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Immunology

Self-reactive T cells can escape clonal deletion in T-cell receptor $V_{\beta}8.1$ transgenic mice

[self-tolerance/minor lymphocyte-stimulating locus (*Mls*)/T cell clones]

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ABSTRACT **To study the mechanisms of tolerance in detail, we have constructed transgenic mice expressing a $V_{\beta}8.1$–$D_{\beta}2$–$J_{\beta}2.3$–$C_{\beta}2$ T-cell receptor (TCR) gene. Since expression of $V_{\beta}8.1$ is known to correlate with reactivity of $CD4^{+}CD8^{-}$ T cells to minor lymphocyte-stimulating locus 1^{a} (*Mls-1*^{a}), we expected to induce tolerance in most $CD4^{+}CD8^{-}$ T cells in $V_{\beta}8.1$ transgenic mice of the *Mls-1*^{a} allele. In one line of *Mls-1*^{b} $V_{\beta}8.1$ transgenic mice, the $V_{\beta}8.1$ TCR was expressed on >98% of mature T cells and their response to Mls-1^{a} was highly enriched. In *Mls-1*^{a} $V_{\beta}8.1$ transgenic mice, $CD4^{+}CD8^{-}$ T cells in these mice were severely reduced among both peripheral T cells and thymocytes. However, the deletion of these cells was not complete, and most of the residual $CD4^{+}CD8^{-}$ mature T cells still expressed normal densities of $V_{\beta}8.1$ TCR. The residual $CD4^{+}CD8^{-}$ T cells did not respond to Mls-1^{a} but were still able to proliferate in response to other stimuli via the TCR. Interestingly, $CD4^{+}CD8^{-}$ $V_{\beta}8.1^{+}$ T-cell clones isolated from *Mls-1*^{a} $V_{\beta}8.1$ transgenic mice could respond to Mls-1^{a}. We suggest that these types of T cells escape clonal deletion in the thymus.**

The T-lymphocyte repertoire can discriminate between foreign and self antigens. This discriminatory feature is known as self-tolerance. Classical experiments have established that self-tolerance is acquired during the development of the immune system (1, 2). However, the general mechanisms underlying tolerance induction are poorly understood. Recently, clonal deletion within the thymus was confirmed as an important form of tolerance in many studies (3–5). These experiments suggest that intrathymic deletion of potentially autoreactive T cells occurs at a time when immature thymocytes are selected to differentiate into mature thymocytes. Clonal anergy, another form of tolerance that is poorly defined, is thought to occur both in the thymus and in the periphery (6–10).

It is difficult to show intrathymic selection of the T-cell repertoire directly, since the T-cell repertoire consists of large numbers of T cells, many of which express distinct T-cell receptor (TCR) configurations and it is impossible to follow development of an individual T cell in detail. One way to overcome this difficulty is to establish mice that express only TCRs having certain α and β chains and follow the development of these T cells with specific anti-TCR antibodies. Transgenic mice provide a powerful system to accomplish this goal (11). Experiments performed by several groups using TCR $\alpha\beta$ transgenic mice support a positive selection mechanism operative on developing T cells and some form of clonal-deletion process occurring before or at the $CD4^{+}CD8^{+}$ thymocyte stage (12–17). In spite of these experimental advantages, $\alpha\beta$ TCR transgenic mice are problematic. Since functionally rearranged TCR α- and β-chain genes were introduced and expressed at abnormal stages of T-cell differentiation, normal T-cell developmental patterns may be altered (18).

To create a more-physiologic transgenic model for T-cell tolerance, we have established transgenic mice carrying a T-cell receptor β-chain gene derived from an Mls-1^{a}-reactive T-cell clone. In this case, the genomic $V_{\beta}8.1$ gene was placed under the control of the normal TCR β-chain promoter and enhancer. Since the expression of the endogenous α chain may affect TCR assembly, configuration, and selection during the development of T cells, these $V_{\beta}8.1$ transgenic mice may more closely resemble normal animals. We have studied tolerance to endogenously expressed Mls-1^{a} in these transgenic mice. In *Mls-1*^{a} $V_{\beta}8.1$ transgenic mice, $CD4^{+}CD8^{-}$ mature T cells were severely deleted while the decrease of $CD4^{-}CD8^{+}$ T cells was less significant. Interestingly, $CD4^{+}CD8^{-}$ $V_{\beta}8.1^{+}$ T cells were readily detected in the periphery but, when initially isolated, were not able to respond to Mls-1^{a} stimulator cells. However, $CD4^{+}CD8^{-}$ $V_{\beta}8.1^{+}$ T-cell clones of cells isolated from the mice responded to Mls-1^{a} stimulator cells. These findings suggest that a subset of $CD4^{+}CD8^{-}V_{\beta}8.1^{+}$ T cells in *Mls-1*^{a} $V_{\beta}8.1$ transgenic mice potentially reactive to Mls-1^{a} escape clonal deletion in the thymus.

MATERIALS AND METHODS

Construction of the Transgene and the Transgenic Mice. The L2 cell clone, selected for reactivity against Mls-1^{a} spleen cells (19), was kindly provided to us by Frank Fitch (Ben May Institute, University of Chicago) and Michael Prystowsky (University of Pennsylvania). High molecular weight DNA was isolated from the L2 clone and a genomic DNA library was constructed. We obtained a functionally rearranged $V_{\beta}8$–C_{β} genomic gene encoding >10 kilobases (kb) of 5′ flanking sequence of $V_{\beta}8$ and part of the $C_{\beta}2$ locus. The fragment containing the $V_{\beta}8$–$J_{\beta}2$ gene was subcloned and ligated to $C_{\beta}2$ and 3′ enhancer (20) sequences previously isolated from a genomic library of the T-cell clone NU1 (K.Y., unpublished work) to create the construct designated pBL2TβEn-1 (Fig. 1). The 18-kb insert of pBL2TβEn-1 was released from the vector sequence and 400–1000 copies were microinjected into the male pronucleus of (SWR/J × CBA/Ca)F_1 fertilized eggs. Three of 52 mice born carried integrated copies of the transgene. Founder mice were crossed with CBA/Ca (*H-2*^{k}, *Mls-1*^{b}) mice, and individual progeny were typed for *H-2* and *Mls-1* loci. For H-2 typing, spleen cells from each animal were stained with the anti-H-2^{q} monoclonal antibody (mAb) 34-1-2 (no cross reactivity with H-2^{k}) (21) plus fluorescein isothiocyanate-conjugated (FITC)-anti-

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Abbreviations: TCR, T-cell receptor; Mls, minor lymphocyte-stimulating locus; mAb, monoclonal antibody; SEB, staphylococcal enterotoxin B; MHC, major histocompatibility complex.
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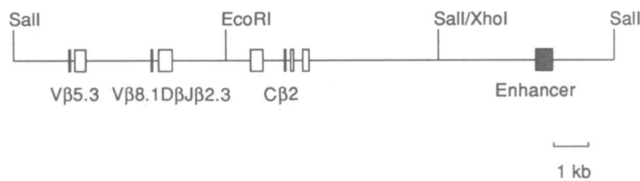

FIG. 1. The TCR β-chain gene construct used to generate the transgenic mice. Only the restriction sites used for ligation of the various gene fragments are shown. The *Sal* I/*Xho* I site was destroyed during plasmid construction.

mouse immunoglobulin antibody. Mls-1^a expression was determined by the ability of spleen cells to stimulate proliferation of the Mls-1^a-reactive T-cell clone BARB12 (a gift of Ryo Abe, National Institutes of Health) (22). Mice were used when 6–10 wk of age.

Flow Cytometric Analysis. Nylon wool-nonadherent lymph node cells were stained with various mAbs in phosphate-buffered saline/0.5% bovine serum albumin/0.1% sodium azide. The following mAbs were used for staining with methods described previously (23): FITC-conjugated anti-CD8 mAb, phycoerythrin-conjugated anti-CD4 mAb (Becton Dickinson), and F23.1 (anti-V_β8) (24). For two-color staining with F23.1, excess mouse immunoglobulin was used to prevent cross reactivity of the FITC-conjugated anti-mouse immunoglobulin secondary antibody as described previously (25).

Cell Culture, T-Cell Lines, and Clones. Separation and culture of T cells were performed as described previously (23). Irradiated CBA/Ca spleen cells were added to the culture when staphylococcal enterotoxin B (SEB) (26) and anti-TCR αβ mAb (H57-597) (27) were used for stimulation of T cells. To establish T-cell lines, lymph node T cells were cultured at 1000 cells per 200 μl with irradiated (2000 rads; 1 rad = 0.01 Gy) C57BL/6 spleen cells (1×10^5) in the presence of 5% rat Con A supernatant. After three cycles of restimulation, cell lines were expanded at 1×10^5 cells per ml with irradiated C57BL/6 spleen cells (2×10^6/ml) in the presence of rat Con A supernatant. T-cell clones were isolated by limiting dilution as described previously (23). For proliferation studies of T-cell lines, 2×10^4 T cells and 5×10^5 irradiated stimulator spleen cells were placed in each well of a microtiter plate and cultured for 3 days. Proliferation was assessed after 16 hr of exposure to 1 μCi of [^{3}H]thymidine (1 Ci = 37 GBq). Results are expressed as the mean or the difference of mean counts between experimental and control responses of triplicate cultures. Standard deviations of most experimental groups were within 10% of the mean and are not shown.

RESULTS

Expression of the Transgenic TCR and T-Cell Subsets. We generated TCR transgenic mice with a functionally rearranged TCR $V_\beta 8.1$–$D_\beta 2$–$J_\beta 2.3$–$C_\beta 2$ gene from a Mls-1^a reactive T-cell clone. Studies of tissue-specific expression of the transgene by Northern (RNA) blotting indicated that the transgene was expressed in a lymphocyte-specific manner (data not shown). In this report, we present data derived from the progeny of one transgenic mouse, founder 21 (SWR × CBA/Ca). The founder mouse was backcrossed to CBA/Ca mice. Since the genotype of the SWR strain is *Mls-1^a H-2^q* and that of CBA/Ca is *Mls-1^b H-2^k*, the offspring were typed for major histocompatibility complex (MHC) and Mls-1.

Expression of the transgenic TCR on the cell surface was analyzed by staining nylon wool-purified T cells with various anti-TCR mAbs (data not shown). Percentages of $V_\beta 8.1^+$ cells were calculated by subtracting the percentage of cells positive for F23.2 (V_β8.2; ref. 4) from those positive for KJ16 (V_β8.1 or V_β8.2; ref. 28). In *Mls-1^b V_β8.1* transgenic mice, >98% of CD3$^+$ (mAb 500A2; ref. 29) cells expressed a V_β8.1-containing TCR. In *Mls-1^a V_β8.1* transgenic mice the proportion was slightly lower (90–95% of CD3$^+$ cells). Expression of other TCR V_β chains, such as V_β8.2 or V_β6 (RR4-7; ref. 30), was not detected.

The total number of lymph node T cells in *Mls-1^b V_β8.1* transgenic mice was equivalent to that in normal mice. The CD4/CD8 ratio in the normal CBA/Ca strain was approximately 2 (Fig. 2*A*). In contrast, numbers of CD4 and CD8 cells in *Mls-1^b V_β8.1* transgenic mice were approximately equivalent (Fig. 2*B*). Also, expression of the transgenic TCR was not homogenous: 4.2% of the CD4$^+$ and 12.4% of the CD8$^+$ T cells were F23.1^{low}, even though two-color staining of the same preparation of T cells revealed that almost all CD4$^+$ and CD8$^+$ cells express CD3 (data not shown). The F23.1^{low} cells probably express a V_β8.1 TCR at low levels, since staining of the T-cell population with anti-V_β8 mAbs revealed that >98% of CD3$^+$ cells were $V_\beta 8.1^+$.

Studies of peripheral T-cell subsets in *Mls-1^a V_β8.1* transgenic mice revealed several striking differences from *Mls-1^b V_β8.1* transgenic mice (Fig. 2*C*). First, the CD4$^+$CD8$^-$ subset of mature T cells was severely decreased. We estimated the absolute number of T cells in lymph nodes of *H-2^{qk} V_β8.1* transgenic mice by using the number of nylon wool-nonadherent lymph node cells and multiplying by the percentage of each T-cell subset. The number of CD4$^+$CD8$^-$ cells in a lymph node of a *Mls-1^a V_β8.1* transgenic mouse ($1.6 \pm 0.3 \times 10^5$) was ≈10% of that of a *Mls-1^b V_β8.1* transgenic mouse ($14.3 \pm 4.8 \times 10^5$). In contrast, the decrease in numbers of CD4$^-$CD8$^+$ T cells was less significant. The total number of CD4$^-$CD8$^+$ cells in a lymph node of a *Mls-1^a V_β8.1* transgenic mouse ($7.8 \pm 1.0 \times 10^5$) was ≈60% of that of a *Mls-1^b V_β8.1* transgenic mouse ($12.4 \pm 2.3 \times 10^5$). The preferential decrease in the CD4$^+$CD8$^-$ subset in the *V_β8.1* transgenic mice was considered a consequence of a self-tolerance mechanism, since CD4$^+$CD8$^-$ cells are reactive to Mls-1^a. The deletion of CD4$^+$CD8$^-$ T cells, however, was not complete, and the majority of the remaining CD4$^+$CD8$^-$ cells still expressed a V_β8.1 TCR at a similar density to T cells in *Mls-1^b* mice. Second, there was a discrete population of CD4$^+$CD8$^-$ T cells that did not express the $V_\beta 8.1^+$ TCR. They expressed an αβ TCR, since all CD4$^+$ cells in the same preparation of T cells expressed αβ TCR (data not shown). This is consistent with our observation that the proportion of T cells in *Mls-1^a V_β8.1* transgenic mice that express a V_β8.1 TCR is lower than that in *Mls-1^b V_β8.1* transgenic mice. In contrast to the CD4$^+$CD8$^-$ subset, expression of the V_β8.1 TCR among CD4$^-$CD8$^+$ cells was comparable to that of CD4$^-$CD8$^+$ lymph node cells in *Mls-1^b* mice.

In the thymus, both CD4$^+$CD8$^-$ and CD4$^-$CD8$^+$ cells were depleted in *Mls-1^a V_β8.1* transgenic mice (data not shown). In addition, thymocytes expressing high levels of the TCR, which are characteristic of mature, functionally active T cells, were selectively decreased.

Function of Mature T Cells in the Transgenic Mice. Since both *Mls-1^b* and *Mls-1^a V_β8.1* transgenic mice expressed a V_β8.1 TCR on mature peripheral T cells, it was of interest to study the reactivity of their T cells to Mls-1^a as well as to other antigens. To compare the response of T cells to TCR occupancy among different mice, we titrated the number of T cells in the proliferation assay and compared the titration curves among nontransgenic, *Mls-1^b V_β8.1* transgenic, and *Mls-1^a V_β8.1* transgenic mouse T cells. Typical results of three experiments are shown in Fig. 3. As expected, T cells in *Mls-1^b V_β8.1* transgenic mice were 3–10 times enriched for Mls-1^a-reactive T cells when compared with control mice. In contrast, no significant proliferative responses to Mls-1^a were observed in the *Mls-1^a V_β8.1* transgenic mice. In spite of the lack of response to Mls-1^a, T cells from *Mls-1^a V_β8.1* trans-

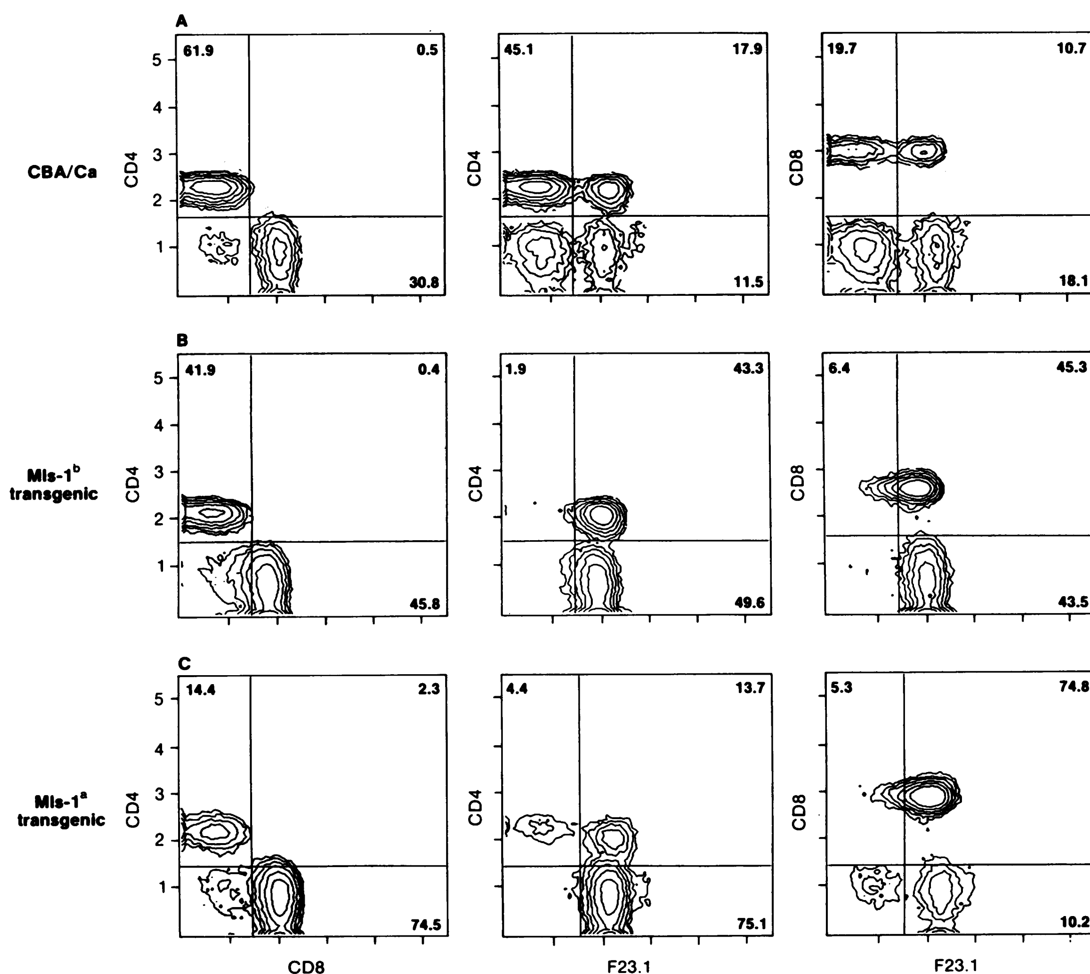

FIG. 2. Cell surface expression of CD4, CD8, and TCR on lymph node T cells from normal, *Mls-1^b^* V_β*8.1* transgenic, and *Mls-1^a^* V_β*8.1* transgenic mice. Nylon wool-nonadherent lymph node lymphocytes of each mouse were stained as described in *Materials and Methods*. The proportion of cells in each quadrant was determined after subtracting percent of control staining from each value. The *Mls-1^a^* and *Mls-1^b^* V_β*8.1* transgenic mice were H-2^{kk} and H-2^{qk}, respectively. No significant differences in the proportion of T-cell subsets or function of T cells were observed between *H-2^kk^* and *H-2^qk^* V_β*8.1* transgenic mice (data not shown).

genic mice showed significant proliferative responses to allogeneic BALB/c and C57BL/6 spleen cells. T cells in *Mls-1^b^* as well as *Mls-1^a^* V_β*8.1* transgenic mice also showed higher than normal proliferative responses to SEB, which activates virtually all T cells bearing $V_\beta8$ TCRs (26) and thus may activate the majority of T cells in these mice.

To study the reactivity of $CD4^+CD8^-$ $V_\beta8.1^+$ T cells in *Mls-1^a^* V_β*8.1* transgenic mice, we treated nylon wool-

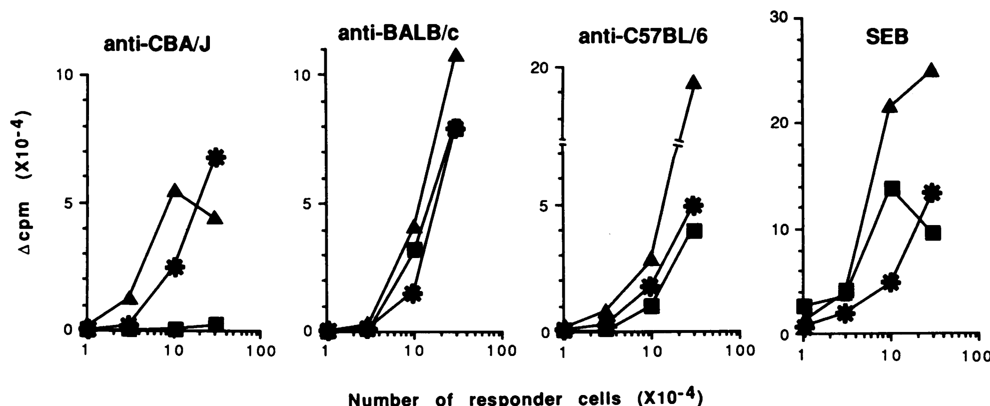

FIG. 3. Proliferation of lymph node T cells from nontransgenicm (*), *Mls-1^b^* V_β*8.1* transgenic (▲), and *Mls-1^a^* V_β*8.1* transgenic (■) mice against Mls-1^a^, allo-MHC, and SEB. Various numbers of nylon wool-nonadherent lymph node T cells (1×10^4–3×10^5 cells) were cultured with 5×10^5 irradiated spleen cells. SEB was added at a final concentration of 10 μg/ml. Cells were cultured for 5 days and incorporation of [^{3}H]thymidine was assessed after a 16-hr incubation with 1 μCi of [^{3}H]thymidine. Data are expressed as the difference (mean cpm) between experimental and control (anti-CBA/Ca) responses (Δcpm). The nontransgenic control was a transgene-negative litter of a founder mouse. H-2 types: nontransgenic, H-2^{kk}; *Mls-1^b^* V_β*8.1* transgenic, H-2^{kk}; *Mls-1^a^* V_β*8.1* transgenic, H-2^{qk}.

Table 1. Proliferative responses of $CD4^+CD8^-$ T cells from *Mls-1*^{a} and *Mls-1*^{b} $V_\beta 8.1$ transgenic mice to TCR occupancy

Stimulator	H-2 type	Mls-1 type	Proliferation of T cells, cpm		
			Nontrans	*Mls-1*^{b}	*Mls-1*^{a}
CBA/Ca	k	b	442	1,787	310
CBA/J	k	a	24,316	96,032	581
C57BL/6	b	b	14,320	9,176	9,642
SEB	—	—	14,646	98,071	62,193
Anti-TCR mAb	—	—	16,633	91,748	50,158

Nylon wool-purified T cells from each mouse were treated with an anti-CD8 mAb and complement for purification of $CD4^+CD8^-$ T cells. Responder cells (1×10^5) were cultured with irradiated spleen cells from various mouse strains, SEB, or anti-TCR $\alpha\beta$ mAb. Results are expressed as mean of triplicate experiments. The nontransgenic (nontrans) mice were littermates of the transgenic founder mouse. The responder mice were H-2^{qk}.

nonadherent T cells from *Mls-1*^{a} $V_\beta 8.1$ transgenic mice with an anti-CD8 mAb and complement and evaluated the remaining $CD4^+CD8^-$ T cells (Table 1). As with the whole T-cell cultures, $CD4^+CD8^-$ T cells from *Mls-1*^{a} $V_\beta 8.1$ transgenic mice proliferated in response to stimulation with alloantigens, SEB, and the anti-TCR mAb and exhibited a selective unresponsiveness to Mls-1^a. The unresponsiveness of the these T cells to Mls-1^a could result from several receptor-related processes. The TCRs on the remaining $CD4^+CD8^-$ $V_\beta 8.1^+$ T cells in *Mls-1*^{a} $V_\beta 8.1$ transgenic mice may simply not have sufficient affinity for Mls-1^a to induce their proliferation. Alternatively, the T cells may be functionally unresponsive to all TCR-mediated stimulation. If this latter situation exists, the proliferation of the T cells to alloantigen, SEB, or the anti-TCR mAb might be mediated by the non-$V_\beta 8.1^+$ T cells that we detected on flow cytometric analysis. A third consideration is that the remaining $CD4^+$-$CD8^-$ $V_\beta 8.1^+$ T cells in *Mls-1*^{a} $V_\beta 8.1$ transgenic mice are specifically unresponsive to stimulation by Mls-1^a but are responsive to other forms of TCR occupancy.

$CD4^+CD8^-$ T-Cell Lines and Clones Isolated from $V_\beta 8.1$ Transgenic Mice. To more closely analyze the reactivity of the remaining $CD4^+CD8^-$ $V_\beta 8.1^+$ T cells in *Mls-1*^{a} $V_\beta 8.1$ transgenic mice, we isolated T cell lines from the transgenic population using coculture with allogeneic C57BL/6 spleen cells. These cell lines were expanded by selection with an antigen unrelated to the antigen of primary interest and represent expanded clones of cells from the $CD4^+CD8^-$ $V_\beta 8.1^+$ subset of *Mls-1*^{a} $V_\beta 8.1$ transgenic mice.

Proliferative responses of the cell lines were tested against CBA/Ca (autologous), CBA/J (Mls-1^a), C57BL/6 (alloantigen), and allogeneic SWR spleen cells (Table 2, experiment 1). To compare the responses of all cell lines, the ratio of anti-CBA/J response versus anti-C57BL/6 response was calculated. The T-cell line derived from *Mls-1*^{b} $V_\beta 8.1$ transgenic mice proliferated in response to both CBA/J and C57BL/6 spleen cells as expected. Surprisingly, the T-cell line derived from *Mls-1*^{a} $V_\beta 8.1$ transgenic mice also proliferated to both CBA/J and C57BL/6 spleen cells, although the ratio of proliferation against CBA/J versus C57BL/6 was less than that of the *Mls-1*^{b}-derived line. More than five independent cell lines isolated from *Mls-1*^{b} or *Mls-1*^{a} $V_\beta 8.1$ transgenic mice showed reactivity patterns similar to those shown in experiment 1 of Table 2 (data not shown).

To determine whether the reduced response of the *Mls-1*^{a}-derived T-cell line was mediated by a small number of T cells capable of responding to Mls-1^a or whether all T cells were reactive to Mls-1^a at a lower level, we isolated >30 clones from the cell line by limiting dilution. Every subclone proliferated in response to CBA/J stimulator cells as well as C57BL/6; data for two of the clones are shown in experiment 2 of Table 2. Thus, it appears that most $CD4^+$ T cells from *Mls-1*^{a} transgenic mice have a positive although reduced responsiveness to Mls-1^a.

DISCUSSION

In *Mls-1*^{a} $V_\beta 8.1$ transgenic mice, $CD4^+CD8^-$ T cells were severely decreased among peripheral T cells as well as thymocytes, consistent with the $V_\beta 8.1$ transgenic mice generated by Pircher *et al.* (31). However, it is intriguing that some $CD4^+CD8^-$ $V_\beta 8.1^+$ T cells persist in the periphery. Escape from clonal deletion has been observed in $\alpha\beta$ TCR transgenic mice that carry TCRs specific for a male antigen in the context of class I MHC molecules (32). In that case, spared T cells in male transgenic mice expressed very low levels of CD8 molecules. In contrast, in our $V_\beta 8.1$ transgenic mice, the remaining $CD4^+CD8^-$ $V_\beta 8.1^+$ T cells showed that they were able to proliferate in response to TCR stimulation with alloantigens, SEB, or anti-TCR mAb but were not reactive to primary stimulation with Mls-1^a. It is conceivable that the T cells do not have sufficient reactivity to Mls-1^a to induce their clonal deletion in the thymus and primary proliferative response *in vitro*. Low affinity for Mls-1^a and MHC may occur by pairing of $V_\beta 8.1$ chains with certain V_α chains, which render the TCR unreactive or poorly reactive to Mls-1^a.

This is an incomplete explanation, since we observed that alloreactive $CD4^+CD8^-$ $V_\beta 8.1^+$ T-cell lines and clones that we established from *Mls-1*^{a} $V_\beta 8.1$ transgenic mice did display significant reactivity to Mls-1^a *in vitro*. It is unlikely that the T-cell clones we have isolated represent rare members of the

Table 2. Proliferative response of T-cell lines and clones from $V_\beta 8.1$ transgenic mice

Stimulator	H-2 type	Mls-1 type	Response, cpm			
			Exp. 1: T-cell lines		Exp. 2: T-cell clones	
			53G-10 (*Mls-1*^{b})	60F10 (*Mls-1*^{a})	4E7	7D6
CBA/Ca	k	b	321	1,718	713	576
CBA/J	k	a	140,783	13,710	9,612	5,920
C57BL/6	b	b	221,483	110,630	37,405	20,950
SWR/J	q	a	1,216	4,758	ND	ND
J/B6 ratio*			0.63	0.11	0.24	0.26

Experiment 1: T-cell lines from $V_\beta 8.1$ transgenic mice (*H-2*^{qk}) were studied after culture for ≈1 month with stimulation by irradiated C57BL/6 spleen cells. Both cell lines were $CD4^+CD8^-$ $F23.1^+$. Responder cells (2×10^4) were cultured with 5×10^5 irradiated spleen cells for 3 days. Experiment 2: proliferative responses of T-cell clones isolated from the 60F10 line were assessed similarly. Results are expressed as mean of triplicate experiments. ND, not done.
*(Anti-CBA/J response − anti-CBA/Ca response)/(anti-C57BL/6 response − anti-CBA/Ca response).

population while other more representative T cells among the $CD4^+CD8^-$ $V_\beta 8.1^+$ subset in *Mls-1^a^ V*$_\beta$*8.1* transgenic mice do not have reactivity to Mls-1^a. We have independently isolated multiple cell lines and clones from *Mls-1*^{a} V_β*8.1* transgenic mice and every one of the clones displayed reactivity to Mls-1^a.

Based on the reactivity pattern of many T-cell clones, we think it is most likely that $CD4^+CD8^-$ $V_\beta 8.1^+$ T cells in *Mls-1*^{a} V_β*8.1* transgenic mice have reduced but significant functional reactivity to Mls-1^a. They have escaped clonal deletion in the thymus and have migrated to peripheral lymphoid organs. These cells are functionally unresponsive in primary *in vitro* assays. Mls-1^a affinity of the TCR on these T cells may not be sufficient to activate virgin T cells or cause their deletion in the thymus, although it is sufficient to induce proliferation of alloantigen-primed T-cell lines. Alternatively, the escaped T cells might have been rendered functionally unresponsive to Mls-1^a.

We believe that this type of escape from clonal deletion may occur in normal animals. Studies of clonal deletion in normal mice using flow microfluorimetric analysis of T-cell populations with anti-TCR V_β-specific mAbs revealed the presence of minor populations of T cells bearing potentially self-reactive TCR V_β in the periphery of "deleted" animals (3–5). In addition, autoreactive cells can be isolated following selection of immune T cells specific for some antigens, and it has been suggested that some autoreactive T cells are derived from antigen-stimulated precursors (33–35).

Our results suggest that potentially self-reactive T cells can escape clonal deletion in the thymus and gain self-reactivity *in vitro* after stimulation with unrelated antigens. It remains to be determined whether the reactivation process occurs frequently *in vivo*. If so, these cells, which are unresponsive to self-antigen *in vivo*, could be activated by environmental antigens and, once activated, would be reactive to self antigens, resulting in autoimmunity.

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