Impaired NK1.1 T Cell Development in Mice Transgenic for a T Cell Receptor \(\beta\) Chain Lacking the Large, Solvent-exposed C\(\beta\) FG Loop

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Summary

A striking feature of the T cell receptor (TCR) \(\beta\) chain structure is the large FG loop that protrudes freely into the solvent on the external face of the C\(\beta\) domain. We have already shown that a transgene-encoded V\(\beta\) 8.2+ TCR \(\beta\) chain lacking the complete C\(\beta\) FG loop supports normal development and function of conventional \(\alpha/\beta\) T cells. Thus, the FG loop is not absolutely necessary for TCR signaling. However, further analysis has revealed that a small population of \(\alpha/\beta\) T cells coexpressing N K1.1 are severely depleted in these transgenic mice. The few remaining N K1.1 T cells have a normal phenotype but express very low levels of TCR. We find that the TCR V\(\beta\) 8.2+ chain lacking the C\(\beta\) FG loop cannot pair efficiently with the invariant V\(\alpha\) 14-J\(\alpha\) 281 TCR \(\alpha\) chain commonly expressed by this T cell family. Consequently, fewer N K1.1 T cells develop in these mice. Our results suggest that expression of the V\(\alpha\) 14+ TCR \(\alpha\) chain is particularly sensitive to TCR-\(\beta\) conformation. Development of N K1.1 T cells appears to need a TCR-\(\beta\) conformation dependent on the presence of the C\(\beta\) loop that is not necessarily required for assembly and function of TCRs on most \(\alpha/\beta\) T cells.

Key words: TCR • C\(\beta\) FG loop • mutagenesis • N K1.1 T cells • V\(\alpha\) 14

Materials and Methods

TCR-\(\beta\) Mutagenesis. The wild-type TCR \(\beta\) chain (V\(\beta\) 8.2-J\(\beta\) 21) cDNA was used as a template for mutagenesis. Deletion of the 14 nucleotides forming the C\(\beta\) FG loop has been described (7). Transgenic vectors have also been described previously (22). Transfection of Cell Lines. Packaging cell lines GP + E-86 (23) were transfected with retroviral vector LXSN expressing the V\(\beta\) 8.2-J\(\beta\) 21+ TCR-\(\beta\) or \(\beta\)-loop chain or LXSP expressing the V\(\alpha\) 4-J\(\alpha\) 47+ or V\(\alpha\) 14-J\(\alpha\) 281+ TCR-\(\alpha\) chain cDNA. The TCR-\(\alpha\) chain (V\(\alpha\) 14-J\(\alpha\) 281) was cloned from N K1.1 \(\alpha/\beta\) T cell hy-
bridoma total RNA provided by R. MacDonald (Ludwig Institute for Cancer Research, Lausanne, Switzerland). After appropriate selection of the packaging cells, the infectious supernatants were used to infect TCR- hybridomas (24) as previously described (25). The TCR-β or β-loop- chain was first introduced into the hybridomas and, after neomycin selection (G418; 1 mg/ml), these cells were superinfected with TCR-α chain by culturing on packaging lines producing LXSP TCR-α Vα4-Jα47 or Vα14-Jα281. The hybridomas were then maintained in IMDM supplemented with 2% FCS, neomycin, and puromycin (10 μg/ml). TCR expression was tested by FACS™ as early as 4 d after selection. Stable transfectants were maintained in G418 and puromycin-containing medium.

TCR Immunoprecipitation and Western Blot Analysis. Hybridomas were lysed at 2 × 10^6 cells/ml in 1% Triton X-100 (Bio-Rad Labs), 150 mM NaCl, 20 mM Tris/HCl, and 5 mM EDTA, pH 7.5, buffer containing complete protease inhibitors (Boehringer Mannheim) for 30 min at 4°C. Lysates cleared of cell debris were immunoprecipitated with purified mAb F23.1 (2 μg/ml) and protein G-Sepharose (Pharmacia). After washing with lys buffer and PBS, the lyophilized pellets were resuspended in reducing SDS buffer, loaded on a 4–12% Bis-Tris precast gel (Novex), and transferred onto nitrocellulose membrane Hybond-C Extra (Amersham). Blots were probed in PBS 6% blotting blocker nonfat milk (Bio-Rad Labs.) and 0.2% Tween with purified mAb H58 (anti-C β), RM4-5 (anti-CD44, Molecular Probes, Inc.), and 0.2% Tween with purified mAb H58 (anti-C-α), followed by goat anti-hamster horseradish peroxidase-labeled mAb (Southern Biotechnology Associates, Inc.) or biotinylated F23.1 (anti-Vβ8) mAb followed by streptavidin-horseradish peroxidase (Southern Biotechnology Associates, Inc.). The proteins were detected with a chemiluminescent detection system (Pierce Chemical Co.).

Results and Discussion

To avoid any influence of the endogenous β locus on the expression of the mutated β chain, mice transgenic for the Vβ8.2- TCR-β chain lacking the Cβ FG loop (β-loop-') were backcrossed to TCR-β- mice (26). T cell development in these mice was compared with that in wild-type Vβ8.2- TCR-β chain transgenic mice, also with a β- chain background. A detailed study of these results was presented in our previous study (7), peripheral T cells from mice transgenic for the TCR-β or β-loop- chain express equal levels of the TCR-CD3 complex, and whereas the anti-Vβ8 F23.1 mAb recognizes all T cells, the Cβ-specific H57 mAb does not stain cells expressing the TCR-β loop- chain (Fig. 1; reference 4). It is worth pointing out that in the absence of the Cβ, FG loop, the anti-CD3e 2C11 mAb stains better, suggesting that the antigen recognized is more accessible, a result that might not be surprising, as one of the CD3e chains is physically adjacent to the β chain in the TCR-CD3 complex (5).

We consistently found that TCR-β loop- transgenic mice have significantly fewer NK1.1α/β^+ T cells in the thymus, liver, and spleen (data not shown) in comparison to TCR-β transgenic mice or wild-type littermates (Fig. 2). Hence, monoclonal expression of this PCR (1 μl) was used for a second round of PCR (35 cycles) to individually reamplify the Vα14^+ TCR-α chain or Vβ8.2- TCR-β chain using the same specific primers.

![Figure 1](https://example.com/figure1.png)

**Figure 1.** T cells expressing the Vβ8.2^+ TCR-β chain lacking the FG loop cannot be stained with the Cβ-specific H57 mAb. LN cell suspensions from TCR-β and β-loop- transgenic mice were stained with anti-CD3 together with anti-Vβ8 or anti-Cβ mAb. 1358 TCR Cβ Mutation Affecting NK1.1 T Cell Development
the wild-type Vβ8.2+ TCR β chain allows NK1.1 T cell development comparable to that of nontransgenic littermates. Characteristically, NK1.1 T cells express intermediate levels of TCR (8). Interestingly, in TCR β-loop− transgenic mice, TCR expression on the few remaining NK1.1 T cells is even lower than in control animals; these cells express about four times less TCR than do those in wild-type β-transgenic mice (Fig. 3). Otherwise, NK1.1 T cells in β-loop− transgenic mice express normal levels of CD4 and are CD44+CD62L− and IL-2Rβ1+, as expected for this T cell population (8). CD1d, a β2 microglobulin–associated molecule required for NK1.1 T cell development (9, 10), is also expressed at normal levels in TCR β-loop− transgenic mice (data not shown).

To determine if development of NK1.1 α/β+ T cells could be rescued by the expression of endogenous β chains, as has been described for other TCR-β transgenic mice (19), we studied NK1.1 T cell frequency in TCR β-loop− transgenic mice on a β1/2 background. We have already observed that in these mice, inhibition of β rearrangements via allelic exclusion is not total, and ~10–20% of peripheral T cells can express endogenous β chains (data not shown). NK1.1 α/β+ T cells expressing endogenous β and β-loop− chains could be distinguished by the Cβ-specific H57 mAb, which cannot stain T cells expressing the mutated β chain (Fig. 1). As shown in Fig. 4, expression of endogenous β chains can rescue NK1.1 T cell development to a certain extent. NK1.1 Cβ+ cells appear in the livers of TCR β-loop− transgenic mice on a β1/2 background. Yet these cells only account for about one-third of the whole NK1.1 T cell population. The NK1.1 Cβ+ T cells are still predominant. There are two populations of NK1.1 Vβ8+ cells, which express either intermediate or low TCR levels in TCR β-loop− transgenic mice on a β1/2− background. Interestingly, expression of endogenous β chains accounts for most of the NK1.1 T cells expressing intermediate TCR levels. Thus, expression of endogenous β chains did rescue some NK1.1 T cell development and restore TCR expression to intermediate levels. This result strongly suggested that the Cβ FG loop is needed for efficient TCR assembly in NK1.1 T cells.

As most NK1.1 α/β+ T cells express an invariant Vα14-Jα281 TCR α chain (20, 21), and the mutant TCR β chain is expressed at normal levels by conventional α/β T cells (Fig. 1) but not by NK1.1 T cells (Fig. 3), we assessed whether the mutant Vβ8.2+ TCR β chain could still pair with the Vα14+ TCR α chain. TCR− hybridomas were
were transfected with cDNAs coding for either the wild-type TCR β or β-loop− chain together with the Vα14+ TCR α chain or Vα4+ TCR α chain (the original partner of the nonmutated β chain) cDNAs. As shown in Fig. 5 A, the TCR β-loop− chain clearly pairs with and is expressed on the cell surface with the Vα4+ TCR α chain but is barely detectable on the cell surface with the Vα14+ TCR α chain. In contrast, the wild-type TCR β chain is expressed on the cell surface with both α chains (Fig. 5). However, the Vα14+ TCR is expressed at lower levels than is the Vα4+ TCR. This observation may reflect the in vivo situation in which a TCR on NK1.1 T cells is expressed at lower levels than on conventional α/β T cells. To assess whether impaired cell surface expression of the TCR wild-type β and β-loop− chain together with the Vα14+ TCR α chain is due to a problem of pairing, TCRs from the transfectants were immunoprecipitated with anti-Vβ8 mAb. As can be seen in Fig. 5 B, the TCR α chain can be immunoprecipitated with the TCR β chain in all transfectants expressing the TCR on the cell surface. In contrast, the Vα14+ TCR α chain cannot be coimmunoprecipitated with the mutant β chain in detectable amounts. This result implies that the Vα14+ TCR α chain pairs very poorly with the Vβ8− TCR β chain lacking the Cβ-FG loop. It is worth pointing out that in the hybridomas producing the wild-type β and Vα14+ chains, many fewer assembled α/β dimers can be immunoprecipitated compared with control Vβ8/ Vα4 dimers. This latter result suggests that mere physical constraints on the assembly of the β chain with the Vα14+ TCR α chain exist and is consistent with the low TCR expression on normal NK1.1 T cells.

Next, we assessed whether the NK1.1 α/β+ T cells that do develop in TCR β-loop− transgenic mice express the Vα14+ TCR α chain by performing RT-PCR on single NK1.1 CD3+ T cells sorted from TCR-β and β-loop− transgenic mice. As summarized in Table I, the frequency of NK1.1 T cells expressing Vα14 is not significantly decreased in TCR β-loop− transgenic mice in comparison to wild-type TCR-β transgenic animals. One has to keep in

**Figure 4.** Expression of endogenous TCR β chains can rescue some NK1.1 T cell development. Liver cell suspensions from TCR β, β-loop− transgenic with a β−/− background (β-loop− β-endo−/−) or β−/− background (β-loop− β-endo+/−) were triple stained with anti-NK1.1, anti-Cβ, and anti-Vβ8 mAbs. Numbers in dot plots are percentages of the total adjacent gated dots. Histograms represent expression of Cβ (in β-loop− transgenic mice with a β−/− or β+/− background) for the NK1.1− gated population expressing either intermediate (NK1.1−Vβ8int) or low levels of TCR (NK1.1−Vβ8low).

**Figure 5.** Inefficient pairing of the Vα14+ TCR α chain with the Vβ8.2+ TCR β chain lacking the complete Cβ FG loop. (A) TCR− hybridomas were transfected with either the Vβ8.2+ wild-type TCR β or β-loop− chain, together with the Vα14+ TCR α chain or Vα4+ TCR α chain. Stable transfectants were stained with biotinylated anti-CD3 mAb, followed by streptavidin–allophycocyanin. Stainings of cells transfected with only the TCR β or β-loop− chain are shown as controls. Numbers in parentheses represent the mean fluorescence intensities of CD3 staining. (B) TCRs of transfected hybridomas or Vβ8+ T hybridoma control (A5) (8.0, 8.0, 10.0, 1.40, 20.0, 20.0, and 8.0 × 107 cells per lane, respectively, from left to right) were immunoprecipitated with anti-Vβ8 mAb (F23.1), electrophoresed on a 4–12% gel in reducing conditions, and blotted with anti-Cα (H58) or anti-Vβ8 mAb as described in Materials and Methods. Numbers represent protein molecular mass (kD).
We have previously shown that in conventional T cells expressing Vβ8.2, deletion of the Cβ FG loop has no effect on Vα (7) and Jα repertoire usage (our unpublished data). This result suggested that no drastic conformational changes in the TCR β chain were created by the mutation. However, in this study we clearly show that expression of the Vα14+ α chain is sensitive to deletion of the Cβ FG loop. Therefore, deletion of the Cβ FG loop must create some subtle change in TCR β chain conformation. It seems that expression of the Vα14+ α chain does not allow much structural flexibility of the TCR, as it is particularly sensitive to TCR β chain conformation. Its expression might impose stringent constraints on α/β assembly. This could at least partially explain why the Vβ repertoire of NK1.1 T cells is relatively limited (30). Pairing with the apparently conformation-sensitive Vα14-Jα281 TCR α chain could be the initial pressure on Vβ usage in NK1.1 T cells (19). Recently, results obtained by using Vα14-transgenic mice suggested that selection was the main force in shaping the NK1.1 T cell repertoire (31). Here we have shown that in addition to selection, differential Vα-Vβ pairing can also potentially influence the NK1.1 T cell diversity. In summary, our data show that subtle changes in the TCR β chain conformation (which do not seem to affect conventional Vβ8.2+ α/β TCRs) can substantially alter pairing with the Vα14+ α chain and impair NK1.1 T cell development.

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