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Immunology

Early expression of a T-cell receptor β-chain transgene suppresses rearrangement of the $V_{\gamma 4}$ gene segment

(transgenic mouse/T-cell receptor $\gamma\delta$ heterodimer/allelic exclusion)

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ABSTRACT **β transgenic mice have a T-cell receptor β-chain gene that is prematurely expressed on the surface of $CD4^-CD8^-$ thymocytes and paired with an uncharacterized non-T-cell receptor α-chain polypeptide. The rearrangement of the T-cell receptor variable region γ chain gene segment $V_{\gamma 4}$, a component of the γ-chain gene that is rearranged and expressed preferentially on thymocytes of normal adult mice, is severely repressed in β transgenic mice. Consequently no $\gamma\delta$ T-cell receptor heterodimers are detectable on the surface of adult thymocytes or splenic T cells. These results indicate that cells expressing $\alpha\beta$ or $\gamma(V_{\gamma 4})$–δ TCRs originate from a common precursor in which the first productive rearrangement of either the β or γ locus determines the further differentiation pathway into either $\alpha\beta$ or $\gamma\delta$ T cells. The repression of $V_{\gamma 4}$ rearrangement by a preexisting β-chain gene may be indicative of one of several mechanisms which ensure that $\gamma\delta$ and $\alpha\beta$ receptors do not as a rule appear on the surface of the same cell.**

T cells expressing $\gamma\delta$ or $\alpha\beta$ heterodimeric T-cell receptors (TCRs) are formed in the thymus where all four receptor loci undergo rearrangement (1–4). Nonproductive, or the occasional productive, γ-chain rearrangement(s) are found in T cells expressing the $\alpha\beta$ TCR ($\alpha\beta$ T cells) and β-chain rearrangement is found in T cells expressing the $\gamma\delta$ TCR ($\gamma\delta$ T cells) (5). This suggests that both types of T cells may originate from a common intrathymic precursor. The argument would be considerably strengthened if it was shown that productive rearrangement of one of the loci would influence rearrangement of other loci. A rearranged TCR β-chain transgene suppresses completely the rearrangement of endogenous genes for the variable region of the β chain (V_β) (6). Here we show that the same transgene suppresses the rearrangement and expression of endogenous γ-chain genes (γ gene).

MATERIALS AND METHODS

Mice. The transgenic founder mice 95 and 93 contain 2 and 20 copies, respectively, of the $V_{\beta 8.2}$ gene segment that codes for an amino acid sequence which can be identified by the monoclonal antibody F23.1. The transgenic mice were backcrossed to C57L mice lacking the $V_{\beta 8.2}$ gene family. Transgenic and nontransgenic mice were identified by Southern blot hybridization of DNA isolated from the tail of the mouse (6). In this report we have analyzed transgenic and nontransgenic littermates as well as normal C57BL/6 mice.

Thymocyte Preparation. Embryonic thymi were prepared as described from time-mated females (7). Thymus lobes were carefully dissected and single-cell suspensions were prepared. $CD4^-CD8^-$ thymocytes were obtained after two cycles of treatment with monoclonal CD4 and CD8 antibodies and complement as described (8).

Cell Surface Staining of Lymphoid Cells. Single-cell suspensions were incubated with CD3, CD4, CD5, and F23.1 antibodies which were uncoupled, biotinylated, or coupled with fluorescein isothiocyanate. Second-step reagents consisted of fluorescein isothiocyanate- or phycoerythrine-coupled anti-mouse or -rat immunoglobulin antibodies. Cells were incubated with first- or second-step reagents for 30 min at 4°C. Cell surface staining was analyzed on a fluorescence-activated cell sorter and data were analyzed and plotted with a Consort C30 program.

Immunoprecipitation. TCRs were precipitated from thymocyte lysates by either the F23.1 antibody, an antiserum specific for the constant region of the α chain (C_α) domain (9) or the anti-γ-chain monoclonal antibody KN365 (anti-γ) (10). Some lysates were cleared by incubation with anti-C_α antibodies and *Staphylococcus* as described (6). Precipitates were analyzed under reducing and nonreducing conditions by polyacryamide gel electrophoresis.

T-Cell Clones. $CD8^+CD4^-$ T-cell clones from β transgenic mice were obtained by stimulating spleen cells from β transgenic mice with allogenic DBA/2 irradiated stimulator cells *in vitro*. T cells were cloned after the second restimulation of interleukin 2-containing medium as described (6).

Southern and Northern Blot Analyses. *Hin*dIII-digested DNAs were electrophoresed into a 0.7% agarose gel, blotted onto a nitrocellulose filter, and hybridized with random-primed probes as described (8). The $J_{\gamma 1}$ probe for the joining (J) region of the γ-chain gene was a *Sty* I–*Hin*dIII fragment from a $V_{\gamma 4}$–$J_{\gamma 1}$ genomic DNA where $V_{\gamma 4}$ is a locus for the variable region of the γ chain and was comprised of $J_{\gamma 1}$ and a part of the flanking intron (11). Assignments of bands to germ-line or rearranged genes was done as described (12). Formaldehyde-treated RNAs were electrophoresed into 1% agarose/formaldehyde gel, blotted onto nylon membranes, and hybridized to random-primed probes as described (8). The $V_{\gamma 2}$ probe is a *Pst* I–*Ava* I fragment from pHDS203 (13).

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Abbreviations: TCR, T-cell receptor; V, J, and C, variable, joining, and constant regions of the TCR genes, respectively; V_β, V_γ, etc., V region of the β chain, γ chain, etc., respectively; C_α C_γ, etc., constant region of the α chain, γ chain, etc., respectively; J_γ, J region of the γ chain.

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RESULTS

Surface Markers on Thymocyte Subsets. In Fig. 1*A* we show that β transgenic mice and nontransgenic littermates contain almost identical populations of thymocytes defined by CD4 and CD8 surface antigens. In addition the CD3 complex is expressed to a similar extent by thymocytes from transgenic and nontransgenic mice: most cells express low levels and ≈20% of the cells expresses higher levels of CD3 molecules. The obvious difference is that practically all transgenic thymocytes express the transgenic β chain on the cells surface: again the majority expresses lower levels while ≈20% of cells, representing the single positive $CD4^+CD8^-$ and $CD4^-CD8^+$ thymocytes (14), shows higher levels of receptor.

We have prepared double-negative cells by cytotoxic treatment of thymocytes from β transgenic as well as C57BL/6 mice with anti-CD4 and -CD8 antibodies. We used C57BL/6 mice because a significant fraction of their T cells expresses TCRs in part encoded by the $V_{\beta 8}$ gene family so that intensity of expression of $V_{\beta 8}$ gene products in transgenic and normal mice could be compared. As shown in Fig. 1*B* the majority of double-negative cells from β transgenic mice expresses low levels of CD3 and, unlike cells from C57BL/6, low levels (comparable to that of $CD4^+CD8^+$ thymocytes) of the transgenic β chain (detected by F23.1) on the cell surface. Staining of these cells with CD5 antibodies shows that they are CD5 dull (Fig. 1*C*). Because CD5-dull $CD4^-CD8^-$ thymocytes contain the precursors for all other thymocyte subpopulations (15), these experiments suggested an "early" expression of a receptor containing the TCR β chain on cells which usually do not express TCRs containing β chain (16, 17). This was verified by the analysis of thymocytes from 14- and 15-day-old embryos: the transgenic β chain could be detected on the surface of ≈10% of thymocytes from 14-day-old embryos and of the majority of thymocytes from 15-day-old embryos (Fig. 2).

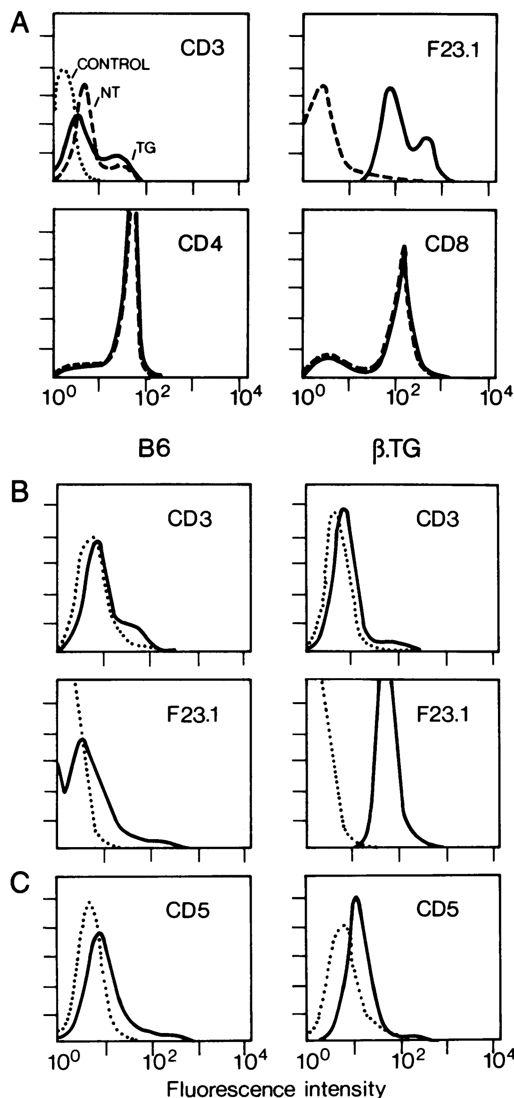

FIG. 1. (*A*) Staining of thymocytes from β transgenic mice (solid line) and from nontransgenic littermates (broken lines) by CD3 and F23.1 (*Upper*) and CD4 and CD8 antibodies (*Lower*). (*B*) Staining of $CD4^-CD8^-$ thymocytes from normal C57BL/6 (B6) and β transgenic (β.TG) mice with CD3 and F23.1 antibodies (broken lines, unstained controls). (*C*) Staining of $CD4^-CD8^-$ thymocytes from normal C57BL/6 (B6) and β transgenic (β.TG) with CD5 antibodies (broken lines, unstained controls).

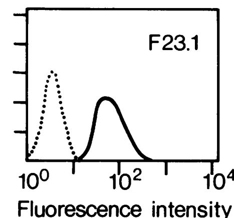

FIG. 2. Staining of thymocytes (solid line) from β transgenic mice from day 15 of gestation by F23.1 antibodies (broken line, unstained cells).

Surface Expression of β Chains Without TCR α Chains. To analyze the chain composition of the receptor expressed on $CD4^-CD8^-$ thymocytes, we precipitated lysates from these cells with either F23.1 or C_α antibodies before and after preclearing of the lysates with C_α antibodies. In control experiments, the lysates from the $\alpha\beta$-TCR-expressing B6.2.16 clone were used. Fig. 3 shows that F23.1 antibodies precipitated a disulfide-linked dimer from the $CD4^-CD8^-$ thymocytes of β transgenic mice as well as the B6.2.16 clone. In contrast C_α antibodies required a similar dimer from the B6.2.16 clone but not from the $CD4^-CD8^-$ thymocytes. Preclearing of the lysates with C_α antibodies removed the dimers precipitated by both the F23.1 as well as the C_α antibody from the lysates of B6.2.16 clone but did not affect the precipitate obtained with the F23.1 antibody and the lysates of $CD4^-CD8^-$ thymocytes from β transgenic mice. This indicates that the transgenic β chain expressed on the surface of $CD4^-CD8^-$ thymocytes is not paired with a TCR α chain.

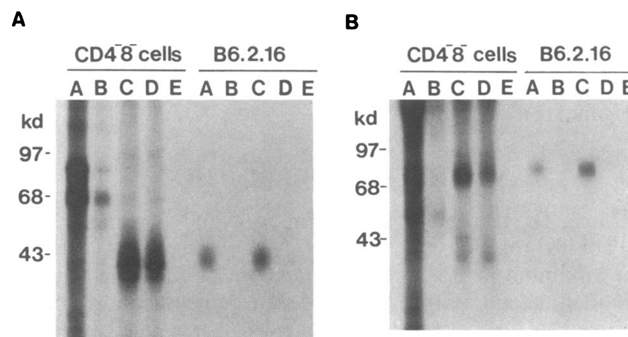

FIG. 3. Association of TCR β chains with molecules other than TCR α chains on $CD4^-CD8^-$ thymocytes in β transgenic mice. TCRs were precipitated before (lanes A and C) or after (lanes B and D) preclearing with anti-C_α antibody from $CD4^-CD8^-$ thymocytes or B6.2.16 cells and analyzed by $NaDodSO_4$/PAGE under reducing (*A*) or nonreducing (*B*) conditions. Lanes: A and B, immunoprecipitates by anti-C_α; C and D, immunoprecipitates by F23.1; E, precipitates without antibody.

Lack of $\gamma\delta$ on Transgenic Thymocytes. The $\gamma\delta$ TCR is normally expressed on about 5% of CD5-dull $CD4^-CD8^-$ thymocytes in adult mice (18). Since in β transgenic mice the developmental timing of the TCR β-chain expression is abnormal and the vast majority of this thymocyte subpopulation express a TCR containing the β transgene, the question arose as to the effect of the β transgene on the generation of $\gamma\delta$-TCR thymocyte population. This was analyzed by immunoprecipitation of lysates prepared from surface-labeled total $CD4^-CD8^-$ thymocyte populations with the anti-γ monoclonal antibody KN365. Lysates from nontransgenic littermates (Fig. 4*a*, lanes A and B, and Fig. 4*b*, lane B) and the control $\gamma\delta$ T-cell hybridoma KN6 (Fig. 4*a*, lanes E, and Fig. 4*b*, lane A) gave characteristic $\gamma\delta$ heterodimers while those from transgenic mice exhibited no detectable $\gamma\delta$ signals (Fig. 4*a*, lanes C and D, and Fig. 4*b*, lane C). As expected, ample $\alpha\beta$ signals were obtained when cell lysates precleared with anti-γ antibody were subsequently immunoprecipitated with a purified anti-α antiserum (Fig. 4*b*, lanes D and E).

γ Gene Rearrangement and Expression in Transgenic Mice. Is the lack of $\gamma\delta$ TCR on transgenic thymocytes due to repression of gene rearrangement? We investigated this question by Southern blot analysis of DNA isolated from thymocyte populations as well as from individual T-cell clones prepared from the β transgenic mice. As shown in Fig. 5*A*, V_4–J_1 γ-chain rearrangement, which is primarily responsible for the surface expression of $\gamma\delta$ TCRs on adult thymocytes and splenic T cells in normal mice (refs. 8, 17, 18, and Y. Takagaki, N. Nakanishi, I.I., O. Kenagawa, and S.T., unpublished observation), is severely hampered in transgenic mice (lanes C and D). By contrast the V_2–J_2 rearrangement (Fig. 5*A*) and the transcription of the resulting $V_2J_2C_2$ γ-chain gene (Fig. 5*B*) are only moderately affected by the presence of the β transgene. The actin probe serves as a control for the amount of total RNA analyzed (Fig. 5*B*).

In further experiments $\alpha\beta$-TCR-bearing T-cell clones from β transgenic mice were obtained by stimulation with allogeneic x-irradiated DBA/2 spleen cells and cloned in interleukin 2-containing medium (6). Analyses of the γ gene loci in at least four of five T-cell clones are in excellent agreement with the population analysis described above. Thus, unlike most $\alpha\beta$ T-cell clones prepared from normal mice (5), all of the five T-cell clones from β-transgenic mice had $J_{\gamma 1}$ in the germ-line configuration while, as in most "normal" T-cell clones, $J_{\gamma 2}$ was rearranged in all clones derived from transgenic mice (four out of five T-cell clones were examined but the fifth one was not).

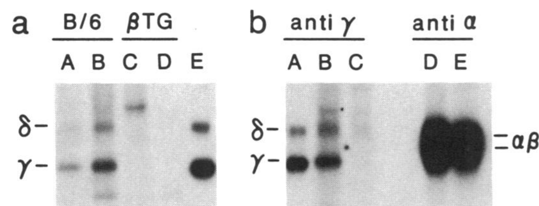

FIG. 4. Analysis of $\gamma\delta$- and $\alpha\beta$-TCR proteins expression on β transgenic thymocytes. (*a*) Cell lysates from total (lanes A and C) or $CD4^-CD8^-$ (lanes B and D) thymocytes were immunoprecipitated with the anti-γ monoclonal antibody KN365 and analyzed by $NaDodSO_4$/PAGE under reducing conditions. Lanes: A and B, C57BL/6; C and D, β transgenic; E, $\gamma\delta$-expressing hybridoma. (*b*) Lysates made from total thymocytes were first immunoprecipitated with protein A-Sepharose beads coated with the anti-γ antibody KN365. The unprecipitated material was then subjected to a second immunoprecipitation with a purified anti-Cα antiserum. Immunoprecipitates from the first (lanes B and C) and the second (lanes D and E) reactions were analyzed by $NaDodSO_4$/PAGE under reducing conditions. Lanes: A, hybridoma KN6; B and D, C57BL/6 thymocytes; C and E, thymocytes from the β transgenic mouse.

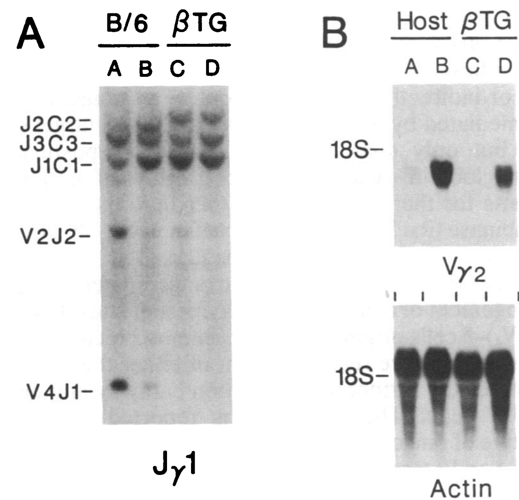

FIG. 5. γ gene rearrangement and expression in β transgenic mice. (*A*) Analysis of γ gene rearrangement in β transgenic mice. *Hin*dIII-digested DNA from thymocytes (lanes A and C) and splenocytes (lanes B and D) of C57BL/6 and β transgenic mice were electrophoresed on agarose gel, transferred onto a nylon filter, and hybridized with the $J_{\gamma 1}$ probe. This probe hybridizes to $J_{\gamma 1}$, $J_{\gamma 2}$, and $J_{\gamma 3}$ gene segments (12). J_2C_2, J_3C_3, and J_1C_1, germ-line position; V_2J_2 and V_4J_1, rearranged position. A strain polymorphism explains the different migration patterns of J_2C_2 germ-line bands of C57BL/6 and β transgenic mice. (*B*) Northern blot analysis of thymocyte and splenocyte RNA from β transgenic mice. Total RNAs from splenocytes (lanes A and C) and thymocytes (lanes B and D) of β transgenic mice and control littermates were electrophoresed on agarose gel, blotted onto nylon filters, and hybridized with $V_{\gamma 2}$ and actin probes.

DISCUSSION

The results reported here indicate that the productive rearrangement of a TCR β-chain gene can lead to the surface expression of that β gene without concomitant expression of a TCR α chain. At present we do not know whether this β chain is paired with another polypeptide chain or expressed as a homodimer. If it is expressed as a heterodimer, it is most likely not paired with TCR γ chain because anti-γ antibodies fail to precipitate a heterodimer from $CD4^-CD8^-$ thymocytes. Pairing with a δ chain is also unlikely because most known mouse δ chains migrate slower than β chains (refs. 8, 10, 17, 18, and Y. Takagaki, N. Nakanishi, I.I., O. Kenagawa, and S.T., unpublished observations) while the precipitation with F23.1 antibodies did not show a band of higher molecular weight than that of the β chain (Fig. 3). It is possible that a new form of a TCR is present in nontransgenic but has escaped detection because of the very similar molecular masses of both chains to the masses of the α and β chains. However, the possibility that the transgenic β chain pairs with a rare or unknown δ chain cannot be ruled out.

Whatever the form of the surface receptor containing TCR β chains, the results reported here suggest that the productive rearrangement of the TCR β-chain gene and possibly its expression on the cell surface are events that have pleiotropic effects on the development of both $\alpha\beta$ and $\gamma\delta$ T cells: not only is the productive rearrangement of endogenous V_β genes completely suppressed (6) but also the rearrangement of certain γ gene segments (V_4), but not other γ gene segments (V_2), is completely suppressed as shown here. One may argue that the negative effect of the β-chain gene on the rearrangement of V_β and V_γ genes may be executed indirectly by accelerated α-chain gene expression leading to an $\alpha\beta$ TCR which shuts off all further rearrangement. Such a feedback

mechanism by a surface-expressed receptor is supported by experiments with the immunoglobulin gene system (19, 20).

Regardless of whether the negative feedback occurs directly or indirectly the suppression of rearrangement seems to be mediated by a mechanism which has its target in all V_{β} genes but only some V_{γ} genes. Thus, as postulated for immunoglobulin genes, the accessibility of certain gene segments for the recombinase rather than the acting of the recombinase itself may be a crucial event in allelic and nonallelic exclusion of TCR genes (21).

The fact that a productively rearranged β gene suppresses rearrangement of a nonallelic $V_{\gamma 4}$ locus indicates that both $\alpha\beta$ and $\gamma(V_4)$–δ cells originate from a common precursor in which the first productive rearrangement of either the β or γ locus determines the further differentiation pathway into either $\alpha\beta$ or $\gamma\delta$ T cells. The observations reported here may be indicative of one of several mechanisms which ensure that as a rule $\gamma\delta$ and $\alpha\beta$ receptors do not appear on the surface of the same cell.

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