCR α-chain can be deleted by this secondary rearrangement and CD4 T cells lose KI TCR α-chain expression without losing GFP protein expression (KI-negative and GFP^{high} CD4 T cells). In RAG I GFP homozygous mice, KI TCR α-chain-positive T cells express GFP with no functional *RAG* gene product. Thus, the KI TCR α-chain is not deleted and T cells remain KI TCR positive with high GFP expression.

To further examine the role of secondary rearrangement of the TCR α-chain in the age-dependent decrease in expression of KI TCR-positive T cells, we compared mouse lines carrying α KI/β TG 2B4 TCR and $\alpha\beta$ TG 2B4 TCR with the B10.BR × CBA/Ca F_1 background. To establish similar conditions for T cells in these mouse lines, bone marrow cells from $\alpha\beta$ TCR TG mice were mixed with those from Vβ3 TCR TG mice (10 $\alpha\beta$ TG bone marrow cells: 1 β-chain TG bone marrow cell) and injected into lethally irradiated recipients as reported previously (30) (all mice have identical B10.BR × CBA/Ca genetic background). Bone marrow cells from KI α/Vβ3 TG mice were also used to reconstitute mice with the same background. Two months after reconstitution, CD4 T cells expressing the 2B4 TCR α-chain were analyzed in the peripheral blood of the chimeric mice (Fig. 5*A*). Mice reconstituted with KI α/Vβ3 TG bone marrow cells had a similar percentage of 2B4 TCR α-chain-positive CD4 T cells to normal KI α/Vβ3 TG mice (Fig. 5*A*). In contrast, the number of 2B4 TCR α-chain-positive CD4 T cells in mice reconstituted with a mixture of $\alpha\beta$ TG bone marrow cells and β TG bone marrow cells varied significantly, and mice in which 30–40% of peripheral blood CD4 T cells were positive for the 2B4 TCR α-chain were selected for further analysis (Fig. 5*A*). The same mice were analyzed for the expression of 2B4 TCR α-chain 8 wk after initial analysis. As shown in Fig. 5*A*, the percentage of CD4 T cells expressing 2B4 TCR α-chain decreased significantly in the mouse that received KI α/Vβ3 TG bone marrow cells. In contrast, there was no change in CD4 T cell population in the mouse received $\alpha\beta$ TG bone marrow cells together with β-chain TG bone marrow cells. These findings are consistent with our previous results (20) that the KI TCR α-chain but not the TG α-chain can be deleted by RAG-mediated recombination activity.

We further examined a second set of KI TCR α-chain/Vβ TG and $\alpha\beta$ TCR TG mouse lines that have identical D^b-restricted and

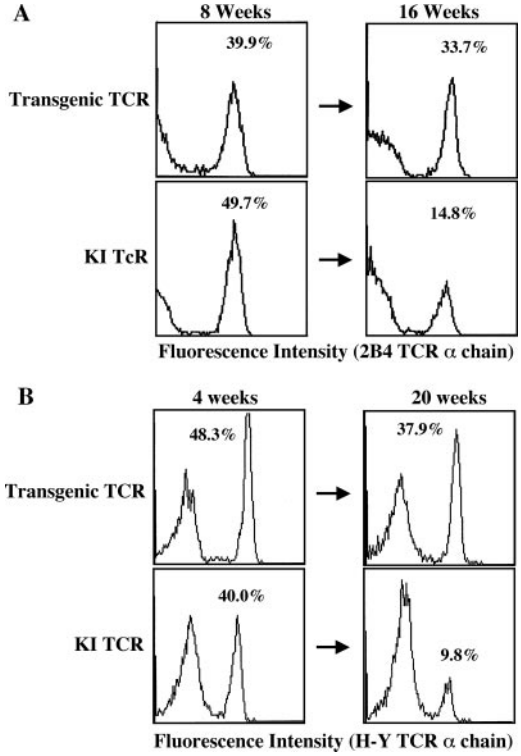

FIGURE 5. Difference between α KI and α TG mice in the decrease in the number of α-chain-positive T cells. *A*, Bone marrow chimeric mice containing 2B4 $\alpha\beta$ TG T cells and 2B4 KI α/Vβ3 TG T cells were established and the number of a2B4 TCR α-chain positive CD4 T cells was monitored 8 and 16 wk after bone marrow reconstitution. Three mice for TG and seven mice for KI TCR were analyzed with similar results (40.2 $\pm$ 5% to 35.1 $\pm$ 4% for TG and 50.5 $\pm$ 6% to 15.3 $\pm$ 3 for KI TCR mice). *B*, Mice containing H-Y-specific TCR-positive CD8 T cells (either $\alpha\beta$ TG or α KI/β TG T cells) were monitored for the expression of Id-positive CD8 T cells at 8 and 20 wk of age. Three mice for each group were analyzed with the similar results (47.5 $\pm$ 6.2% to 40.8 $\pm$ 6.2% for TG and 38.9 $\pm$ 3.7% to 10.7 $\pm$ 2.1% for KI mice).

H-Y-specific TCR. In young mice (4 wk old), 40–50% of CD8 T cells expressed the H-Y-specific TCR α-chain. The percentage of TG $\alpha\beta$ TCR-positive CD8 T cells did not change for >15 wk in all the four mice analyzed (a representative result is shown in Fig. 5*B*). Approximately 40% of CD8 T cells expressed H-Y-specific $\alpha\beta$ TCR in young H-Y-specific TCR α-chain KI/Vβ8 TG mice, similar to that in $\alpha\beta$ TCR TG mice. However, in old H-Y-specific TCR α-chain KI/Vβ8 TG mice (20 wk of age), <10% of CD8 T cells were positive for H-Y-specific TCR. This decline was observed in all the five mice examined in our colony. These results clearly demonstrate that changes in the T cell repertoire in the periphery are not limited to 2B4 TCR-positive CD4 T cell populations. Furthermore, difference between the KI TCR α-chain and the TG TCR α-chain in both 2B4 and H-Y T cell systems suggests the importance of the location of TCR α-chain gene in the change of the T cell repertoire in peripheral lymphoid organs.

Discussion

We have demonstrated that the interaction between $\alpha\beta$ TCR and endogenous gene products decreases the number of T cells expressing the cytochrome *c*-specific I-E^k-restricted 2B4 $\alpha\beta$ TCR. This is not due to the deletion of self-reactive T cells by negative selection in the thymus, because there is no evidence of deletion of double-positive T cells expressing 2B4 $\alpha\beta$ TCR in the thymus and a large fraction of mature peripheral CD4 T cells express the same $\alpha\beta$ TCR in young mouse. However, at ~4 wk of age, mice carrying genes from at least three mouse strains, 129, CBA/Ca, and AKR/J, start to lose CD4 T cells expressing 2B4 $\alpha\beta$ TCR. We have also demonstrated that similar age-dependent changes occur in CD8 T cells expressing H-Y-specific TCR. These results clearly suggest that the age-dependent change in the T cell repertoire is mediated by the interaction between endogenous Ag and $\alpha\beta$ TCR in the periphery.

The decrease in percentage of T cells expressing the KI TCR α-chain is mediated by the re-expression of *RAG* gene activity and the secondary rearrangement of TCR, similar to previous reports of receptor revision in peripheral T cells (16–19, 33). There are, however, notable differences between our findings and previous ones. First, peripheral TCR revision was found using TG mouse lines carrying either $\alpha\beta$ or β-chain TCR transgenes. In these TG mouse lines, TCR transgenes are integrated randomly in the genome and cannot be deleted by secondary rearrangement. Thus, changes in TCR expression (receptor revision) can be observed only when newly generated TCRs compete with TG TCR for the paring/expression of $\alpha\beta$ TCR (34). In contrast, the KI α-chains used in our study can be deleted by TCR α-chain secondary rearrangement, as described previously (20, 21). The fact that the change in TCR repertoire was found only in TCR α KI/β TG mice but not in TCR $\alpha\beta$ TG mice (Fig. 5) clearly demonstrates the importance of the physiological location of expressed TCR α-chain for the detection of peripheral TCR revision. Thus, previous reports of TCR revision using TG TCR mice may represent rare TCR revision occurring in vivo. Second, TCR revision in the periphery was observed in T cells expressing TCRs reactive to self-Ags, regardless of whether they were nominal Ags or superantigens. Thus, this phenomenon was thought to be unique for the down-regulation of autoimmune response in vivo. However, in this study, we demonstrated TCR revision in T cells expressing well-characterized foreign Ag-specific TCRs with no apparent self-reactivity. Taken together, these findings raise an intriguing possibility that TCR revision under physiological conditions may be a common phenomenon in the peripheral T cell pool. This issue can be clarified by establishing more TCR α-chain KI mice.

From the results, it is clear that all three mouse strains, 129, AKR/J, and CBA/Ca, contain genetic elements that cause a decrease in the percentage of KI TCR α-chain-positive CD4 T cells in the periphery. It is difficult to evaluate other mouse lines for the presence of genetic elements, because the majority of mouse lines contain endogenous MTV superantigens that delete T cells expressing Vβ3 TCR in the thymus. Using CBA/Ca and AKR/J mice, we have observed reproducible differences between B10.BR $\times$ AKR/J and B10.BR $\times$ CBA/Ca F_1 mice in terms of the decrease in percentage of KI TCR α-chain-positive CD4 T cells. The decrease in CBA/Ca F_1 mice is consistently faster than that found in AKR/J F_1 mice. This may be due to the difference in genetic elements or in the number of genetic elements present in these mouse lines. Our analysis of backcrossed mice demonstrated significant heterogeneity in the loss of KI TCR α-chain-positive CD4 T cells (Table II). Although the number of mice analyzed in this study is limited, these results suggest that multiple genetic loci are involved in the decrease in percentage of KI TCR α-chain-positive CD4 T cells in CBA/Ca $\times$ B10.BR F_1 mice. We have established B10.BR congenic lines carrying 129 (see Fig. 3) derived genes that decrease the number of KI TCR-positive T cells. These congenic mouse lines (after eight generations of backcrossing) carry two 129-derived chromosome segments (~5 cM in size): chromosome 4 and the TCR α locus in chromosome 14 from the 129 mouse (35) (data not shown). Further analysis of this congenic mouse line will reveal the nature of genetic elements responsible for the decrease in percentage of KI TCR α-chain-positive CD4 T cells in the periphery. At present, we have no information

concerning genetic elements that interact with H-Y-specific TCR. We are currently attempting to establish a congenic mouse line to identify such genetic elements.

The role of endogenous Ag in T cell repertoire formation has been well demonstrated for both negative and positive thymic selections (36). Besides these thymic selection events, the additional role of the self-peptide/MHC complex in the maintenance and survival of T cells has been demonstrated in vivo (12, 13). Furthermore, Stefanova et al. (15) demonstrated that the interaction between T cells and self-peptide/MHC complexes is required to maintain readiness of the T cell response to foreign Ags. These findings clearly demonstrate the importance of the self-peptide/MHC-TCR interaction (37, 38) in the maintenance of T cell repertoire size and function in vivo. Our findings provide new evidence that the interaction between T cells and endogenous Ags via surface $\alpha\beta$ TCR can negatively regulate T cells in the periphery in an age-dependent manner.

The biological significance of this age-dependent decrease in the number of T cells expressing known foreign Ag specificity is not clear at present. However, if this age-dependent change is common to a large fraction of T cells and is mediated by RAG-dependent receptor revision, such a change should generate a large number of peripheral T cells that express TCR without thymic selection. Then, such a change in T cell specificity may account for the decline in the number of T cells capable of responding to foreign Ags in the elderly (22–24, 39). It is also possible that receptor revision may generate self-reactive T cells responsible for the autoimmune phenomenon in adults (40, 41).

For both thymic and extrathymic interactions between T cells and endogenous Ags, no specific Ags have been identified so far. The lack of information concerning self-Ags interacting with TCR has made it difficult to elucidate mechanisms by which the T cell repertoire is generated in the thymus and maintained in the periphery. The availability of congenic mouse lines carrying genetic elements that interact with 2B4 $\alpha\beta$ TCR will allow us to identify endogenous Ags, and the identification of such Ags will provide a tool to reveal molecular mechanisms for the induction of the *RAG* gene in peripheral mature T cells, as well as new insights to further our understanding of the maintenance of peripheral T cell repertoire and function.

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Disclosures

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