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Different Development of Myelin Basic Protein Agonist- and Antagonist-Specific Human TCR Transgenic T Cells in the Thymus and Periphery[1]

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Myelin basic protein (MBP)-specific T cells are thought to play a role in the development of multiple sclerosis. MBP residues 111–129 compose an immunodominant epitope cluster restricted by HLA-DRB1*0401. The sequence of residues 111–129 of MBP ($MBP_{111–129}$) differs in humans (MBP122:Arg) and mice (MBP122:Lys) at aa 122. We previously found that ~50% of human $MBP_{111–129}$ (MBP122:Arg)-specific T cell clones, including MS2-3C8 can proliferate in response to mouse $MBP_{111–129}$ (MBP122:Lys). However, the other half of T cell clones, including HD4-1C2, cannot proliferate in response to $MBP_{111–129}$ (MBP122:Lys). We found that $MBP_{111–129}$ (MBP122:Lys) is an antagonist for HD4-1C2 TCR, therefore, MS2-3C8 and HD4-1C2 TCRs are agonist- and antagonist-specific TCRs in mice, respectively. Therefore, we examined the development of HD4-1C2 TCR and MS2-3C8 TCR transgenic (Tg) T cells in the thymus and periphery. We found that dual TCR expression exclusively facilitates the development of $MBP_{111–129}$ TCR Tg T cells in the periphery of HD4-1C2 TCR/HLA-DRB1*0401 Tg mice although it is not required for their development in the thymus. We also found that MS2-3C8 TCR Tg $CD8^+$ T cells develop along with MS2-3C8 TCR Tg $CD4^+$ T cells, and that dual TCR expression was crucial for the development of MS2-3C8 TCR Tg $CD4^+$ and $CD8^+$ T cells in the thymus and periphery, respectively. These results suggest that thymic and peripheral development of MBP-specific T cells are different; however, dual TCR expression can facilitate their development. ***The Journal of Immunology,*** **2008, 181: 5462–5472.**

The human HLA-DRB1*0401 allele is associated with the development of autoimmune diseases including rheumatoid arthritis and multiple sclerosis (MS)[4] (1–4). Thymic development of T cells is determined by the interaction of thymocytes with an Ag/MHC. Furthermore, the homeostatic proliferation and survival of T cells in the peripheral tissues is determined by the interaction of the TCR with a self-Ag/MHC complex (5). Thus, autoantigens associated with HLA-DRB1*0401 could tend to promote the development of rheumatoid arthritis- or MS-associated pathogenic T cells during the development of $CD4^+$ T cells in the thymus and peripheral lymphoid organs. To study the involvement of HLA-DRB1*0401 in the development of autoimmune disease, HLA-DRB1*0401 transgenic (Tg) mice expressing human-mouse chimeric MHC class II composed of the Ag-binding domain of HLA-DRB1*0401 and the CD4 association domain of I-E^d (see Fig. 1*A*) were generated (6). The HLA-DRA1*0101-I-E^d/HLA-DRB1*0401-I-E^d chimeric Tg mice were further crossed with MHC class II knockout (KO) mice to replace the endogenous mouse MHC class II gene with the human-mouse chimeric MHC class II gene, resulting in HLA-DRB1*0401 Tg mice. This human-mouse chimeric MHC class II allows mouse CD4 molecules to associate with the MHC class II and also allows Ags to bind to the Ag-binding groove of the HLA-DRB1*0401. In the HLA-DRB1*0401 Tg mouse, self-Ags associated with HLA-DRB1*0401 can facilitate the development of HLA-DRB1*0401-restricted $CD4^+$ T cells expressing diverse TCRs (6). Thus, this Tg mouse model has been used to identify autoantigens involved in the development of autoimmune diseases (7–11).

The HLA-DRB1*0401 Tg mouse was used to generate HLA-DRB1*0401-restricted human TCR Tg mice. Because myelin basic protein (MBP)-reactive T cells have been suggested to be involved in the development of MS and MBP residues 111–129 constitute an immunodominant epitope of MBP in humans expressing the HLA-DRB1*0401 haplotype, Tg mice expressing the HLA-DRB1*0401-restricted $MBP_{111–129}$-specific human TCR gene (see Fig. 1*A*) were created (12). The $MBP_{111–129}$ epitope sequence is different between humans and mice [human MBP122:Arg (122R) and murine MBP122:Lys (122K)]. In previous experiments, screening of a panel of DRB1*0401-restricted $MBP_{111–129}$-specific T cell clones (TCCs) with altered peptide ligands identified position 122 as an essential TCR contact residue for approximately half of the clones tested (11 of 19) as the conservative substitution MBP122R→K completely abrogated proliferative response of these clones (13). In the remaining eight TCCs with the same HLA-DRB1*0401 restriction, substitution of 122R

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[4] Abbreviations used in this paper: MS, multiple sclerosis; MBP, myelin basic protein; TCC, T cell clone; Tg, transgenic; KO, knockout; DP, double positive; HA, hemagglutinin.

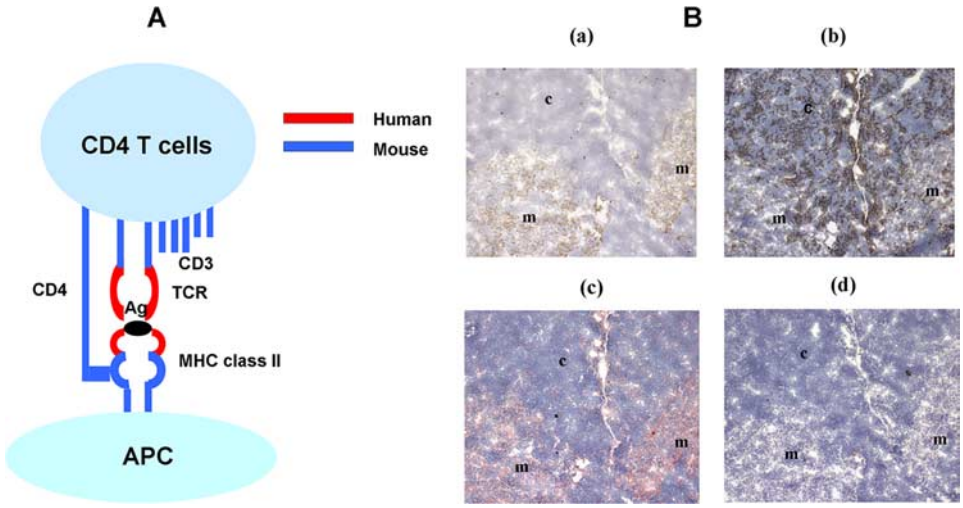

FIGURE 1. Schematic representation of the humanized TCR/MHC class II Tg animal model and thymic expression of humanized MHC class II. *A*, Ag presentation and recognition are mediated by human MHC class II and TCR components. *B*, Thymic sections from the HLA-DRA1*0101/HLA-DRB1*0401 Tg mouse were stained by Abs against MTS10 for detection of medullary epithelial cells (a), anti-CD205 Ab for detection of dendritic cells and some cortical epithelial cells (b), anti-HLA-DRα Ab (c), and anti-mouse IgG2a Ab (isotype control of anti-HLA-DRα) (d). The medulla (m) and cortex (c) are stained for immunoassay.

did not affect proliferation (13) (P. A. Muraro, unpublished observations).

Previously, we selected two TCRs with a distinct response to MBP at position 122 to generate humanized TCR Tg mice. The HD4-1C2 TCC proliferated in response only to human $MBP_{111–129}$, whereas the MS2-3C8 TCC proliferated in response to both human and mouse $MBP_{111–129}$. Due to the cross-reactivity of MS2-3C8 TCR with mouse $MBP_{111–129}$, the MS2-3C8 TCR Tg T cells can induce experimental autoimmune encephalomyelitis in HLA-DRB1*0401 Tg mice, whereas HD4-1C2 TCR Tg T cells cannot (12).

Although allelic exclusion of the TCR-β gene locus leads to the expression of a single TCR β-chain on T lymphocytes, around 10–25% of mouse and human T lymphocytes express dual TCR α-chains due to incomplete allelic exclusion at the TCR-α locus (14, 15). It has been suggested that expression of dual TCRs is involved in the development of foreign Ag-specific T cells as well as autoreactive T cells (16–19). We found that $MBP_{111–129}$ (122K) is an antagonist for the clone HD4-1C2 TCR and an agonist for clone MS2-3C8 TCR. In this study, we examined the thymic and peripheral development of $MBP_{111–129}$ TCR Tg T cells in MS2-3C8 and HD4-1C2 TCR Tg mice and the influence of dual TCR expression on their development.

Materials and Methods

Animals

C57BL/6 mice and RAG1-deficient mice were purchased from The Jackson Laboratory. HLA-DRB1*0401, HLA-DRB1*0404, MS2-3C8 TCR, and HD4-1C2 TCR Tg mice were generated in our laboratory previously (6, 12, 20) and maintained in our animal facility. These mice were housed in specific pathogen-free facilities.

Flow cytometry

Anti-I-A^b, anti-CD4, anti-CD8, and anti-CD5 mAbs, a panel of anti-mouse TCR-α Abs, and streptavidin-CyChrome were all purchased from BD

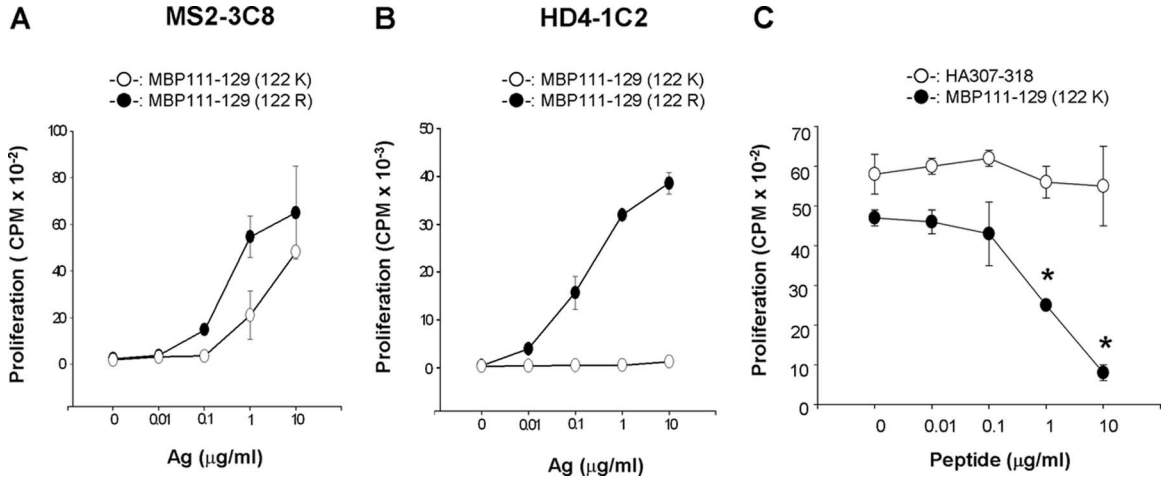

FIGURE 2. Antigenic specificity of human TCCs used for generation of human TCR Tg mice. Different antigenic specificity of human TCCs MS2-3C8 (*A*) and HD4-1C2 (*B*) tested in a proliferation assay with human $MBP_{111–129}$ (122R) (-●-) and murine $MBP_{111–129}$ (122K) (-○-). *C*, Murine $MBP_{111–129}$ (122K) exhibited antagonism for the HD4-1C2 TCC. The human HD4-1C2 TCC was stimulated with APC prepulsed with the agonist peptide human $MBP_{111–129}$ (122R) in the presence of different concentrations of an antagonist peptide, murine $MBP_{111–129}$ (122K) (-●-), and a control peptide $HA_{307–318}$ (-○-), which is a strong binder to HLA-DRB1*0401. *, $p < 0.05$ compared with inhibition by $HA_{307–318}$. Data shown are representative of at least three independent experiments.

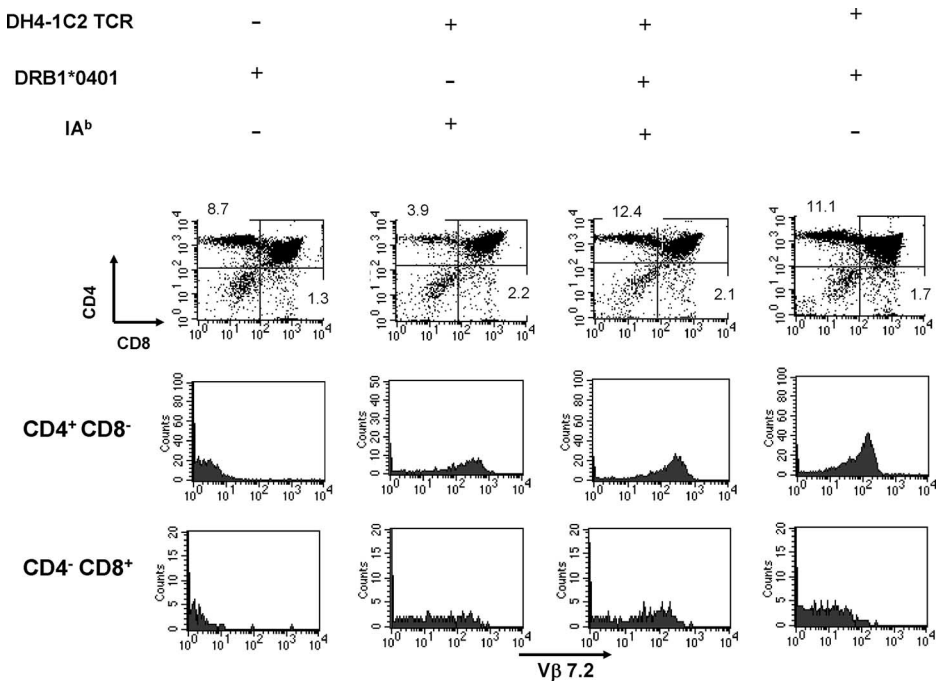

FIGURE 3. Skewing of HD4-1C2 Tg T cells into the CD4 T cell subset by thymic expression of the HLA-DRB1*0401 gene. Thymocytes of HD4-1C2 Tg mice were stained with anti-CD4, anti-CD8, and anti-Vβ7.2 Abs. Vβ7.2 expression is gated for the $CD4^+CD8^-$ and $CD4^-CD8^+$ T cell populations. The number of thymocytes in these mice is as follows: Non-TCR Tg mouse (1.4×10^8), HD4-1C2 TCR^+/I-A^{b+}/DRB1*$0401^{-/-}$ (2.2×10^8), HD4-1C2 TCR^+/I-A^{b+}/DRB1*0401^+ (1.4×10^8), and HD4-1C2 TCR^+/I-A^{b-}DRB1*0401^+ (1.5×10^8). Three to five mice of each genotype were analyzed. FACS plots are representative of three independent experiments.

Pharmingen. Monoclonal Abs against HLA-DRα, human TCR Vβ2.1, and human TCR Vβ7.2 were purchased from Beckman Coulter. Flow cytometry analysis was performed on a FACSCalibur instrument using CellQuest software (BD Biosciences) and a Cytomics FC500 using CXP software (Beckman Coulter). For analysis, a live gate was set around viable lymphocytes based on their forward and side scatter light FSC/SSC profile.

T cell proliferation assay

$CD4^+$ or $CD8^+$ T cells from naive mice (>95% pure by flow cytometry analysis) were obtained by positive selection using anti-CD4- or anti-CD8-coated microbeads (Miltenyi Biotec). $CD4^+$ and $CD8^+$ Tg T cell lines were established by cultivation of these naive Tg T cells with $MBP_{111–129}$ (10 μg/ml) and irradiated (2000 rad) HLA-DRB*0401 transgenic spleen cells (4×10^5/well) for 4 days and then further cultured with murine IL-2 (PeproTech) at 3 ng/ml for 10 days. The Tg naive T cells or T cell lines (4×10^4/well) were cultured with irradiated HLA-DRB*0401 or HLA-DRB*0404 transgenic spleen cells (4×10^5/well) for the proliferation assays. These cells were incubated at 37°C for 72 h, and [^{3}H]thymidine (1 μCi) was added to each well for the last 12 h of culture. The cells were harvested and counted on a TriLux liquid scintillation counter (Wallac).

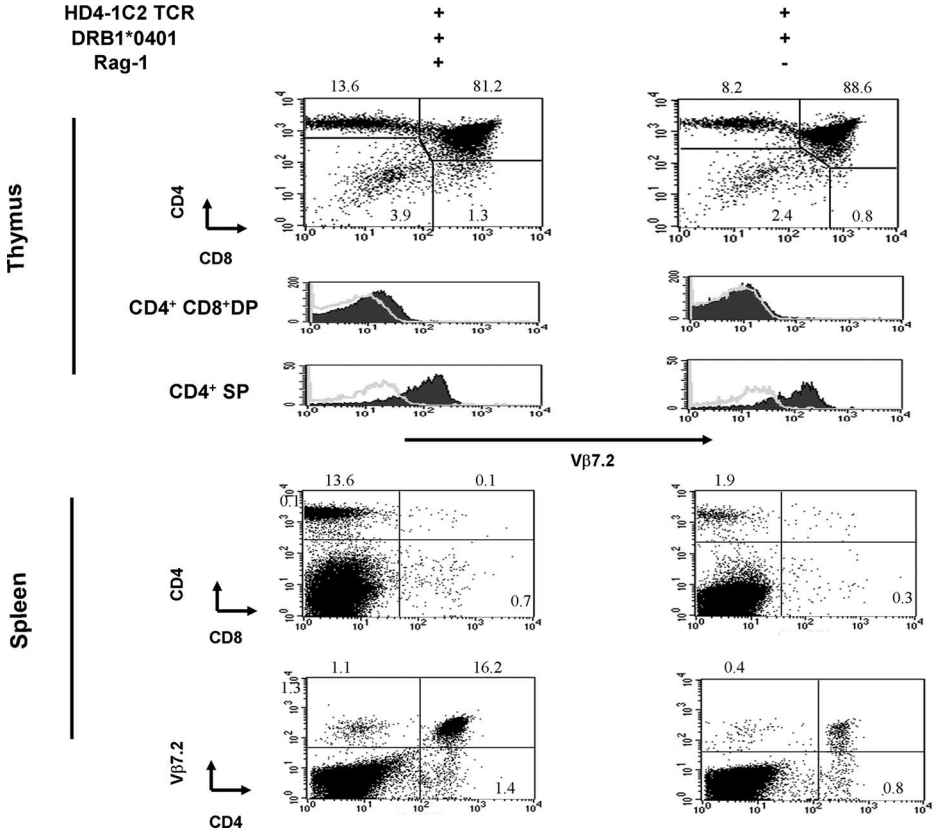

FIGURE 4. Thymic and splenic profile of Tg T cells in HD4-1C2 TCR/HLA-DRB1*0401 and HD4-1C2 TCR/HLA-DRB1*0401/RAG1 KO mice. The number of thymocytes and splenocytes in these mice is as follows: 1.5×10^8 thymocytes and 4.7×10^7 splenocytes (HD4-1C2 TCR/HLA-DRB1*0401 Tg mouse), and 3.3×10^8 thymocytes, 4.6×10^7 splenocytes (HD4-1C2 $TCR/RAG1^{-/-}$/HLA-DRB1*0401 Tg mouse). Three of each genotype were analyzed. FACS plots are representative of three independent experiments.

Antagonism assay

HLA-DRB1*0401/HLA-DRA1*0101-transfected bare lymphocyte syndrome cells were pulsed for 2 h with 1 μg/ml agonist peptide human $MBP_{111–129}$ (122R, LSRFSWGAEGQRPGFGYGG). Bare lymphocyte syndrome cells were simultaneously treated with mitomycin C to suppress their spontaneous proliferation and washed three times before they were used as APCs in the proliferation assay. The APCs (5×10^4/well) were seeded together with 2×10^4 cells from human TCC HD4-1C2 in duplicate wells. The candidate mouse antagonist peptide $MBP_{111–129}$ (122K, LSRF SWGAEGQKPGFGYGG) and the control peptide hemagglutinin $(HA)_{307–318}$ (PKYVKQNTLKLA) were immediately added to the wells at different concentrations (0, 0.01, 0.1, 1.0, and 10 μg/ml). As a control for the specificity of proliferation, APCs that had not been prepulsed with the agonist peptide were also included in the assay. For an antagonism assay of HD4-1C2 TCR Tg T cells, $CD11c^+$ dendritic cells were isolated using the MACS beads column (Miltenyi Biotec) and then pulsed with $MBP_{111–129}$ (10 μg/ml) at 37°C for 2 h following irradiation at 1200 rad. The pulsed APCs were cocultured with a HD4-1C2 TCR Tg $CD4^+$ T cell line in the presence of $MBP_{111–129}$ (122K) or $HA_{307–318}$. After 72 h of incubation, proliferation was measured by tritiated thymidine incorporation according to standard methods.

Immunohistology

Immunohistochemical analysis was performed on frozen sections that were fixed with acetone, rehydrated in PBS, and incubated with 0.3% H_2O_2 for 10 min to eliminate endogenous peroxidase. Sections were incubated with Abs against MTS10 (BD Pharmingen), CD205 (Serotec), HLA-DRα (BD Pharmingen), or a mouse IgG2a isotype control Ab (BD Pharmingen) for 1 h at room temperature, and biotinylated secondary Ab was applied after washing. Sections were treated with streptavidin-HRP and stained with diaminobenzidine chromogen and counterstained for analysis.

Cytotoxicity assay

Cytotoxicity was quantified by a time-resolved fluorometric assay. Murine B lymphoma cells coexpressing the HLA-DRB1*0401 and HLA-DRA1*0101 genes were used as target cells. The target cells were pulsed with peptides ($MBP_{111–129}$ or $HA_{307–319}$) at 10 μg/ml, for 1 h at 37°C, washed twice in CTL assay medium and then added to $CD4^+$ Tg and $CD8^+$ Tg effector cells. Supernatants were harvested from each well after 4 h of incubation and added to wells containing Europium solution in 0.3 M acetic acid (pH 4). The fluorescence of the Europium-TDA chelates formed was quantified in a time-resolved fluorometer (DELFIA 1234; Wallac) as previously described (21).

Statistical analyses

Statistical analyses were performed with the Student *t* test using GraphPad Prism software. A value of $p < 0.05$ was considered statistically significant.

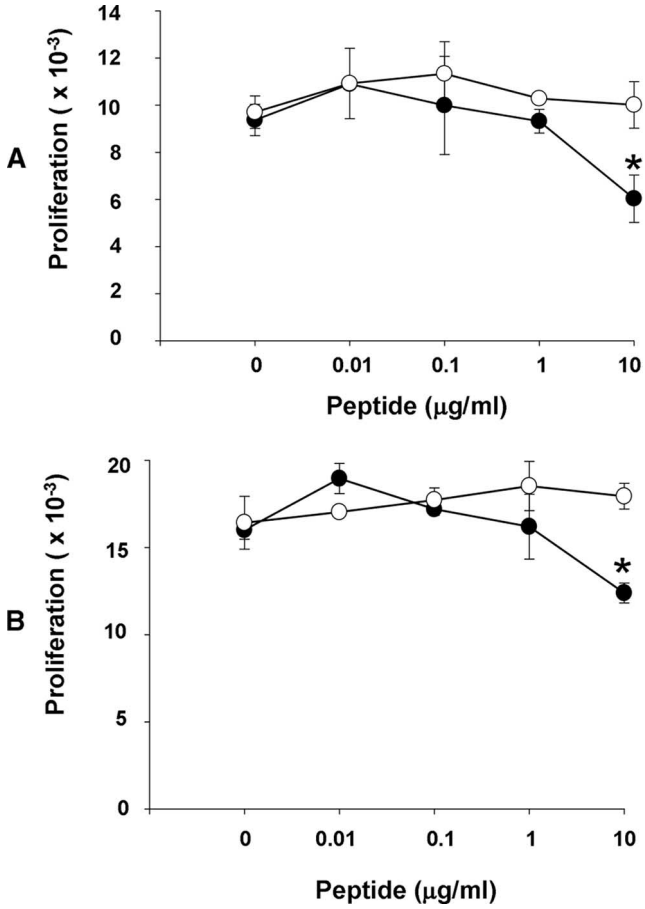

FIGURE 5. Murine $MBP_{111–129}$ (122K) exhibited antagonism for HD4-1C2 TCR Tg T cells. $CD11c^+$ APCs isolated from HLA-DRB1*0401 Tg mice were prepulsed with the agonist peptide, human $MBP_{111–129}$ (122R), and then $CD4^+$ T cells isolated from HD4-1C2 TCR Tg mice (*A*) or $CD4^+$ T cell line established from HD4-1C2 TCR/RAG1 KO Tg mice (*B*) were stimulated with the prepulsed $CD11c^+$ APCs in the presence of different concentrations of murine $MBP_{111–129}$ (122K) (-●-) or $HA_{307–318}$ (-○-). *, $p < 0.05$ compared with inhibition by $HA_{307–318}$. Data shown are representative of at least three independent experiments.

Results

*The HLA-DRB1*0401/HLA-DRA1*0101 transgene is expressed in the thymus in a tissue-specific manner*

Thymic expression of HLA-DR genes is crucial for development of humanized TCR Tg T cells in the thymus of mice. The tissue-specific expression of MHC class II transgenes is more tightly regulated in the thymic epithelial cells compared with peripheral lymphoid cells (22). Therefore, we first examined the expression of chimeric class II Tg genes in the thymic cortex and medulla by staining with anti-HLA-DR mAbs even though tissue-specific expression of the chimeric MHC class II genes had previously been observed in the spleen (6). The humanized MHC class II gene was expressed strongly on medullary epithelial cells and weakly on cortical epithelial cells (Fig. 1*B*), similar to the thymic expression of mouse MHC class II genes (23), indicating that the humanized MHC class II Tg gene is expressed in a tissue-specific manner in the mouse thymus.

Murine $MBP_{111–129}$ (122K) is an antagonist for the HD4-1C2 TCR and the development of HD4-1C2 TCR Tg T cells differs between the thymus and periphery

HLA-DRB1*0401-restricted human $MBP_{111–129}$ (122R)-specific TCC MS2-3C8 proliferated in response to the murine $MBP_{111–129}$ (122K) epitope (Fig. 2*A*). In contrast, HD4-1C2 TCC did not proliferate in response to $MBP_{111–129}$ (122K) (Fig. 2*B*). Because MBP at position 122 is a TCR contacting residue for HLA-DRB1*0401-restricted $MBP_{111–129}$-specific T cells (13), we examined whether murine $MBP_{111–129}$ (122K) possesses antagonist activity against the HD4-1C2 human TCC. As a control peptide, we used $HA_{307–319}$, which binds strongly to HLA-DRB1*0401. $MBP_{111–129}$ (122K) inhibited proliferation of HD4-1C2 TCC in response to human $MBP_{111–129}$ (122R) and the control peptide $HA_{307–319}$ did not inhibit proliferation (Fig. 2*C*). This result indicates that murine $MBP_{111–129}$ (122K) was an antagonist for the HD4-1C2 TCC.

Because MBP is expressed in the lymphoid organs (24), the influence of the $MBP_{111–129}$ antagonist on the development of MBP-TCR Tg T cells in the HD4-1C2 TCR Tg mice was examined. Thymic development of human TCR Vβ7.2$^+$ Tg T cells in both the $CD4^+$ and $CD8^+$ T cell subsets in the HD4-1C2 TCR Tg mice that do not express the HLA-DRB1*0401/HLA-DRA1*0401 genes was observed, whereas the results were skewed to the $CD4^+$ T cell subset when HLA-DRA1*0101/HLA-DRB1*0401 genes were expressed (Fig. 3) as expected. To eliminate the influence of endogenous mouse TCR on the development of HD4-1C2 TCR Tg

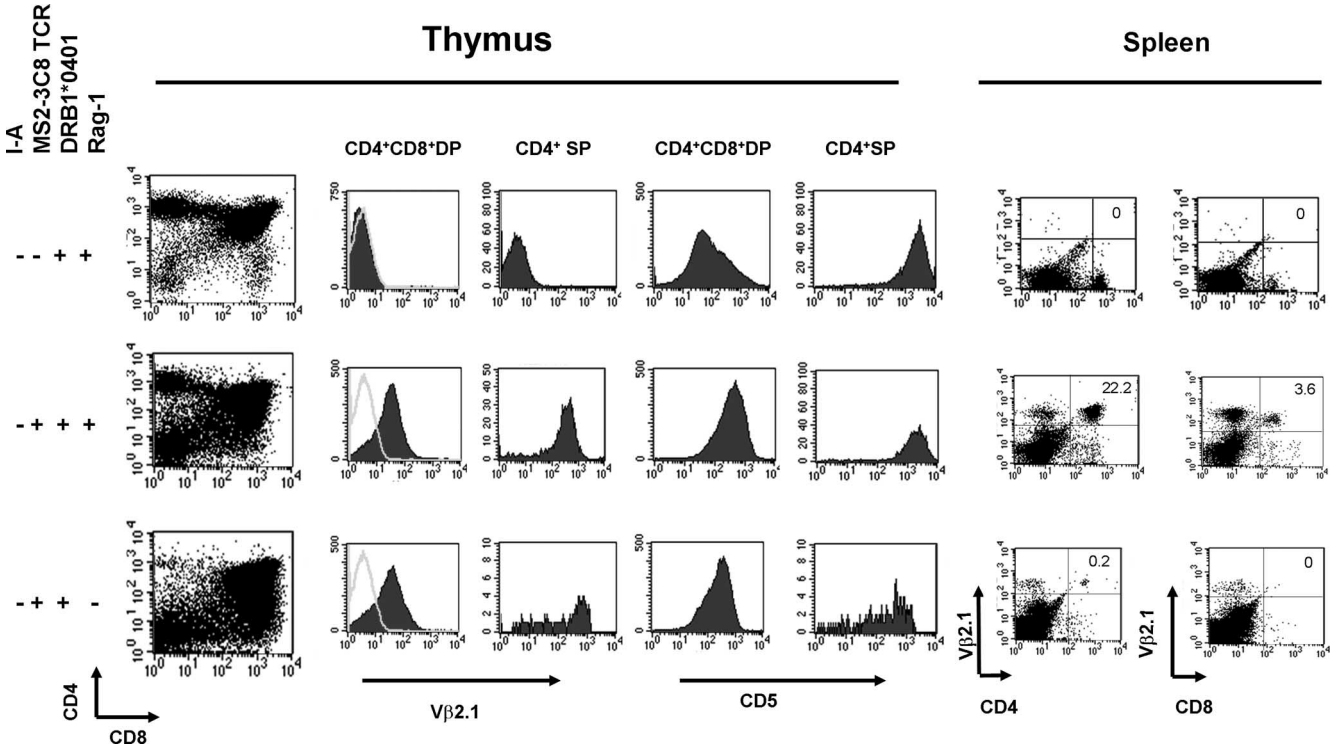

FIGURE 6. Tg T cells were negatively selected in MS2-3C8/HLA-DRB1*0401/RAG1 KO mice. CD5 expression was up-regulated on $CD4^+CD8^+$ DP thymocytes in MS2-3C8/HLA-DRB1*0401/RAG1 KO mice. Thymocytes from 6-wk-old mice were stained with anti-CD4, anti-CD8, anti-CD5, and anti-Vβ2.1 Abs. The number of thymocytes in these mice are as follows: 8.2×10^7 (HLA-DRB1*0401 Tg thymus), 4.6×10^6 (MS2-3C8 TCR/HLA-DRB1*0401 Tg thymus), and 6.8×10^6 (MS2-3C8 TCR/RAG1$^{-/-}$/HLA-DRB1*0401 thymus). The expression of Vβ2 and CD5 was gated for $CD4^+CD8^+$ DP and $CD4^+CD8^-$ ($CD4^+$ SP) thymocytes. Spleen cells were stained with anti-human TCR Vβ2.1, anti-CD4, and anti-CD8 Abs. FACS plots are representative of at least three independent experiments.

T cells, the HD4-1C2 TCR Tg mice were crossed with RAG1 KO mice. HD4-1C2 TCR Tg/RAG1 KO $CD4^+$ T cells were positively selected in the thymus. However, their development was dramatically reduced from 16.2% to 1.3% in the spleen (Fig. 4). These results indicate that HD4-1C2 TCR Tg/RAG1 KO T cells can be positively selected by $MBP_{111–129}$ (122K) associated with HLA-DRB1*0401 in the thymus, whereas their development was inefficient in the periphery.

We next examined whether $MBP_{111–129}$ (122K) was also an antagonist for HD4-1C2 TCR Tg T cells. As shown in Fig. 5, $MBP_{111–129}$ (122K) inhibited the proliferation of the HD4-1C2 TCR Tg and HD4-1C2 TCR Tg/RAG1 KO T cells in response to $MBP_{111–129}$ (122R), indicating that endogenous $MBP_{111–129}$ (122K) epitope acted as an antagonist for HD4-1C2 TCR Tg T cells.

MS2-3C8 TCR Tg T cells undergo negative selection in the thymus

Thus far, we have shown that murine $MBP_{111–129}$ (122K) is an agonist for MS2-3C8 TCR Tg T cells (Fig. 2*A*). Therefore, we next examined thymic and peripheral development of MS2-3C8 TCR Tg T cells. In accordance with our previous report (12), development of MS2-3C8 TCR Tg $CD4^+$ T cells was reduced in the thymus of MS2-3C8 TCR Tg/RAG1 KO mice, although the cells were positively selected in the MS2-3C8 TCR Tg/RAG1 wild-type mice (Fig. 6). This result suggests that expression of endogenous mouse TCR can facilitate the development of MS2-3C8 TCR Tg T cells in the thymus. The inefficient thymic development of Tg T cells in the MS2-3C8 TCR Tg/RAG1 KO mice was possibly due to negative selection or inefficient positive selection. To address this question, we examined whether $CD4^+CD8^+$ double positive (DP) thymocytes can be signaled in the thymus for positive selection. Because expression of CD5 on $CD4^+CD8^+$ thymocytes is up-regulated when they recognize thymic Ags (25, 26), we examined CD5 expression on $CD4^+CD8^+$ DP Tg T cells to assess whether the DP Tg T cells could recognize an Ag in the thymus of MS2-3C8 TCR Tg/RAG1 KO mice. In HLA-DRB1*0401 Tg mice, CD5 expression was up-regulated in a certain population of

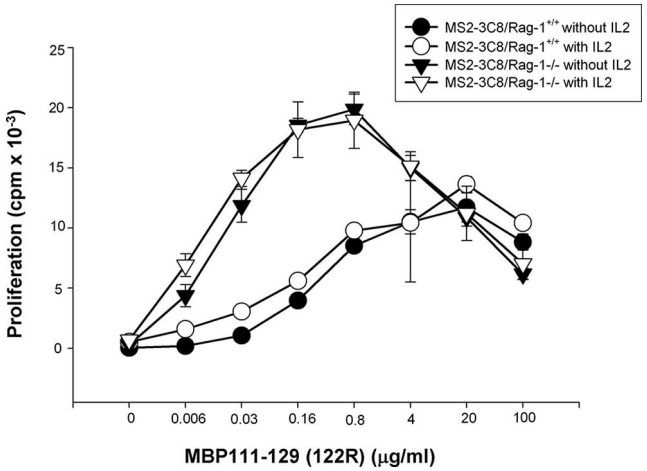

FIGURE 7. MS2-3C8 TCR Tg T cells that escape negative selection are not rendered anergic. Spleen cells isolated from the MS2-3C8 TCR/HLA-DRB1*0401/RAG1 wild-type mice and MS2-3C8 TCR/HLA-DRB1*0401/RAG1 KO mice were cultured with $MBP_{111–129}$ (122R) (10 μg/ml) in the absence or presence of IL-2 (10 ng/ml) for 3 days. The proliferation of cultured cells was analyzed by incorporation of [^{3}H]thymidine. Data shown are representative of two independent experiments.

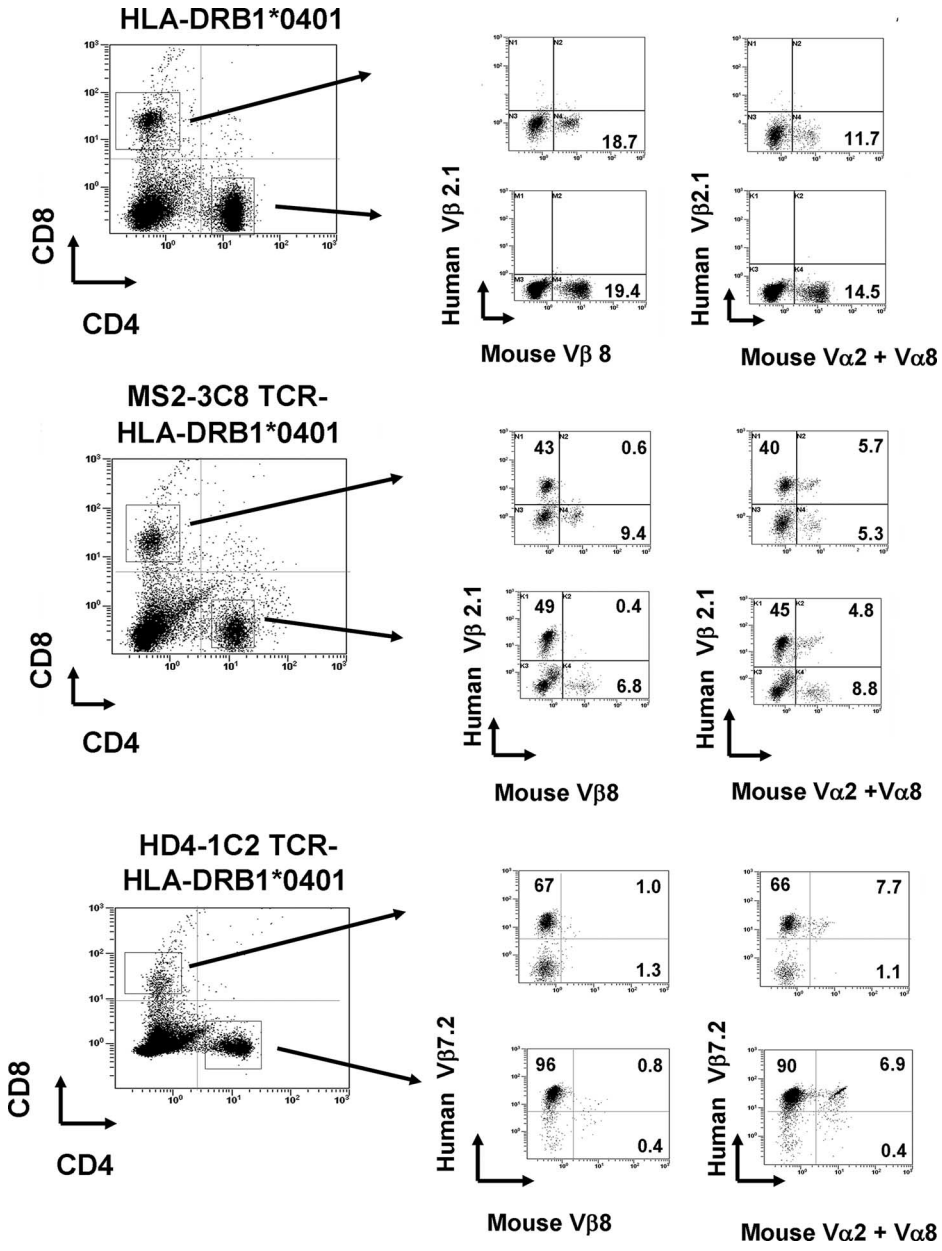

FIGURE 8. Allelic inclusion of mouse TCR-α genes in the humanized TCR Tg mice. Spleen cells isolated from HLA-DRB1*0401 Tg mice, MS2-3C8 TCR/HLA-DRB1*0401 Tg mice, and HD4-1C2 TCR/HLA-DRB1*0401 Tg mice were analyzed by four-color staining using anti-CD4 Alexa Fluor 647, anti-CD8 PE-Cy7, anti-human TCR Vβ2.1 PE, anti-mouse TCR Vα2-FITC, anti-mouse TCR Vα8-FITC, and anti-mouse TCR Vβ8-FITC. The CD4$^+$ and CD8$^+$ T cell population was gated for analysis of the expression of human TCR Vβ2.1 and mouse TCR Vβ8 or mouse TCR Vα2 and TCR Vα8. FACS plots are representative of at least three independent experiments.

DP T cells (Fig. 6, *upper plots*) as observed in conventional mice (22). In accordance with the positive selection of Tg T cells in the MS2-3C8 TCR Tg mice, CD5 expression on DP Tg T cells was up-regulated (Fig. 6, *middle plots*). Similarly, CD5 expression on the DP T cells was up-regulated in the MS2-3C8 TCR Tg/RAG1 KO mice (Fig. 6, *lower plots*). These data suggest that DP thymocytes were signaled in vivo by intrathymic ligands, indicating that the inefficient development of MBP-TCR Tg CD4$^+$ T cells in the MS2-3C8 TCR Tg/RAG1 KO mice is most likely due to negative selection. This thymic-negative selection was suppressed by expression of endogenous TCR.

MS2-3C8 TCR Tg T cells that survive thymic-negative selection are not rendered anergic

Although CD4$^+$ T cells were deleted in the thymus of the MS2-3C8 TCR Tg/RAG1 KO mice, the thymic deletion was incomplete. MS2-3C8 TCR Tg CD4$^+$ T cells still developed in the spleen of the MS2-3C8 TCR Tg/RAG1 KO mice (Fig. 6). We examined whether the Tg T cells that escape from thymic-negative selection undergo anergy (an unresponsive stage). Spleen cells isolated from the MS2-3C8 TCR Tg/RAG1 KO mice were cultured with $MBP_{111–129}$ (122R). As a control, spleen cells isolated from the MS2-3C8 TCR Tg mice were used for this proliferation assay. Because clonal anergy can be reversed by addition of IL-2 (27, 28), these spleen cells were cultured with $MBP_{111–129}$ in the presence or absence of IL-2. We observed that spleen cells isolated from the MS2-3C8 TCR Tg/RAG1 KO mice proliferated in response to $MBP_{111–129}$ without exogenous IL-2 and their response was more efficient than those isolated from the MS2-3C8 TCR Tg mice (Fig. 7). These results indicate that MS2-3C8 TCR Tg/RAG1 KO T cells that escaped the thymic-negative selection were not rendered anergic.

Expression of MS2-3C8 TCR transgenes induces allelic inclusion and exclusion of endogenous TCR α- and β-chains, respectively

Because allelic exclusion is very strict at the TCR-β locus, the majority of T cells express a single TCR β-chain. In contrast, the

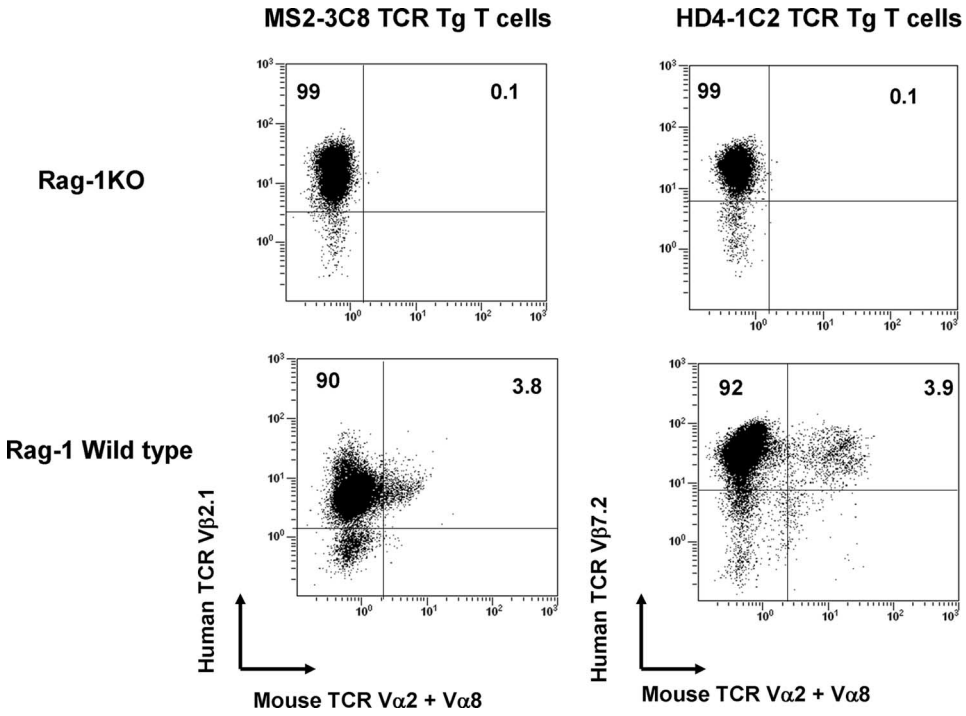

FIGURE 9. Expression of endogenous mouse TCR-α in the $MBP_{111–129}$-specific human TCR Tg T cells. Spleen cells isolated from the MS2-3C8 TCR/HLA-DRB1*0401,HD4-1C2TCR/HLA-DRB1*0401, MS2-3C8 TCR/HLA-DRB1*0401/RAG1 KO, and HD4-1C2 TCR/HLA-DRB1*0401/RAG1 KO mice were cultured with $MBP_{111–129}$ (10 μg/ml) for 4 days and further cultured with IL-2 (10 ng/ml) for 10 days. The growing cells were stained with anti-human TCR Vβ2.1 and anti-mouse TCR Vα2 and Vα8 Abs. Data shown are representative of two independent experiments.

TCR-α locus allelic exclusion is extremely inefficient. Thus, dual TCR-α chains are expressed on mouse and human T cells (14, 15). We examined how the expression of human MS2-3C8 TCR transgenes affects the expression of endogenous mouse TCR α- and β-chains. Because TCR Vα2, Vα8, and Vβ8 were the major T cell subsets in HLA-DRB1*0401 Tg mice (Fig. 8, *upper plots*) (6), we examined the influence of MS2-3C8 TCR transgene expression on these T cell subsets. As shown in Fig. 8 (*middle plots*), expression of mouse TCR Vβ8 was exclusively inhibited in the MS2-3C8 TCR Tg T cells (human TCR Vβ2.1-positive cells), although it was detected in non-TCR Tg T cells (human TCR Vβ2.1-negative cells). In contrast, allelic exclusion was not observed in TCR α-chain as expression of mouse TCR Vα2 and Vα8 were detected in human Vβ2.1-positive cells in the MS2-3C8 TCR Tg mice (Fig. 8, *middle plots*). Similar results were observed in HD4-1C2 TCR Tg mice (Fig. 8, *lower plots*). Expression of mouse TCR Vα2 and Vα8 were detected in human Vβ7.2-positive cells. We next generated $MBP_{111–129}$-specific T cell lines from the MS2-3C8 TCR Tg, HD4-1C2 TCR Tg, MS2-3C8 TCR Tg/RAG1 KO, and HD4-1C2 TCR Tg/RAG1 KO mice by stimulation with $MBP_{111–129}$ (122R) and then examined the expression of endogenous mouse TCR-α on these Tg T cell lines. As shown in Fig. 9, expression of mouse TCR Vα2 and Vα8 were detected on the MS2-3C8 TCR and HD4-1C2 TCR Tg T cell line but not in the MS2-3C8 TCR Tg/RAG1 KO and HD4-1C2 TCR/RAG1 KO T cell lines. These data suggest that, similar to the mouse TCR transgene (29), expression of human TCR transgenes induces allelic inclusion and exclusion of the TCR-α and -β locus, respectively. Thus, these humanized TCR Tg T cells express dual TCR that is composed of human TCR α/β heterodimer and mouse TCR-α/human TCR-β heterodimer.

*Dual TCR expression promotes the development of MHC-mismatched $CD8^+$ T cells in the periphery of MS2-3C8 TCR/HLA-DRB1*0401 Tg mice*

We have shown that MS2-3C8 TCR Tg $CD8^+$ T cells still develop in the MS2-3C8 TCR/HLA-DRB1*0401Tg mice (3.6% of whole spleen cells), although the population was skewed to the $CD4^+$ T cell subset (Fig. 6). Therefore, we examined whether MS2-3C8 TCR Tg $CD8^+$ T cells can proliferate in response to $MBP_{111–129}$. As a control, MS2-3C8 TCR Tg $CD4^+$ T cells were used. In this proliferation assay, both naive MS2-3C8 TCR Tg $CD4^+$ and Tg $CD8^+$ T cells proliferated in response to $MBP_{111–129}$ (Fig. 10*A*).

We next examined whether the $CD8^+$ Tg T cells possess cytotoxic activity. B lymphoma cells expressing the HLA-DRB1*0401/HLA-DRA1*0101 transgenes were pulsed with $MBP_{111–129}$ (122R) or $HA_{307–319}$ and these peptide-pulsed cells were used as target cells. The MS2-3C8 TCR Tg $CD8^+$ T cells killed the B lymphoma cells pulsed with $MBP_{111–129}$ (122R), but they did not kill the B lymphoma cells pulsed with $HA_{307–319}$ (Fig. 10*B*). These results indicate that MS2-3C8 TCR Tg $CD8^+$ T cells possessed cytotoxic activity and this activity was mediated through recognition of $MBP_{111–129}$ (122R) associated with HLA-DRB1*0401.

To examine the restriction element of the MS2-3C8 TCR Tg $CD8^+$ T cells, $CD4^+$ and $CD8^+$ T cells isolated from the MS2-3C8 TCR/HLA-DRB1*0401 Tg mice were cultured with $MBP_{111–129}$ (122R), using spleen cells isolated from the HLA-DRB1*0401 or HLA-DRB1*0404 Tg mice as APCs. HLA-DRB1*0404 is another subtype of the HLA-DR4 allele and its Ag-binding motif is different from that of HLA-DRB1*0401 molecules (30). Both MS2-3C8 TCR Tg $CD4^+$ and Tg $CD8^+$ T cells proliferated in response to $MBP_{111–129}$ (122R) when HLA-DRB1*0401 Tg spleen cells were used as APCs, but neither proliferated when HLA-DRB1*0404 Tg spleen cells were used (Fig. 10*C*). Thus, both MS2-3C8 TCR Tg $CD4^+$ and Tg $CD8^+$ T cells proliferated in response to $MBP_{111–129}$ (122R) in a HLA-DRB1*0401-restricted manner. These data indicate that MHC-mismatched $CD8^+$ T cells developed in the MS2-3C8 TCR Tg mice.

We examined whether the HLA-DRB1*0401 transgene was required for thymic-positive selection of MS2-3C8 TCR Tg $CD8^+$ T cells. In the thymus and spleen of the MS2-3C8 TCR Tg mice that do not express the HLA-DRB1*0401 gene, we observed that $MBP_{111–129}$ TCR Tg $CD8^+$ T cells still develop but $CD4^+$ T cells do not (Fig. 11). This result indicates that HLA-DRB1*0401 was not required for thymic selection of the MS2-3C8 TCR Tg $CD8^+$ T cells. To eliminate the influence of endogenous TCR expression

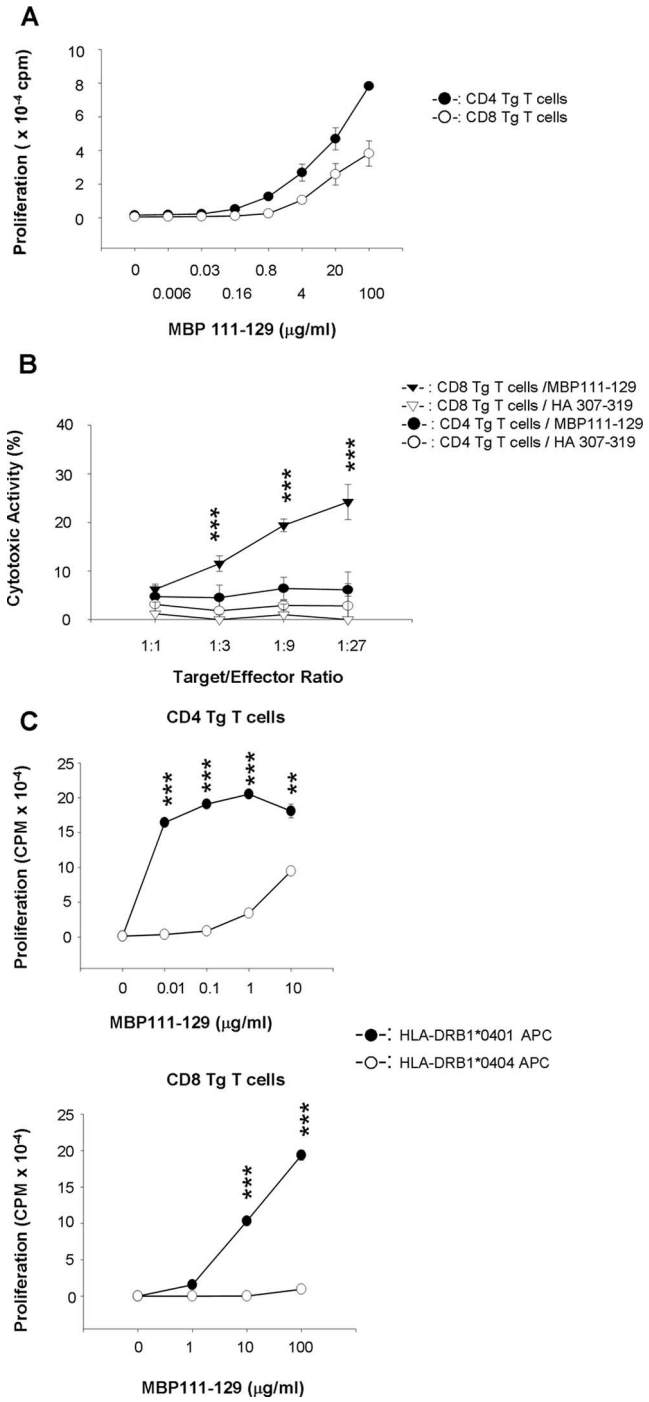

FIGURE 10. Development of HLA-DRB1*0401-restricted $CD8^+$ Tg T cells in MS2-3C8 TCR Tg mice. *A*, $CD8^+$ Tg T cells proliferate in response to $MBP_{111–129}$. $CD8^+$ and $CD4^+$ T spleen cells isolated from the MS2-3C8 TCR/HLA-DRB1*0401 Tg mice were cultured with $MBP_{111–129}$ (122R) and irradiated HLA-DRB1*0401 Tg spleen cells. *B*, Cytotoxic activity of MS2-3C8 TCR Tg $CD4^+$ and $CD8^+$ T cells. Murine B lymphoma cells coexpressing the HLA-DRB1*0401 and HLA-DRA1*0101 genes were pulsed with $MBP_{111–129}$ (122R) or $HA_{307–319}$, and the cytotoxic activity of the MS2-3C8 TCR Tg $CD4^+$ and $CD8^+$ T cells against these pulsed cells was measured. ***, $p < 0.001$ compared with CD8 Tg T cells/$HA_{307–319}$. *C*, The restriction element of $CD4^+$ and $CD8^+$ Tg T cells is HLA-DRB1*0401. $CD4^+$ and $CD8^+$ Tg T cell lines established from MS2-3C8 TCR/HLA-DRB1*0401 Tg mice were stimulated with $MBP_{111–129}$ (122R) by using HLA-DRB1*0401 (-●-) and HLA-DRB1*0404 (-○-) Tg spleen cells as APCs. **, $p < 0.01$ and ***, $p < 0.001$ compared with HLA-DRB1*0404 APC. Data shown are representative of at least three independent experiments.

on the development of MS2-3C8 TCR Tg $CD8^+$ T cells, we examined the development of $CD8^+$ Tg T cells in the MS2-3C8 TCR/HLA-DRB1*0401/RAG1 KO mice. Although we observed the development of $CD8^+$ Tg T cells in the thymus, their development was not detectable in the periphery (Fig. 6). Costaining with anti-CD4 and anti-CD8 mAbs indicated that CD4-negative Tg T cells developing in the MS2-3C8 TCR/HLA-DRB1*0401/RAG1 KO mice was $CD4^-CD8^-$ Tg T cells (data not shown). This result indicates that dual TCR expression was crucial for the development of MS2-3C8 TCR Tg $CD8^+$ T cells in the periphery.

Discussion

Development of $CD4^+$ T cells is determined by the interaction of their TCRs with the cognate Ags associated with HLA-DR expressed in the thymus and peripheral lymphoid organs. MBP is a putative autoantigen involved in the development of MS. Golli-MBP and classical MBP are expressed in the lymphoid organs including the thymus, spleen, and lymph nodes in addition to the CNS (24, 31). Thus, MBP-specific T cells could be deleted in the thymus or rendered anergic in the periphery. Nevertheless, MBP-specific T cells are detected in the periphery in humans and animals. To investigate the mechanism of development of MBP-specific T cells, MBP-TCR Tg mice have been generated (32, 33). In B10PL mice, low avidity TCR Tg T cells for MBP Ac1–12 associated with $I\text{-}A^u$ escape thymic-negative selection and are not rendered anergic in the periphery (33). In contrast, high avidity Tg T cells specific for $MBP_{121–151}/I\text{-}A^u$ were partially deleted in the thymus and rendered less responsive in the periphery (34). These results suggest that the development of MBP-specific T cells is dependent on the avidity of TCR to the MBP Ag associated with MHC class II. In humans expressing the HLA-DRB1*0401 haplotype, $MBP_{111–129}$ is an immunodominant epitope and its binding affinity to HLA-DRB1*0401 is weak (13, 35). To investigate the development of HLA-DRB1*0401-restricted $MBP_{111–129}$-specific human TCR Tg T cells, we generated humanized Tg mice coexpressing HLA-DRB1*0401 and $MBP_{111–129}$-specific human TCR genes. Although a previously described humanized TCR animal model has been made that expresses human CD4 and whole human MHC class II genes (23), this animal model cannot be used for the study of thymic selection of human TCR Tg T cells due to the expression of the human CD4 transgene. We crossed human-mouse chimeric TCR Tg mice with human-mouse chimeric MHC class II Tg mice. In the resultant Tg mouse, thymic and peripheral development of $CD4^+$ T cells is determined by the interaction of human TCRs with the Ag associated with HLA-DR molecules because mouse CD4 can associate with the chimeric MHC class II. Thus, these humanized MHC class II/TCR mice can allow us to investigate thymic and peripheral development of human TCR Tg T cells.

We observed a skewing of Tg T cells into the $CD4^+$ T cell subset when HD4-1C2 TCR Tg mice were crossed with HLA-DRB1*0401 Tg mice. To examine the influence of endogenous mouse TCRs on the development of Tg T cells, we crossed these Tg mice to RAG1 KO mice. We found that $MBP_{111–129}$ (122K) associated with HLA-DRB1*0401 can facilitate thymic-positive selection of HD4-1C2 TCR Tg/RAG1 KO T cells, but it cannot facilitate their peripheral development. These data suggest that peripheral development of antagonist-specific T cells is stricter than their thymic development. Antagonist signal may lead to failure in homeostatic proliferation or survival of the HD4-1C2 TCR Tg T cells in the periphery as suggested previously (36). However, we show that expression of dual TCRs can facilitate the development of HD4-1C2 TCR Tg T cells in the periphery. Because interaction of TCR with cognate Ags associated with MHC class II facilitates

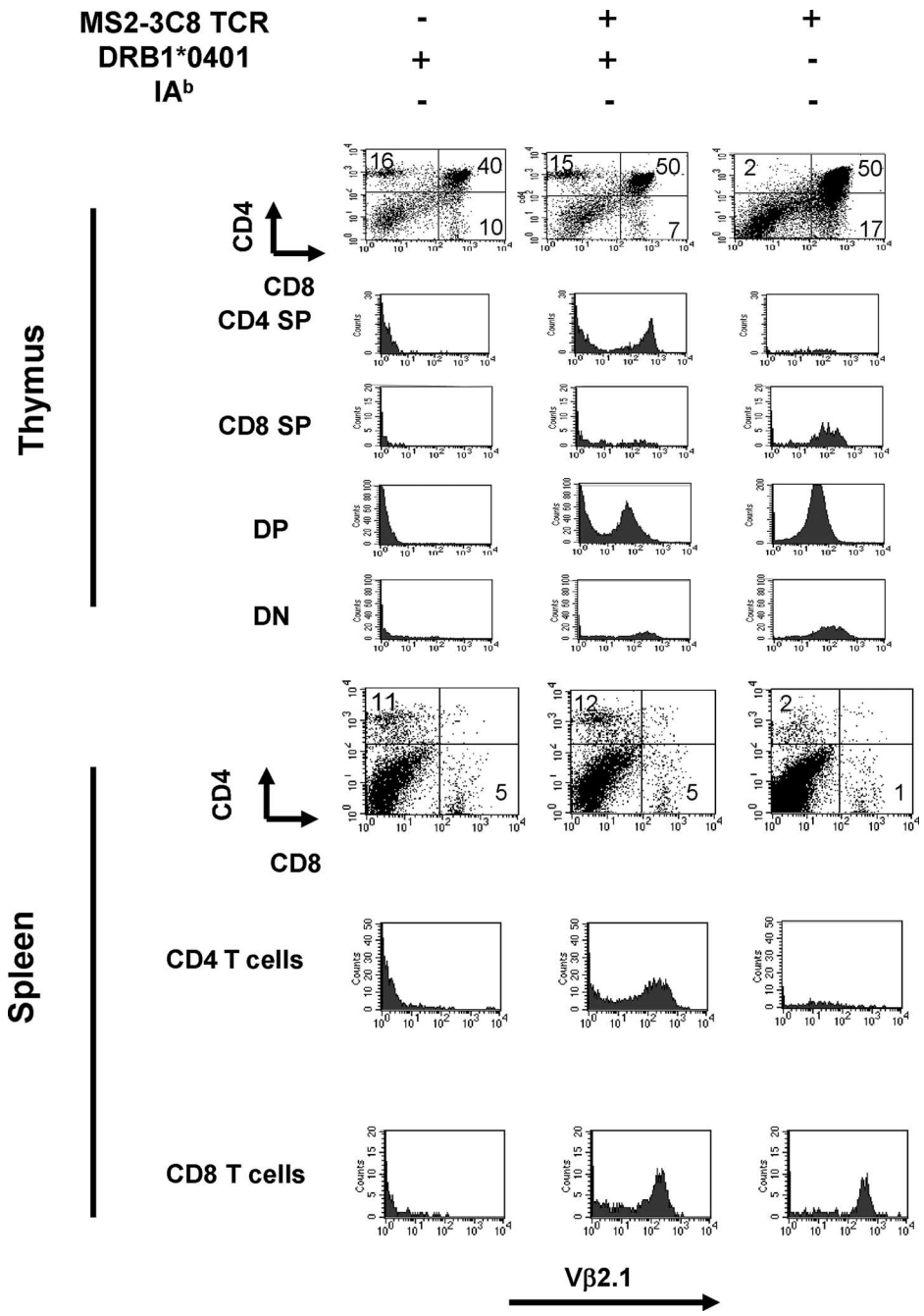

FIGURE 11. The expression of HLA-DRB1*0401 is not necessary for the development of MS2-3C8 TCR Tg $CD8^+$ T cells. Thymocytes and splenocytes were isolated from HLA-DRB1*0401/I-$A^{b-/-}$ mice,MS2-3C8TCR/HLA-DRB1*0401/I-$A^{b-/-}$ mice, and MS2-3C8 TCR/HLA-DRB1*$0401^{-/-}$/I-$A^{b-/-}$ mice and stained with anti-CD4, anti-CD8, and anti-Vβ2.1 Abs. Two to three mice of each genotype were analyzed. FACS plots are representative of two independent experiments.

development of $CD4^+$ T cells in the periphery (37–41), dual TCRs expressed on the HD4-1C2 TCR Tg T cells may interact with other peripheral self-Ags and promote their development.

We also investigated thymic and peripheral development of $MBP_{111–129}$ agonist-specific human TCR Tg T cells. Despite positive thymic selection of MS2-3C8 TCR Tg/RAG1 wild-type T cells, MS2-3C8 TCR Tg/RAG1 KO T cells are exclusively deleted in the thymus. This thymic-negative selection may lead to inefficient development of MS2-3C8 TCR Tg/RAG1 KO T cells in the periphery. Because HD4-1C2 TCR Tg/RAG1 KO T cells do not undergo thymic-negative selection, signal through MS2-3C8 TCR leads to the negative selection. However, endogenous mouse TCRs prevents negative selection and facilitates the peripheral development of MS2-3C8 TCR Tg T cells. The association of endogenous TCR α-chains with human Vβ2.1 Tg TCR could decrease the density of Tg TCR-α/β heterodimer and this reduction may allow MS2-3C8 Tg T cells to escape thymic deletion. A similar observation was reported in serum protein C5 Tg mice (16, 26). Interestingly, MS2-3C8 TCR Tg/RAG1 KO T cells that escape thymic-negative selection are not rendered anergic, even though autoreactive T cells that escape massive thymic-negative selection tend to be rendered anergic (42, 43). Although Golli-MBP is expressed in the thymus and peripheral lymphoid tissues, it does not encode $MBP_{111–129}$ (44). However, classical MBP, which encodes $MBP_{111–129}$, is expressed in the lymphoid tissues as well as in the CNS (24). Thus, classical MBP expressed in the thymus or released from the CNS could be involved in the thymic-negative selection of MS2-3C8 TCR Tg T cells. However, expression of classical MBP in the periphery may not be sufficient to induce anergy in the $MBP_{111–129}$-specific T cells.

Interestingly, we detected the development of HLA-DRB1*0401-restricted $MBP_{111–129}$ TCR Tg $CD8^+$ T cells in the MS2-3C8 TCR Tg mice. We therefore examined the involvement of HLA-DRB1*0401 in the development of the $CD8^+$ Tg T cells in the MS2-3C8 TCR Tg mice that do not express HLA-DRB1*0401 transgene. Although $CD4^+$ Tg T cells did not develop in the MS2-3C8 TCR Tg mice that do not express HLA-DRB1*0401, $CD8^+$ Tg T cells still developed in the thymus and periphery. These data indicate that HLA-DRB1*0401-restricted $CD8^+$ Tg T cells were not positively selected by HLA-DRB1*0401 and another MHC restriction element is involved in the selection of MHC-mismatched $CD8^+$ T cells. A similar phenomenon was observed previously in certain MHC class II-restricted $CD8^+$ Tg T cells (27–29). Importantly, we could not detect $CD8^+$ Tg T cells in the periphery of the MS2-3C8 TCR Tg/RAG1 KO mice, although we detected $CD4^+$ and $CD4^-CD8^-$ Tg T cells. Thus, dual TCR expression can facilitate the development of MHC class II-restricted MS2-3C8 $CD8^+$ Tg T cells in the periphery. We also detected MBP-TCR Tg $CD8^+$ T cells in HLA-DR2a-restricted $MBP_{83–99}$-specific human TCR (3A6) Tg mice (our unpublished observation). Interestingly, 3A6 TCR Tg $CD8^+$ T cells do not proliferate in response to $MBP_{83–99}$ (our unpublished observation). Thus, the proliferative capacity of MHC class II-restricted $CD8^+$ T cells may be dependent on their antigenic epitope and restriction elements. Although MHC class II-restricted cytotoxic $CD8^+$ T cells develop in normal mice (45, 46), their immunologic role is still unknown. We are currently investigating a regulatory activity of the MS2-3C8 TCR Tg $CD8^+$ T cells.

Because allelic exclusion is incomplete in TCR-α chains (29), two TCR α-chains are expressed together with single TCR β-chain and 10–25% of T cells express dual TCRs in humans and mice (14, 47). This allelic inclusion of TCR-α can be induced by interaction of primary TCR with thymic and peripheral Ags followed by TCR internalization and activation of RAG1 and RAG2 (18). Similarly, expression of rearranged human Tg TCR-β gene induces allelic exclusion of mouse TCR-β gene, whereas the rearranged human Tg TCR-α gene cannot affect allelic exclusion of mouse TCR-α gene. These data suggest that allelic exclusion by TCR-β gene can be inducible by xenotypic TCR-β gene.

To our knowledge, this analysis is the first precise study of thymic and peripheral development of MBP-specific human TCR Tg T cells in humanized TCR/MHC class II Tg mice. Our data suggest that development of MBP antagonist-specific $CD4^+$ T cells and MBP-specific MHC-mismatched $CD8^+$ T cells are inefficient in the periphery, but dual TCR expression can facilitate their development.

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Disclosures

The authors have no financial conflict of interest.

References

1. Stastny, P. 1978. Association of the B-cell alloantigen DRw4 with rheumatoid arthritis. *N. Engl. J. Med.* 298: 869–871.
2. Grasso, M. G., F. Cutrupi, S. Bernardi, S. Trabace, C. Pozzilli, S. Cappellacci, and C. Fieschi. 1992. HLA determinants in familial multiple sclerosis. *Neuroepidemiology* 11: 85–89.
3. Marrosu, M. G., F. Muntoni, M. R. Murru, G. Spinicci, M. P. Pischedda, F. Goddi, P. Cossu, and M. Pirastu. 1988. Sardinian multiple sclerosis is associated with HLA-DR4: a serologic and molecular analysis. *Neurology* 38: 1749–1753.
4. Kurdi, A., I. Ayesh, A. Abdallat, and U. Maayta. 1977. Different B lymphocyte alloantigens associated with multiple sclerosis in Arabs and North Europeans. *Lancet* 1: 1123–1125.
5. Dorfman, J. R., and R. N. Germain. 2002. MHC-dependent survival of naive T cells? A complicated answer to a simple question. *Microbes Infect.* 4: 547–554.
6. Ito, K., H. J. Bian, M. Molina, J. Han, J. Magram, E. Saar, C. Belunis, D. R. Bolin, R. Arceo, R. Campbell, et al. 1996. HLA-DR4-IE chimeric class II transgenic, murine class II-deficient mice are susceptible to experimental allergic encephalomyelitis. *J. Exp. Med.* 183: 2635–2644.
7. Touloukian, C. E., W. W. Leitner, S. L. Topalian, Y. F. Li, P. F. Robbins, S. A. Rosenberg, and N. P. Restifo. 2000. Identification of a MHC class II-restricted human gp100 epitope using DR4-IE transgenic mice. *J. Immunol.* 164: 3535–3542.
8. Meyer, A. L., C. Trollmo, F. Crawford, P. Marrack, A. C. Steere, B. T. Huber, J. Kappler, and D. A. Hafler. 2000. Direct enumeration of Borrelia-reactive CD4 T cells ex vivo by using MHC class II tetramers. *Proc. Natl. Acad. Sci. USA* 97: 11433–11438.
9. Forsthuber, T. G., C. L. Shive, W. Wienhold, K. de Graaf, E. G. Spack, R. Sublett, A. Melms, J. Kort, M. K. Racke, and R. Weissert. 2001. T cell epitopes of human myelin oligodendrocyte glycoprotein identified in HLA-DR4 (DRB1*0401) transgenic mice are encephalitogenic and are presented by human B cells. *J. Immunol.* 167: 7119–7125.
10. Wang, D., J. A. Hill, A. M. Jevnikar, E. Cairns, and D. A. Bell. 2002. Induction of transient arthritis by the adoptive transfer of a collagen II specific Th1 clone to HLA-DR4 (B1*0401) transgenic mice. *J. Autoimmun.* 19: 37–43.
11. Tarcha, E. J., V. Basrur, C. Y. Hung, M. J. Gardner, and G. T. Cole. 2006. Multivalent recombinant protein vaccine against coccidioidomycosis. *Infect. Immun.* 74: 5802–5813.
12. Quandt, J. A., M. Baig, K. Yao, K. Kawamura, J. Huh, S. K. Ludwin, H. J. Bian, M. Bryant, L. Quigley, Z. A. Nagy, et al. 2004. Unique clinical and pathological features in HLA-DRB1*0401-restricted MBP 111–129-specific humanized TCR transgenic mice. *J. Exp. Med.* 200: 223–234.
13. Muraro, P. A., M. Vergelli, M. Kalbus, D. E. Banks, J. W. Nagle, L. R. Tranquill, G. T. Nepom, W. E. Biddison, H. F. McFarland, and R. Martin. 1997. Immunodominance of a low-affinity major histocompatibility complex-binding myelin basic protein epitope (residues 111–129) in HLA-DR4 (B1*0401) subjects is associated with a restricted T cell receptor repertoire. *J. Clin. Invest.* 100: 339–349.
14. Padovan, E., G. Casorati, P. Dellabona, S. Meyer, M. Brockhaus, and A. Lanzavecchia. 1993. Expression of two T cell receptor α chains: dual receptor T cells. *Science* 262: 422–424.
15. Casanova, J. L., P. Romero, C. Widmann, P. Kourilsky, and J. L. Maryanski. 1991. T cell receptor genes in a series of class I major histocompatibility complex-restricted cytotoxic T lymphocyte clones specific for a *Plasmodium berghei* nonapeptide: implications for T cell allelic exclusion and antigen-specific repertoire. *J. Exp. Med.* 174: 1371–1383.
16. Zal, T., S. Weiss, A. Mellor, and B. Stockinger. 1996. Expression of a second receptor rescues self-specific T cells from thymic deletion and allows activation of autoreactive effector function. *Proc. Natl. Acad. Sci. USA* 93: 9102–9107.
17. Sarukhan, A., C. Garcia, A. Lanoue, and H. von Boehmer. 1998. Allelic inclusion of T cell receptor α genes poses an autoimmune hazard due to low-level expression of autospecific receptors. *Immunity* 8: 563–570.
18. McGargill, M. A., J. M. Derbinski, and K. A. Hogquist. 2000. Receptor editing in developing T cells. *Nat. Immunol.* 1: 336–341.
19. He, X., C. A. Janeway, Jr., M. Levine, E. Robinson, P. Preston-Hurlburt, C. Viret, and K. Bottomly. 2002. Dual receptor T cells extend the immune repertoire for foreign antigens. *Nat. Immunol.* 3: 127–134.
20. Gebe, J. A., K. A. Unrath, B. A. Falk, K. Ito, L. Wen, T. L. Daniels, A. Lernmark, and G. T. Nepom. 2006. Age-dependent loss of tolerance to an immunodominant epitope of glutamic acid decarboxylase in diabetic-prone RIP-B7/DR4 mice. *Clin. Immunol.* 121: 294–304.
21. Ding, Y. H., K. J. Smith, D. N. Garboczi, U. Utz, W. E. Biddison, and D. C. Wiley. 1998. Two human T cell receptors bind in a similar diagonal mode to the HLA-A2/Tax peptide complex using different TCR amino acids. *Immunity* 8: 403–411.
22. Giacomini, P., A. Ciucci, M. R. Nicotra, C. Nastruzzi, G. Feriotto, E. Appella, R. Gambari, L. Pozzi, and P. G. Natali. 1991. Tissue-specific expression of the HLA-DRA gene in transgenic mice. *Immunogenetics* 34: 385–391.
23. Fehling, H. J., W. van Ewijk, J. L. Pasquali, C. Waltzinger, M. Le Meur, P. Gerlinger, C. Benoist, and D. Mathis. 1990. Functional consequences of overexpressed Ia antigens in AKα/AKβ transgenic mice. *J. Immunol.* 144: 2865–2874.
24. Liu, H., A. J. MacKenzie-Graham, K. Palaszynski, S. Liva, and R. R. Voskuhl. 2001. "Classic" myelin basic proteins are expressed in lymphoid tissue macrophages. *J. Neuroimmunol.* 116: 83–93.
25. Sosinowski, T., N. Killeen, and A. Weiss. 2001. The Src-like adaptor protein downregulates the T cell receptor on $CD4^+CD8^+$ thymocytes and regulates positive selection. *Immunity* 15: 457–466.
26. Azzam, H. S., A. Grinberg, K. Lui, H. Shen, E. W. Shores, and P. E. Love. 1998. CD5 expression is developmentally regulated by T cell receptor (TCR) signals and TCR avidity. *J. Exp. Med.* 188: 2301–2311.
27. Beverly, B., S. M. Kang, M. J. Lenardo, and R. H. Schwartz. 1992. Reversal of in vitro T cell clonal anergy by IL-2 stimulation. *Int. Immunol.* 4: 661–671.
28. Schwartz, R. H. 2003. T cell anergy. *Annu. Rev. Immunol.* 21: 305–334.
29. Borgulya, P., H. Kishi, Y. Uematsu, and H. von Boehmer. 1992. Exclusion and inclusion of α and β T cell receptor alleles. *Cell* 69: 529–537.
30. Hammer, J., F. Gallazzi, E. Bono, R. W. Karr, J. Guenot, P. Valsasnini, Z. A. Nagy, and F. Sinigaglia. 1995. Peptide binding specificity of HLA-DR4 molecules: correlation with rheumatoid arthritis association. *J. Exp. Med.* 181: 1847–1855.

31. Fritz, R. B., and I. Kalvakolanu. 1995. Thymic expression of the Golli-myelin basic protein gene in the SJL/J mouse. *J. Neuroimmunol.* 57: 93–99.
32. Goverman, J., A. Woods, L. Larson, L. P. Weiner, L. Hood, and D. M. Zaller. 1993. Transgenic mice that express a myelin basic protein-specific T cell receptor develop spontaneous autoimmunity. *Cell* 72: 551–560.
33. Lafaille, J. J., K. Nagashima, M. Katsuki, and S. Tonegawa. 1994. High incidence of spontaneous autoimmune encephalomyelitis in immunodeficient anti-myelin basic protein T cell receptor transgenic mice. *Cell* 78: 399–408.
34. Harrington, C. J., A. Paez, T. Hunkapiller, V. Mannikko, T. Brabb, M. Ahearn, C. Beeson, and J. Goverman. 1998. Differential tolerance is induced in T cells recognizing distinct epitopes of myelin basic protein. *Immunity* 8: 571–580.
35. Bielekova, B., M. H. Sung, N. Kadom, R. Simon, H. McFarland, and R. Martin. 2004. Expansion and functional relevance of high-avidity myelin-specific CD4+ T cells in multiple sclerosis. *J. Immunol.* 172: 3893–3904.
36. Williams, C. B., D. L. Engle, G. J. Kersh, J. Michael White, and P. M. Allen. 1999. A kinetic threshold between negative and positive selection based on the longevity of the T cell receptor-ligand complex. *J. Exp. Med.* 189: 1531–1544.
37. Ernst, B., D. S. Lee, J. M. Chang, J. Sprent, and C. D. Surh. 1999. The peptide ligands mediating positive selection in the thymus control T cell survival and homeostatic proliferation in the periphery. *Immunity* 11: 173–181.
38. Viret, C., F. S. Wong, and C. A. Janeway, Jr. 1999. Designing and maintaining the mature TCR repertoire: the continuum of self-peptide:self-MHC complex recognition. *Immunity* 10: 559–568.
39. 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.
40. 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.
41. 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.
42. Kisielow, P., H. Bluthmann, U. D. Staerz, M. Steinmetz, and H. von Boehmer. 1988. Tolerance in T-cell-receptor transgenic mice involves deletion of nonmature $CD4^+8^+$ thymocytes. *Nature* 333: 742–746.
43. Lanoue, A., C. Bona, H. von Boehmer, and A. Sarukhan. 1997. Conditions that induce tolerance in mature $CD4^+$ T cells. *J. Exp. Med.* 185: 405–414.
44. Campagnoni, A. T., T. M. Pribyl, C. W. Campagnoni, K. Kampf, S. Amur-Umarjee, C. F. Landry, V. W. Handley, S. L. Newman, B. Garbay, and K. Kitamura. 1993. Structure and developmental regulation of Golli-MBP, a 105-kilobase gene that encompasses the myelin basic protein gene and is expressed in cells in the oligodendrocyte lineage in the brain. *J. Biol. Chem.* 268: 4930–4938.
45. Golding, H., T. Mizuochi, S. A. McCarthy, C. A. Cleveland, and A. Singer. 1987. Relationship among function, phenotype, and specificity in primary allospecific T cell populations: identification of phenotypically identical but functionally distinct primary T cell subsets that differ in their recognition of MHC class I and class II allodeterminants. *J. Immunol.* 138: 10–17.
46. Miller, R. A., and O. Stutman. 1982. Monoclonal antibody to Lyt 2 antigen blocks H-2I- and H-2K- specific mouse cytotoxic T cells. *Nature* 296: 76–78.
47. Heath, W. R., F. R. Carbone, P. Bertolino, J. Kelly, S. Cose, and J. F. Miller. 1995. Expression of two T cell receptor alpha chains on the surface of normal murine T cells. *Eur. J. Immunol.* 25: 1617–1623.