An Unusual Lineage of α/β T Cells that Contains Autoreactive Cells

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Summary

In male mice that express a transgenic α/β T cell receptor (TCR) specific for a male-specific peptide presented by class I D^b major histocompatibility complex (MHC) molecules, we describe an unusual lineage of α/β T cells that are thymus dependent but do not require selection by D^b MHC molecules on thymic epithelium in the absence of the specific peptide (positive selection). These cells express the transgenic α/β TCR and have the $CD4^-8^-$ or $CD4^-8^{low}$ phenotype. Cells with the latter phenotype are only detected when hemopoietic cells express both the male-specific peptide as well as D^b MHC molecules. In fact, these cells are autoreactive, as they expand relatively slowly after transfer into male nude mice. Also in male but not female α/β TCR transgenic mice, the $CD8^+$ cells with the transgenic TCR bear the Pgp1 marker characteristic of mature T cells activated by antigen. $CD4^-8^-$ as well as $CD4^-8^{low}$ cells do not respond significantly when cultured with male stimulator cells but proliferate vigorously when stimulated by TCR antibodies. By this latter criterion, cells in the periphery of male α/β TCR transgenic mice differ from mature male-specific T cells from female α/β TCR transgenic, which become intrinsically anergic when transferred into male nude mice and cannot be stimulated significantly by TCR antibodies. Thus, intrathymic deletion does not eliminate all autoreactive T cells and it is possible that cells with an apparently "benign" autoreactivity may be involved in certain forms of autoimmunity.

A series of experiments in TCR transgenic as well as normal mice has delineated a maturation pathway of developing T cells that includes the selection of cells according to the specificity of their receptor for antigen (reviewed in references 1 and 2). It has been established that the maturation of α/β T cells that express normal levels of CD4 and CD8 coreceptors requires the binding of the α/β TCR to thymic MHC ligands. This has been most clearly shown in TCR transgenic SCID mice (3), as well as in mice that lack class I (4) or class II MHC molecules (5). This selection step does not require the presence of the specific peptide recognized by the TCR but does require the presenting MHC molecule, probably occupied by some other peptide on thymic epithelial cells (6–8). Another selective step that occurs in the presence of both the specific peptide as well as the presenting MHC molecule is the deletion of developing T cells that express the relevant TCR (9–11). For deletion to occur, it is sufficient that the complete ligand is expressed by cells of hemopoietic origin (1).

When analyzing TCR transgenic mice expressing a TCR specific for male-specific (HY) peptides presented by class I D^b MHC molecules, we were surprised to find normal numbers of Thy-1-positive cells in the periphery of male $H\text{-}2^b$ mice, even though their thymus was very small (11). Moreover, we showed that most of the peripheral T cells in these mice were either $CD4^-8^-$ or $CD4^-8^{low}$, with very few $CD4^+8^-$ T cells present in lymph node and spleen. It turned out that the vast majority of the peripheral T cells expressed the transgenic TCR consisting of transgenic β_T and α_T TCR chains, while the few $CD4^+8^-$ T cells expressed $\beta_T\alpha_E$ receptors, due to the lack of "allelic exclusion" of TCRα chains in TCR transgenic mice (1, 12). The origin of the $CD4^-8^-$ and $CD4^-8^{low}$ $\beta_T\alpha_T$ cells in these mice was not obvious: one possibility was that these cells had escaped negative selection in the thymus, undergone positive selection, and on confrontation with the male-specific peptides presented by D^b MHC molecules in peripheral lymphoid tissue, had downregulated their CD8 coreceptor molecules. An alternative possibility was that some of these cells had intrinsically low levels of coreceptor molecules sufficiently high for positive but insufficiently high for negative selection, and that cells with this phenotype had expanded considerably in the periphery of male $H\text{-}2^b$ TCR transgenic mice. A third possibility was that T cells with this phenotype represented a distinct lineage of α/β T cells that could accumulate in peripheral lymphoid organs.

To discriminate between the various possibilities, we conducted experiments on the origin and selection of $CD4^-8^-$

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and $CD4^-8^{low}$ T cells bearing a TCR specific for self antigens. Our experiments show that the $CD4^-8^-$ and $CD4^-8^{+low}$ T cells represent a thymus-dependent population of T cells that does not require positive selection by MHC molecules on thymus epithelium. Also, these cells are not susceptible to deletion. On the contrary, the $CD4^-8^{low}$ cells appear to be activated and expanded in vivo by hemopoietic cells expressing both the specific peptide as well as presenting MHC molecules. While these cells appear unreactive in vitro, their expansion potential can be revealed by transfer in adoptive hosts. Because of this anomalous behavior and the similarity to some T cells found in autoimmune strains of mice, we think it possible that T cells of this unusual lineage could be involved in certain forms of autoimmunity.

Materials and Methods

Mice. The α/β TCR transgenic mice were described previously (11). B10.D2 mice were obtained from the animal colony of the Basel Institute for Immunology. CD57Bl/6 nude mice were obtained from the CNRS, Centre de sélection de d'élevage d'animaux de laboratoire (Orléans, France).

Bone Marrow Chimeras. Female B10.D2 recipient mice were lethally X-irradiated with 950 rad. Afterwards, the mice were injected intravenously with 10^7 T-depleted bone marrow cells from either female or male α/β TCR transgenic H-2^b SCID mice (3).

Injection into Nude Mice. 5.5 × 10^6 spleen cells from male C57Bl/6 Thy-1.1 α/β TCR transgenic mice were depleted of sIg-positive B cells and injected intravenously into either male or female C57Bl/6 Thy-1.2 nude mice. The absolute number of recovered T cells of different phenotypes recovered from the recipient spleen at different timepoints after transfer was calculated as described previously (13, 14).

Purification of Cells Expressing the Transgenic TCR from Various Mice. Cells from lymphoid organs were stained with monovalent Fab fragments of the T3.70 mAb directed against the TCR α chain of the transgenic TCR. These fragments were coupled with carboxyfluorescein-*N*-hydroxysuccinimide ester (FLUOS) and because of their monovalent nature did not induce proliferation of T cells. The stained cells were purified by cell sorting and subsequently cultured with various stimulator cells or TCR antibodies.

In Vitro Proliferation. Various numbers of responder cells were cultured with 1–5 × 10^5 X-irradiated (2,500 rad) female or male spleen cells from nude mice. Cultures were set up either in the presence or absence of exogenous interleukin 2 (15). Some cultures contained 10 μg of the T3.70 antibody directed against the transgenic α TCR chain (16) in addition to female spleen cells from nude mice.

Antibodies. For cell surface staining, we used CD4 (No. 1447; Becton Dickinson & Co., Mountain View, CA) (PE-coupled or GK1.5 [17] FITC-conjugated), CD8α (No. 1353; Becton Dickinson & Co., fluoresceine conjugated), T3.70 anti-TCR α chain (18), CD8β (31.1.1) (19), and Pgp1 (CD44) (20) antibodies.

Surface Staining. 5 × 10^4 up to 5 × 10^6 lymphocytes were pelleted in round-bottomed wells of 96-well microtiter plates. Cells were resuspended in 50 μl of PBS containing the described antibodies in optimal concentrations and incubated for 15 min on ice. After incubation, cells were washed twice in PBS containing 2% FCS and resuspended either in 100 μl of the same solution (in case of direct staining) or in 50 μl of PBS containing PE-streptavidine (Southern Biotechnology Associates, Inc., Birmingham, AL) and incubated for further 15 min on ice. After washing, the cells were analyzed on a FACScan®.

Results

The $CD4^-8^-$ and $CD4^-8^{low}$ α/β T cells in TCR Transgenic Mice Are Thymus Dependent. To find out whether Thy-1^+, $CD4^-8^-$, and $CD4^-8^{low}$ T cells expressing a transgenic α/β TCR are thymus dependent, we crossed H-2^b α/β TCR transgenic mice with H-2^b nu^+/nu^- mice and crossed the resulting F_1 generation back with H-2^b nu^+/nu^- mice. Male and female nu^+ mice harboring the TCR transgenes were selected from the backcross generation, and cells from peripheral lymphoid organs as well as bone marrow were analyzed. As shown in Fig. 1, the staining of various cells with CD4, CD8, and T3.70 (anti-α_T) antibodies was not different from that obtained with cells from nontransgenic nu^+ mice. This indicates that rearranged TCR genes present in the germline cannot bypass the requirement of a thymus in the development of α/β T cells that are normally present in the spleen, axillary, and inguinal lymph nodes and bone marrow of mice. In addition, we conclude that α/β T cells with the abnormal $CD4^-8^-$ and $CD4^-8^{low}$ phenotype, which are predominant in male H-$2D^b$ α/β TCR transgenic mice, are also thymus dependent, since we do not detect significant numbers of these cells in male TCR transgenic nu^+ mice.

$CD4^-8^-$ α/β T Cells Do Not Require Positive Selection by Thymic MHC Molecules. We have reported previously that we could detect $CD4^-8^-$ α/β T cells bearing the transgenic TCR in peripheral lymphoid organs of H-2^d α/β TCR transgenic SCID mice, indicating that these cells do not require positive selection by thymic H-$2D^b$ MHC molecules (3). This finding could be reproduced in hemopoietic X-irradiation chimeras constructed by injecting T cell–depleted bone marrow from female H-$2^{d/b}$ or male H-$2^{d/d}$ α/β TCR transgenic SCID mice into female lethally X-irradiated B10.D2 (H-2^d) recipient mice. Fig. 2 (*middle* and *bottom*) shows that in this transfer experiment, host cells of hemopoietic origin survived the X-irradiation and reconstituted the lymphnodes of recipient mice to some extent. It is also clear that cells that expressed the transgenic TCR were present in peripheral lymph nodes. These cells expressed neither CD4 nor CD8 coreceptors in contrast to $\beta_T\alpha_T$ cells in male H-2^b α/β TCR transgenic mice, which were comprised of both $CD4^-8^-$ as well as $CD4^-8^{low}$ cells. This raised the possibility that the latter population was dependent on the presence of both the male-specific peptide as well as the presenting D^b MHC molecules.

$CD4^-8^{low}$ α/β T Cells Are Selected by Nominal Antigen on Hemopoietic Cells. To test whether the presence of $CD4^-8^{low}$ $\beta_T\alpha_T$ T cells required selection by H-$2D^b$ MHC molecules on thymic epithelial cells and/or the presence of nominal antigen on hemopoietic cells, we prepared hemopoietic X-irradiation chimeras by injecting T cell–depleted bone marrow cells from male H-$2^{d/b}$ α/β TCR transgenic SCID mice into lethally X-irradiated B10.D2 recipients. In this sit-

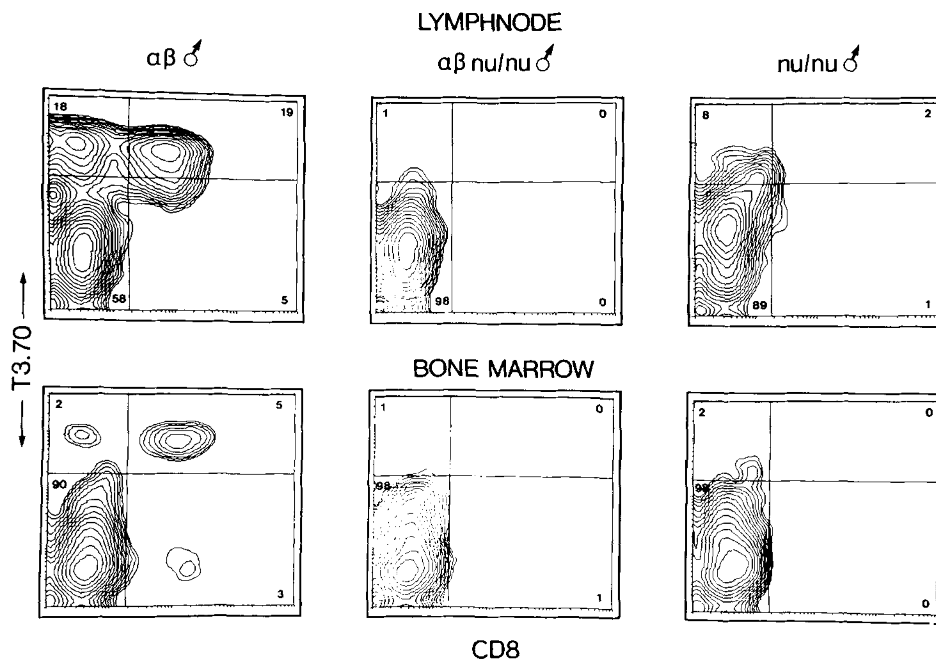


Figure 1. Staining of lymph node and bone marrow cells from male H-2^b α/β TCR transgenic mice (α/β ♂; *left*), from male H-2^b α/β TCR transgenic *nu/nu* mice (α/β *nu/nu* ♂; *middle*), and male C57Bl/6 nude mice (*nu/nu* ♂; *right*) by CD8 and T3.70 (transgenic TCR α chain) antibodies. Numbers in quadrants represent the proportion of all gated cells in each quadrant.

uation, the developing T cells do not encounter D^b MHC molecules on epithelial cells in the thymus but do encounter nominal antigen presented by D^b MHC molecules on cells of hemopoietic origin. As shown in Fig. 2 (*top*), the presence of the male peptide on hemopoietic cells was responsible for the appearance of $CD4^-8^{low}$ $\beta_T\alpha_T$ T cells, which could not be detected in B10.D2 recipient mice reconstituted with bone marrow from female H-$2^{b/d}$ or male H-$2^{d/d}$ α/β TCR transgenic SCID mice (Fig. 2). This indicates that $CD4^-8^{low}$ $\beta_T\alpha_T$ cells like $CD4^-8^-$ $\beta_T\alpha_T$ cells are generated independently of positive selection by D^b MHC molecules on thymus epithelium but require selection by the complete ligand, i.e., the specific peptide and presenting MHC molecules on hemopoietic cells. This may be so either because the complete ligand induces the expression of CD8 coreceptor molecules and/or because it induces the expansion of $CD4^-8^{low}$ cells.

$CD4^-8^{low}$ Cells Can Expand upon Transfer into nu^+ Mice. To test whether T cells with the transgenic α/β TCR would expand in vivo, we transferred T cells from male H-2^b α/β TCR transgenic mice into male H-2^b nu^+ recipient mice (Fig. 3). As observed in previous experiments (13), T cells expressing endogenous *TCR* α chains and normal levels of CD8 molecules grew rapidly after transfer and did not expand after reaching maximal levels 1–2 wk after injection (13; and data not shown). In contrast, $CD4^-8^{low}$ $\beta_T\alpha_T$ cells grew very slowly. 1–2 wk after injection, the number of $CD4^-8^{low}$ $\beta_T\alpha_T$ cells in the spleen of recipient mice was similar to the number of injected cells with that phenotype. From then on, the number of $CD4^-8^{low}$ $\beta_T\alpha_T$ cells increased progressively. Further expansion could be observed when $CD4^-8^{low}$ $\beta_T\alpha_T$ were collected 3 mo after the first transfer and were injected again in male nu^+ mice (Fig. 3). In contrast, $CD4^-8^-$ $\beta_T\alpha_T$ showed no expansion potential in the same assay (data not shown). These results indicate that $CD4^-8^{low}$ $\beta_T\alpha_T$ cells are capable of significant expansion in vivo but that their expansion has much slower kinetics than that of $\beta_T\alpha_T$ $CD8^+$ cells from female α/β TCR transgenic mice and that of $\beta_T\alpha_E$ or $\beta_E\alpha_E$ $CD8^+$ cells from either male or female α/β TCR transgenic mice (13).

$CD4^-8^{low}$ $\beta_T\alpha_T$ T Cells May Be Derived from $CD4^-8^-$ $\beta_T\alpha_T$ Precursors. We prepared $CD4^-8^-$ $\beta_T\alpha_T$ T cells from male H-$2D^b$ α/β TCR transgenic mice by rigorous depletion of cells expressing CD8 coreceptors. When these cells were propagated in vitro by stimulation with T3.70 antibodies and IL-2, no $CD8^+$ cells were recovered after 1 wk of culture (Fig. 4). However, when such cells were not cultured, but injected into male nu^+ recipients, and lymph nodes were analyzed 2–7 wk weeks after the injection, a significant number of $CD4^-8^{low}$ T cells that bore the transgenic TCR were detected (Fig. 5). While this result is consistent with a precursor-product relationship of $CD4^-8^-$ and $CD4^-8^{low}$ $\beta_T\alpha_T$ T cells, we can at the present time not exclude the possibility of selective expansion of a very minor contaminant present in the inoculum.

The Surface Phenotype of $CD4^-8^{low}$ $\beta_T\alpha_T$ T Cells in Male H-2^b α/β TCR Transgenic Mice. If $CD4^-8^{low}$ $\beta_T\alpha_T$ T cells were indeed antigenically stimulated cells, one might expect to find markers typical for activated or memory T cells like Pgp1 (CD44) (20). As shown in Fig. 6, many $CD4^-8^{low}$ T cells are in fact Pgp1 positive, while $CD4^-8^+$ $\beta_T\alpha_T$ cells from female α/β TCR transgenic mice are Pgp1 negative (Fig. 6) and remain negative when they are transferred into female but not male H-2^b nu^+/nu^+ mice (our unpublished results). This may suggest that the Pgp1 expression of $CD4^-8^+$ T cells results from antigenic stimulation. It was also interesting to see whether $CD4^-8^{low}$ T cells were expressing $CD8\alpha$ homodimers rather than $CD8\alpha/\beta$ hetero-

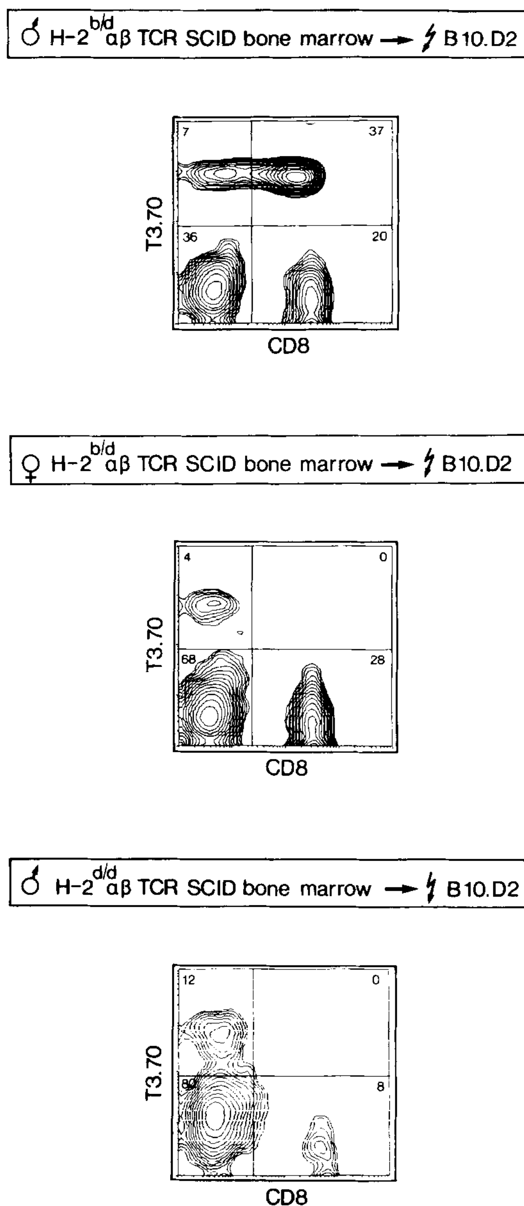


Figure 2. Staining of lymph node cells by CD8 and T3.70 (transgenic α TCR chain) antibodies 2 mo after reconstitution of lethally X-irradiated female B10.D2 recipients with T cell–depleted bone marrow cells from either male (*top*) or female (*middle*) H-$2^{d/b}$ or male H-$2^{d/d}$ (*bottom*) α/β TCR transgenic SCID/SCID donor mice. The T3.70-negative $CD8^+$ and $CD8^-$ cells are mostly derived from the X-irradiated host as defined by staining with H-2^d and H-2^b antibodies (data not shown). The experiment shown at the bottom was done on a different day, hence the slight difference in the intensity of staining.

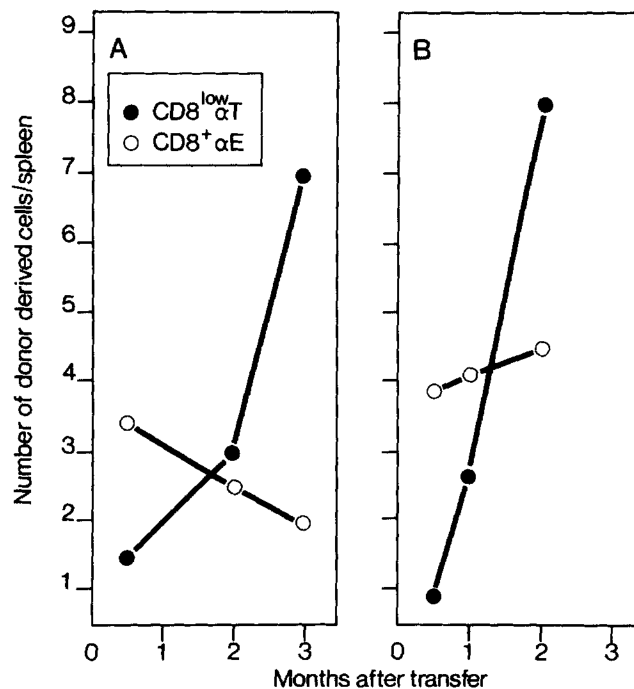


Figure 3. Expansion of $CD4^-CD8^{low}$ T cells from male α/β TCR transgenic mice after transfer into male nu^+ mice. Results represent the absolute number ($\times 10^{-6}$) of donor cells of each phenotype recovered in recipient spleen at different time points after transfer. The are the mean of two (1 wk and 2 mo after first transfer, all time points of second transfer) and four (3 mo after first transfer) mice in each group. (*A*) First transfer: spleen cells from Thy-1.1 male α/β TCR transgenic mice were injected intravenously into Thy-1.2 male nu^+ mice. The number ($\times 10^{-6}$) of donor cells of each phenotype injected simultaneously in each recipient was: $CD4^-8^-$ α_T, 2.3; $CD4^-8^{low}$ α_T, 1.4; $CD4^-8^+$ α_E, 0.7. (*B*) Second transfer: Thy-1.1 donor cells were recovered from the hosts 3 mo after the first transfer and injected into a second set of male nu^+ recipients. The number ($\times 10^{-6}$) of donor cells of each phenotype injected simultaneously into each recipient were: $CD4^-8^-\alpha_T$, 0.05; $CD4^-8^{low}$ α_T, 0.9; $CD4^-8^+$ α_E, 0.2. $CD4^-8^-\alpha_T$ cells did not expand in any of the recipients and are therefore not shown.

dimers in light of the finding that "activated" γ/δ T cells express predominantly CD8α homodimers. As shown in Fig. 7, this is not the case for $CD4^-8^{low}$ $\beta_T\alpha_T$ T cells that do express some very reduced levels of CD8β proteins on the cell surface.

$CD4^-8^-$ and $CD4^-8^{low}$ Cells from Male α/β TCR Transgenic Mice Are Not Intrinsically Anergic. In recent experiments, we have shown that mature malespecific $CD4^-8^+$ $\alpha_T\beta_T$ T cells from female transgenic mice proliferate strongly when injected into male but not female nu^+ recipients such that after 5 d, the recovery of malespecific cells from male recipients is 50 times that from female recipients. Thus, the male-specific proliferation detected in vitro in the presence (see Table 2) or absence (13) of exogenously added IL-2 is reflected in a significant expansion potential in vivo. We observed that after the initial wave of proliferation, the number of male-specific $\beta_T\alpha_T$ cells decreased in the male nu^+ recipients and that the remaining cells with the transgenic TCR became refractory to antigenic stimulation in the presence or absence of exogenous IL-2. We wanted to compare the functional capabilities of T cells with the transgenic receptor obtained from male nu^+ mice injected with T cells from female transgenic mice and the T cells present in the periphery of male transgenic mice in order to see whether the different selection of these cells was also reflected in a different functional phenotype. As shown in Tables 1–3, this is clearly the case: to obtain the same number of cells with the transgenic TCR from male

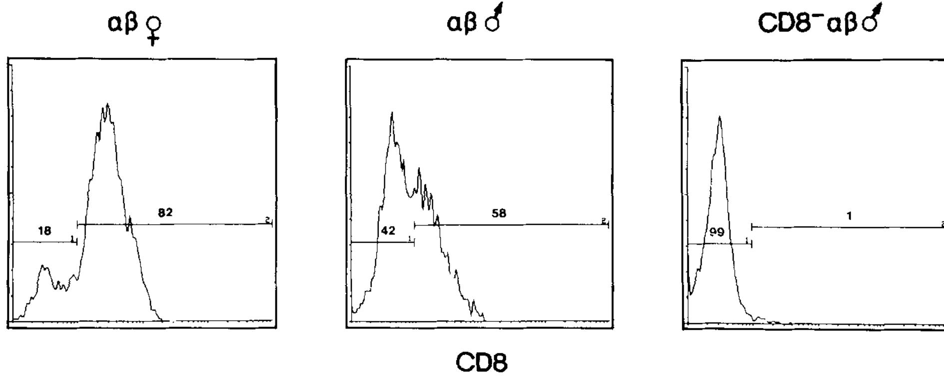


Figure 4. Propagation of T cells from female H-2^b α/β TCR transgenic mice (*left*), from male H-2^b α/β TCR transgenic mice (*middle*), and from male mice after depletion of $CD8^+$ cells by T3.70 antibodies and IL-2 in culture. Cells were stained after 1 wk in culture with CD8 antibodies. Numbers represent the proportion of all gated cells in the various fractions.

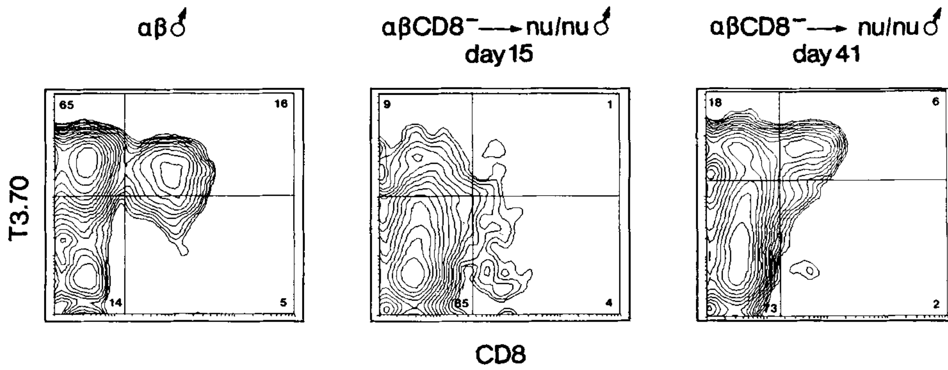
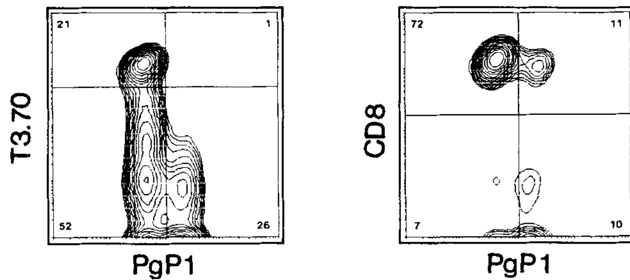


Figure 5. Staining of lymph node cells from male H-2^b α/β TCR transgenic mice (*left*) and from male H-2^b nude mice at day 15 (*middle*) and day 41 (*right*) after injection with $CD4^-8^-$ T cells from male H-2^b α/β TCR transgenic mice with CD8 and T3.70 (transgenic α TCR chain) antibodies.



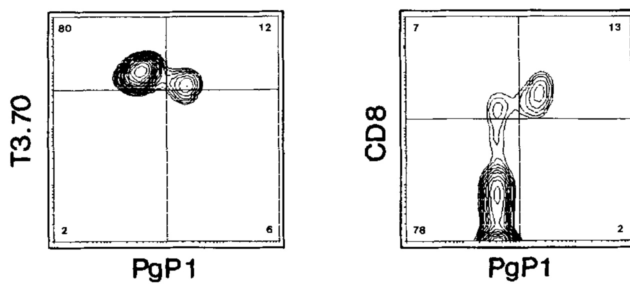


Figure 6. Staining of lymph node T cells from male and female H-2^b α/β TCR transgenic mice with CD8, T3.70 (α_T = antibody directed against the transgenic TCR α chain) and Pgp1 antibodies. Cell suspensions were depleted of $CD4^+$ cells before the analysis.

or female nu^+ mice injected with T cells from female transgenic mice, the cells were stained with a monovalent FITC-conjugated Fab fragment of the T3.70 TCR antibody. This fragment did not induce any measurable activation of T cells expressing the transgenic TCR. The T3.70-positive cells were sorted and stimulated in culture either with antigen (male spleen cells) or the T3.70 antibody. It can be seen in Table 1 that sorted cells from female nude mice injected with lymph node cells from female α/β TCR transgenic mice responded with proliferation when stimulated by male antigen or T3.70 and CD3 antibodies. The stronger response to the antibodies is probably due to the high affinity of the antibodies to the TCR while the receptor may have relatively low affinity for male antigen (which, however, is sufficient to lead to massive clonal expansion in vivo). In contrast, cells recovered from recipient male mice did not respond significantly to male stimulator cells and also their response to TCR and CD3 antibodies was greatly diminished. This indicates that the latter cells are intrinsically anergic. In contrast, $CD4^-8^-$ and $CD4^-8^{low}$ cells from male α/β TCR transgenic mice have a different functional phenotype (Table 2): while they do not proliferate when stimulated by male antigen, they proliferate vigorously when stimulated by TCR antibodies, i.e., they are not intrinsically anergic because their responses to stimulation by TCR antibodies is very similar to that of cells from female α/β TCR transgenic mice even when the dose of TCR antibodies is varied (Table 3).

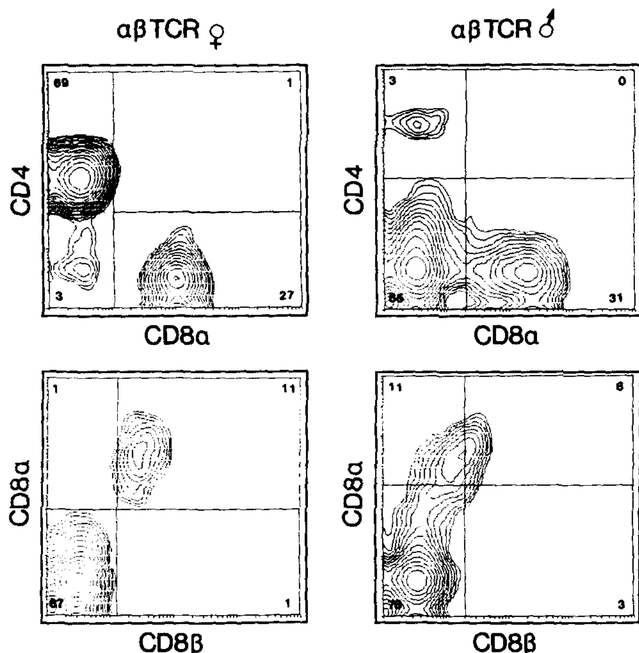


Figure 7. Staining of lymph node cells from female (*left*) and male (*right*) α/β TCR transgenic mice with CD4, CD8α, and CD8β antibodies.

Table 1. *Intrinsic Anergy of CD4⁻8⁺ $\beta_{T\alpha T}$ Cells after Confrontation with Antigen*

Stimulators	Responders	
	nu/nu ♀ recipients	*nu/nu* ♂ recipients
	cpm	
nu/nu ♀	200	150
nu/nu ♂	7,350	300
nu/nu ♀ + T3.70	81,850	2,250
nu/nu ♀ + CD3	82,000	5,700

10^7 lymph node cells from female C57Bl/6 α/β TCR transgenic mice were injected intravenously in either female or male C57Bl/6 *nu/nu* mice. After 2 wk, single cell suspensions from lymph node and spleen were prepared, depleted of B cells (αIg-coupled dynabeads), and stained with FITC-conjugated Fab fragments of the T3.70 (anti-α_T) antibody as well as PE-coupled L3T4 (anti-CD4) antibodies. CD4⁻, T3.70⁺ cells were sorted, and 7 × 10^3 cells were cultured with 10^5 various stimulator cells and antibodies. Responses to male stimulator cells were obtained in the presence (above) or absence of exogenously added IL-2 (12), and were in the same order of magnitude. The TCR transgenes were derived from the B6.2.16 clone, which showed a strong male-specific proliferative response but relatively weak male-specific killing (<30%), presumably because of a relatively low affinity of the TCR. The same level of killing is observed with lymph node cells from female α/β TCR transgenic mice when stimulated with male cells, and for this reason, we only provide data on proliferative responses. The data shown here are representative of three independently carried out experiments.
* All cultures contained IL-2 (15).

Table 2. *CD4⁻8⁻ and CD4⁻8^low $\beta_{T\alpha T}$ Cells from Male Transgenic Mice Are Not Anergic*

Stimulators* (X-irradiated)	Responders		
	α/β ♀ CD8⁺	α/β ♂ CD8⁻	α/β ♂ CD8^low
		cpm	
nu/nu ♀	500	400	300
nu/nu ♂	13,200	500	300
nu/nu ♀ + T3.70	105,000	80,000	81,000

5 × 10^4 lymph node cells from either female or male C57Bl/6 α/β TCR transgenic mice were cultured with various stimulator cells and antibodies. CD8⁻ and CD8^low T cells from male α/β TCR transgenic mice were obtained by staining the cells with the FITC-conjugated 53-6.7 (anti-CD8) antibody and sorting the cells. The data shown here are representative of five independently carried out experiments.
* All cultures contained IL-2 (15).

Discussion

The lineage of α/β T cells described in this report is different from that which undergoes positive selection in the thymus and contains mature T cells that can become intrinsically anergic when confronted with antigen in peripheral lymphoid organs (13): the CD4⁻8⁻ and CD4⁻8^low cells expressing a male-specific TCR in male α/β TCR transgenic mice appear not to undergo positive selection induced by D^b MHC molecules on thymic epithelium and have not become intrinsically anergic on contact with male antigen in peripheral lymphoid organs. Rather, the CD4⁻8^low cells appear to

Table 3. *$\beta_{T\alpha T}$ Cells from Female and Male α/β Transgenic Mice Respond to Similar Doses of Receptor Antibodies*

Stimulators	Responders	
	α/β ♀	α/β ♂
nu/nu ♀	1,800	900
nu/nu ♂	15,300	1,200
nu/nu ♀ + T3.70		
4 ng/ml	51,000	80,000
15 ng/ml	68,000	100,000
60 ng/ml	86,000	129,000
250 ng/ml	103,000	141,000
1,000 ng/ml	111,000	149,000
4,000 ng/ml	105,000	135,000

5 × 10^4 CD4⁻ and sIg-depleted spleen and lymphnode cells from female and male α/β TCR transgenic mice were cultured with 3.5 × 10^5 X-irradiated (2,200 rad) female or male nu^+ spleen cells or with the former and various concentrations of the T3.70 antibody. All cultures contained IL-2 (15).

be antigenically stimulated and their (relatively slow) expansion potential in response to male antigen can be revealed in vivo but not in vitro. Thus, these cells show some "benign" autoreactivity in vivo, and one may wonder whether this can develop into a more malicious form when, for instance, in a certain microenvironment lymphokines upregulate the level of antigen or upregulate the effector function of these autoreactive T cells.

The lineage of α/β T cells described in this report does not appear to be an artifact observed only in α/β TCR transgenic mice for the following reasons. The $CD4^-8^-$ T cells correspond very closely to a population of α/β T cells detected in the thymus of normal mice with regard to surface markers, functional phenotype, appearance during ontogeny, and expression of autospecific receptors. In normal mice as well as α/β TCR transgenic mice, α/β-bearing $CD4^-8^-$ cells lack J11d surface molecules and are readily induced to proliferation by lectins or antireceptor antibodies. In both types of mice, these cells develop relatively late during ontogeny (16, 21), i.e., around birth, and they can express autospecific TCRs. It would appear from our results that these cells do not require positive selection by MCH molecules on thymic epithelium. Also, cells with this phenotype may take part in lymphoproliferation in the *gld* and *lpr* autoimmune strains.

We think it is possible that the $CD4^-8^{low}$ cells are products of $CD4^-8^-$ precursors and that they are selected when both the specific peptide and the presenting MHC molecules are present. It is also possible that these ligands induce CD8 expression. We cannot detect such precursor-product relationship in vitro after stimulation with TCR antibodies, but we are detecting $CD4^-8^{low}$ cells several weeks after injection of $CD4^-8^-$ T cells into male nu^+ mice. These cells bear markers (Pgp1) that are believed to be the result of antigenic stimulation while the $CD4^-8^-$ T cells do not.

The origin of this lineage of α/β T cells remains obscure at present. One of us has speculated (1) that these cells are in fact cells of the γ/δ lineage that may occasionally express α/β TCRs due to some leakiness in the mechanisms controlling their development. There is no clear argument for or against such an hypothesis, and one reason for this speculation is missing experimental evidence that γ/δ cells require positive selection by ligands on thymic epithelium.

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