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Engagement of the T-cell receptor during positive selection in the thymus down-regulates *RAG-1* expression

(allelic exclusion/T-cell receptor rearrangement/positive selection/T-cell receptor transgenic mice)

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ABSTRACT **We have examined the expression of the recombination activating gene *RAG-1* by *in situ* hybridization to thymi from mice bearing transgenes for the T-cell receptor (TCR) α chain, TCR β chain, or both TCR α and β chains. *RAG-1* transcription was found in the thymic cortex of transgenic mice carrying a single TCR α- or TCR β-chain transgene, comparable to normal mice. However, *RAG-1* transcription was strikingly reduced in the thymic cortex from transgenic mice carrying both TCR α- and β-chain genes and expressing major histocompatibility complex (MHC) class I (H-2^b) molecules necessary for positive selection of the transgenic TCR. In contrast, thymi of transgenic mice also carrying both TCR α- and β-chain genes but expressing MHC molecules (H-2^d) that did not positively select the transgenic TCR displayed high levels of *RAG-1* transcription. The low thymic *RAG-1* expression coincided with high transgenic TCR α-chain surface expression and with inhibition of endogenous TCR α-chain rearrangement. Our findings suggest that binding of the TCR to self MHC molecules during positive selection down-regulates *RAG-1* transcription in cortical thymocytes and thereby prevents further TCR α-chain rearrangements.**

The T-cell receptor (TCR) is composed of disulfide-linked α and β glycoproteins, which are made up of variable (V) and constant (C) regions. During intrathymic development, TCR β-chain genes are formed first by somatic rearrangements of interchangeable germ-line-encoded β-chain V (Vβ), diversity (Dβ), and joining (Jβ) gene segments. Next, TCR α-chain genes are assembled from V (Vα) and J (Jα) gene segments (for review, see ref. 1). The recombination activating genes *RAG-1* and *RAG-2* have been cloned (2, 3) and shown to be crucially involved in these V-(D)-J recombination processes of TCR and immunoglobulin genes (4, 5).

The αβ TCR repertoire is shaped in the thymus by both positive and negative selection events: only thymocytes with a TCR specific for self major histocompatibility complex (MHC) molecules are selected for further maturation and may eventually leave the thymus as mature T cells (for review, see ref. 6). Although individual T cells have the potential to generate two functional rearrangements at both alleles of their TCR α and their TCR β loci, T cells express only one type of TCR β chain on their cell surface (for review, see ref. 7). In addition, several studies have demonstrated that the presence of a transgenic TCR β-chain gene prevents rearrangement and expression of endogenous TCR β-chain genes (8–10). Thus, a mechanism must exist to ensure allelic exclusion of TCR β-chain genes. In contrast to TCR β-chain genes, allelic exclusion of TCR α-chain genes appears less stringent: (*i*) several T-cell clones have been described that bear two functional TCR α chains (11, 12) and (*ii*) the suppression of endogenous TCR α-chain expression in TCR α-chain transgenic mice is incomplete (13). These observations led to the hypothesis that neither a productive TCR α-chain gene rearrangement nor cell surface expression of TCR αβ polypeptides but rather the interaction of the TCR with self MHC molecules during positive thymic selection prevents further TCR α-chain gene rearrangements (11, 14, 15).

To test this hypothesis, we have examined *RAG-1* expression in transgenic mice bearing a functional TCR α-, TCR β-, or both TCR α- and TCR β-chain genes derived from a virus-specific CD8$^+$ T-cell clone. The results support the view that TCR engagement during positive thymic selection prevents TCR α-chain rearrangements by down-regulation of the recombination activating genes.

MATERIALS AND METHODS

Transgenic Mice, Virus, and Peptides. Generation of the TCR α (line 335), the TCR β (line 128), and the TCR αβ (line 327) transgenic mice has been described (16–18). The TCR transgenic founder mice were backcrossed to either C57BL/6 (H-2^b) or BALB/c (H-2^d) mice, which were purchased from the Institut für Zuchthygiene (Tierspital Zurich). Mice at 4–6 weeks of age were used. Transgenic mice were typed for the presence of the transgene by PCR analysis of tail DNA by using Vα2 (5′-CTGACCTGCAGTTATGAGGACAGCAC) and Cα (5′-CGAGGATCCTTTAACTGGTACACAG-CAGG) or Vβ8 (5′-CATGGAGGCTGCAGTCACCC) and Cβ (5′-GTTTGTTTGCGAGCTCTGTTTTGATGGCTC) as specific primers. The PCR was done for 30 cycles (1 min at 95°C, 1 min at 37°C, and 3 min at 72°C).

(TCR α × TCR β)F$_1$ double-transgenic mice were derived from $\alpha_T \times \beta_T$ matings. The WE strain of the lymphocytic choriomeningitis virus (LCMV) was originally obtained from Fritz Lehmann-Grube (Heinrich-Pette-Institut für Experimentelle Virologie und Immunologie an der Universität Hamburg, Hamburg, F.R.G.). The peptide IKAVYNFATCG (amino acids 32–42) (19) of the LCMV-WE glycoprotein recognized by the transgenic αβ TCR was synthesized by the solid-phase technique and purchased from Neosystem Laboratoire (Strasbourg, France).

***In Situ* Hybridization.** *In situ* hybridization was performed on 5-μm-thick frozen sections of the thymus as described (20). Hybridized tissue sections were dipped into NTB2 nuclear track emulsion (Kodak) diluted 1:2 with 600 mM ammonium acetate. Sections hybridized with a ^{35}S-labeled

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Abbreviations: TCR, T-cell receptor; MHC, major histocompatibility complex; LCMV, lymphocytic choriomeningitis virus; V, variable; C, constant; J, joining; mAb, monoclonal antibody; FITC, fluorescein isothiocyanate; PE, phycoerythrin.

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RNA antisense or sense probe of the *RAG-1* gene [1363-base-pair (bp) *Eco*RI fragment of the *RAG-1* gene cloned into the *Eco*RI site of pSPT-18 (Boehringer Mannheim)] were exposed for 21 days at 4°C in a light-tight box. Slides were developed with Kodak developer PL-12 for 2.5 min and fixed with Kodak fixer for 5 min at room temperature. Counterstaining was done with nuclear fast red [0.05% in 5% (wt/vol) aluminium sulfate].

Cytofluorometric Analysis. Single-cell suspensions were prepared from mesenteric lymph nodes and thymus. Cells were stained in balanced salt solution (0.01 g of phenol red/0.14 g of $CaCl_2 \cdot 2H_2O$/8.00 g of NaCl/0.40 g of $MgSO_4 \cdot 7H_2O$/0.40 g of KCl/0.20 g of $MgCl_2 \cdot 6H_2O$/0.06 g of KH_2PO_4/0.24 g of $Na_2HPO_4 \cdot 2H_2O$/1.00 g of D-glucose, to a final volume of 1 liter of double-distilled H_2O, filter-sterilize) containing 2% fetal calf serum and 0.2% NaN_3 with hybridoma culture supernatant (1:1) of the monoclonal antibody (mAb) KT3 (rat IgG anti-CD3) (21), mAb B20.1 (rat IgG anti-TCR Vα2) (22), or mAb KJ16 (rat IgG anti-TCR Vβ8.1+8.2) (23) for 30 min at 4°C, followed by fluorescein isothiocyanate (FITC)-conjugated goat anti-rat IgG antibodies (Tago). For double staining, the cells were further incubated with biotinylated anti-CD8 antibodies (Becton Dickinson), followed by streptavidin-phycoerythrin (PE; Tago) or PE-conjugated anti-CD4 antibodies (Becton Dickinson). CD4/CD8 surface expression was analyzed by incubating cells with FITC-labeled anti-CD8 (Becton Dickinson) and PE-conjugated anti-CD4 antibodies. Cells were analyzed by flow cytometry on an Epics profile analyzer with four logarithmic scales (Coulter).

Southern Blot Analysis. Isolated total genomic DNA (15 μg) was digested with *Eco*RI (25). DNA was separated on a 0.8% agarose gel and transferred to Biodyne membranes (Pall). The filters were prehybridyzed for 6 h in 5× standard saline citrate (SSC)/10× Denhardt's solution/0.1% SDS/10% (wt/vol) dextran sulfate/salmon sperm DNA (100 μg/ml) at 65°C and hybridized overnight in the prehybridization mixture containing the following ^{32}P-labeled probes (10^6 cpm/ml): a

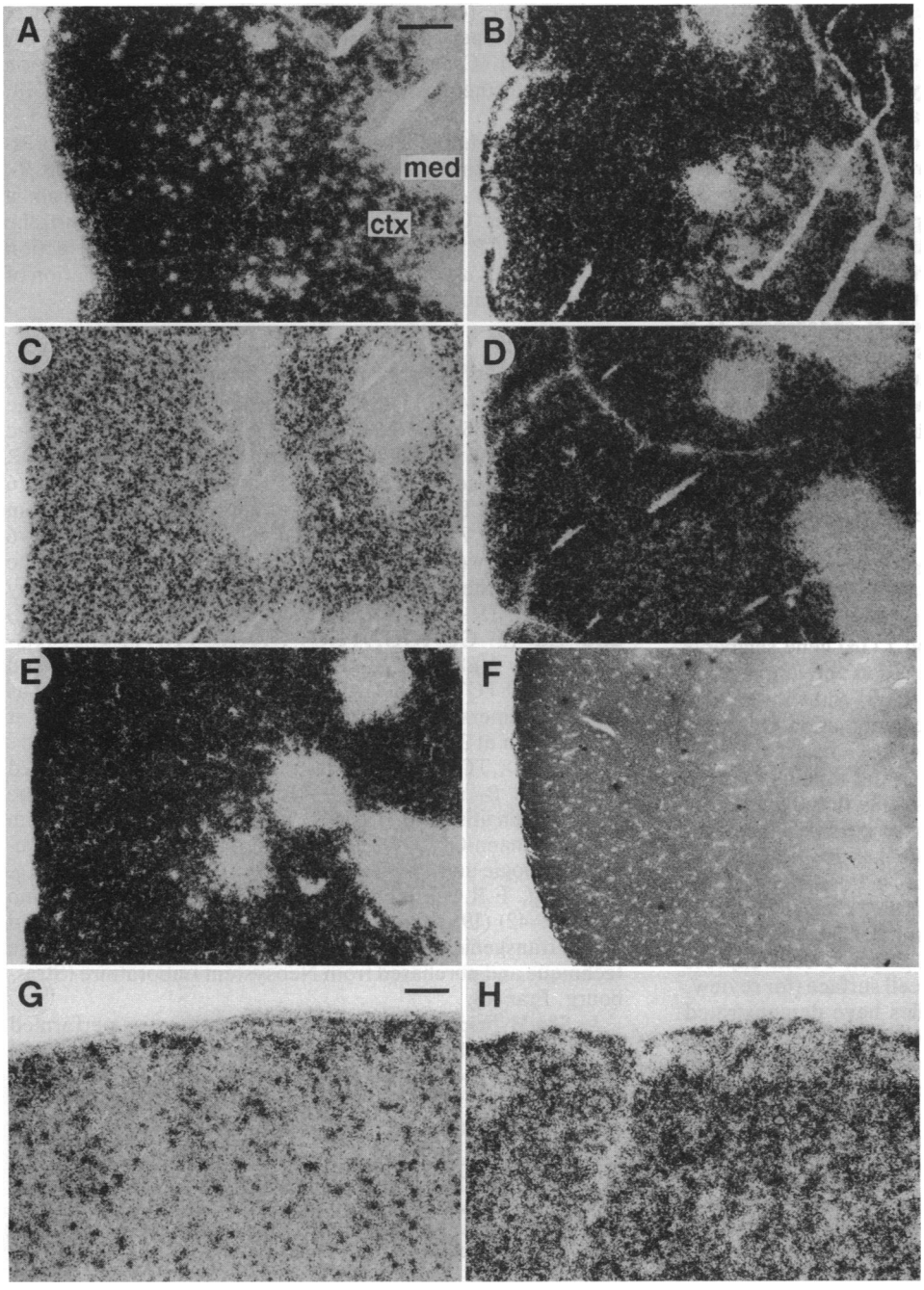

FIG. 1. *RAG-1 in situ* hybridization of thymus sections from TCR transgenic mice. Tissue sections of thymi from the following TCR transgenic mice were hybridized with a radiolabeled RNA antisense probe for the *RAG-1* gene. (*A*) TCR α, H-2^b. (*B*) TCR β, H-2^b. (*C*) (TCR α × TCR β)F$_1$, H-2^b. (*D*) (TCR α × TCR β)F$_1$, H-2^d. (*E*) C57BL/6. (*G*) TCR $\alpha\beta$ (line 327), H-2^b. (*H*) TCR $\alpha\beta$ (line 327), H-2^d. (*F*) As a negative control, an adjacent tissue section of the thymus shown in *E* was hybridized with a radiolabeled RNA sense probe of the *RAG-1* gene. Cortical (ctx) and medullary (med) regions are defined by counterstaining. (*A–F*, bar in *A* = 0.2 mm; *G* and *H*, bar in *G* = 0.05 mm.)

300-bp *Eco*RI fragment of the Cδ gene (pC9δ4.1) (26) or a 1000-bp *Eco*RI–*Sal* I fragment of the human β-actin gene (pSPT672-hu β-actin) (27). The filter was washed for two 15-min periods in 2× SSC/0.1% SDS at room temperature and for a 30-min period in 0.2× SSC/0.1% SDS at 65°C.

Northern Blot Analysis. Total RNA was prepared from intact thymi by guanidinium isothiocyanate extraction (28) and purified on CsCl gradients (29). Total RNA was separated on a 1% agarose/glyoxal gel (25) and transferred to a Biodyne membrane (Pall). Filters were prehybridized and hybridized using the conditions described above for Southern blots. The following cDNA probes were used: a mixture of 527-, 640-, and 1363-bp *Eco*RI fragments of the *RAG-1* gene (M6-BSK$^+$) (2) and a 1000-bp *Eco*RI–*Sal* I fragment of the human β-actin gene (pSPT672-hu β-actin) (27).

RESULTS

***RAG-1* Expression in Thymi of TCR Transgenic Mice.** We have used transgenic mice carrying the P14 TCR α-, the P14 TCR β-, or both the P14 TCR α- and β-chain genes derived from the LCMV/H-2D^b-specific CD8$^+$ T-cell clone P14 to examine the influence of thymic selection processes on *RAG-1* expression in maturing thymocytes. Previous studies (30) have demonstrated that "positive selection" of T cells bearing this transgenic TCR was dependent on the presence of the H-2D^b molecules. *RAG-1* expression in the thymi of the TCR transgenic mice was analyzed by *in situ* hybridization using a radiolabeled *RAG-1* antisense RNA probe. A strong signal for *RAG-1* was observed exclusively in the thymic cortex from H-2^b single TCR α (Fig. 1*A*) and single TCR β (Fig. 1*B*) transgenic mice comparable to that of nontransgenic mice (Fig. 1*E*). The hybridization appeared to be specific since no significant labeling with the corresponding RAG-1 sense probe was obtained (Fig. 1*F*). In contrast to single TCR α- or β-chain transgenic mice, *RAG-1* transcription was strongly reduced in thymi from (P14 TCR α × P14 TCR β)F$_1$ double transgenic mice of H-2^b type (Fig. 1*C*), which allowed positive selection of the transgenic αβ TCR. High levels of *RAG-1* transcription were detected in thymi from (TCR α × TCR β)F$_1$ hybrid mice that also carried both TCR α- and β-chain genes but expressed nonselective H-2^d MHC molecules (Fig. 1*D*). The same results with a low *RAG-1* expression in H-2^b (Fig. 1*G*) and with high expression in H-2^d (Fig. 1*H*) were obtained when thymi from the P14 αβ TCR transgenic line 327 were examined that had cointegrated both TCR α and TCR β transgenes. The low level of *RAG-1* expression in H-2^b thymi of (TCR α × TCR β)F$_1$ hybrid and αβ TCR transgenic mice was not due to a decreased number of CD4$^+$ CD8$^+$ thymocytes in these mice since the percentages of CD4$^+$ CD8$^+$ thymocytes among the examined mice were between 68 and 82% (Table 1).

The clustered distribution of the silver grains in H-2^b thymi from transgenic mice carrying both P14 TCR α- and β-chain genes (Fig. 1 *C* and *G*) indicated that most cortical thymocytes were *RAG-1*-negative and a few expressed *RAG-1* at a normal level.

The results of the *in situ* hybridization were confirmed by Northern blot analysis. Densitometric measurement revealed that the level of *RAG-1* transcription was decreased 4-fold in H-2^b thymi of αβ TCR transgenic mice, which allow positive selection of the transgenic TCR when compared to nonselective αβ transgenic H-2^d thymi (Fig. 2). Similar results were obtained when a *RAG-2*-specific cDNA probe was used (data not shown).

The Extent of Transgenic TCR α-Chain Surface Expression Depends on Thymic Selection. Several independent transgenic lines harboring the P14 α-chain transgene expressed the transgenic TCR Vα2 chain only on a low percentage of peripheral T cells (data not shown). The P14 α-chain transgenic line 335 used in this study behaved similarly, and only

Table 1. Surface expression of CD4 and CD8 on thymocytes from TCR transgenic mice

Mouse	H-2	% of total thymocytes CD4$^+$ CD8$^+$	CD4$^+$ CD8$^-$	CD4$^-$ CD8$^+$	CD4$^-$ CD8$^-$
α_T (line 335)	b/b	78 ± 1	9 ± 0	4 ± 0	9 ± 2
β_T (line 128)	b/b	77 ± 5	14 ± 2	6 ± 2	3 ± 2
$\alpha_T \times \beta_T$	b/b	76 ± 3	10 ± 1	10 ± 3	4 ± 1
$\alpha_T \times \beta_T$	d/d	77 ± 6	13 ± 3	5 ± 1	5 ± 1
$\alpha_T\beta_T$ (line 327)	b/b	68 ± 2	5 ± 2	21 ± 2	6 ± 2
$\alpha_T\beta_T$ (line 327)	d/d	77 ± 2	12 ± 0	4 ± 2	7 ± 0
C57BL/6	b/b	82 ± 8	10 ± 6	4 ± 2	4 ± 0

Total thymocytes were stained with FITC-conjugated anti-CD8 and PE-conjugated anti-CD4 antibodies and analyzed by flow cytometry. The percentages (mean ± SEM) of at least two mice per group are shown.

17% of peripheral T cells were Vα2$^+$ when compared to endogenous TCR Vα2 expression in C57BL/6 mice, which ranged from 10 to 14% (Table 2). Cell surface expression of the transgenic P14 TCR α chain was also low on thymocytes (Fig. 3*A*). The P14 TCR α transgenic mice were crossed with P14 TCR β transgenic mice, and the surface expression of the transgenic TCR chains was analyzed in double-transgenic F$_1$ hybrid mice carrying selective (H-2^b) or nonselective (H-2^d) MHC haplotypes. As shown in Table 2, the number of Vα2$^+$ cells was increased in the peripheral CD8$^+$ T-cell subset from 17% in single TCR α transgenic mice to 76% in H-2^b F$_1$ hybrid mice. In contrast, the number of Vα2$^+$ T cells in the CD8 subset was not increased in double-transgenic F$_1$ hybrid mice carrying the nonselective H-2^d haplotype. Accordingly, a substantial subset of thymocytes in the selective H-2^b F$_1$ hybrid mice expressed the transgenic TCR α and β chains at an intermediate density (Fig. 3*C*). This population was absent in single TCR α transgenic mice or in transgenic F$_1$ hybrid mice carrying the nonselective H-2^d haplotype (Fig. 3*D*). Thus, thymocytes bearing both transgenic TCR α and β chains were predominantly selected in the presence of H-2^b MHC molecules and, subsequently, gave rise to the most prominent T-cell population in these mice.

To confirm the cytofluorometric analysis, the functional activities of T cells from P14 TCR α, P14 TCR β, and double-transgenic F$_1$ hybrids were tested in a primary anti-LCMV mixed lymphocyte culture *in vitro*. Only effector cells from H-2^b double-transgenic F$_1$ hybrid mice generated a LCMV-specific cytotoxic T-cell response under the conditions used (Fig. 4).

The Extent of Endogenous TCR α-Chain Rearrangements Depends on Thymic Selection. It has been demonstrated that TCR Vα–Jα rearrangement involves the deletion of the TCR Cδ locus. Therefore, rearrangement of endogenous TCR

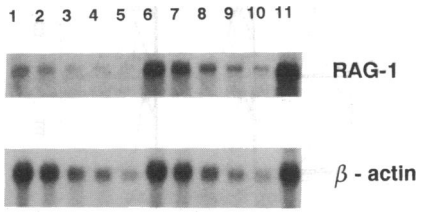

FIG. 2. Transcription of *RAG-1* in thymocytes from P14 αβ TCR transgenic mice. Total RNA was prepared from four pooled thymi from P14 αβ TCR transgenic mice of the H-2^b (lanes 1–5) and the H-2^d (lanes 6–10) haplotypes and from C57BL/6 mice (lane 11). Total RNA (10 μg; lanes 1, 6, and 11) and 1:2 dilution steps thereof (lanes 2 and 7, lanes 3 and 8, lanes 4 and 9, lanes 5 and 10) were hybridized with a *RAG-1*-specific probe (*Upper*). To control the quality and quantity of the examined RNA, the filter was washed and rehybridized with a β-actin-specific probe (*Lower*).

Table 2. Surface expression of CD4, CD8, TCR Vα2, and TCR Vβ8 on lymph node cells from TCR transgenic mice

Mouse	H-2	% of total lymph node cells		CD4/CD8 ratio	% of CD8+ T cells		% of CD4+ T cells	
		CD4+	CD8+		TCR Vα2	TCR Vβ8	TCR Vα2	TCR Vβ8
α_T	b/b	34 ± 1	23 ± 6	1.5	17 ± 2	14 ± 1	17 ± 2	18 ± 5
β_T	b/b	28 ± 5	32 ± 9	0.9	9 ± 1	95 ± 3	10 ± 3	86 ± 5
$\alpha_T \times \beta_T$	b/b	20 ± 5	56 ± 9	0.4	76 ± 7	97 ± 1	14 ± 2	82 ± 2
$\alpha_T \times \beta_T$	d/d	41 ± 1	31 ± 0	1.3	19 ± 5	96 ± 3	10 ± 3	84 ± 5
C57BL/6	b/b	37 ± 2	24 ± 4	1.5	10 ± 1	18 ± 2	14 ± 1	19 ± 2

Total lymph node cells were stained with FITC-conjugated anti-CD8 and PE-conjugated anti-CD4 antibodies and analyzed by flow cytometry. The percentages (mean ± SEM) of at least two mice per group are shown. Total lymph node cells were stained with mAb B20.1 (anti-TCR Vα2) or mAb KJ16 (anti-TCR Vβ8.1/8.2) and goat anti-rat IgG-FITC antibodies. The staining was followed by biotinylated mAb specific for CD8 plus PE-conjugated avidin or PE-conjugated mAb specific for CD4. The percentages (mean ± SEM) of at least two mice per group are shown.

α-chain genes in peripheral CD8+ T cells from P14 TCR α, P14 TCR β, and (P14 TCR α × P14 TCR β)F_1 hybrid mice was monitored by the presence of Cδ sequences. Peripheral CD8+ T cells from single TCR α (Fig. 5, lane A), TCR β (lane B), and H-2^d F_1 hybrid mice (lane D) had deleted most of their Cδ genes, indicating a high frequency of endogenous TCR α-chain rearrangements. In contrast, a strong Cδ hybridization signal was obtained with DNA from CD8+ T cells that were derived from H-2^b double-transgenic F_1 hybrid mice (lane C). Thus, endogenous TCR α-chain rearrangements were suppressed in CD8+ T cells from those mice in which positive selection of the transgenic αβ TCR pair was possible.

DISCUSSION

The present report demonstrates that the transcription of the recombination activating gene *RAG-1* was decreased in immature thymocytes from mice expressing both transgenic TCR α and TCR β chains and with a thymus bearing MHC molecules that allowed positive selection of the transgenic TCR. The low thymic *RAG-1* expression in these mice correlated with suppression of endogenous TCR α-chain gene rearrangements and with high transgenic TCR α-chain surface expression. Thus our data support the hypothesis that the interaction of the αβ TCR on immature CD4+ CD8+ thymocytes with thymic self MHC molecules induces down-regulation of *RAG-1* transcription and thereby prevents further TCR α-chain rearrangements.

It is possible that engagement of the TCR with thymic self MHC molecules directly inhibits *RAG-1* transcription. Alternatively, one could imagine that the differentiation stage and/or the phenotypic changes (i.e., up-regulation of TCR density) associated with positive selection leads to the termination of *RAG-1* transcription. The first possibility of signal transduction is supported by the *in vitro* study (31) that demonstrated that cross-linking of the TCR–CD3 complex of immature thymocytes with anti-CD3 antibodies strongly reduced *RAG-1* and *RAG-2* transcription. However, it is doubtful whether the anti-CD3 antibody treatment is an appropriate *in vitro* model for positive selection since TCR cross-linking by CD3-specific antibodies is generally assumed to provide negative selection signals (32, 33). At present we do not know whether down-regulation of *RAG-1* expression represents an irreversible one-step process or whether continuous triggering by the TCR is needed to prevent *RAG-1* transcription in CD4+ CD8+ thymocytes. Further experiments using antibodies to block the interaction of the TCR with the self MHC during positive selection may help to address this question.

This laboratory has reported (34) that expression of the transgenic P14 TCR was found on CD4− CD8− thymocytes (which were HSA+, CD25−, and CD44−) irrespective of presence (H-2^b) or absence (H-2^d) of the selecting haplotype. Thus, cell surface expression of TCR molecules on CD4− CD8− thymocytes alone is not sufficient to prevent *RAG-1* expression in CD4+ CD8+ thymocytes.

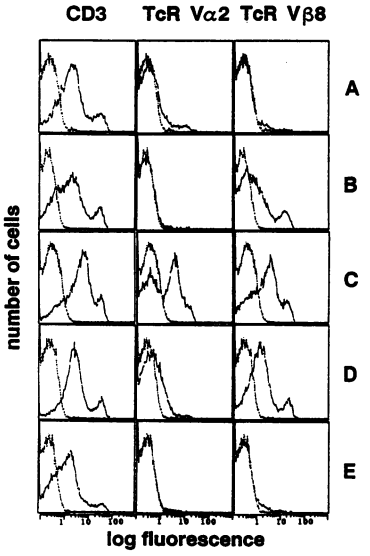

FIG. 3. Surface expression of CD3, TCR Vα2, and Vβ8 on thymocytes from TCR transgenic mice. Thymocytes from the indicated TCR transgenic mice were labeled with anti-CD3, anti-TCR Vα2, or anti-TCR Vβ8.1+8.2 antibodies and analyzed by flow cytometry (solid lines). Negative controls with the fluorescent conjugate alone are shown as dotted lines. The flow cytometry profile of one representative example from each group is shown. (*A*) TCR α, H-2^b. (*B*) TCR β, H-2^b. (*C*) (TCR α × TCR β)F_1, H-2^b. (*D*) (TCR α × TCR β)F_1, H-2^d. (*E*) C57BL/6.

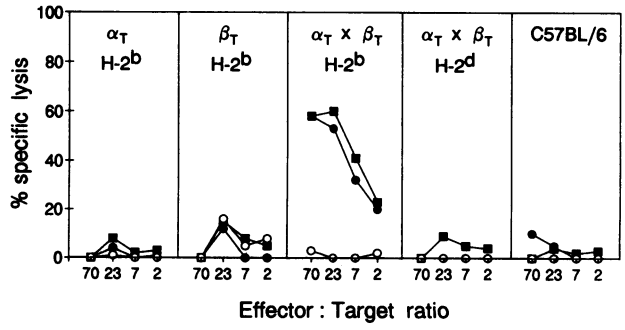

FIG. 4. Induction of primary anti-LCMV cytotoxic T-cell response *in vitro*. Spleen cells from unprimed TCR α transgenic (α_T, H-2^b), TCR β transgenic (β_T, H-2^b), and (TCR α × TCR β)F_1 double-transgenic mice ($\alpha_T \times \beta_T$, either H-2^b or H-2^d) were cultured with irradiated LCMV-infected peritoneal macrophages from C57BL/6 mice. After 3 days, the lytic activities of the LCMV-stimulated cultures were determined on LCMV-infected (solid squares), LCMV peptide (100 μM)-coated (solid circles), and uninfected (open circles) MC57G (H-2^b) target cells in a 4- to 5-h ^{51}Cr release assay as described (24). Spontaneous release values were 26–30%.

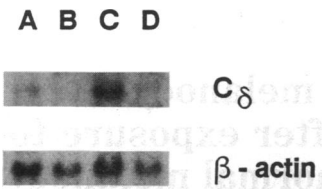

FIG. 5. TCR α-chain rearrangement in transgenic mice examined by Southern blot analysis of the TCR δ-chain locus. Genomic DNA (15 μg) extracted from peripheral CD8+ T cells of the indicated TCR transgenic mice was digested with *Eco*RI and analyzed with a Cδ-specific probe (*Upper*). The same filter was stripped and rehybridyzed with a β-actin-specific probe (*Lower*). Lanes: A, TCR α, H-2^b; B, TCR β, H-2^b; C, (TCR α × TCR β)F_1, H-2^b; D, (TCR α × TCR β)F_1, H-2^d.

Our data suggest that the studied transgenic P14 TCR undergoes positive selection efficiently in the presence of H-2^b MHC molecules. This is based on the finding that transgenic TCR α-chain surface expression was strongly increased in the (P14 TCR α × P14 TCR β)F_1 hybrid mice when compared to single P14 TCR α-chain transgenic mice. The efficient selection of the transgenic TCR in our system may be due to an effective pairing of the used transgenic TCR α and β chains that leads to the formation of a high-affinity receptor. This view is supported by our previous study (16) that revealed a preferential selection of P14 types of αβ TCR in single P14 TCR α and P14 TCR β transgenic mice after antigenic stimulation. It is also possible that the number of selecting "niches" for the P14 TCR in the thymus is not as limited as observed with the HY male-specific transgenic TCR (35). We therefore conclude that the clearly visible down-regulated *RAG-1* expression in the thymic cortex of our mice was due to the efficient positive selection of P14-TCR-bearing thymocytes in the presence of H-2^b MHC molecules. The extent of *RAG-1* down-regulation may vary in other TCR transgenic models depending upon the TCR–MHC affinities involved.

The present study illustrates the differences in the control of TCR α- and β-chain gene rearrangements. We (10) and others (8, 9) have shown that expression of a transgenic TCR β chain almost completely inhibited productive endogenous TCR β-chain rearrangements. Thus, allelic exclusion of the TCR β-chain genes appears to be tight. In contrast, our data indicate that the TCR α-chain rearrangements are controlled by the thymic positive selection mechanism: Immature thymocytes that fail to be positively selected may continue to recombine Vα–Jα gene segments, whereas the recombination machinery is turned off in cells bearing a selectable TCR on their surface. The frequent occurrence of two productively rearranged TCR α-chain loci in T-cell clones is compatible with the scenario summarized above (11, 12, 36, 37).

Note Added in Proof. Borgulya *et al.* (38) have recently demonstrated that CD4+ CD8+ TCR^{hi} thymocytes from TCR transgenic mice transcribe *RAG-1* and *RAG-2* at a low level.

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