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A cortisone sensitive $CD3^{low}$ subset of $CD4^+CD8^-$ thymocytes represents an intermediate stage in intrathymic repertoire selection

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Abstract

Two populations of CD4 single positive (SP) thymocytes were found in transgenic mice bearing class I-restricted Mls-1^a reactive ($V_\beta 8.1$) TCR genes in the absence of the restriction element. $CD3^{high}$ CD4 SP cells were deleted in the presence of Mls-1^a and were cortisone resistant, whereas $CD3^{low}$ CD4 SP cells were not deleted in the presence of Mls-1^a and were cortisone sensitive. Intravenous transfer of $CD3^{low}$ CD4 SP cells into nude mice resulted in significant peripheral expansion of these cells with apparent upregulation of CD3. These data indicate that $CD3^{low}$ CD4 SP thymocytes represent an intermediate stage in the transition from $CD3^{low}$ double positive (DP) to $CD3^{high}$ SP thymocytes and raise the possibility that these cells may have undergone positive but not negative selection events (at least to Mls-1^a). Furthermore the fact that $CD3^{high}$ DP thymocytes were also deleted by Mls-1^a in these mice suggests strongly that sensitivity to Mls-1^a deletion is dependent upon stage of thymic maturation (as revealed by TCR density) rather than CD4/CD8 phenotype.

Introduction

During T cell development in the murine thymus the T cell repertoire is both negatively selected to delete potentially self reactive clones (1–3) and positively selected to allow antigen recognition in the context of self MHC molecules in the periphery (4–6). In order to study the developmental sequence of such T cell repertoire selection, we have produced transgenic mice carrying the TCR $\alpha\beta$ chains $V_\alpha 2-J_\alpha TA31$ (α_T) and $V_\beta 8.1DJ_\beta 2.4$ (β_T) specific for lymphocytic choriomeningitis virus (LCMV) presented in the context of H-$2D^b$ (7). Positive selection of the transgenic TCR and the resultant skewing of thymocytes and peripheral T cells to the CD8 lineage in these mice had previously been shown to be dependent on the presence of the H-2^b molecule (7,8). Furthermore, negative selection via clonal deletion was found to occur at an early stage of intrathymic development when the transgenic TCR was expressed in H-2^b LCMV carrier mice (7,8). Collectively these data are compatible with other recent studies of repertoire selection employing MHC restricted transgenic TCR specific for antigens such as H-Y and H-2 L^d (reviewed in ref. 9).

In the present study, we have extended our analysis of these TCR transgenic mice by crossing them on a BALB/c (H-2^d, i.e. non-selective) background in the presence or absence of the minor lymphocyte stimulatory antigen Mls-1^a. Since the β_T encodes a Mls-1^a reactive $V_\beta 8.1$ domain (10,11), such mice should allow analysis of the developmental sequence of Mls-1^a specific clonal deletion in the absence of a dominant positive selection. We show here that $CD4^+CD8^+$ 'double positive' (DP) as well as $CD4^+CD8^-$ or $CD4^-CD8^+$ 'single positive' (SP) thymocytes expressing high levels of β_T are deleted by Mls-1^a. In addition we document the existence of a cortisone sensitive subset of CD4 SP thymocytes expressing low levels of β_T that is not deleted by Mls-1^a. The implications of this latter subset for T cell repertoire selection and lineage commitment will be discussed.

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Methods

Animals

Congenic BALB.D2.Mls^a (H-2^d, Mls-1^a) mice were maintained from breeding pairs kindly provided by Dr. H Festenstein (12). BALB/c nude mice and C57BL/6 nude mice were supplied by BOM (Denmark). TCR$\alpha\beta$ transgenic mice were as described (7). Severe combined immunodeficiency (SCID) mice on a H-2^d (BALB/c) background were supplied by Iffa Credo. F$_1$ and F$_2$ hybrids were bred locally.

mAbs and FACS analysis

Cytotoxic rat IgM mAbs against CD8 (3.168.1) and against Thy-1 (AT83) were used in the presence of rabbit complement to deplete thymocytes of CD8$^+$ cells prior to sorting for CD4 SP thymocytes and to deplete spleen cell suspensions of T cells respectively.

For three-colour immunofluorescence, aliquots of thymocytes or lymph node (LN) cells were stained at 4°C with rat mAbs KJ16 133 (anti-V$_\beta$8.1/8.2; ref. 13), 17A2 (anti-CD3; ref. 14), or B20.1 (anti-V$_\alpha$2; B. Malissen, unpublished data), followed by a FITC-conjugated goat anti-rat second reagent. An additional incubation with phycoerythrin (PE)-conjugated anti-CD4 and biotinylated anti-CD8 (Becton-Dickinson) was followed by avidin-TANDEM (Southern Biotechnology). Samples were analysed on a FACScan (Becton-Dickinson). Fluorescent histograms for V$_\beta$, V$_\alpha$, or CD3 staining were obtained after gating on thymocyte subpopulations defined by CD4 and CD8 staining. In all cases representative histograms are shown from an analysis of at least four mice. Only data of viable cells were collected, using a live gate by a combination of forward and 90° light scatter.

Cell Culture

All cell culture was carried out in Dulbecco's modified Eagle's medium supplemented with 5% FBS and 5×10^{-5} M 2-mercaptoethanol. Sorted CD4 SP thymocytes were cultured for 20 or 40 h at a concentration of 1.5×10^6 cells/ml in 250 μl medium.

Hydrocortisone injection

Two week old mice were injected i.p. with 150 μl hydrocortisone acetate suspension at 25 mg/ml (Steuli & Co., Switzerland) and killed 2 days later. Six week old mice were injected i.p. with 250 μl suspension and were killed at 2, 5, 6, and 7 days after injection.

IL-2 and IFN-γ production

Responder cells from LN were cultured at 2×10^6 cells/ml in 2 ml in 24-well plates. Cultures were stimulated with 4.5×10^6 T cell depleted irradiated (1000 rad) spleen cells from BALB.D2.Mls^a or BALB/c mice. Supernatants were collected after 72 h for analysis of their IL-2 or IFN-γ content.

IL-2 assay

IL-2 levels were determined using an IL-2-dependent (IL-4 non-responsive) subline of CTLL-2 and a colorimetric assay based on the production of hexosaminidase. Units per millilitre of sample were calculated by comparing titrations of test samples with a standard curve of human recombinant IL-2.

IFN-γ assay

IFN-γ levels were determined using an ELISA assay (15). The rat mAb R4-6A2 was used as the coating antibody and the mAb AN-18.17.24 was used as the detecting antibody. Horseradish peroxidase labelled streptavidin–biotin complex (BRL, Gaithersburg, MD) was used to detect binding of biotin-labelled AN-18.17 24 to IFN-γ. Recombinant mouse IFN-γ was used as a standard.

Injection of nude mice

Five week old nude mice were injected i.v. with 5×10^5 CD4 SP thymocytes in 250 μl PBS, prepared from 1 month old BALB.D2.Mls^a TCR transgenic mice by antibody and complement deletion of CD8$^+$ cells followed by sorting for CD4$^+$ cells on a FACS. Nude mice were killed 6 weeks after injection and LN cell suspensions were analysed for TCR expression by three-colour flow cytometry as described above.

Results

Two subsets of CD4 SP thymocytes in TCR transgenic mice

Three-colour immunofluorescence revealed two distinct populations of CD4 SP thymocytes in 2 week old BALB/c transgenic mice (non-selective background) that differed in CD3 and β_T density (Figs 1 and 2). Of the CD4 SP cells, 50% were CD3/β_T^{high} and 45% were CD3/β_T^{low}. In the CD8 SP subset 80–90% of cells were CD3/β_T^{high} and the remainder CD3/β_T^{low}. The proportion of CD3/β_T^{low} cells in both SP subsets decreased with age (data not shown; see also Fig. 5). Expression of α_T was very low on most SP thymocytes (Fig. 2).

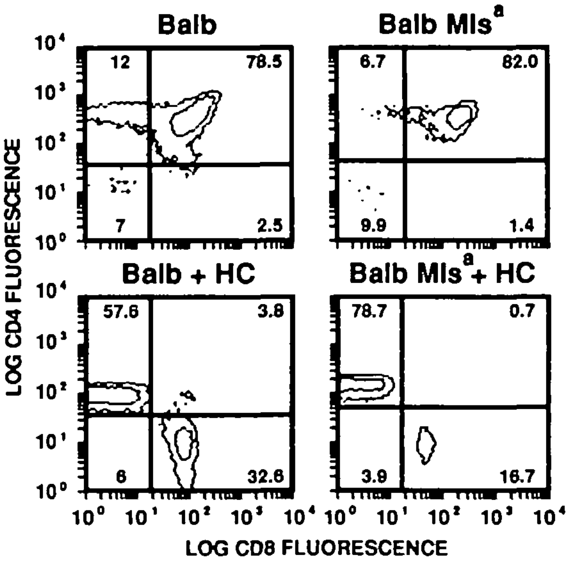

Fig. 1. Thymus subsets of TCR transgenic mice. Control or hydrocortisone (HC) resistant thymocytes from 2 week old TCR transgenic mice on a BALB/c or BALB.D2.Mls^a background were stained with mAbs directed against CD4 and CD8, and analysed by flow cytometry. The average cellularity of the thymuses were 1.7×10^8 (BALB/c), 1.7×10^8 (BALB.D2.Mls^a), 5.6×10^6 (BALB/c + HC), and 4.2×10^6 (BALB.D2 Mls^a + HC).

Deletion of CD3/β_T^{high} SP thymocytes in the presence of Mls-1^a

The CD3/β_T^{high} cells in both CD4 and CD8 SP thymocyte populations were deleted when the BALB/c transgenic mice were crossed with BALB.D2.Mls^a, whereas the CD3/β_T^{low} SP thymocytes were not deleted (Fig. 2).

Hydrocortisone sensitivity of CD3/β_T^{low} SP thymocytes

All CD3^{low} SP thymocytes were cortisone sensitive and those SP thymocytes remaining after cortisone treatment of BALB/c transgenic mice expressed high levels of both β_T and CD3 (Fig. 3). In contrast, most of the cortisone resistant SP thymocytes expressed low to intermediate levels of α_T and $<10\%$ stained brightly with the antibody against V$_\alpha$2 (Fig. 3). Assuming that each TCR β-chain and CD3 molecule must be associated with an α-chain, it follows that at least 90% of the cortisone resistant SP thymocytes in these mice expressed two α-chains on their cell surface: low to intermediate levels of the transgenic α-chain in addition to high levels of an endogenously rearranged α-chain(s). The presence of two α-chains on the cell surface of hybridomas derived from T cell clones from TCR transgenic mice has previously been demonstrated by immunoprecipitation (9).

When cortisone was administered to the Mls-1^a transgenic mice all remaining cells were CD3^{high} but there were few CD4 SP cells which stained brightly with the antibodies against the transgenic α- or β-chains (Fig. 3). These few bright CD4 SP cells (10 – 15%) might in fact express endogenously rearranged V$_\beta$8 or V$_\alpha$2 genes recognized by the same antibodies, since CD4 SP thymocytes from non-transgenic littermates expressed V$_\beta$8 and V$_\alpha$2 at similar levels (data not shown). The greater proportion of V$_\beta$8^{high} cells remaining in the CD8 SP population after cortisone treatment may reflect the tendency of cells expressing this V$_\beta$8 gene segment to be positively selected towards the CD8 lineage (11).

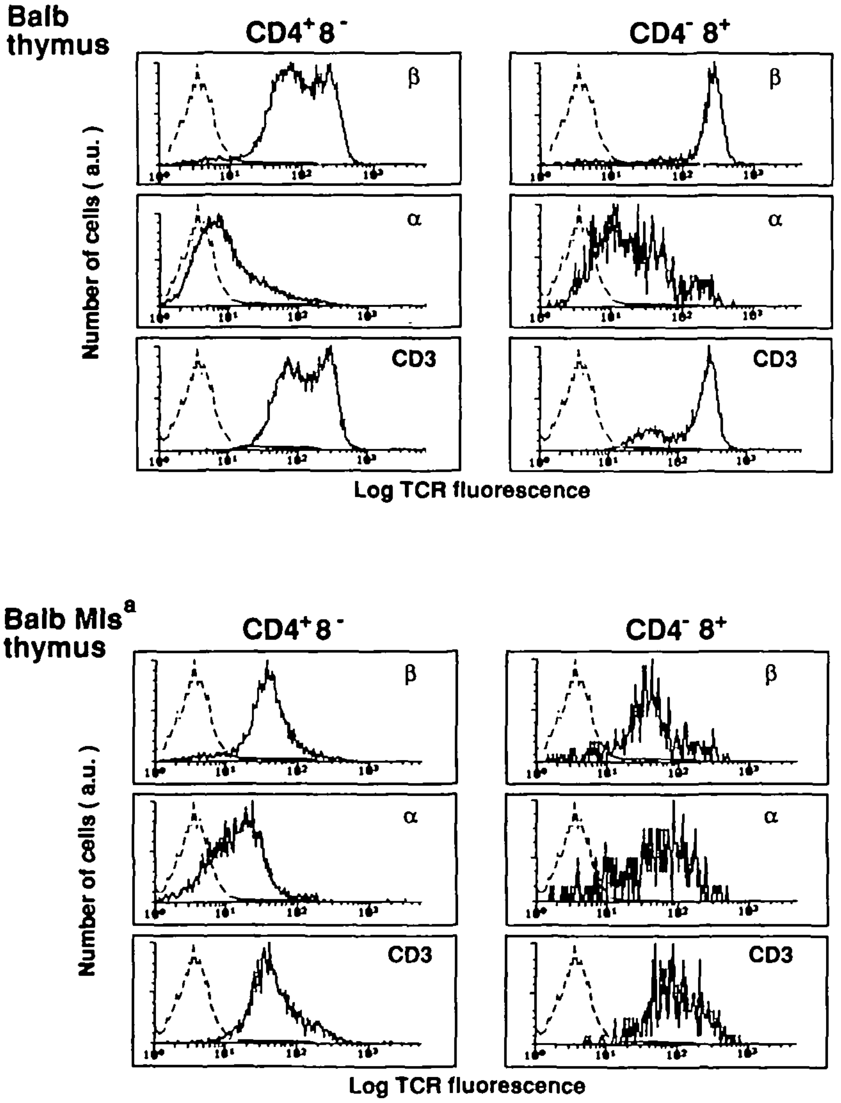

Fig. 2. Expression of transgenic TCRα and TCRβ chain and CD3 by SP thymus subsets Thymocytes from 2 week old TCR transgenic BALB/c or BALB.D2.Mls^a mice were triple stained with mAbs against CD4, CD8, and either TCRα chain, TCRβ chain, or CD3. Data shown are gated for CD4$^+$CD8$^-$ or CD4$^-$CD8$^+$ subsets (see Fig. 1).

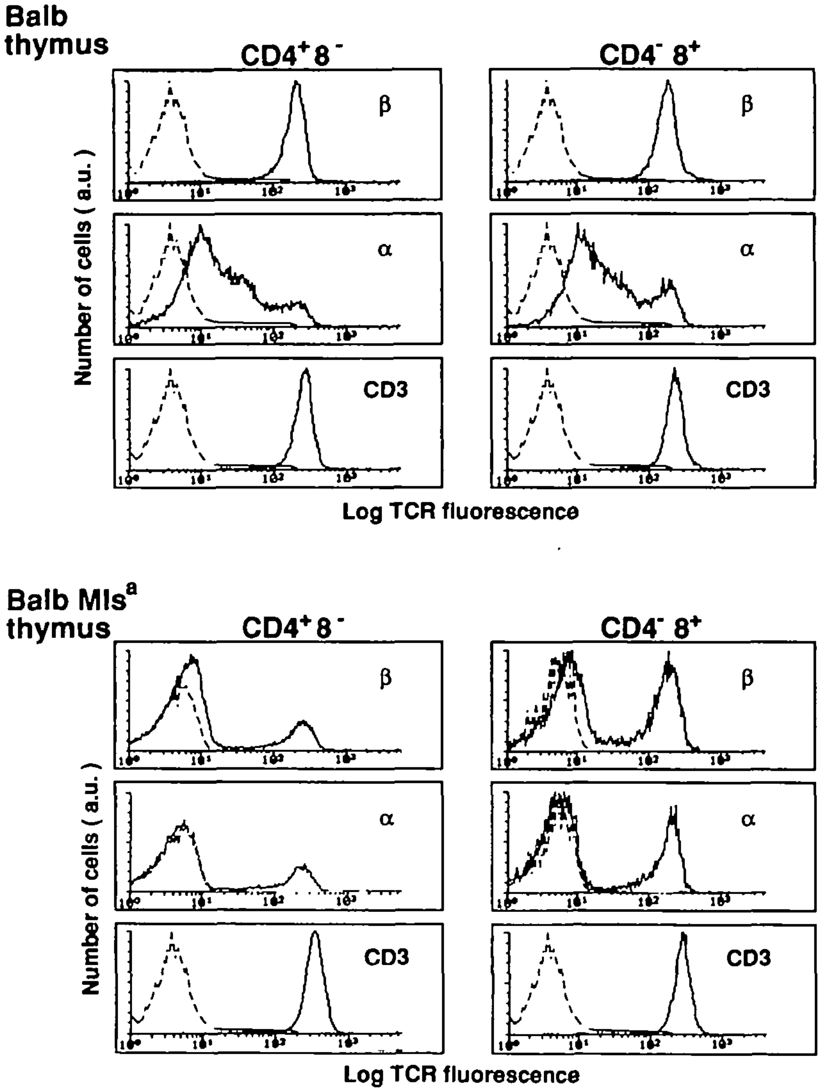

Fig. 3. Expression of transgenic TCRα and TCRβ chain and CD3 by SP thymus subsets of hydrocortisone-treated TCR transgenic mice on a BALB/c or BALB.D2.Mls^a background. Thymocytes were stained and gated as described in Fig 2

CD3^{low} CD4 SP cells are a post-DP stage of development

The cortisone sensitivity of the CD3^{low} CD4 SP cells led us to ask if these cells in fact represented a pre-DP stage of development (16,17), which expressed the transgenic TCR abnormally early. Three pieces of evidence suggested that this was not the case. First, when sorted CD4 SP thymocytes from a BALB.D2.Mls^a transgenic mouse were cultured for 20 or 40 h no differentiation into DP thymocytes was observed (data not shown). Second, no CD3^{low} CD4 SP thymocytes were found in 2 week old H-2^d SCID-TCR transgenic mice (Fig. 4) and if such cells were pre-DP and hence not dependent on positive selection there is no *a priori* reason why they should be absent from such mice. Third, we compared the kinetics of regeneration of thymocyte populations after cortisone treatment of adult BALB/c transgenic mice (Fig. 5). Two days after cortisone treatment only CD3/β_T^{high} SP and DP cells were present. By day 5, however, the majority of DP thymocytes expressed low levels of TCR, as did 15% of CD8 SP, but the CD4 SP cells were still CD3^{high}. By the seventh day after treatment a CD3^{low} CD4 SP population was clearly visible and TCR expression in the DP thymocytes had returned to the usual distribution, with ~15% CD3/β_T^{high}. Thus, CD3^{low} DP cells reappeared well before the CD3^{low} CD4 SP cells, suggesting that the CD3^{low} CD4 SP cells were not at a pre-DP stage of development.

Deletion of Mls-1^a can occur when thymocytes are DP

Between 10 and 15% of DP thymocytes of all BALB/c transgenic mice examined were CD3/β_T^{high} and α_T^{low}, suggesting that they expressed endogenously rearranged α-chains on their cell surfaces. These cells were nearly all deleted in the presence of Mls-1^a (Fig. 6) and a proportion were cortisone resistant (Fig. 5).

TCR expression in LN T cells of transgenic mice

In an attempt to assess the developmental potential of the CD3^{low} SP thymocytes, we next examined TCR expression in

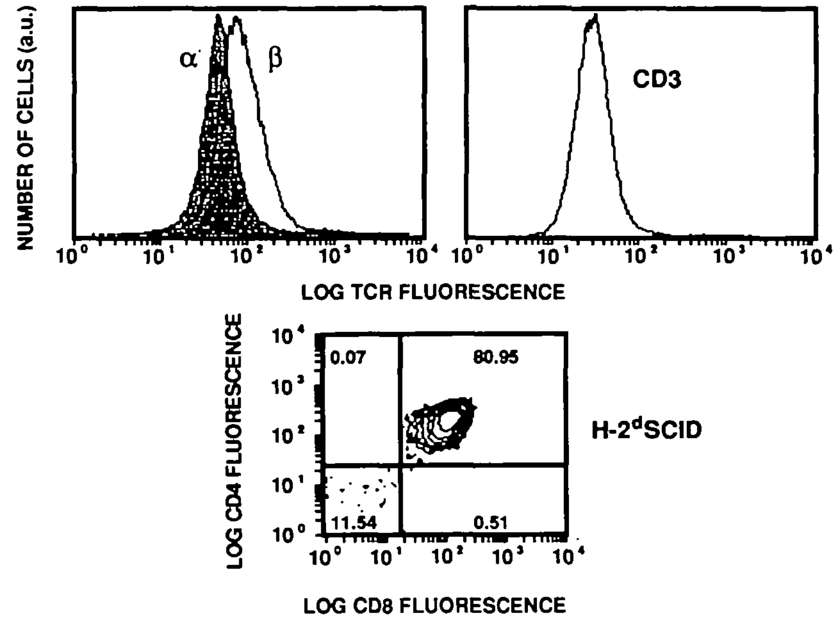

Fig. 4. Expression of transgenic TCRα (shaded) and β (open) chains and CD3 by DP thymocytes from SCID mice on a BALB/c background. Thymocytes from 2 week old TCR transgenic SCID mice were triple stained with mAbs against CD4, CD8, and TCR (α, β, or CD3). The upper panels are gated on the DP subset.

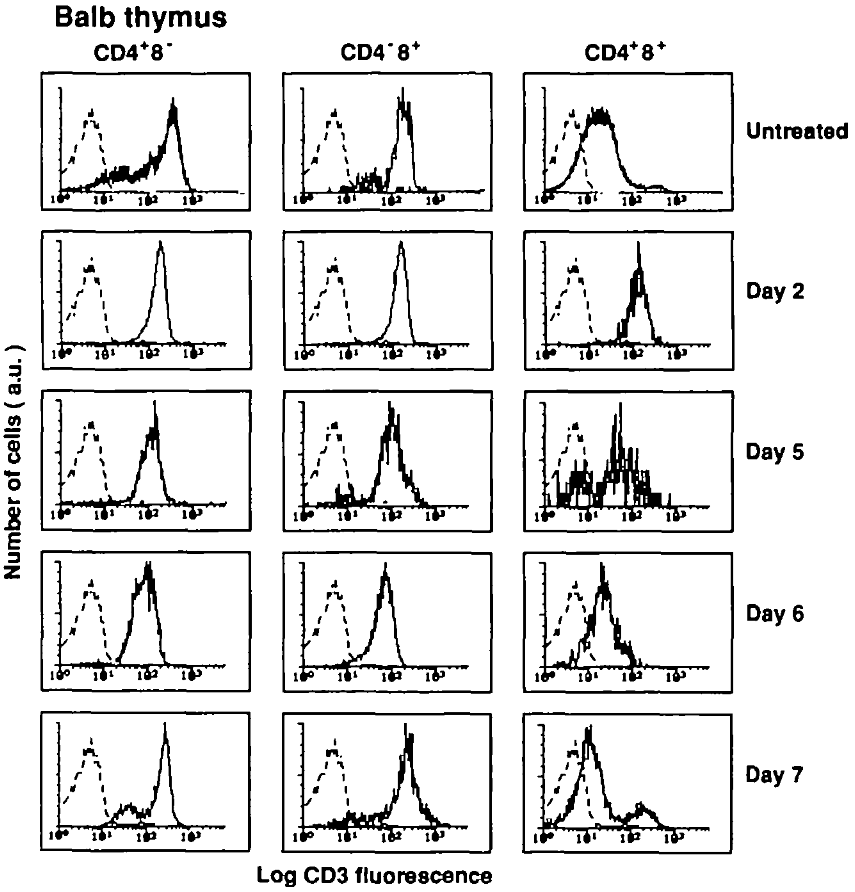

Fig. 5. Expression of CD3 by SP and DP thymocytes of control or hydrocortisone-treated TCR transgenic BALB/c mice (2, 5, 6, and 7 days after cortisone treatment). Thymocytes from adult (6 week old) mice were triple stained with mAbs against CD4, CD8, and CD3, and data are shown gated for CD4$^+$CD8$^-$, CD4$^-$CD8$^+$, and CD4$^+$CD8$^+$ subsets.

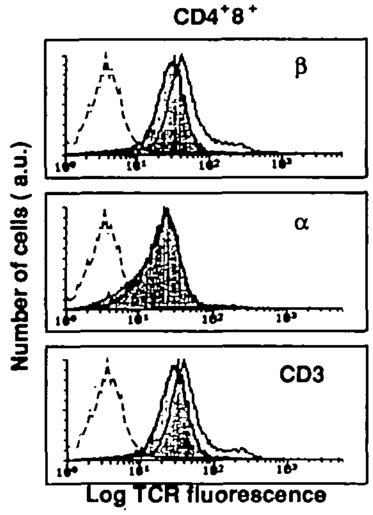

Fig. 6. Expression of transgenic TCRα and TCRβ chain and CD3 by DP thymocytes from BALB.D2.Mls^a (shaded) and BALB/c (open) mice, stained as in Fig. 2 The proportion of cells with high transgenic β-chain expression was 13% in BALB/c mice and 1.5% in BALB.D2 Mls^a mice.

LN T cells of adult Mls-1^a transgenic mice and control BALB/c transgenic mice (Fig. 7). Surprisingly, in the Mls-1^a transgenic mice, 55% of CD4$^+$ and 85% of CD8$^+$ LN T cells expressed high levels of β_T. As expected, α_T expression was low on most of these cells (Fig. 7) and CD3 expression was homogeneously high. The ratio of CD4:CD8 in BALB.D2.Mls^a LN T cells was 1:3.5 (compared to 1.8:1 in control BALB/c). Despite expression of high levels of β_T, LN T cells from the Mls-1^a transgenic mice were non-responsive to Mls-1^a, as assessed by their abilities to proliferate and to produce both IFN-γ and IL-2 (Table 1). Thus it is possible that these CD3/β_T^{high} SP cells in LN were derived from CD3/β_T^{low} SP cells that escaped deletion in the Mls-1^a thymus and subsequently upregulated their TCR in the periphery.

Transfer of CD3^{low} CD4 SP thymocytes to nude mice

More direct evidence that CD3^{low} CD4 SP thymocytes were not just a dead-end lineage, but were capable of some maturation outside the thymus, was obtained from experiments in which sorted CD4 SP thymocytes from Mls-1^a transgenic mice were transferred into C57BL/6 (H-2^b) nude mice or BALB/c (H-2^d) nude mice. Six weeks after i.v. injection of 5 × 10^5 cells, the

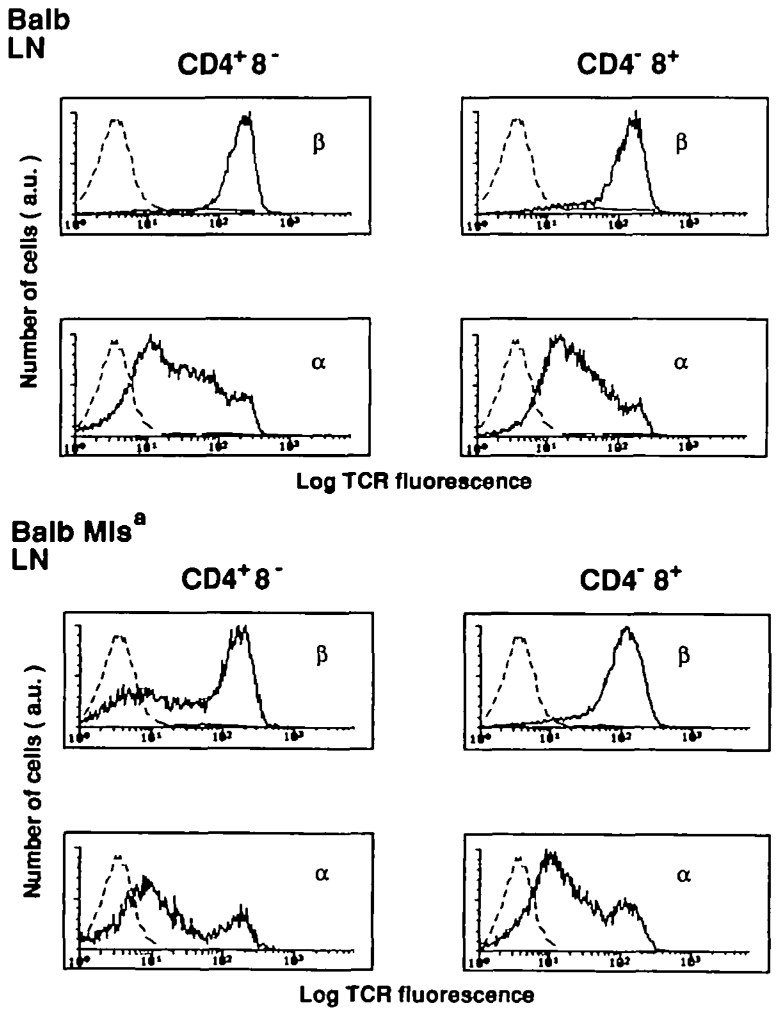

Fig. 7. Expression of transgenic TCRα and TCRβ chain by CD4$^+$ and CD8$^+$ T cells from LN of BALB.D2.Mls^a and BALB/c mice. Lymphocytes were triple stained as in Fig. 2 and data are shown gated for CD4$^+$ or CD8$^+$ populations. All CD4$^+$ and CD8$^+$ T cells were CD3^{high} (not shown).

mice were killed and LN cells were analysed for transgenic TCR expression. On average 9.8 × 10^6 (±6 × 10^5) cells were recovered from the C57BL/6 nude mice and 2.9 × 10^7 (±4 × 10^5) cells were recovered from the BALB/c nude mice, as opposed to 6 × 10^6 and 5 × 10^6 from the respective non-injected control mice. Double staining with antibodies against Thy-1 and H-2K^b indicated that in the C57BL/6 nude mice at most 6% of T cells were of transgenic origin. About 5% of the recovered T cells expressed low levels of α_T and β_T, and there were few CD3^{high} cells. In the BALB/c nude mice, however, half of CD4$^+$ T cells expressed high levels of CD3 and the remainder were CD3^{low} (Fig. 8). The β_T expression followed a similar pattern, with 40% high and 50% low. Approximately 80% of these cells expressed low levels of α_T. In contrast CD4$^-$CD8$^-$ cells in the injected BALB/c nude LNs were negative for staining with V$_\beta$8 and V$_\alpha$2 specific mAbs (data not shown). Furthermore, no significant V$_\beta$8 or V$_\alpha$2 staining was seen in CD4$^+$ LN cells from age-matched control (uninjected) BALB/c nude mice. Given these data, it seems reasonable to conclude that the CD4$^+$ T cells recovered after transfer were of transgenic donor origin, despite the absence of a strain specific marker.

Discussion

The data presented here demonstrate that two subsets of CD4 SP thymocytes can be clearly distinguished in TCRαβ transgenic mice in the absence of the appropriate positively selecting MHC restriction element. Comparison of staining patterns of mAbs directed against the transgenic α- or β-chains with CD3 indicated that one subset expresses CD3 and β_T at high levels (paired with a putative endogenous α-chain) while the other subset expresses low levels of CD3 and β_T. The CD3/β_T^{high} CD4 SP subset is completely deleted in the thymus of mice expressing Mls-1^a but is not sensitive to *in vivo* cortisone treatment. Reciprocally the CD3/β_T^{low} subset resists deletion by Mls-1^a but is ablated by cortisone.

The relationship of these CD4 SP subsets to those defined so far in normal mice is not clear. Normal CD4 SP thymocytes are heterogeneous with respect to expression of a number of phenotypic markers (reviewed in ref. 18) but are generally considered to be homogeneously CD3^{high} and cortisone resistant. Nevertheless several studies have documented a partial sensitivity of CD4 SP thymocytes to hydrocortisone *in vivo* (19,20). Of particular relevance to the data presented here, Guidos *et al.* (21) have recently used intrathymic transfer of purified DP blast cells to identify a CD4 SP thymocyte subset that expresses low levels of CD3 (see below).

The timing of intrathymic deletion by Mls-1^a has been a subject of some controversy. In this regard, recent studies from two groups (22,23) have shown that DP V$_\beta$6^{high} cells are deleted in the thymus of normal Mls-1^a bearing mice, whereas DP V$_\beta$6^{low} cells are unaffected, thus arguing in favour of deletion occurring at a late DP stage. On the other hand, Guidos *et al.* (21) have identified CD4 SP thymocytes with a V$_\beta$6^{low} phenotype that are not deleted in Mls-1^a mice following intrathymic transfer of DP blasts, and Schneider *et al.* (24) have found cells with a similar phenotype (V$_\beta$6^{low} CD4 SP) in the early post-natal Mls-1^a thymus. In both instances V$_\beta$6^{high} CD4 SP thymocytes were deleted by Mls-1^a, supporting models in which Mls-1^a deletion occurs at a SP stage. In transgenic models, Pircher *et al.* (7,11) observed significant deletion of CD3/V$_\beta$8.1^{high} T cells by Mls-1^a in the thymus and periphery of both β and αβ transgenic mice on a positively selecting (H-2$^{b/d}$) background; however, these cells were not further analysed for CD4/CD8 phenotype. In con-

Table 1. LN T cells from TCR transgenic BALB.D2.Mls^a mice are non-responsive to Mls-1^a *in vitro*

Responding LN cells	IL-2 (U/ml)		IFN-γ (U/ml)	
	BALB.D2.Mls^a	BALB/c	BALB.D2.Mls^a	BALB/c
BALB/c	42.8 ± 6.7	<0.1	39.6 ± 5 4	<1
BALB.D2 Mls^a	0.4 ± 0.2	<0.1	<1	<1

LN cells from 6 week old TCR transgenic mice on a BALB/c or BALB.D2.Mls^a background were cultured with irradiated T cell depleted spleen cells from normal BALB/c or BALB.D2.Mls^a mice. After 48 h supernatants (SNS) were collected and assayed for IL-2 or IFN-γ. Data are presented as mean ± SD of four individual mice.

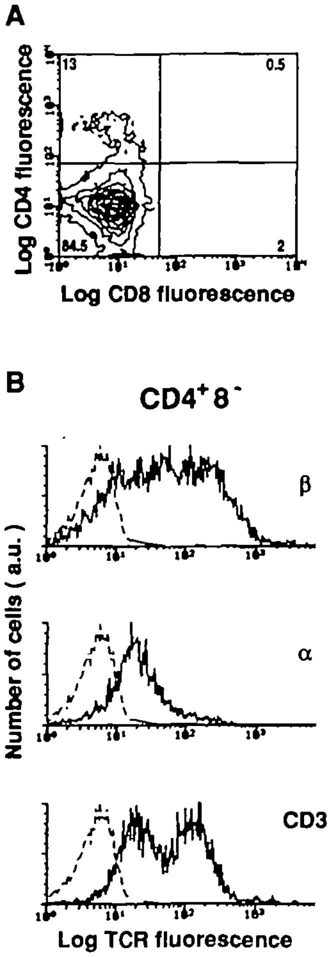

Fig. 8. (A) Expression of CD4 and CD8 by LN cells from BALB/c nude mice 6 weeks after injection with CD3^{low} CD4 SP thymocytes from BALB.D2.Mls^a TCR transgenic mice. (B) Expression of transgenic TCRα and TCRβ chain and CD3 by CD4$^+$CD8$^-$ cells as above. Cells were triple stained as in Fig. 2 and data are shown gated for CD4$^+$CD8$^-$.

trast, Blackman *et al.* (25) found little evidence for clonal deletion by Mls-1^a in an independent V$_\beta$8.1 transgenic model. The present data confirm and extend these previous reports by demonstrating that CD3/β_T^{high} (but not CD3/β_T^{low}) cells are deleted by Mls-1^a irrespective of their DP or SP phenotype. We therefore favour the hypothesis that clonal deletion by self superantigens such as Mls-1^a occurs at a stage of thymic development corresponding to the acquisition of high TCR density.

Although CD3/β_T^{high} SP cells were not detectable in the thymus of BALB.D2.Mls^a mice such cells accounted for the majority of both CD4 SP and CD8 SP subsets in the periphery. Nevertheless no specific response of these CD3/β_T^{high} cells to Mls-1^a (assessed by proliferation or lymphokine production) could be detected *in vitro*. These findings are reminiscent of other recent reports in which encounter of T cells with Mls-1^a, either in the thymus or periphery, was found to result in subsequent functional non-responsiveness (25 – 27). In the present case lack of Mls-1^a responsiveness could reflect either anergy or functional immaturity of the CD3/β_T^{high} population.

An interesting property of CD3/β_T^{low} CD4 SP thymocytes was their ability to partially reconstitute young athymic mice upon i.v. transfer. Previous studies have shown that mature peripheral T cells are capable of extensive proliferation in syngeneic athymic mice, ultimately accounting for 10 – 15% of total cells in spleen or LN (28). In the present study, 13% ($\sim 4 \times 10^6$) of recovered nude LN cells were CD4 SP following transfer of 5×10^5 CD3/β_T^{low} BALB.D2.Mls^a CD4 SP thymocytes to congenic BALB/c nude mice, indicating that considerable expansion of the inoculum had occurred. About 50% of the recovered CD4 SP cells were CD3/β_T^{high} and the remainder were CD3/β_T^{low}. Thus it is clear that at least some cells with a CD3/β_T^{low} CD4 SP phenotype are capable of survival (and expansion) in the periphery. Furthermore it seems likely that CD3/β_T^{high} CD4 SP cells are derived from these CD3/β_T^{low} CD4 SP cells via TCR upregulation (although it cannot be formally excluded that these cells arise from rare CD3/β_T^{high} cells that escape deletion in the Mls-1^a thymus). It is worth noting that the failure of CD3/β_T^{low} CD4 SP thymocytes to survive upon transfer to C57BL/6 (H-2^b) nude mice raises the possibility that these cells had already become restricted to H-2^d (presumably class II MHC) molecules in the BALB.D2.Mls^a thymus. However, other interpretations of these data (including peripheral clonal deletion upon exposure to the H-2D^b restriction element) remain tenable.

If CD3/β_T^{low} CD4 SP thymocytes are in fact positively selected (as suggested by their CD4 SP phenotype and ability to expand in a H-2^d environment), it would follow that these cells represent a stage of thymic development that has undergone positive but not negative selection events. A similar 'transitional phenotype' has been noted by Guidos *et al.* (21) for CD3^{low} CD4 SP thymocytes developing in normal mice shortly after intrathymic transfer of DP blasts. In contrast, Benoist and Mathis (29) could not find any evidence for positive selection (assessed by V$_\beta$ usage) among CD3^{low} CD4 SP thymocytes in normal mice, although positive selection was evident in the CD3^{high} CD4 SP subset. Our own previous studies in normal mice (6) could not directly address this issue since we only looked for positive selection among the CD3^{high} cortisone-resistant thymocyte population.

Finally, it should be noted that the identification of CD3^{low} and CD3^{high} subsets of CD4 SP thymocytes has implications for lineage models of thymocyte development. Current evidence from a variety of sources favours the hypothesis that CD4 SP and CD8 SP thymocytes are derived from a common DP precursor (reviewed in ref. 18). Within the framework of such a model, two (non-mutually exclusive) alternative pathways of thymic development can be envisaged to explain our data. Assuming that TCR upregulation is irreversible during development, fully mature CD3^{high} SP thymocytes could be derived from CD3^{low} DP precursors via either CD3^{high} DP or CD3^{low} SP intermediates. The coexistence of both pathways would suggest that positive selection (as manifested by DP → SP transition) does not necessarily require concomitant upregulation of surface TCR expression and that the latter phenomenon may occur as a rather late event in T cell development.

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Abbreviations

α_T	transgenic α-chain
β_T	transgenic β-chain
DP	double positive
LCMV	lymphocytic choriomeningitis virus
LN	lymph node
PE	phycoerythrin
SCID	severe combined immunodeficiency
SP	single positive

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