**T Cell Receptor \(\beta\) Chain Lacking the Large Solvent-exposed C\(\beta\) FG Loop Supports Normal \(\alpha/\beta\) T Cell Development and Function in Transgenic Mice**

By Sylvie Degermann, Giuseppina Sollami, and Klaus Karjalainen

From the Basel Institute for Immunology, CH-4005 Basel, Switzerland

**Summary**

The striking and unique structural feature of the T cell receptor (TCR) \(\beta\) chain is the bulky solvent-exposed FG loop on the C\(\beta\) domain, the size of almost half an immunoglobulin domain. The location and size of this loop suggested immediately that it could be a crucial structural link between the invariant CD3 subunits and antigen-recognizing \(\alpha/\beta\) chains during TCR signaling. However, functional analysis does not support the above notion, since transgene coding for TCR \(\beta\) chain lacking the complete FG loop supports normal \(\alpha/\beta\) T cell development and function.

**Key words:** T cell receptor • C\(\beta\) domain • mutagenesis • transgenic mice • T cell development and function

Here we report our finding that the TCR \(\beta\) chain lacking the complete 14-amino acid FG loop is able to support normal T cell development and function in transgenic mice.

**Materials and Methods**

TCR-\(\beta\) Mutagenesis. A retroviral expression vector LXSN coding for the wild-type TCR \(\beta\) chain (V\(\beta\)8.2-J\(\beta\)2.1) cDNA was used as template for mutagenesis. Deletion of the region corresponding to the 14-amino acid FG loop of the C\(\beta\) domain was performed by linking PCR. A 1:1 ratio of the products from PCR 1 (5’ oligo of V\(\beta\)8.2 GATTCCTTACCAAGATGGCTCATCAGGCCTTC [oligo A] and 3’ oligo spanning the deletion GTCACACAGAACATCAGTGCAGAG and 3’ oligo containing the stop codon AGGATCCTCATGAGTTTTTTCTTTTGAC [oligo B]) was used as template for PCR 3 (oligo A and B). The PCR product was digested with EcoRI and BamHI and cloned into an EcoRI and BamHI–opened retroviral vector LXSN. Deletion (underlined amino acids 231–244) GLSEEDKWPEGSPKPV was then verified by DNA sequencing. Transgenic vectors were as described previously (8).

Transfection of Cell Lines. Infectious retroviral stocks were generated by transfecting packaging cell lines GP + E-86 (9) with retroviral expression vectors LXSN (neo/mycin resistant) coding for wild-type or mutant TCR \(\beta\) chain, or vectors LXSP (puromycin resistant) coding for wild-type TCR \(\alpha\) chain (V\(\alpha\)4-J\(\alpha\)47). The supernatants from appropriately selected packaging cell lines were used to infect TCR– hybridomas. The wild-type \(\beta\) or mutant \(\beta\) chain were first introduced into the hybridomas, and after neomycin selection (G418, 1 mg/ml) these lines were superinfected separately with TCR \(\alpha\) chain as described previously (10).
The cell lines were then cultured in IMDM supplemented with 2% FCS, G418, and puromycin (10 μg/ml). TCR expression was tested by FACS as soon as 4 d after selection. Stable transfectants were maintained in G418 and puromycin-containing medium.

Mice. BALB/c and C57BL/6 mice were purchased from IFFA-Credo. The TCR-β knockout mice used in this study have already been described (11), and were bred in our specific pathogen–free animal facility with the wild-type TCR-β or mutant TCR-β transgenic mice.

Flow Cytometry and Antibodies. Immunofluorescence stainings were done as described previously (12). Flow cytometric analysis was performed with a FACScalibur equipped with CellQuest software (Becton Dickinson). The reagents used were mAbs biotinylated 145-2C11 (anti-CD3ε), PE-labeled RM4-5 (anti-CD4) and FITC-labeled H57-597 (anti-CD8) (Molecular Probes, Inc.). The RR8-1, RR3-16, B20.1, B21-14, and B21-121 (anti-CD8), and RR8-1, RR3-16 (anti-Vα3.2), B20.1 (anti-Vα2), and RR8-1, RR3-16 (anti-Vα11.1, 2) (all seven mAbs purchased from PharMingen), Cy5-labeled 55-6.7 (anti-CD8), fluorescein-succinimidyl-ester (FLUOS)-labeled F23.1 (anti-Vβ8.1, 2, 3) (14), and second-step reagent streptavidin-allophycocyanin (APC) (Molecular Probes, Inc.).

Figure 1. The mutant TCR β chain is functionally expressed on the cell surface. (A) Cells transfected with TCR α chain together with a control β (αβ) or mutant β chain (αβ-loop-1) were stained with biotinylated anti-CD3ε mAb followed by streptavidin-APC. Staining of cells transfected with mutant β chain only is shown as negative control. Numbers represent the mean of fluorescence intensity of CD3 staining. (B and C) Functional response of 10^7 TCR transfectants (αβ, αβ-loop-1, αβ-loop-2) cultured with 10^6 irradiated antigen-presenting cells (B cell lymphomas A20) and the indicated concentrations of influenza hemagglutinin peptide HA 110-119 (B) or superantigen SEC 3 (C). After 20 h, the culture supernatant was collected and tested for the presence of lymphokines using the IL-2–dependent proliferation assay of HT2 cell lines.
orously assess the functional potential of the TCR containing the mutant β chain in normal physiological settings in vivo, we generated transgenic mice expressing either a wild-type or a loop-deleted version of the TCR β chain. The β transgenes, as in the above transfection studies, were derived from 14.3d T cell hybridoma expressing the TCR specific for influenza hemagglutinin peptide HA 110-119 in the context of I-E^d MHC class I molecules (16). In fact, it was the very same β chain (Vβ8.2-Jβ2.1) whose three-dimensional structure was first solved, thus providing us with the inspiration for the current study (3). Two characteristics of the transgenic lines used here were considered essential for straightforward interpretation of the data. First, the level of α/β TCR expression was identical in both lines (Fig. 2). Presumably the small handicap of the mutant β chain in the TCR assembly could be compensated by higher intracellular expression. Second, both transgenes were bred to TCR-β2/2 background to avoid any contribution of endogenous β chains for the observed α/β T cell behavior (11).

Mutant TCR β Chain Supports Normal α/β T Cell Development. α/β T cell development proceeds undisturbed and similarly in both TCR β chain transgenic lines as shown by flow cytometric analysis of thymic and lymph node cells (Fig. 2). Even the skewing into single positive CD4 thymocytes, as noted earlier for our wild-type TCR-β transgenic mice (8), occurs to the same extent in both lines. As predicted, mAb H57-597 (anti-Cβ [13]) does not bind to mutant TCR β chain (Fig. 2 H [6]). Interestingly, mAb F23.1 (anti-Vβ8.1, 2, 3 [14]) binds equally well to both β chains, whereas mAb MR5-2 (anti-Vβ8.1, 2 [17]) fails to react with the mutant, suggesting that the FG loop may form part of the MR5-2 epitope (not shown). Since the cellularity of thymi is normal in both cases, we assume that pre-TCR-mediated T cell expansion occurs normally in these mice.

Normal α/β T Cell Responses in Mutant β Chain Transgenic Mice. Peripheral T cell responses were measured in several types of assays, and none of them, to our disappointment, showed any significant differences between mice of the different transgenic lines. The in vitro responses to anti-TCR antibodies (not shown) and to SEB and SEB superantigens were repeatedly similar in all mice tested (Fig. 3, A and B). In addition, the in vivo CD4^+ T cell responses measured by T cell help for hapten-specific IgG production were basically indistinguishable between control and mutant mice (Fig. 3 C). Finally, α/β T cells from mutant TCR β chain transgenic mice made vigorous cytotoxic T cell responses against allogeneic targets as their control counterparts (Fig. 3 D). We also monitored the representation of four different Vα families by flow cytometry in peripheral T cells in order to reveal any subtle in vivo biases, but none were found (Table I). In addition, limited DNA sequence analyses of Vα 2 and 8 families from single α/β T cells revealed no obvious “mutant”-specific features (data not shown).

Concluding Remarks. Thus far, we have found only a quantitative role in the TCR assembly process for the large solvent-exposed FG loop on the Cβ domain. In transfectants, the TCR will assemble in the absence of the loop in the β chain but slightly less efficiently compared with the wild-type structure. Of course, the reduced surface expression leads to somewhat impaired function. However, we were able to show in vivo that TCRs are functionally ex-
pressed at the same level with or without the FG loop, and we did not find any qualitative or quantitative differences in their activity. This finding seems to rule out the models where the FG loop has an absolute role in TCR signaling. However, the apparent absence of any effect in vivo could also be due to the fact that some subtle compensatory mechanisms have been turned on in vivo (but not in cell lines), e.g., TCR affinities could be modulated, or new carbohydrate structures on the Cβ domain could partially replace the FG loop functionally. Interestingly, all nonmammalian species studied to date, including birds, amphibians, reptiles, and fish, do not have the FG loop on their Cβ domain (18); hence, our in vivo findings may not be that surprising.

Table 1. TCR Vα Usage in Mutant TCR-β Transgenic Mice

| Percent of CD4⁺ | Vα2⁺ | Vα3.2⁺ | Vα8⁺ | Vα11⁺ |
|-----------------|------|--------|------|-------|
| β               | 22.7 ± 1.0 | 0.7 ± 0.2 | 3.0 ± 0.1 | 1.5 ± 0.4 |
| β-loop⁻        | 22.7 ± 0.9 | 0.5 ± 0.1 | 2.6 ± 0.3 | 2.3 ± 0.3 |

| Percent of CD8⁺ | Vα2⁺ | Vα3.2⁺ | Vα8⁺ | Vα11⁺ |
|-----------------|------|--------|------|-------|
| β               | 7.7 ± 1.8 | 1.0 ± 0.1 | 4.5 ± 0.1 | 1.2 ± 0.2 |
| β-loop⁻        | 7.6 ± 1.1 | 1.4 ± 0.6 | 3.6 ± 0.7 | 2.0 ± 0.3 |

Lymph node cells from TCR-β (β) or mutant TCR-β (β-loop⁻) transgenic mice were triple stained with the FITC-labeled anti-Vα Abs, anti-CD4-PE, and anti-CD8-Cy5. Results (three mice per group) are expressed as the percentage of stained cells ± SD.
The Basel Institute for Immunology was founded and is supported by F. Hoffmann-La Roche Ltd., Basel, Switzerland.

Address correspondence to Sylvie Degermann or Klaus Karjalainen, Basel Institute for Immunology, Grenzacherstrasse 487, CH-4005 Basel, Switzerland. Phone: 41-61-605-1249; Fax: 41-61-605-1364; E-mail: degermann@bii.ch, or karjalainen@bii.ch

Received for publication 30 November 1998 and in revised form 21 January 1999.

References
1. Klausner, R.D., J. Lippincott-Schwartz, and J.S. Bonifacino. 1990. The T cell antigen receptor: insights into organelle biology. Annu. Rev. Cell Biol. 6:403–431.
2. Letourneur, F., and R. Klausner. 1992. Activation of T cells by a tyrosine kinase activation domain in the cytoplasmic tail of CD3 epsilon. Science. 255:79–82.
3. Bentley, G., G. Boulot, K. Karjalainen, and R. Mariuzza. 1995. Crystal structure of the beta chain of a T cell antigen receptor. Science. 267:1984–1987.
4. Garcia, K., M. Degano, R. Stanfield, A. Brunmark, M. Jackson, P. Peterson, L. Teyton, and I. Wilson. 1996. An alpha beta T cell receptor structure at 2.5 Å and its orientation in the TCR-MHC complex. Science. 274:209–219.
5. Garboczi, D., P. Ghosh, U. Utz, Q. Fan, W. Biddison, and D. Wiley. 1996. Structure of the complex between human T-cell receptor, viral peptide and HLA-A2. Nature. 384:134–141.
6. Wang, J., K. Lim, A. Smolyar, M. Teng, J. Liu, A. Tse, R. Hussey, Y. Chishti, C. Thomson, R. Sweet, et al. 1998. Atomic structure of an alpha beta T cell receptor (TCR) heterodimer in complex with an anti-TCR Fab fragment derived from a mitogenic antibody. EMBO J. 17:10–26.
7. Ghandeharizadeh, Y., A. Smolyar, C. Chang, and E. Reinherz. 1998. One of the CD3e subunits within a T cell receptor complex lies in close proximity to the Cβ FG loop. J. Exp. Med. 187:1529–1536.
8. Kirberg, J., A. Baron, S. Jakob, A. R. Olink, K. Karjalainen, and H. von Boehmer. 1994. Thymic selection of CD8+ single positive cells with a class II major histocompatibility complex-restricted receptor. J. Exp. Med. 180:25–34.
9. Markowitz, D., J. Lippincott-Schwartz, J. S. Bonifacino, and E. Palmer. 1996. A motif within the T cell receptor alpha chain constant region connecting peptide domain controls antigen responsiveness. Immunity. 5:437–447.
10. Degermann, S., C. Surh, L. Glimcher, J. Sprent, and D. Lo. 1994. B7 expression on thymic medullary epithelium correlates with epithelium-mediated deletion of V beta 5+ thymocytes. J. Immunol. 153:2624–2635.
11. Kubo, R., W. Born, J. Kappler, P. Marrack, and M. Pigeon. 1989. Characterization of a monoclonal antibody which detects all murine αβ T cell receptors. J. Immunol. 142:2736–2742.
12. Ghandeharizadeh, Y., H. Rammensee, J. Benedetto, and M. Bevan. 1985. Characterization of a murine monoclonal antibody specific for an allootypic determinant on T cell antigen receptor. J. Immunol. 134:3994–3999.
13. Andersson, J., A. Smolyar, and C. Reinherz. 1995. Stimulation by T cell independent antigens can relieve the arrest of differentiation of immature auto-reactive B cells in the bone marrow. Scand. J. Immunol. 42:21–33.
14. Weber, S., A. Traunecker, F. Oliveri, W. Gerhard, and K. Karjalainen. 1992. Specific low-affinity recognition of major histocompatibility complex plus peptide by soluble T-cell receptor. Nature. 356:793–796.
15. Kanagawa, O. 1988. Antibody-mediated activation of T cell clones as a method for screening hybridomas producing antibodies to the T cell receptor. J. Immunol. Methods. 110:169–178.
16. Chretien, I., A. M. Sarrazin, C. Charlemagne, and L. Du Pasquier. 1997. The T cell receptor β genes of Xenopus. Eur. J. Immunol. 27:763–771.