Interleukin 7 Induces Preferential Expansion of Vβ8.2+CD4-8- and Vβ8.2+CD4+8- Murine Thymocytes Positively Selected by Class I Molecules

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Summary

We analyzed the phenotype and VB-TCR repertoire, together with interleukin 7 receptor (IL-7R) expression in unfractionated thymocytes stimulated in vitro with IL-7. This culture system results in a specific proliferation of mature thymocytes belonging to the CD3+CD4-, CD4+8-, and CD4-8+ subsets. IL-7 induced a preferential expansion of Vβ8.2+CD4-8- and Vβ8.2+CD4+8- thymocytes. This phenomenon is not observed in β2-microglobulin-deficient mice, showing that a fraction of CD4+8- thymocytes, enriched in Vβ8.2+ cells, is selected by class I molecules in normal mice, as are a large proportion of CD4-8-αβTCR + thymocytes. Our findings also establish that IL-7 plays a major role in the expansion of rare thymocyte subsets, which could exert important functions in inflammatory and immune responses.

T lymphocyte maturation involves coordinate rearrangement of TCR genes in committed precursor cells which will be selected to differentiate into mature T cells. Those steps are closely dependent on the thymic microenvironment and implicate cell-to-cell interactions or cytokines (1). Concerning the latter, it is now generally acknowledged that they induce a preferential expansion of particular thymocyte subsets (1).

Recent data point to the involvement of IL-7 in intrathymic development in adult mice. Its implication in thymopoiesis is suggested both by its predominant mRNA expression in the thymus and the studies on IL-7 transgenic mice (2, 3, 4). This notion is also supported by in vitro experiments showing that IL-7 exerts biological effects on distinct thymic subsets. The earliest IL-7-responsive cells are the immature CD44+CD25+, CD3-4-8- thymocytes, whose viability is maintained, apparently without proliferation or differentiation (5, 6). Furthermore, IL-7 exerts a proliferative effect on mature CD3+ thymocytes, namely CD4-8-TCR+, CD4+8-, and CD4-8+ thymocyte subsets (6-8).

A particular feature of CD4-8-TCRαβ+ thymocytes, relative to mature CD4-8- and CD4+8- thymocytes and peripheral T lymphocytes, consists of overexpression of the Vβ8.2 gene family (9, 10). It has recently been shown that the majority of these supernumerary CD4-8-Vβ8.2+ thymocytes are positively selected by class I molecules, since they are absent from β2-microglobulin-deficient mice (11). In keeping with these data, a particular subset of CD4-8- thymocytes, called Thy0, also presents overusage of the Vβ8.2 gene family, but little is known about the way they are selected (12). No such subset has been described among CD4-8- thymocytes.

We have recently found that stimulation of total thymocytes by IL-7 results in preferential proliferation of CD4-8-TCRαβ+ and CD4+8-TCRγδ+ thymocytes and, to a lesser extent, in CD4-8- and CD4+8- cell expansion (13, 13a). The aim of the present study was to analyze the IL-7R expression in relation to the VβTCR repertoire in IL-7-cultured thymocytes. We demonstrate herein that IL-7 preferentially expands CD4-8-Vβ8.2+ and CD4+8- Vβ8.2+ thymocytes in normal but not in β2-microglobulin-deficient mice, indicating that IL-7 plays a major role in the expansion of rare thymocyte subsets.

Materials and Methods

Mice. 4- to 6-wk-old male C3H/HeJ low endotoxin-responder mice were purchased from CSEAL (Orléans, France). β2-microglobulin-deficient mutant mice of the 129 strain (−/−) (14), hereafter referred to as β2m-−, and wild-type littermates, referred to as β2m+−, were bred in our own facilities by backcross generation from heterozygous β2m+/− mice. First, β2m+/− breeders and
mice lacking MHC class II molecules (A12/2) (15) (referred to as Class II') were obtained from CSEAL (Orléans, France).

In Vitro Culture of Thymic Cells. Single-cell thymocyte suspensions were prepared in RPMI 1640 medium (GIBCO BRL, Gaithersburg, MD) containing 10% heat-inactivated FCS (Flow Laboratories, Irvine, Scotland), 10 mM Hepes buffer, 2 mM L-glutamine, 100 IU/ml penicillin, 100 µg/ml streptomycin (all from GIBCO BRL), and 0.05 mM 2-ME (culture medium). Cultures were carried out at 37°C in 24-well plates (Costar, Cambridge, MA) using 15 × 10^6 unseparated thymocytes/well in a total volume of 0.5 ml. In some experiments, 10 × 10^6 of both β2m- and β2m+ thymocytes were mixed in a total volume of 0.5 ml, and then incubated for 4 or 9 d with 100 U/ml human recombinant IL-7 (hrIL-7, specific activity of 8.8 × 10^6 U/mg) kindly provided by Sterling Winthrop, Inc. (Collegeville, PA). At the end of culture, thymocytes were harvested and dead cells were removed by density gradient centrifugation (Lympholyte M; Cedarlane, Ontario, Canada).

Depletion of CD8+ Thymocytes. To analyze the VB-TCR repertoire of freshly isolated CD4-8- and CD4+8- thymocytes, together with IL-7R expression, we depleted thymocyte suspensions of CD8+ cells. Briefly, total thymocytes were incubated with purified anti-CD8 (clone 3155) followed by low-tox-M rabbit complement (Cedarlane), washed extensively, and then incubated with another purified anti-CD8 (clone 53-6.7), followed by depletion with anti-rat Ig coated magnetic beads (Dynal, Oslo, Norway). Purification was assessed by staining the remaining cells with a third anti-CD8 (clone YTS-169.4). CD8+ contaminants were routinely <2%.

Immunofluorescence Labeling and Flow Cytometry. For surface antigen detection, cells were resuspended in PBS containing 5% FCS and 0.02% sodium azide and incubated for 30 min with an appropriate dilution of antibody. The following antibodies were used for double or triple labeling: anti-oβTcR (clone H57-97), anti-Vβ6 (clone 44.22.1), anti-Vβ8.2 (clone P23.2), anti-Vβ14 (clone 14-2), anti-γδTCR (clone GL3), anti-HSA (clone J1Id), anti-CD44 (clone 1M781), anti-CD25 (clone PC61), anti-IA (clone M5-114), anti-H-2Kb (clone 34-1-2S), anti-CD4 (clone GK1.5), and anti-CD8 (clone 53-6.7), coupled to biotin, FITC or PE. For triple labeling, biotinylated antibodies were revealed using Tricolor-streptavidin (Caltag Labs, San Francisco, CA).

Flow cytometry was performed using a FACSscan apparatus (a registered trademark of Becton Dickinson, Mountain View, CA) equipped with a 488-nm argon laser. In double-labeling experiments, cell suspensions were analyzed after propidium iodide exclusion of dead cells. At least 10^5 lymphoid cells were acquired in each run and the results were analyzed using Lyys II software.

IL-7R Detection. We used biotinylated murine IL-7 to detect IL-7R, by means of flow cytometry (16). Briefly, 10^6 cells were incubated for 60 min on ice with 10 µl of a 10 µg/ml biotinylated murine IL-7 solution (British Biotechnology, Oxon, UK). Unbound cytokine was removed by washing the cells twice followed by another incubation for 30 min with 10 µl of PE-streptavidin second-step reagent (British Bio-technology) or Tricolor-streptavidin. The specificity of biotinylated murine IL-7 labeling was assessed by its reversal in the presence of a 80-fold molar excess of unlabeled human IL-7 (data not shown).

Analysis of DNA-synthesizing cells. Total thymocytes were incubated with IL-7 for 4 d and with 100 µg/ml bromodeoxyuridine (BrdUrd) for the last 15 h of culture. BrdUrd incorporation by DNA synthesizing cells was analyzed by flow cytometry with a three color labeling technique (17). Briefly, cultured cells were surface stained with appropriate combinations of anti-CD4, anti-CD8, anti-αβTCR, anti-Vβ6, anti-Vβ8.2, anti-Vβ14, or anti-γδTCR, coupled to either biotin or PE. Biotinylated antibodies were revealed with Tricolor-streptavidin. Cells were then fixed for 48 h at 4°C in 1% paraformaldehyde containing 0.01% Tween 20 and incubated with pancreatic deoxyribonuclease I (Pharmacia, Uppsala, Sweden) for 1 h at 37°C. BrdUrd incorporated into DNA was detected with the 76/7 antibody and FITC-conjugated anti-mouse IgG1. The negative control consisted of cultured cells labeled by the same procedure but without BrdUrd incubation.

Results

The CD4-CD8- Thymocyte Subset Is the Predominant Cell Population Recovered after IL-7 Stimulation. We have previously described that IL-7 induces a strong proliferative response of unseparated thymocytes, that peaks after 3-4 d of culture (13). As illustrated in Table 1, a 4-d incubation with IL-7 results in a preferential expansion of CD4-CD8- thymocytes, the second major subset being CD4+CD8-. Some CD4+CD8+ thymocytes were also present, while nearly all CD4+CD8- cells had disappeared.

Phenotypic Analysis of CD4-CD8- and CD4+CD8- IL-7-stimulated Thymocytes. We analyzed the expression of CD44, CD25, HSA, αβTCR, γδTCR, and IL-7R in CD4-8- and CD4+8- thymocyte after 4-d stimulation of total thymocytes with IL-7 relative to freshly isolated thymocytes. Table 2 shows that all CD4-8- thymocytes recovered after 4 d of IL-7 stimulation expressed either αβTCR or γδTCR. The vast majority were mature HSA- and activated CD44 + T cells bearing IL-7R. They also homogeneously expressed intermediate CD25 levels, i.e., at a lower intensity than fresh CD4-8- thymocytes. All CD4-8- thymocytes were mature αβTCR T cells, as defined by low HSA expression. About one third were IL-7R+, while only 10-20% were CD25+ or CD44+. In both CD4-8- and CD4+8- thymocyte populations, IL-7R expression corresponded to a larger cell size (data not shown).

IL-7 Promotes the Preferential Expansion of Vβ8.2- T Thymocytes from Normal Mice. We analyzed the actual cell recoveries of CD4-8- and CD4+8- thymocytes expressing different Vβs after 4 d of IL-7 stimulation and found a preferential recovery of Vβ8.2+ cells (Table 3). More precisely, CD4-8-Vβ8.2- thymocytes expanded approximately 10-fold when CD4-8 thymocytes expressing other Vβs expanded approximately three fold. Concerning CD4-8- thymocytes, we did not observe enhanced recovery of any particular subset, but the recovery of CD4+CD8+Vβ8.2- thymocytes was only slightly diminished whereas those of CD4-8+ thymocytes expressing other Vβs decreased by twofold. This result may reflect the heterogeneity of the sensitivity of CD4+8- thymocytes to IL-7 rather than a selective survival, as only a fraction of them expressed IL-7R (Table 2) and as a notable proportion of CD4+8- thymocytes died during the first days of culture (data not shown). We inves-

1 Abbreviations used in this paper: BrdUrd, bromodeoxyuridine; hrIL-7, human recombinant IL-7.

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Table 1. Recovery of CD4- and CD8-defined Thymocyte Subsets after 4 d of Culture with IL-7

|                | CD4+CD8- | CD4+CD8+ | CD4+CD8- | CD4+CD8+ |
|----------------|-----------|-----------|-----------|-----------|
| Day 0          | 12.2 ± 1.1| 83.1 ± 1.2| 9.7 ± 1.0 | 4.6 ± 1.0 |
| Day 4 + IL-7   | 8.5 ± 3.5 | 0.7 ± 0.8 | 4.6 ± 0.9 | 2.6 ± 0.8 |

* Day 0 or day 4 IL-7-stimulated thymic cells from C57Bl/6 mice were stained with fluorescently labeled antibodies specific for CD4 and CD8 and analyzed by two-color flow cytometry. Dead cells and debris were eliminated by propidium iodide exclusion. Cultures were set up with 15 x 10^6 unseparated thymic cells in 0.5 ml of culture medium containing 100 U/ml hrIL-7.

† Results are expressed as mean ± SD of five separate experiments. They represent viable cell recovery calculated from 10^5 cells at the beginning of culture.

Table 2. Phenotype of CD4-8- and CD4+8- Thymocytes before and after 4 d of IL-7 Stimulation

|                | CD4+8- | CD4+8- | CD4+8- | CD4+8- |
|----------------|--------|--------|--------|--------|
| Percentage of positive cells in gated populations |         |        |        |        |
| αβ-TCR +       | 132    | 99     | 76     | 99     |
| γδ-TCR +       | 20     | 0      | 23     | 0      |
| CD25 +         | 34     | 6      | 62     | 10     |
| CD44 +         | 25     | 8      | 85     | 13     |
| HSA +          | 80     | 56     | 10     | 18     |
| IL7R +         | 44     | 25     | 95     | 32     |

* Freshly isolated or cultured thymocytes exposed for 4 d to IL-7 were stained with anti-CD4-PE, anti-CD8-FITC, and the appropriate biotinylated antibody or biotinylated mAb to IL-7, and then analyzed by three-color flow cytometry. Cultures were set up with 15 x 10^6 unfractionated thymic cells in 0.5 ml of culture medium containing 100 U/ml hrIL-7.

† Results are expressed as the percentage of positive cells compared to staining with Tricolor-streptavidin alone, in one of three independent experiments.

Table 3. Recovery of CD4-8- and CD4+8- Thymocytes Expressing Different Vβs after 4 d of Culture with IL-7

|                | CD4+8- | CD4+8- | CD4+8- | CD4+8- |
|----------------|--------|--------|--------|--------|
| Vβ6 +          | 10.7 ± 0.3| 10.9 ± 0.8| 2.3 ± 0.5| 4.5 ± 0.5|
| Vβ8.2 +        | 2.7 ± 0.8 | 12.7 ± 1 | 28.4 ± 1.1 | 10.4 ± 1.2 |
| Vβ14 +         | 0.04 ± 0.2 | 5.9 ± 0.8 | 0.13 ± 0.2 | 2.4 ± 0.3 |

* Freshly isolated or cultured thymocytes exposed for 4 d to IL-7 were stained with anti-CD4-PE, anti-CD8-FITC, and the appropriate biotinylated anti-Vβ antibody plus Tricolor-streptavidin. Cultures were set up with 15 x 10^6 unfractionated thymic cells in 0.5 ml of culture medium containing 100 U/ml hrIL-7.

† Results are expressed as mean ± SD of three separate experiments. They represent viable cell recovery in each subpopulation calculated from 10^5 cells at the beginning of culture.
Table 4. Relation between Cycling Cells and Vβ-TCR Repertoire in IL-7-stimulated Thymocytes

| Gated in      | CD4-8-        | CD4+8-        |
|---------------|---------------|---------------|
| Total         | 69 ± 3        | 12 ± 2        |
| αβTCR+        | 70 ± 2        | 12 ± 2        |
| γδTCR+        | 68            | ND            |
| Vβ6+          | 65 ± 1        | 12 ± 2        |
| Vβ8.2+        | 73 ± 2        | 22 ± 3        |
| Vβ14+         | 65 ± 6        | 10 ± 1        |

* Cultures were performed with 15 × 10^6 unseparated thymic cells from C3H/HeJ mice, incubated for 4 d in 0.5 ml of culture medium containing 100 U/ml rhIL-7. BrdUrd (100 μM) was incorporated during the last 15 h of culture. Cells were stained with anti-CD4-PE plus anti-CD8-PE, the appropriate biotinylated antibody, and Tricolor-streptavidin. BrdUrd-incorporating cells were revealed using a standard technique described in Materials and Methods. The percentage of BrdUrd-incorporating cells was determined by comparison with the staining of cells not incubated with BrdUrd. Results are expressed as mean percentages ± SD from three separate experiments, except for γδTCR+ (two experiments).

Figure 1. Vβ-TCR repertoire of CD4-8-αβTCR+ and CD4+8- thymocytes according to IL-7R expression before and after 4-d IL-7 stimulation. The Vβ-TCR repertoire was analyzed by three-color flow cytometry in CD8- depleted thymocytes before (Day 0) and after exposure to IL-7 (Day 4 + IL-7). In CD4-8- αβTCR+ (A) and CD4+8- (B) thymocytes. The Vβ-TCR repertoire is represented as the percentage of positive cells among IL-7R- (hatched) and IL-7R+ (solid) populations. Results are mean percentages of two separate experiments for Day 0 and mean percentages ± SD of three separate experiments for Day 4.

Table 5. IL-7R Expression According to the Vβ-TCR Repertoire in CD4-8- and CD4+8- Thymocytes before and after 4 d of IL-7 Stimulation

|                      | Day 0 | Day 4 + IL-7 | Percentage of IL-7R+ in CD4-8- | Day 0 | Day 4 + IL-7 |
|----------------------|-------|--------------|-------------------------------|-------|--------------|
| αβTCR+               | 93    | 93           | 29                            | 32    | 32           |
| Vβ6+                 | 92    | 90           | 32                            | 28    |
| Vβ8.2+               | 98    | 96           | 31                            | 49    |
| Vβ14+                | 98    | 95           | 32                            | 32    |

* Day 0 CD8- depleted or 4-d IL-7-stimulated thymic cells from C3H/HeJ mice were stained with anti-CD4PE, the appropriate antibody coupled to FITC and biotinylated mIg-7, revealed by Tricolor-streptavidin, then analyzed by three-color flow cytometry. Cultures were performed with 15 × 10^6 unseparated thymic cells incubated for 4 d in 0.5 ml of culture medium containing 100 U/ml rhIL-7.

† Results are expressed as the percentage of positive cells for IL-7R compared to control, in one representative experiment out of three.
expression of IL-7R on V\(^{38.2}\) cells at the onset of culture exposure to Ib7 is apparently not due to a more important expression of the V\(^{38.2}\) gene family among ID7R + thymocytes (Fig. 1). This suggests a significant bias in the repertoire towards overusage of the V\(^{38.2}\) gene family by CD4-8- \(~\beta\)TCR + thymocytes, whereas no such bias existed in the V\(\beta\) repertoire of cultured CD4-8- thymocytes (data not shown).

**Table 6. Recovery of CD4-8-, CD8- and \(\alpha\beta\) TCR+-defined Thymocyte Subsets after a 4-d Exposure to IL-7 in \(\beta\)2m+ and \(\beta\)2m- Mice**

|                | CD4-8- | CD4+8+ | CD4-8- | CD4+8+ | CD4-8- \(\alpha\beta\)TCR+ |
|----------------|--------|--------|--------|--------|---------------------------|
| Day 0 \(\beta\)2m+ | 13.3   | 84.6   | 10.3   | 1.8    | 1.1                       |
| Day 0 \(\beta\)2m- | 5.3    | 87.6   | 6.4    | 0.7    | 1.0                       |
| Day 4 + IL-7 \(\beta\)2m+ | 13.6   | 1.6    | 7.6    | 2.2    | 8.1                       |
| Day 4 + IL-7 \(\beta\)2m- | 9.4    | 1.3    | 11.5   | 0.7    | 5.2                       |

* Day 0 or 4-d IL-7-stimulated thymic cells from \(\beta\)2m+ or \(\beta\)2m- mice were stained with fluorescence-labeled antibodies specific for CD4, CD8, \(\alpha\beta\)TCR, and analyzed by three-color flow cytometry. Cultures were performed with \(15 \times 10^6\) unseparated thymic cells incubated for 4 d in 0.5 ml of culture medium containing 100 U/ml hrIL-7.

† Results are expressed as viable cell recovery in each subset calculated from \(10^6\) cells at the beginning of culture, in one representative experiment out of three.

and CD4+8- thymocytes before and after 4 d of IL-7 stimulation of total thymocytes, according to their IL-7R expression (Fig. 1). Before stimulation, V\(\beta\) expression was not significantly different between CD4-8- \(\alpha\beta\)TCR+ and CD4-8- subpopulations whether they expressed IL-7R or not (Fig. 1). After a 4-d stimulation with IL-7, there appeared a significant bias in the repertoire towards overusage of the V\$8.2$ gene family among IL-7R+ thymocytes (Fig. 1). This was the case for both CD4-8- \(\alpha\beta\)TCR+ and CD4+8- thymocytes, whereas no such bias existed in the V\(\beta\) repertoire of cultured CD4-8- thymocytes (data not shown).

**IL-7R Expression Among CD4-8- \(\alpha\beta\)TCR+ and CD4+8- Thymocytes According to V\(\beta\) Usage.** The overexpression of the V\$8.2$ gene family by IL-7R+ thymocytes upon exposure to IL-7 is apparently not due to a more important expression of IL-7R on V\$8.2+$ cells at the onset of culture since we could not detect significant differences in this respect between V\(\beta\)$, V\$8.2+$, and V\$14+$, CD4-8-, or CD4+8- thymocytes (Table 5). After 4 d of IL-7 stimulation, the vast majority of CD4-8- cells were IL-7R+ cells, whatever the V\(\beta\) subset considered (Tables 2 and 5). Among CD4+8- thymocytes, the proportion of IL-7R+ cells was significantly higher in the V\$8.2+$ than in the V\$6+$, V\$14+$, or the total CD4+8- subset (Table 5).

**IL7 Does Not Promote Preferential Expansion of V\$8.2+$ Thymocytes from \(\beta\)2 Microglobulin-deficient Mice.** It has recently been demonstrated that overexpression of the V\$8.2$ gene family among CD4-8- \(\alpha\beta\)TCR+ thymocytes is largely due to positive selection of V\$8.2+$ cells by \(\beta\)2-microglobulin-associated class I molecules (11). This finding together with the preferential growth of CD4-8- thymocytes in response to IL-7 prompted us to analyze the V\(\beta\)-TCR repertoire of CD4-8- and CD4+8- cells from \(\beta\)2-microglobulin-deficient mice (\(\beta\)2m-), as compared to control \(\beta\)2m+ littermates, before and after IL-7 stimulation for 4 or 9 d. IL-7 promoted the expansion of CD4-8- \(\alpha\beta\)TCR+ and CD4+8- thymocytes from both \(\beta\)2m+ and \(\beta\)2m- thymocytes and with similar efficacy (Table 6). In addition, CD4+8- \(\alpha\beta\)TCR+ and CD4+8- thymocytes from \(\beta\)2m+ and \(\beta\)2m- mice expressed similar levels of IL-7R in vivo (data not shown).

On analyzing the repertoire of freshly isolated CD4-8- \(\alpha\beta\)TCR+ thymocytes from \(\beta\)2m+ mice, we found, as described by others (11), no bias towards V\$8.2$ expression (Fig. 2). Similarly to C3H/HeJ mice, V\$8.2$ expression by both CD4-8- and CD4+8- \(\alpha\beta\)TCR+ IL-7-stimulated thymocytes from \(\beta\)2m+ mice, is enhanced. Yet, the progressive overexpression of V\$8.2$ was more pronounced in CD4-8- thymocytes. In \(\beta\)2m- mice, the repertoire of CD4-8- \(\alpha\beta\)TCR+ and CD4+8- thymocytes was unchanged, even after 9 d of IL-7 stimulation (Fig. 2). These results show that in our model IL-7 does not induce a specific bias towards V\$8.2$ expression in CD4-8- \(\alpha\beta\)TCR+ or CD4+8- thymocytes from \(\beta\)2m- mice. They thus provide evidence that either CD4-8- \(\alpha\beta\)TCR+ or CD4+8- thymocytes selected by class I molecules preferentially expand in response to IL-7.

**CD4+8- Thymocytes and Peripheral Lymphocytes from Class II-deficient Mice Are Enriched in V\$8.2+$ Cells.** Mice lacking MHC class II molecules are not completely devoid of CD4-8- thymocytes or peripheral T lymphocytes (15). Therefore, the rare CD4-8- thymocytes could have been selected in the absence of class II molecules, and may represent a subpopulation of class I-selected thymocytes. We found that not only peripheral CD4+8- lymphocytes (15) but also CD4-8- thymocytes from class II-deficient mice were enriched in V\$8.2+$ cells (Fig. 3). These in vivo findings strengthen our in vitro demonstration that a fraction of normal CD4-8- thymocytes characterized by overexpression of the V\$8.2$ gene family are selected by class I molecules. Moreover, class I-selected cells could also represent a fraction of peripheral CD4+8- lymphocytes.

**Co-culture of \(\beta\)2m+ and \(\beta\)2m- Thymocytes Does Not Change V\$8.2$ Expression in CD4-8- \(\alpha\beta\) TCR+ or CD4+8- Thymocytes from \(\beta\)2m- Mice.** It has previously been demonstrated that V\$8.2$, CD4-8- \(\alpha\beta\)TCR+ thymocytes are selected by class I molecules expressed by bone marrow-de-
Interleukin 7 Stimulates Class I-selected CD4+8- and CD4+8+ Thymocytes

Figure 2. Vβ-TCR repertoire of CD4+8-αβ TCR+ and CD4+8+ thymocytes in β2m+ and β2m- mice before and after 4- and 9-d IL-7 stimulation. The Vβ-TCR repertoire was analyzed by three-color flow cytometry in total thymocytes from β2-microglobulin-deficient mice (β2m-, hashed) and control littermates (β2m+, solid) before (Day 0) or after IL-7 stimulation (Day 4 + IL-7). This was done in CD4+8- αβ TCR+ (A) and CD4+8+ (B) thymocytes. The Vβ-TCR repertoire is represented as the percentage of positive cells. Results are mean percentages ± SD of three separate experiments.

Figure 3. Vβ8.2 expression in thymic and peripheral CD4+8- lymphocytes from class II-deficient mice. Three color flow cytometry was used to analyze Vβ8.2 expression among CD4+8- lymphocytes (corresponding to the gated R1 population in CD4 versus CD8 dot plots) in the thymus (A) and lymph nodes; (B) of class II-deficient (II-, right) and wild-type control mice (left). Vβ8.2 expression is indicated in each log scale histogram as the percentage of positive cells compared to negative control.

Discussion

In the thymus, a small population of CD4+8+ immature thymocytes gives rise to mature CD4+8- and CD4+8+ thymocytes. During this development process, the vast majority of the TCR repertoire is positively selected so that CD4+8- and CD4+8+ thymocytes express TCRs capable of interaction with self MHC class II and I molecules, respectively (18). However, the striking finding that distinct, although rare, populations of CD4+8- thymocytes and peripheral T lymphocytes are present in mice rendered deficient in MHC class II expression supports the notion that not all CD4+8- thymocytes are positively selected by class II MHC...
molecules (15, 19). Class I selection of a minute fraction of CD4+8- thymocytes could explain this phenomenon. However, it is not known if such cells exist in normal mice.

We observed preferential expansion of normal CD4+8- thymocytes expressing the Vβ8.2 gene family in a culture system of total thymocytes stimulated by IL-7. This preferential expansion generated a bias in the Vβ-TCR repertoire of CD4+8- thymocytes towards overexpression of Vβ8.2. The Vβ8.2 bias did not exist among both CD4+8- and CD4+8- αβTCR+ IL-7-stimulated thymocytes from β2m-deficient mice, despite a similar response to IL-7 stimulation. This finding strongly suggests that the fraction of CD4+8- thymocytes overexpressing Vβ8.2 that expand in our system have previously been selected by class I molecules in vivo. Furthermore, we found that CD4+8- thymocytes and peripheral T lymphocytes from class II-deficient mice were enriched in Vβ8.2+ cells. Taken together, these data provide evidence that, in normal mice, a fraction of CD4+8- thymocytes may be selected by class I molecules. Moreover, it has been reported that rare subsets of CD4+8- thymocytes from normal mice overexpress Vβ8.2. These cells were found in the Ly6C*, NK1.1+ and HSA-3G11-defined subsets of CD4+8- thymocytes, the latter being referred to as the Thy0 population (12, 20, 21). Thy0 thymocytes share other properties with CD4+8- αβTCR+ thymocytes, including an enrichment in CD44+ cells (22). We therefore conclude that class I-positive selection may contribute to the development of the Thy0 CD4+8- thymocyte subset.

Several mechanisms could account for the preferential IL-7-induced growth of class I-selected CD4+8- αβTCR+ and CD4+8- thymocytes in our culture conditions. We showed that this phenomenon was not due to an in vitro selection process. It is also not explained by overexpression of IL-7R on Vβ8.2+CD4+8- thymocytes before culture, though it cannot be excluded that an undetectable fraction of these Vβ8.2+CD4+8- thymocytes are enriched in IL-7R- cells in vivo. The same conclusion applies to the Vβ8+CD4+8- population since the vast majority of CD4+8- αβTCR+ cells were IL-7R before culture, whatever the Vβ expressed. It is thus possible that CD4+8- αβTCR+ thymocytes bearing other Vβs are also selected by class I molecules and are therefore sensitive to IL-7 stimulation. Indeed, Vβ8.2 overexpression among IL-7-stimulated CD4+8- αβTCR+ thymocytes was only slightly enhanced. In addition, our method of IL-7R detection does not discriminate between IL-7 receptors of different affinities. Finally, CD4+8- or CD4+8- αβTCR+ thymocytes selected by class I molecules could have expanded preferentially because they were more mature or in an activated state before culture. Indeed, it has been shown that both CD4-8- αβTCR+ and Thy0 CD4+8- thymocytes are mature cells that respond better to various stimuli, including CD3 cross-linking and mitogens, than other thymocyte subsets (23, 24). Moreover, it has been suggested that the Thy0 subset is composed of activated cells (22).

The particular sensitivity of class I-selected CD4+8- and CD4+8- αβTCR+ thymocytes to IL-7 stimulation leads us to speculate on the possible physiological roles of IL-7. It has been proposed that murine CD4+8- Vβ8+ T cells recognize and are selected by a specific set of nonclassical class I molecules, such class Ib or class I-like CD1 molecules (11, 25). If so, one possible role for these CD4+8- Vβ8+ T cells would be to recognize stress- or inflammation-induced autologous antigens bound preferentially to class I-like molecules, as originally proposed for various subsets of CD4+8- γδTCR+ T cells (11, 26-28). It is thus conceivable that a particular subset of T cells, including class I-selected CD4+8- αβTCR+ and possibly CD4+8- T cells, recognize a defined set of peptides related to inflammation. Interestingly, we have previously found a synergistic effect of IL-1 on IL-7-dependent thymocyte proliferation (13). IL-7 is mainly expressed by thymic stromal cells (2), but its level is probably low in healthy animals and this could explain why these populations are not expanded in normal mice. In contrast, the release of IL-7 cytokines like IL-1 during inflammation may promote their preferential expansion in other organs, together with the release of IL-7 by particular cells, e.g., keratinocytes (29). Another function of CD4-8- αβTCR+ and Thy0 CD4+8- thymocytes is related to their ability to produce IL-4 upon stimulation (12, 23, 24). IL-4 is a major

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**Figure 4.** Vβ-TCR repertoire of CD4+8- αβTCR+ and CD4+8- thymocytes in mixed cultures of β2m+ and β2m- thymocytes after a 9-d IL-7 stimulation. The Vβ-TCR repertoire was investigated by three-color flow cytometry in CD4+8- αβTCR+ (A) and CD4+8- (B) thymocytes from cultures of β2m+, β2m- and mixed β2m+ + β2m- thymocytes after 9-d IL-7 stimulation. In mixed cultures, thymocytes from β2m+ and β2m- were discriminated by the use of anti-H-2Kb mAb, as all β2m+ stimulated thymocytes were H-2Kb+ and all β2m- stimulated thymocytes were H-2Kb- in independent cultures (data not shown). Results are mean percentages of two separate experiments.
regulatory molecule of peripheral immune responses, modulating Th2 versus Th1 responses (30). Interestingly, IL-4 itself seems to be necessary to induce IL-4 production by conventional peripheral CD4+8- T cells (31). IL-4–producing thymocytes could therefore be a source of IL-4 which may influence the peripheral Th1/Th2 balance. If so, the role of IL-7 in the proliferation of thymic IL-4 producers, which remains to be fully demonstrated, would be of major relevance.

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