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An NK1.1$^+$ CD4$^+$8$^-$ single-positive thymocyte subpopulation that expresses a highly skewed T-cell antigen receptor V_β family

(T lymphocytes/bone marrow cells/aging)

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ABSTRACT **In the present report we describe a CD4$^+$8$^-$ heat stable antigen-negative (HSA$^-$) thymocyte subpopulation that expresses a distinguishably low density of $\alpha\beta$ T-cell antigen receptors (TCR^{lo}) from the majority of CD4$^+$8$^-$ high-density TCR (TCR^{hi}) mature-type thymocytes. This subpopulation appears relatively late in life. Analysis of MEL-14, Pgp-1 (CD44), ICAM-1 (CD54), and NK1.1 expression on this subpopulation revealed that the CD4$^+$8$^-$ TCR^{lo} population was a population having unique characteristics (MEL-14$^-$, CD44$^+$, ICAM-1$^+$, and NK1.1$^+$) compared to the CD4$^+$8$^-$ TCR^{hi} thymocytes, most of which are MEL-14$^+$, CD44$^-$, ICAM-1$^-$, and NK1.1$^-$. When TCR β-chain variable region (V_β) usage was analyzed, this thymic population expressed predominantly products of V_β7 and V_β8.2 TCR gene families. Interestingly, cells with V_β8.1 TCRs, which are reactive to Mls-1^a antigens, were not eliminated from the CD4$^+$8$^-$ HSA$^-$ TCR^{lo} subpopulation but had been eliminated from the major CD4$^+$8$^-$ HSA$^-$ TCR^{hi} subpopulation in Mls-1^a strains. A subset with a phenotype similar to the CD4$^+$8$^-$ HSA$^-$ TCR^{lo} thymocytes was also identified primarily in bone marrow, and this subset constituted approximately half of the CD4$^+$ T cells in the bone marrow. The CD4$^+$8$^-$ HSA$^-$ TCR^{lo} cells showed extremely high proliferative responses to immobilized anti-TCR antibody but generated negligible responses to allogeneic H-2 antigens compared to the responses generated by the major CD4$^+$8$^-$ HSA$^-$ CD3^{hi} cells. However, the CD4$^+$8$^-$ HSA$^-$ TCR^{lo} cells in Mls-1^b mice mounted vigorous proliferative responses to Mls-1^a antigens but not in Mls-1^a mice. The properties of this T-cell subset suggest that these cells belong to a lineage distinct from the major T-cell population.**

In previous studies, we (1) and others (2–4) have shown that phenotypically mature CD4$^+$ single-positive thymocytes contain heat stable antigen (HSA)-dull and HSA-negative subpopulations. Sequential analysis of the thymocyte maturation revealed that the CD4$^+$8$^-$ HSA^{dull} thymocytes appeared earlier than CD4$^+$8$^-$ HSA$^-$ thymocytes. These findings suggest that the former population matures into the latter thymocyte population and that the CD4$^+$8$^-$ HSA$^-$ thymocytes are likely to be direct progenitors of peripheral CD4$^+$ T cells (1).

However, even among the CD4$^+$8$^-$ HSA$^-$ seemingly mature thymocyte subpopulation, heterogeneity was found. When these cells were analyzed for CD3 or T-cell antigen receptor (TCR) expression, 10–20% of these cells expressed a distinguishably low-density TCR phenotype (TCR^{lo}). Furthermore, the population expressed the NK1.1 antigen, which is generally expressed only on natural killer cells (5, 6). In the present study, we focused our investigation on this NK1.1$^+$ CD4$^+$8$^-$ TCR^{lo} subpopulation. We were especially concerned with the ontogeny of these cells, their surface phenotype, tissue distribution, and responsiveness to several stimulations.

MATERIALS AND METHODS

Mice. AKR/J mice were obtained from The Jackson Laboratory. C57BL/10 SnSlc (B10), B10.BR SgSnSlc (BR), B10.D2/nSnSlc (D2), DBA/2CrSlc, and CBA/NSlc mice were obtained from the Shizuoka Laboratory Animal Cooperation (Hamamatsu, Japan). CBA/Jjcl mice were obtained from Japan Clea Cooperation (Tokyo). Mice were used at 10–14 weeks of age unless otherwise indicated.

Antibodies and Flow Cytometry. Primary monoclonal antibodies (mAbs) used for immunofluorescence staining and flow cytometry in these experiments and their β-chain variable region (V_β) and β-chain constant region (C_β) specificities were KJ-25 (anti-TCR V_β3) (7), KT4 (anti-TCR V_β4) (8), MR9-4 (anti-TCR V_β5) (9), 44.22.1 (anti-TCR V_β6) (10), TR310 (anti-TCR V_β7) (11), F23.1 (anti-TCR V_β8.1, -2, -3) (12), KJ-16 (anti-TCR V_β8.1, -2) (13), F23.2 (anti-TCR V_β8.2) (14), MR10-2 (anti-TCR V_β9) (15), KT10b (anti-TCR V_β10) (16), RR3-15 (anti-TCR V_β11) (17), 2C11 (anti-CD3-ε) (18), H57.597 (anti-TCR C_β) (19), KM201 [anti-CD44 (Pgp-1)] (20), MEL-14 (21), J11d (anti-HSA) (22), PK-136 (anti-NK1.1) [purchased from American Type Culture Collection (ATCC), Rockville, MD], MALA-2 [anti-CD54 (ICAM-1)] (23), FD441.8 [anti-LFA-1 (CD11a)] (purchased from ATCC), F7D5 (anti-Thy-1.2) (Olac, Bichester, U.K.), 020-210 [anti-CD5 (Lyt1.2)] (Meiji Institute of Health, Japan), and 7D4 [anti-interleukin 2 (IL-2) receptor α chain] (purchased from ATCC). Phycoerythrin (PE)-anti-CD4 was purchased from Becton Dickinson. Fluorescence-labeled secondary antibodies and avidin used herein were fluorescein isothiocyanate (FITC)-anti-mouse IgG, FITC-anti-rat IgG, FITC-anti-hamster IgG, biotin-anti-mouse IgG, biotin-anti-rat IgG (Cappel Laboratories), PE-anti-rat IgG (Jackson ImmunoResearch), and PE-streptavidin and Tandem-streptavidin (Southern Biotechnology Associates, Birmingham, AL). Flow cytometry analysis was performed on a FACScan (Becton Dickinson), and cell sorting was performed on a FACStar (Becton Dickinson). Data acquisition and analysis of the data were basically according to Arase *et al.* (1).

Cell Preparation. Fresh thymocytes from each group of two to three mice were treated with anti-Lyt2.2 or anti-Lyt2.1 mAbs (Meiji Institute of Health) and in some experiments with additional J11d mAb followed by rabbit complement (24). To completely remove the remaining CD8$^+$ or HSA$^+$ cells, we mixed the resultant cells with Dynabeads M-450 (Dynal, Oslo). Purity of the cell preparation was checked by

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Abbreviations: BM, bone marrow; HSA, heat-stable antigen; LN, lymph node; mAb, monoclonal antibody; Spl, spleen; TCR, T-cell antigen receptor(s); PE, phycoerythrin; FITC, fluorescein isothiocyanate; V_β, β-chain variable region; C_β, β-chain constant region; IL-2, interleukin 2.

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fluorescence-activated cell sorting and was always shown to be >98%. Spleen (Spl), lymph node (LN), and bone marrow (BM) T cells were purified by using a nylon wool column.

TCR Stimulation. Purified anti-TCR C_β antibody (H57.597) was immobilized on flat-bottomed 96-well microculture plates (Falcon). Thereafter, 5×10^4 T cells sorted by fluorescence-activated cell sorting were cultured in 200 μl of RPMI 1640 supplemented with 10% fetal calf serum, penicillin (100 units/ml), streptomycin (100 μg/ml), and 50 μM 2-mercaptoethanol. The plates were incubated at 37°C in a humidified 5% CO_2/95% air atmosphere in an incubator for 3 days. Twelve hours prior to harvesting, 1 μCi (1 Ci = 37 GBq) of [^{3}H]thymidine was added to each well. The cultures were harvested and assayed in a liquid scintillation counter. For assessment of IL-2 production by these cells, the culture supernatants taken before addition of [^{3}H]thymidine were added to cultures of an IL-2-dependent cell line, CTLL-2, for 1 day. Thereafter, [^{3}H]thymidine uptake of the cultures was analyzed as described above.

Mixed Lymphocyte Reaction. CD8$^-$ HSA$^-$ thymocytes were stained with FITC-anti-CD44 and PE-anti-CD4. Stained cells were sorted into CD4$^+$ CD44$^+$ and CD4$^+$ CD44$^-$ populations. In purification of AKR thymocytes, CD8$^-$ HSA$^-$ thymocytes were mixed with MEL-14-coated Dynabeads to enrich the CD44$^+$ population before sorting. Splenic CD4$^+$ T cells were prepared by sorting the nylon wool-purified spleen cells after staining with PE-anti-CD4. The purity of sorted cells was always >98%. These sorted responder T cells (8×10^4) and spleen cells (2×10^5) treated with mitomycin c at 50 μg/ml for 30 min were mixed together in 200 μl of RPMI 1640 medium supplemented as described above. These cells were cultured in a 96-well round-bottomed microculture plate (Falcon) for 4 days. To evaluate cell proliferation, [^{3}H]thymidine incorporation was measured as described above.

RESULTS

A Thymocyte Subpopulation with Low TCR Expression Among CD4$^+$8$^-$ Single-Positive Thymocytes. In contrast to peripheral T cells, adult murine CD4$^+$8$^-$ single-positive thymocytes consist of heterogeneous subpopulations when further classified by HSA expression. Namely, ≈50% of CD4$^+$8$^-$ thymocytes are weakly HSA positive (1–4). When TCR expression on CD4$^+$8$^-$ HSA$^-$ thymocytes was analyzed, a cell population expressing a low level of CD3 (CD3^{lo}) was detected among the HSA$^-$ cells (Fig. 1). The proportion of the CD3^{lo} population among the CD4$^+$8$^-$ HSA$^-$ thymocytes was 21.1%. Similar cells with the TCR^{lo} phenotype were observed when the anti-TCR C_β (H57.597) or the anti-TCR V_β8 (F23.1) mAb was used (data not shown). We then analyzed the expression of various kinds of surface antigens on the CD4$^+$8$^-$ HSA$^-$ thymocytes in B10.BR mice (12 weeks old) (Fig. 1). The majority of the cells in the population with the CD4$^+$8$^-$ high-density CD3 (CD3^{hi}) phenotype expressed MEL-14, but only a small proportion expressed the CD44, NK1.1, or ICAM-1 antigens. To the contrary, a majority of the CD3^{lo} population expressed CD44, NK1.1, and ICAM-1, but only a small proportion expressed MEL-14. No obvious difference was observed in expression of the LFA-1, K^k, Thy-1, or CD5 antigens between CD3^{hi} and CD3^{lo} populations. No significant expression of the IL-2 receptor α chain was observed on either subpopulation.

TCR V_β Usage in CD4$^+$8$^-$ HSA$^-$ TCR^{lo} Thymocytes. We then analyzed TCR V_β usage in the CD4$^+$8$^-$ HSA$^-$ CD3^{lo} thymocytes from B10 mice. Since CD4$^+$8$^-$ HSA$^-$ CD3^{lo} cells were all CD44$^+$ (Fig. 1), we analyzed the CD4$^+$8$^-$ HSA$^-$ CD44$^+$ (CD44$^+$) population as representative of the CD4$^+$8$^-$ HSA$^-$ CD3^{lo} population and compared the V_β usage with the CD4$^+$8$^-$ HSA$^-$ CD44$^-$ (CD44$^-$) population, which appeared to represent the CD4$^+$8$^-$ HSA$^-$ CD3^{hi} population. Fig. 2 shows that a high proportion of CD44$^+$ thymocytes express V_β7 and V_β8.2 TCRs as compared to CD44$^-$ thymocytes. In contrast, significantly lower proportions of the CD44$^+$ cells were $V_\beta3^+$, $V_\beta4^+$, $V_\beta6^+$, $V_\beta10^+$, and $V_\beta11^+$ as compared to CD44$^-$ cells. Next, we analyzed TCR V_β expression in seven different strains to see the efficiency of negative selection of thymocytes expressing self-reactive TCRs (Table 1). $V_\beta5^+$ or $V_\beta11^+$ thymocytes were eliminated in most I-E$^+$ strains (9, 17), and $V_\beta6^+$ or $V_\beta8.1^+$ thymocytes were eliminated in I-E$^+$ and Mls-1^a strains (10, 14). Table 1 shows that the $V_\beta5^+$ or $V_\beta11^+$ cells had been eliminated or reduced in the both CD44$^+$ and CD44$^-$ populations of I-E$^+$ strains. Similarly, $V_\beta6^+$ cells were eliminated from both populations in I-E$^+$ and Mls-1^a strains. By contrast, considerable proportions of $V_\beta8.1^+$ cells were seen in the CD44$^+$ populations of Mls-1^a strains, even though in the CD44$^-$ populations the $V_\beta8.1^+$ cells were completely eliminated.

Tissue Distributions of CD4$^+$8$^-$ HSA$^-$ CD3^{lo} Thymocytes. We then tried to identify the CD4$^+$8$^-$ CD3^{lo} population in various lymphoid tissues. Fig. 3 demonstrates the expression of CD3 and MEL-14 or CD3 and CD44 antigens on the CD4$^+$ cells from Spl, LN, or BM. The CD4$^+$ T cells with the CD3^{lo} and CD44$^+$ phenotype were found most frequently in BM (they comprise 37.8% of the entire CD4$^+$ T cells in this location). To the contrary, in LN or Spl only a very small proportion of such CD3^{lo} CD44$^+$ T cells could be detected. Similarly CD4$^+$ CD3^{lo} MEL-14$^-$ T cells were detected most frequently in BM (39.2%) but were hardly detectable in LN or Spl. No significant numbers of T cells from peripheral blood, Peyer's patches, or intestinal LN showed the phenotype of the CD4$^+$ CD3^{lo} CD44$^+$ or MEL-14$^-$ population (data not shown). When $V_\beta8$ expression on the CD4$^+$ CD44$^+$ or CD4$^+$ MEL-14$^-$ T cells in Spl, LN, or BM was analyzed and compared with those in the thymus, high proportions of $V_\beta8^+$ cells, similar to those in the thymus, were noted only in BM T cells (Table 2).

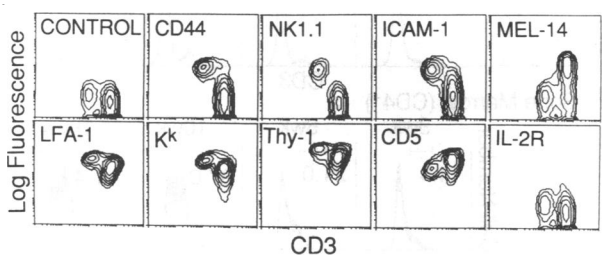

FIG. 1. Phenotypic analysis of CD4$^+$8$^-$ HSA$^-$ thymocytes. CD4$^+$8$^-$ HSA$^-$ thymocytes were stained with mAbs that recognize the antigens indicated in the figure followed by Tandem-streptavidin, FITC-anti-CD3, and PE-anti-CD4. Expression of CD3 (x axis) and the other antigens indicated (y axis) on the CD4$^+$8$^-$ HSA$^-$ population is illustrated.

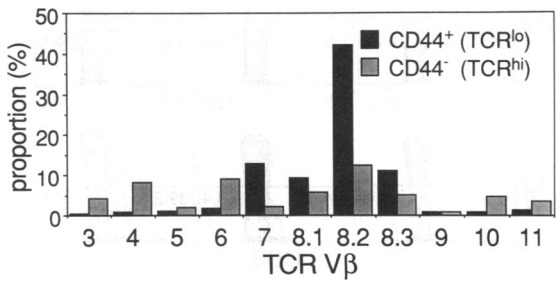

FIG. 2. TCR V_β expression on CD4$^+$8$^-$ HSA$^-$ CD44$^+$ or CD4$^+$8$^-$ HSA$^-$ CD44$^-$ thymocytes. Pooled CD8$^-$ HSA$^-$ thymocytes from three B10 mice were stained with FITC-anti-TCR V_β, PE-anti-CD4, and Tandem-anti-CD44 mAbs. The proportion of each type of TCR V_β-positive cells in CD4$^+$ CD44$^+$ or CD4$^+$ CD44$^-$ cells is illustrated.

Table 1. TCR V_β usage of CD44$^+$ or CD44$^-$ CD4$^+$8$^-$ HSA$^-$ thymocytes

Mouse strain	I-E	Mls-1	CD44	TCR V_β -5	-6	-8.1*	-8.2	-11
B10	−	b	+	1.5	4.0	7.8	34.3	2.1
			−	1.8	9.2	5.9	13.7	3.8
B10.BR	+	b	+	0.3	2.8	5.1	39.1	0.0
			−	0.0	9.2	2.4	16.8	0.2
CBA/N	+	b	+	0.4	5.0	7.5	37.2	0.5
			−	0.2	12.2	4.0	17.6	0.3
CBA/J	+	a	+	0.4	1.1	5.7	35.9	0.6
			−	0.3	0.1	0.1	19.7	0.2
DBA/2	+	a	+	0.6	0.6	5.3	37.5	0.8
			−	1.0	0.1	0.0	16.6	1.7
AKR/J	+	a	+	0.6	0.4	3.2	20.0	0.3
			−	0.0	0.0	0.1	12.7	0.1

CD8$^-$ HSA$^-$ thymocytes pooled from three mice were stained with PE-anti-CD4, Tandem-anti-CD44, and FITC-anti-TCR V_β antibodies. Data were calculated by subtracting the background staining. Underlined values indicate that these populations have not been eliminated significantly.

*Frequencies of V_β8.1$^+$ cells were calculated by subtracting the frequencies of V_β8.2$^+$ cells (F23.2$^+$) from V_β8.1,2$^+$ cells (KJ-16$^+$).

Ontogenetic Study of the CD4$^+$8$^-$ HSA$^-$ CD3^{lo} Thymocytes. To examine the appearance of the CD4$^+$8$^-$ HSA$^-$ CD3^{lo} thymocytes in ontogenetic terms, we analyzed thymocytes from B10.BR mice of various ages (10 days and 3, 6, 10, and 18 weeks). As shown in Fig. 4*A*, only a small proportion of CD3^{lo} cells was detected in the thymus of 10-day-old mice. Thereafter, the proportion of the CD3^{lo} cells gradually increased, and rather high proportions were demonstrated in the thymus after 10 weeks. These findings indicate that the CD4$^+$8$^-$ HSA$^-$ CD3^{lo} cells appear at a late stage compared to the CD4$^+$8$^-$ HSA$^-$ CD3^{hi} population. Similarly, the proportion of CD4$^+$ CD3^{lo} cells in BM gradually increased with age, although the population size was much larger than that of the thymus (Fig. 4*B*).

Functional Analysis of the CD4$^+$8$^-$ HSA$^-$ CD3^{lo} Thymocytes. In the next set of experiments, we analyzed responsiveness of CD4$^+$8$^-$ HSA$^-$ CD3^{lo} cells sorted from B10 thymocytes by the immobilized anti-TCR C_β mAb. As shown in Fig. 5, CD4$^+$8$^-$ HSA$^-$ CD3^{lo} thymocytes generated proliferative responses through perturbation of the TCR that were almost 10 times greater than those of normal splenic CD4$^+$ T cells. Similarly, significantly high levels of IL-2 were detected in the supernatant of the CD4$^+$8$^-$ HSA$^-$ CD3^{lo} thymocytes stimulated with anti-TCR C_β mAb compared to those of CD4$^+$ splenic T cells.

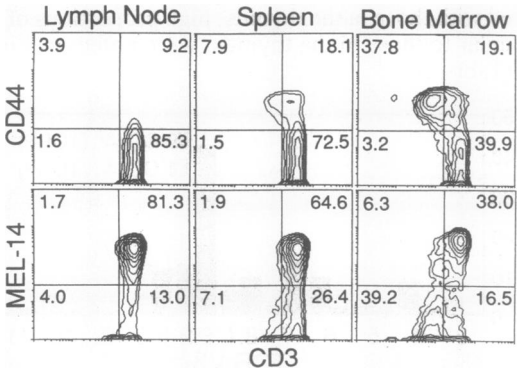

FIG. 3. Tissue distributions of CD4$^+$8$^-$ TCR^{lo} CD44$^+$ MEL-14$^-$ T cells. Cells from B10.BR (12-week-old) mice were stained with Tandem-anti-CD4, FITC-anti-CD3, and PE-anti-MEL-14 or PE-anti-CD44. Expression of CD3 and MEL-14 or CD44 on the CD4$^+$ T cells is illustrated. The proportions of each population are indicated.

Table 2. Proportions of V_β8$^+$ cells in CD4$^+$ T cells from various tissues

Tissue	CD44$^+$	CD44$^-$	MEL-14$^+$	MEL-14$^-$
Thymus	53.0	21.4	20.7	40.9
LN	24.5	25.8	24.1	26.9
Spl	27.2	24.6	23.5	28.3
BM	44.1	22.7	23.9	45.1

Each tissue was pooled from three mice. CD8$^-$ HSA$^-$ thymocytes or nylon wool-purified T cells were stained with PE-anti-CD44, Tandem-anti-CD4, and FITC-anti-CD3 or anti-V_β8 mAbs. The proportion of CD3$^+$ cells in each population was >99%. Underlined values are significantly higher than those of LN or Spl.

Next, we carried out syngeneic and allogeneic mixed lymphocyte reactions in addition to anti-TCR stimulation with H57.597 in B10.BR (Mls-1^b), B10 (Mls-1^b), AKR (Mls-1^a), and CBA/J (Mls-1^a) mice. As shown in Table 3, the CD44$^+$ thymocytes showed only negligible responses to allogeneic H-2 antigens as compared to the CD44$^-$ thymocytes or splenic CD4$^+$ T cells. When reactivity to AKR stimulator cells was analyzed in B10 or B10.BR mice, the CD44$^+$ thymocytes showed vigorous and comparable proliferation to those of CD44$^-$ thymocytes or CD4$^+$ splenic T cells. This finding indicates that the reactivity of the CD44$^+$ thymocytes to Mls-1^a antigens is comparable to that of the CD44$^-$ thymocytes or CD4$^+$ splenic T-cell populations. However, in AKR/J and CBA/J mice (Mls-1^a) no greater responses to the syngeneic stimulators could be detected among CD44$^+$ thymocytes than with CD44$^-$ thymocytes or splenic CD4$^+$ T cells. This finding is of interest, since significant proportions of V_β8.1$^+$ cells were present in the CD44$^+$ population of the AKR/J and CBA/J thymocytes (see Table 1).

DISCUSSION

In the present study, we have demonstrated a thymocyte subset characterized by the surface phenotype CD4$^+$8$^-$, HSA$^-$, and CD3^{lo} or $\alpha\beta$ TCR^{lo}. The CD4$^+$8$^-$ HSA$^-$ TCR^{lo} subset made up about 10% of the whole CD4$^+$8$^-$ thymocytes or about 20% of the CD4$^+$8$^-$ HSA$^-$ thymocytes in adult B10.BR mice. Furthermore, a similar population was demonstrated to comprise about 40% of BM CD4$^+$ T cells. By contrast, these CD3^{lo} T cells could not be detected in significant numbers in LN or Spl. Most of the CD4$^+$8$^-$ HSA$^-$ TCR^{lo} cells were shown to be NK1.1$^+$, CD44$^+$, ICAM-1$^+$, and MEL-14$^-$. The expression pattern of the CD44, MEL-14, and ICAM-1 on the CD4$^+$8$^-$ HSA$^-$ TCR^{lo} population is identical to that expressed on memory T cells as reported (23,

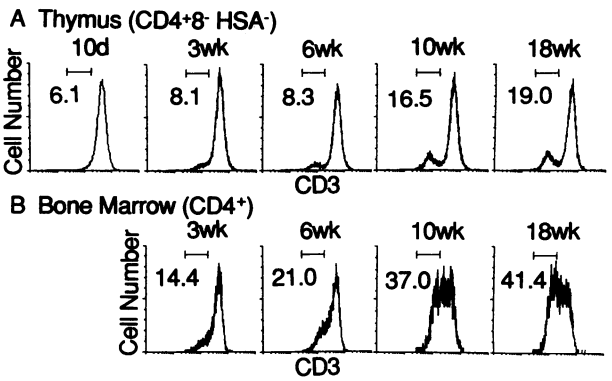

FIG. 4. Ontogenetic analysis of CD4$^+$8$^-$ HSA$^-$ TCR^{lo} thymocytes (*A*) or CD4$^+$ TCR^{lo} BM cells (*B*). CD3 expression on the CD4$^+$8$^-$ HSA$^-$ thymocytes or CD4$^+$ BM cells from B10.BR mice of various ages is illustrated. The proportions of CD3^{lo} cells are indicated.

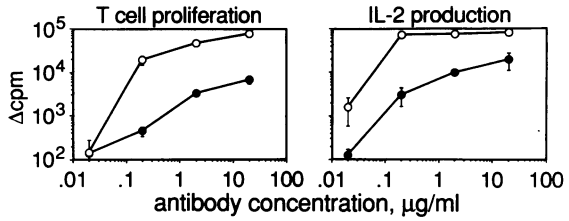

FIG. 5. Proliferative responses and IL-2 production by CD4+8− HSA− CD3lo thymocytes and splenic CD4+ T cells to stimulation with immobilized anti-TCR C_β mAb. CD4+8− HSA− CD3lo thymocytes were prepared by sorting CD8− HSA− thymocytes into CD4+ CD3lo population (○). Splenic CD4+ T cells were prepared by sorting the splenic T cells into CD4+ cells (●). Data are presented as the mean change in cpm ± SD of triplicate determinations.

25–28). However, the expression of the NK1.1 antigens is generally limited to natural killer cells (5, 6), and antigen-specific CD4+ T cell lines did not express NK1.1 antigen (unpublished observation). From this finding, we infer that the CD4+8− HSA− TCRlo population represents a lineage different from the major T-cell population and that this lineage may play a distinct role, which is also reflected by its restricted tissue location.

This CD4+8− HSA− TCRlo population highly expresses the $V_\beta7$ and $V_\beta8$ TCR repertoire. Furthermore, in Mls-1a strains, the $V_\beta8.1$ TCR+ cells that are reactive to Mls-1a antigens were not eliminated from this population. On the other hand, self-reactive $V_\beta6^+$, $V_\beta5^+$, or $V_\beta11^+$ cells had been almost completely eliminated. These differences in efficacy of negative selection of self-reactive thymocytes observed between the $V_\beta6$, $V_\beta5$, or $V_\beta11$ TCR and the $V_\beta8.1$ TCR repertoire may be due to the difference in affinity to self-antigens or to a difference in maturation stages (29). Recently, Takahama *et al.* (30) reported Ly6C+ CD4+8− thymocytes that express a high proportion of $V_\beta8.2$ TCR. However, when Ly6C expression on CD4+8− HSA− TCRlo thymocytes was analyzed, these thymocytes did not necessarily express Ly6C antigen (57% were Ly6C+ in CD4+8− HSA− TCRlo thymocytes). Furthermore, in an Mls-1a strain, self-reactive $V_\beta6^+$ cells were eliminated from CD4+8− HSA− CD44+ thymocytes, unlike CD4+8− Ly6C+ thymocytes.

The CD4+8− HSA− TCRlo population appeared in the thymus or BM later than other T-cell populations. Furthermore, a forbidden clone ($V_\beta8.1^+$) was shown not to have been eliminated from the CD4+8− HSA− TCRlo population as described above. Thus, one possible origination of the population may be from extrathymic tissues such as BM. Actually, we (31) and others (32) have reported that activated T cells can reenter the thymus. However, the possibility that this population has been generated in the thymus in a manner different from the major thymocyte population cannot be eliminated by the present studies.

Functional analysis demonstrated that the CD4+8− HSA− TCRlo thymocytes elicit negligible allogeneic H-2 responsiveness compared to the major CD4+8− thymocytes or the splenic CD4+ T cells, although they responded vigorously to stimulation with immobilized anti-TCR mAb. On the contrary, when the responsiveness of the CD4+8− HSA− TCRlo population to Mls-1a antigens was quantified in Mls-1b strains, cells of this population showed comparable responses to those exhibited by the major CD4+8− thymocytes or the splenic CD4+ T cells. This unique responsiveness is presumably attributable to the highly skewed TCR repertoire. However, in Mls-1a strains the CD4+8− HSA− TCRlo thymocytes mounted no responsiveness to Mls-1a antigens, even though they contained a considerable proportion of $V_\beta8.1^+$ cells. Thus, the forbidden clones bearing self-reactive $V_\beta8.1$ TCR in this population seem to be rendered tolerant to the self-antigens in a manner distinct from the usual clonal elimination.

From the clinical point of view, the feature of the relatively abundant CD4+ CD44+ MEL-14− TCRlo T cells in BM seems important. T cells that represent a very small fraction of total marrow cells (1–2%) exert significant influences on the prognosis of patients transplanted with allogeneic BM cells. BM T cells induce a graft versus host reaction that has been shown to be lethal, depending on the combination of donors and recipients. On the other hand, graft versus host reaction becomes a benefit especially when BM transplantation is performed for treatment of leukemia patients (33, 34) (graft versus leukemia reaction). Thus, it seems critical to determine what functional significance the CD4+8− HSA− TCRlo population has *in vivo*, especially when BM transplantation is employed in appropriate patients.

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Table 3. Proliferative responses of CD4+8− HSA− CD44+ thymocytes to stimulation with allogeneic H-2 antigens, Mls-1a antigens, or anti-TCR mAb

Exp.	Mouse strain	Tissue	Cell	Stimulator cells: MC	B10	BR	CBA/N	D2	AKR	CBA/J	anti-TCR mAb*
1	B10	Thy	CD44−	88 ± 14	150 ± 20	3506 ± 720			44,977 ± 3944		1,636 ± 149†
	B10	Thy	CD44+	77 ± 28	177 ± 107	289 ± 20			22,072 ± 60		50,154 ± 455
	B10	Spl	CD4+	103 ± 20	176 ± 55	2485 ± 223			28,363 ± 1286		11,050 ± 2,367
2	BR	Thy	CD44−	164 ± 59		129 ± 12		3679 ± 627	24,232 ± 2437		1,647 ± 740
	BR	Thy	CD44+	147 ± 62		135 ± 11		613 ± 336	14,067 ± 1234		48,247 ± 18,714
	BR	Spl	CD4+	170 ± 76		192 ± 157		1991 ± 453	14,117 ± 1345		8,234 ± 2,772
3	AKR/J	Thy	CD44−	80 ± 65		ND		9994 ± 2076	316 ± 72		1,662 ± 343
	AKR/J	Thy	CD44+	27 ± 4		ND		268 ± 147	163 ± 132		71,330 ± 2,984
	AKR/J	Spl	CD4+	91 ± 31		247 ± 214		5705 ± 534	435 ± 417		10,623 ± 2,412
	BR	Spl	CD4+	107 ± 55		208 ± 202		3153 ± 607	18,710 ± 4216		6,353 ± 866
4	CBA/J	Thy	CD44−	414 ± 56			240 ± 86	5794 ± 569		1,580 ± 315	6,082 ± 1,630
	CBA/J	Thy	CD44+	406 ± 188			ND	1079 ± 229		540 ± 140	53,998 ± 3,235
	CBA/J	Spl	CD4+	203 ± 124			199 ± 45	4834 ± 703		1,070 ± 616	34,339 ± 6,934
	CBA/N	Spl	CD4+	138 ± 124			205 ± 177	3256 ± 288		33,837 ± 3,520	5,235 ± 957

The data are the mean cpm ± SD of triplicate determinations. ND, not determined; Thy, thymus.
*Thirty thousand cells were stimulated with immobilized anti-TCR mAb.
†Data are the mean change in cpm ± SD of triplicate determinations.

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