

## **A $\gamma/\delta$ T Cell Receptor Heterodimer Induces the Expression of CD4 and CD8 in Thymocytes**

By Makio Iwashima,\* Mark M. Davis,\*<sup>†</sup> and Yueh-hsiu Chien\*

From the \*Department of Microbiology and Immunology; and <sup>†</sup>The Howard Hughes Medical Institute, Beckman Center, Stanford University, Stanford, California 94305

### **Summary**

CD4 and CD8 have been useful surface markers for  $\alpha/\beta$  T cell maturation. In an  $\alpha/\beta$  T cell receptor (TCR) transgenic SCID mice system, it has been shown that  $\alpha/\beta$  TCR alone is sufficient to induce CD4 and CD8 surface expression on thymic T cells. Although the late embryonic thymic  $\gamma/\delta$  T cells are predominately single and double positive, it has not been clear if  $\gamma/\delta$  TCR has a similar capacity. In this study, we show that when transgenes encoding the earliest embryonic  $\gamma/\delta$  TCR are coexpressed with the SCID defect, the  $\gamma/\delta$  transgenes promote the appearance of both the CD4<sup>-</sup>8<sup>-</sup> and CD4<sup>+</sup>8<sup>+</sup> T cells in the thymus. Furthermore, the expression of CD4 and CD8 does not require continuous surface  $\gamma/\delta$  TCR expression. These results indicate that  $\gamma/\delta$  TCR alone can promote the CD4/8 surface expression, and may suggest a role for  $\gamma/\delta$  T cells in initiating normal thymic ontogeny.

There are two major types of TCR-bearing T cells in vertebrate animals:  $\alpha/\beta$  T cells and  $\gamma/\delta$  T cells (1-3). In the thymus of adult animals, most of the  $\alpha/\beta$  cells are believed to be derived from double-negative (CD4<sup>-</sup>8<sup>-</sup>) precursor populations, and differentiate through the double-positive (CD4<sup>+</sup>8<sup>+</sup>) stages where various selective events occur. They then downregulate either the CD4 or CD8 surface markers and become mature single-positive (CD4<sup>+</sup>8<sup>-</sup> or CD4<sup>-</sup>8<sup>+</sup>) cells (4-10). Unlike  $\alpha/\beta$  T cells, the adult thymic  $\gamma/\delta$  T cells are almost all double negative (CD4<sup>-</sup>8<sup>-</sup>). Although earlier works also implied that fetal  $\gamma/\delta$  thymocytes are CD4<sup>-</sup>8<sup>-</sup> (11, 12), Itohara et al. (13) showed that through late embryonic life and persistent until 1 wk after birth, the major thymic  $\gamma/\delta$  T cells are both single positive and double positive. While it is clear, that  $\alpha/\beta$  TCR alone can induce the surface expression of CD4 and CD8 (14), it has not been shown whether  $\gamma/\delta$  TCRs have the same capacity. Instead, recent experiments (15) raise the possibility that these CD4 and CD8 expressed on the late embryonic  $\gamma/\delta$  T cells may be induced by  $\alpha/\beta$  T cells. Another possibility is that these surface CD4, CD8 expressions on  $\gamma/\delta$  T cells were induced by cytoplasmic functional  $\alpha/\beta$  TCRs. We have shown that transgenes encoded the earliest  $\gamma/\delta$  TCR can be expressed on the surface of thymic double-positive as well as double-negative cells (16). In this study, we further demonstrated that when the transgenes are coexpressed with the SCID mouse defect, the  $\gamma/\delta$  transgene promotes the appearance of both CD4<sup>-</sup>8<sup>-</sup> and CD4<sup>+</sup>8<sup>+</sup> cells. In addition, the expression of CD4 and CD8 does not require surface  $\gamma/\delta$  TCR expression. These results indicate that  $\gamma/\delta$  TCR alone

can promote the appearance of CD4 and CD8 on thymocytes, and may suggest a role for  $\gamma/\delta$  T cells in initiating normal thymic ontogeny.

### **Materials and Methods**

**Animals.** C3H/HeJ mice were purchased from The Jackson Laboratory (Bar Harbor, ME) and maintained in our animal facility. CB.17 Icr SCID mice were purchased from the Department of Radiation Biology, Stanford University.

**Staining Reagents.** The following reagents were used in this study. mAbs: anti-mouse V $\gamma$ 3 (536) (17), anti-mouse CD3  $\epsilon$  chain (500A2) (18), anti-mouse CD4 (PE-conjugated GK1.5) (Becton Dickinson & Co., Mountain View, CA), anti-mouse CD8 (FITC or biotin-conjugated 53-6.7) (Becton Dickinson & Co.). Others include goat anti-hamster antiserum (Texas Red [TR] conjugated) (Cappel Laboratories, Malvern, PA), goat anti-mouse IgG (allophycocyanin [APC] conjugated) (Bio Meda, Foster City, CA), TR-conjugated avidin (Cappel Laboratories), APC-conjugated avidin (Bio Meda) normal hamster IgG (Cappel Laboratories), goat anti-rat IgG TR conjugated (Caltag, South San Francisco, CA).

**TCR Constructs and Transgenic Mice.** The functional  $\gamma$  and  $\delta$  chain constructs represented those isolated from the Thy-1<sup>+</sup> dEC clone 717 (19). The  $\gamma$  chain construct contains an EcoR1 fragment with the rearranged V $\gamma$ 3J $\gamma$ 1C $\gamma$ 1 gene isolated from a phage library derived from the clone 717. The  $\delta$  chain construct contains a Cla1 fragment with the V $\delta$ 1D $\delta$ 2 J $\delta$ 2 sequence from a cosmid library. The generation of the constructs and transgenic mouse line C3H(T) from C3H/HeJ that expresses the transgenic  $\gamma/\delta$  TCR on T cell surface are described in detail elsewhere (16).

**FACS<sup>®</sup> Analysis.** For FACS<sup>®</sup> analysis (Becton Dickinson & Co., Mountain View, CA), single cell suspensions of thymocytes were

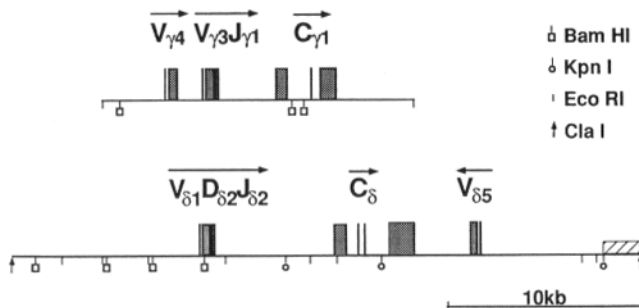
stained as described (17). Detailed combinations of reagents are described in the legend to Fig. 2. Data analysis was performed on 30,000–50,000 cells after proper size gating using the FACS®/DESK Program (Department of Genetics, Stanford University).

**SCID Mouse Screening.**  $\gamma/\delta$  SCID mice were produced by backcrossing (C3H(T)  $\times$  CB17 Icr SCID)F<sub>1</sub> mice with CB17 Icr SCID (20). The mice were screened by ELISA for their serum IgM level using goat anti-mouse IgG (Sigma Chemical Co., St. Louis, MO)-coated plates and alkaline phosphatase-conjugated goat anti-mouse IgM ( $\mu$  chain specific, Sigma Chemical Co.).

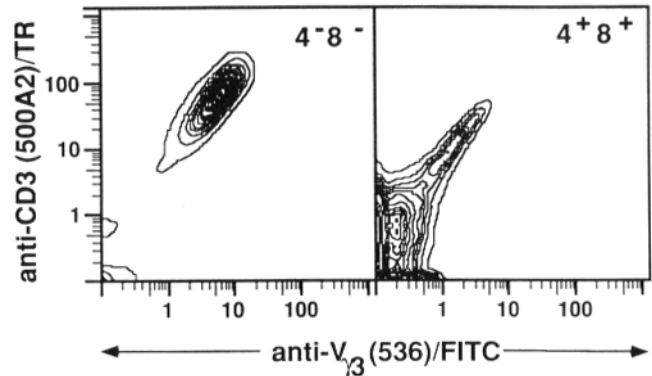
## Results and Discussion

Rearranged  $\gamma$  and  $\delta$  chains genes with TCR coding sequences typical of those found in the earliest fetal thymocytes and adult dendritic epidermal cells (19) (Fig. 1) were co-introduced into the germline of C3H/HeJ mice. Cell surface expression of the transgene was determined by FACS® analysis with a mAb (536) reactive to V $\gamma$ 3 (17). The transgenic  $\gamma$  and  $\delta$  chains cosegregate in all lineages (data not shown). Two independent lines showing transgene expression in T cells were obtained, but only the experimental results from one is reported in detail (16).

In the adult transgenic thymus, the CD4/CD8 staining pattern is very similar to that of nontransgenic littermates. The transgene-encoded  $\gamma$  chain, and thus, presumably, the heterodimer, are expressed on all double-negative (CD4<sup>-</sup>CD8<sup>-</sup>) cells. 30% of the double-positive (CD4<sup>+</sup>CD8<sup>+</sup>) cells also express only the transgenes (Fig. 2). This is not entirely unexpected, because although normal adult thymic  $\gamma/\delta$  T cells are CD4<sup>-</sup>CD8<sup>-</sup>, >35% of the late fetal  $\gamma/\delta$  T cells are CD4<sup>+</sup>CD8<sup>+</sup> (13). One possibility is that these CD4, CD8 antigens expressed on the double-positive  $\gamma/\delta$  cells are induced by the endogenous  $\alpha/\beta$  TCR, as it has been shown that  $\alpha/\beta$  TCR alone can induce CD4 and CD8 surface expression on thymic T cells (14). Alternatively, they can be induced by  $\alpha/\beta$  T cells (15). To clarify this issue, we decided to express the  $\gamma/\delta$  transgenes on a SCID mouse background. The SCID mouse has defect(s) in Ig and TCR gene assembly, and therefore cannot produce functional Ig or TCR gene products. Because the SCID defect is recessive, we first crossed the transgenic mouse line C3H(T) with SCID, then backcrossed the F<sub>1</sub> mice to SCID and analyzed only the progeny that were



**Figure 1.** TCR  $\gamma$  and  $\delta$  gene chain constructs. Maps of the rearranged TCR  $\gamma$  and  $\delta$  chain gene from Thy-1<sup>+</sup> dEC clone 717 used for the generation of transgenic mice (16).

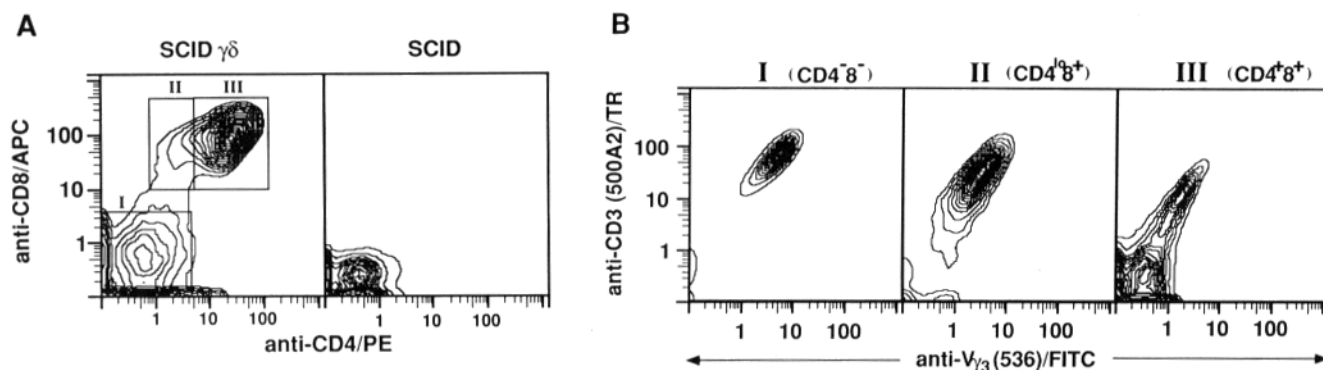


**Figure 2.** CD3 and V $\gamma$ 3 expression in CD4<sup>-</sup>CD8<sup>-</sup>, CD4<sup>+</sup>CD8<sup>+</sup> transgenic thymic subpopulations. Total thymocytes C3H(T) transgenic mice were stained as follows. Cells were first incubated with anti-CD3 antibody (500 A-2), followed by Texas Red (TR)-conjugated goat anti-hamster IgG (Caltag). Residual binding sites on anti-hamster antibody were saturated by incubation with 100  $\mu$ g/ml hamster IgG (Cappel Laboratories). After washing, cells were further stained with FITC-conjugated 536 (anti V $\gamma$ 3), biotin-conjugated anti-CD8, and PE-conjugated anti-CD4. APC-conjugated avidin was added at the end. The thymic population was divided into four subgroups according to surface CD4,CD8 expression. The 500A-2 (y-axis) vs. 536 (x-axis) expression of the two subgroups (CD4<sup>-</sup>CD8<sup>-</sup>, CD4<sup>+</sup>CD8<sup>+</sup>) is presented. Similar results have been observed in at least three independent experiments.

transgene positive and homozygous for the SCID defect (as assayed by low serum Ig levels).

Introducing this pair of  $\gamma/\delta$  TCR genes into SCID mice restores the size of SCID thymus. This is largely, but not exclusively, due to the generation of surface  $\gamma/\delta$ -positive cells. The total number of thymocytes in these  $\gamma/\delta$  SCID mice is 10–30 times greater than that in the SCID mice. About 75% of the cells express the transgenic  $\gamma/\delta$  TCR. There are three major types of thymocytes as defined by surface CD4 CD8 expression (Fig. 3 A); CD4<sup>+</sup>CD8<sup>+</sup>, CD4<sup>lo</sup>CD8<sup>+</sup>, and CD4<sup>-</sup>CD8<sup>-</sup>. They represent 50%, 10%, and 40% of the total thymocytes, respectively. Transgene-positive cells can be found in all these compartments (Fig. 3 B). In the  $\gamma/\delta$  SCID thymus, no single-positive (CD4<sup>+</sup>CD8<sup>-</sup>, or CD4<sup>-</sup>CD8<sup>+</sup>) cells were present. This is consistent with the supposition that these cells are composed mainly of positively selected  $\alpha/\beta$  T cells. While the number of thymocytes in the  $\gamma/\delta$  SCID mouse is similar to that of a normal mouse, the peripheral lymphoid organs remain underdeveloped. This is also consistent with the idea that most of the T cells in these organs are  $\alpha/\beta$  T cells. The lymph node  $\gamma/\delta$  T cells in the  $\gamma/\delta$  transgenic mice as well as in the  $\gamma/\delta$  SCID mice are all CD4<sup>-</sup>CD8<sup>-</sup>. Our results show that a rearranged TCR  $\alpha$  or TCR  $\beta$  gene is not a prerequisite for CD4/CD8 expression, and that  $\gamma/\delta$  TCR alone can induce the expression of CD4 and CD8 on thymic T cells.

In a normal adult mouse thymus, at least 40% of the CD4<sup>+</sup>CD8<sup>+</sup> cells do not show surface TCR expression (17). However, in either  $\alpha/\beta$  TCR transgenics (21–23) or  $\alpha/\beta$  TCR/SCID mice (14), these TCR<sup>-</sup>CD4<sup>+</sup>CD8<sup>+</sup> cells are not generally observed. Instead, all the CD4<sup>+</sup>CD8<sup>+</sup> cells in those systems express only the transgenes. In our  $\gamma/\delta$  transgenics



**Figure 3.** Expression of (A) CD4/CD8 and (B) TCR on the surface of  $\gamma/\delta$  SCID mouse thymocytes. Thymocytes from  $\gamma/\delta$  SCID transgenic mouse derived from backcrossing C3H(T)  $\times$  CB17 Icr SCID<sup>F1</sup> into CB17 Icr SCID were stained with anti-CD4, anti-CD8, anti-CD3, and anti-V $\gamma$ 3, and analyzed as described in Fig. 2. The thymic CD4 CD8 staining pattern is shown in (A). The anti-CD3 (500A-2) vs. anti-V $\gamma$ 3 (536) expression of all three subgroups, as defined in A, is shown. All tested mice showed identical phenotype, and the representative pattern is shown in B.

and  $\gamma/\delta$  SCID mice system,  $\sim 60\%$  of the CD4<sup>+</sup>8<sup>+</sup> thymocytes are TCR<sup>-</sup>, a situation similar to that observed for normal mouse, again reinforcing the idea that the expression of surface CD4 and CD8 does not require persistent surface TCR expression. The fact that not all of the CD4<sup>+</sup>8<sup>+</sup> cells express the transgenic  $\gamma/\delta$  TCR raises the possibility that some of these cells are strictly in the  $\alpha/\beta$  lineage and thus for some reason cannot support  $\gamma/\delta$  TCR surface expression.

Recently, Shores et al. (15) transferred normal mouse bone marrow cells to SCID mouse, and observed the appearance of TCR<sup>-</sup>CD4<sup>+</sup>8<sup>+</sup> and CD4<sup>-</sup>8<sup>+</sup> cells that are of SCID origin. They postulate that the expression of the CD4,CD8 antigens on these cells is induced by the TCR<sup>+</sup> bone marrow-derived cells, and speculate that during fetal ontogeny,

similar mechanisms may perpetuate the generation of CD4<sup>+</sup>8<sup>+</sup>  $\alpha/\beta$  lineage cells. While in their experimental system most of the bone marrow-derived cells are  $\alpha/\beta$  TCR<sup>+</sup>, they suggest that during fetal ontogeny, the proposed "inducers" might be CD4<sup>-</sup>8<sup>-</sup>  $\gamma/\delta$  T cells.

In our  $\gamma/\delta$  SCID system, we do not know if the cell surface CD4/CD8 expression is induced by the  $\gamma/\delta$  transgenes within the cell, or if they were induced by other cells that express surface  $\gamma/\delta$  TCR. Nevertheless, our data indicate that the  $\gamma/\delta$  TCR derived from d15 fetal thymocytes can promote the entry of immature CD4<sup>-</sup>8<sup>-</sup> cells into the CD4<sup>+</sup>8<sup>+</sup> compartment and thus provide useful experimental system for dissecting this process.

We thank S. Eriksson for technical assistance, and B. E. Robertson for preparation of the manuscript.

This work was supported in part by the National Institutes of Health and the Howard Hughes Medical Institute. M. Iwashima was supported in part by a fellowship from the Fulbright Foundation.

Address correspondence to Yueh-hsiu Chien, Department of Microbiology and Immunology, Stanford University School of Medicine, Stanford, CA 94305. M. Iwashima's current address is Department of Medicine, Howard Hughes Medical Institute, University of California, San Francisco, CA 94143.

Received for publication 1 November 1990 and in revised form 26 February 1991.

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