Brief Definitive Report

Signaling Efficiency of the T Cell Receptor Controlled by a Single Amino Acid in the β Chain Constant Region

By B. Thomas Bäckström, Barbara T. Hausmann, and Ed Palmer

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

Summary

A single amino acid residue, Gln_{136}, located within the connecting peptide domain of Cβ controls the ability of the α/β TCR to transmit a full signal. TCRs in which this Cβ residue is mutated to Phe, the residue found in TCR-γ, are unresponsive to antigenic ligands. Interestingly, this Cβ residue is either polar or charged in every species studied thus far, including the trout and the skate. In contrast, the analogous residue in Cγ is always hydrophobic. In spite of their compromised antigen responsiveness, the mutant TCR complex contains the CD3-γ, -δ, -ϵ, and -ζ chains, and undergoes ζ chain phosphorylation and ZAP-70 recruitment. However, the biological response of the mutant TCR could be rescued with a calcium ionophore, implying that mutant TCRs are defective in generating a calcium-mediated signal. The implications of the differences between Cβ and Cγ are considered.

One of the primary goals of immunologists over the last 10 yr has been to understand how the TCR transduces a signal (1) . The TCR complex consists of an antigen-binding α/β heterodimer supported by noncovalently associated CD3-γ, -δ, and -ϵ as well as ζ chains (2). The α and β constant regions are presumably required to propagate a signal to the CD3 and ζ components of the complex.

Considered together, the cytoplasmic tails of the CD3 and ζ chains contain 10 immunoreceptor tyrosine-based activation motifs (ITAMs), which are rapidly phosphorylated upon TCR engagement (3, 4). Subsequently, the protein tyrosine kinase ZAP-70 is recruited to the TCR complex and activated (5, 6). Thereafter, large multimolecular complexes of proteins participating in the signaling cascades assemble around the cytoplasmic domains of activated TCRs (7), which leads to the initiation of intracellular signaling pathways.

Although homologous to its α/β relative, the γ/δ TCR is expressed in a separate lineage of T lymphocytes (8). While it has been generally assumed that both classes of TCR use similar mechanisms to generate a signal, this may not be the case, as there are several features that distinguish these two types of T cell receptors. The CD3-ϵ chains contained within human α/β receptors express an epitope which is masked in γ/δ receptors (9, 10). Furthermore, unlike α/β receptors, γ/δ TCRs can be expressed in the absence of CD3-δ, can develop in MHC deficient mice, and can recognize unprocessed antigens in the absence of MHC presentation (11–14). Finally, the TCR-α chain contains a motif within its connecting peptide (CP) domain that is required for the transduction of antigenic signals (15). α/β TCRs lacking this α chain–connecting peptide motif (α-CPM) do not properly interact with the CD3 complex and fail to generate Ca^{2+}-mediated signals. The α-CPM is specific for the α/β TCR and seems to have evolved after the divergence of TCR-α and -δ chains. That this motif is found within α/β but not γ/δ TCRs implies that these two types of TCRs may use subtly different signaling mechanisms.

We wondered whether there exists a region within the TCR-β chain, analogous to the α-CPM, which is required to produce a functional α/β receptor. The experiments reported here identify a single amino acid within the β chain–CP domain that controls TCR signaling efficiency. Mutation of this β chain amino acid to a residue commonly seen in TCR-γ chains generates an α/β receptor which is particularly inefficient in transducing signals from antigenic ligands.

Materials and Methods

Generation of DNA Constructs. The Vα2.1 and Vβ8.1 TCR cDNAs were isolated from the T cell hybridoma, 3BBM74, and confer reactivity to the $I\text{-}A^{bm12}$ alloantigen and the staphylococcal enterotoxin B (SEB) superantigen (16). The wild-type (wt) and mutated TCR constructs were generated using overlapping oligo nucleotides and PCR as previously described (15). All constructs were verified by DNA sequencing using the SequiTherm™ cycle sequencing kit (Epicentre Technologies Corp., Madison, WI) and the deduced amino acid sequences of the mutant β chains are shown in Fig. 1.

Production of Retroviral Supernatants and Infection of T Cell Hybridomas. Retroviruses encoding wt or chimeric TCR chains were generated and used to infect the α^-/β^- T cell hybridoma, 58hCD4, as previously described (15).

 J. Exp. Med. © The Rockefeller University Press • 0022-1007/97/12/1933/06 $2.00
Volume 186, Number 11, December 1, 1997 1933–1938
http://www.jem.org

Cell Lines. The 58hCD4 cell line has been previously described (15, 17) and was provided by O. Acuto (Pasteur Institute, Paris, France). The murine fibroblast cell line DAP.3 transfected with human HLA class II DR1 molecules (18) or the human, DR1-expressing, B lymphoblastoid cell line .221 (19) was used to present SEB. The use of the cytokine indicator cell lines HT-2 and FDC-P1 have been previously described (15).

Antibodies. The use of anti-Vα2 (B20.1), anti-Vβ8.1 (F23.1), and anti–CD3-ε (145-2c11) mAbs, as well as anti–CD3-γ, anti–CD3-δ, and anti–CD3-ε rabbit antisera have been previously described (20). The anti–ζ chain mAb, H146-968 (21), was purified from culture supernatants using protein G–Sepharose beads (Pharmacia Biotech AB, Uppsala, Sweden). The rabbit anti–ZAP-70 antiserum was provided by Dr. L. Samelson (National Institutes of Health, Bethesda, MD). The antiphosphotyrosine mAb 4G10 was purchased from Upstate Biotechnology Inc. (Lake Placid, NY).

Flow Cytometry, T Cell Stimulation, and IL-2 and IL-3 Assays. The levels of cell-surface antigens were detected by immunofluorescence and analyzed on a FACScan® using the CELLQuest analysis software (Becton Dickinson, Rutherford, NJ). T cell stimulation and IL-2 and IL-3 assays were performed as previously described (15).

Immunoprecipitation and Western Blotting. T hybridoma cells (2×10^7/sample) were lysed in buffers containing 1% digitonin and the TCR complex immunoprecipitated with 2 μg of B20.1 (anti Vα2). Immunoprecipitations, Western blotting, and detection of CD3 chains were carried out as previously described (20).

ζ Chain Tyrosine Phosphorylation Analysis. For superantigen stimulation, 10^6 .221 APCs were incubated with or without SEB (10 μg/ml) for 2 h in 1 well of a 24-well plate. 10^7 T hybridoma cells were added to the APCs, centrifuged at 1,000 rpm for 30 s, and then incubated at 37°C for 20 min. Cells were washed twice with ice cold PBS containing 0.4 mM Na_3VO_4 and 0.4 mM EDTA, and were then lysed in 0.5 ml lysis buffer (10 mM Tris/HCl, pH 7.5, 150 mM NaCl, 1 mM PMSF, 10 μg/ml leupeptin, and 10 μg/ml aprotinin) containing 2 mM Na_3VO_4, 25 mM NaF, and 1% Triton X-100. Lysates were centrifuged at 12,000 *g* for 10 min, and the relevant proteins in the supernatant were immunoprecipitated with 1.5 μg of H146-968 (anti-ζ) mAb or 3 μl of a rabbit polyclonal anti–ZAP-70 antiserum. Immunoprecipitates were recovered using protein G–Sepharose beads. The tyrosine phosphorylated proteins present in these immunoprecipitates were analyzed by Western blotting and detected using the antiphosphotyrosine mAb 4G10.

Results

To better understand the functional relationships between the α/β heterodimer and the CD3 and ζ components, we made a number of chimeric TCR-β chains by replacing domains from the β chain constant region with homologous domains from the TCR-γ chain. We first generated a chimeric β chain, βVII, which contained TCR-β–derived V, D, J, and C region sequences up to and including the interchain Cys, followed by Cγ sequences encoding the COOH-terminal part of the CP domain, the transmembrane domain, and the cytoplasmic tail (Fig. 1 *A*). As seen in Fig. 1 *B*, this chimeric βVII chain was not expressed at the cell surface when paired with a wt α chain. However, Cβ and Cγ differ in the length of their CP domains. There are 13 amino acids between the interchains Cys_{127} and Ala_{141} in all β chains and 16 amino acids between Cys_{117} and Ala_{134} in all mammalian γ chains (Fig. 1 *A* and reference 22). Thus, the fact that the βVII chimera could not be expressed at the cell surface might have been due to the possibility that the Cγ length in this region (16 amino acids) was incompatible with surface expression in the context of an α/β TCR.

To test this idea, we constructed two additional chimeras, βV and βVI, which respected the conserved β chain length (13 amino acids) in this region (αwt; Fig. 1 *A*). Both the βV and the βVI chimeras were expressed at the cell surface in conjunction with a wt α chain (αwt; Fig. 1 *B*). In fact, the αwt/βV and the αwt/βVI chimeric TCRs were expressed at two- to threefold higher levels on the cell surface than were the αwt/βwt receptor. Hybridomas expressing these chimeric TCRs were stimulated with the superantigen, SEB bound to APCs. As seen in Fig. 2 *A*, the αwt/βV TCR responded to SEB about as well as the wt TCR, while the αwt/βVI TCR was clearly less sensitive (~100-fold) to this superantigen. This signaling deficit was seen even more clearly when the SEB response of a hybridoma expressing the αwt/βVI mutant receptor was compared to that of an αwt/βwt hybridoma sorted for an equivalently high level of TCR expression (Fig. 2 *B*). Furthermore, transgenic mice expressing the αwt/βVI TCR were defective in responding to SEB and the I-A^{bm12} alloantigen (data not shown). Thus, the signaling defect of this mutant TCR was not limited to superantigens. On the other hand, these chimeric TCRs could be activated by plate-bound anti-TCR mAbs (Fig. 2 *C* and data not shown), indicating that the hybridomas expressing the αwt/βVI TCR were not intrinsically defective. Therefore, TCRs comprised of the chimeric βVI chain seemed to be specifically deficient in transducing signals from antigenic ligands.

TCRs from these hybridomas were immunoprecipitated, and the associated polypeptides were analyzed by Western blotting (data not shown). The CD3-γ, -δ, and -ε chains as well as the ζ polypeptides were coprecipitated from hybridomas expressing either the wt TCR or the signaling-defective, αwt/βVI TCR (data not shown). In spite of a pronounced signaling defect (Fig. 2), the interactions with the CD3 and ζ chains were preserved in complexes containing the functionally defective, αwt/βVI heterodimer.

The tyrosine phosphorylation of the ζ chain was examined in these hybridomas as well. From the data in Fig. 3 *A*, it was apparent that the ζ chains in the αwt/βwt and αwt/βVI TCRs could be tyrosine phosphorylated and that both forms (p21 and p23) of the phosphorylated ζ chain could be generated. Although the tyrosine phosphorylation of ZAP-70 could not be demonstrated even in hybridomas expressing the αwt/βwt receptor (data not shown), an anti–ZAP-70 antiserum was used to evaluate ZAP-70 recruitment (Fig. 3 *B*). In superantigen-stimulated cells expressing either wt or mutant TCRs, the p21 and p23 forms of the ζ chain as well as the p34 phosphoprotein were coprecipitated with ZAP-70. These experiments indicated that even in hybridomas expressing the signaling-defective

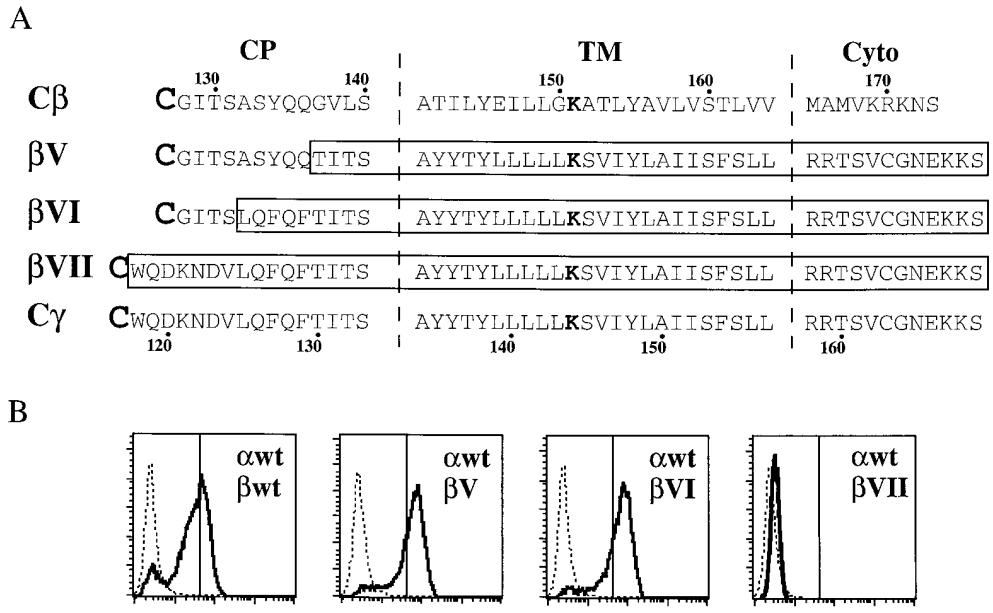


Figure 1. Amino acid sequences and surface expression of chimeric TCR-β chains. (*A*) The sequences of the wt TCR-β chain (Cβ), wt TCR-γ chain (Cγ), and the 3 chimeric TCR-β γ chains (βV-βVII) are shown using the single letter amino acid code. The boxes indicate the TCR-γ chain–derived amino acids. Only the CP, transmembrane, and Cyto domains of the TCR constant regions are shown. The complete α and β chain cDNAs have been previously described (16). The NH_2-terminal amino acid in *A* represents the interchain Cys_{127} of the TCR-β constant region. The dotted lines indicate the approximate boundary of the TM domain, defined using the Lasergene Navigator Protean Software program (DNASTAR, Inc., Madison, WI). (*B*) 58hCD4 (α^-/β^-)T cell hybridomas expressing wt α chains and wt β or chimeric TCR-β γ chains were stained with the biotinylated anti-Vβ8 mAb, F23.1, and SAPE, and then analyzed by flow cytometry. Dashed lines represent fluorescence of the same cells stained with streptavidin-phycoerythrin alone. The solid vertical lines indicate the mean fluorescence intensity of 58hCD4 cells expressing the wt α/β TCR. Similar results were obtained using anti-Vα2, anti–CD3-ϵ, or anti–Cβ specific mAbs (data not shown).

αwt/βVI TCR, ZAP-70 was nevertheless recruited to the phosphorylated ζ chains. Thus, the signaling defect in hybridomas expressing this mutant TCR was likely downstream from ZAP-70 recruitment.

Binding of an appropriate ligand to the TCR eventually leads to the activation of the protein kinase C/ras and the calcium intracellular signaling pathways (1, 7). Deficient activation of the PKC/ras pathway can be compensated for by the addition of the phorbol ester, PMA, whereas deficient activation of the calcium pathway can be compensated for by the addition of a calcium ionophore, such as ionomycin. In Fig. 4, hybridomas expressing the wt or the defective αwt/βVI TCR were stimulated with SEB in the presence of PMA or ionomycin. The impaired response of the αwt/βVI TCR was only marginally affected by several different concentrations of PMA, but was rescued in the presence of ionomycin used at several different concentrations (Fig. 4 and data not shown). These findings suggested that the mutant TCR was defective or inefficient in activating the calcium pathway.

The functional chimera, βV, contains the β chain sequence ASYQQ, which has been replaced with the γ chain sequence, LQFQF, in the nonfunctional chimeric chain, βVI (Fig. 1 *A*). The most striking amino acids in this region are Tyr_{134} and Gln_{136}, both of which are encoded by Phe in murine Cγ1. Point mutations were introduced into the wt β chain cDNA, and the resulting mutant β chains were paired with an αwt chain and were tested for their reactivity to SEB. As seen in Fig. 5, *A* and *B*, the $Tyr_{134} \rightarrow$ Phe mutation had a negligible effect on the response to SEB, whereas TCRs carrying the $Gln_{136} \rightarrow$ Phe mutation had a marked and reproducible effect on SEB responsiveness. Considering the pronounced effect of the $Gln_{136} \rightarrow$ Phe exchange on superantigen responsiveness, we examined the amino acid residues present at this position in all Cβ and Cγ sequences, some of which are shown in Fig. 5 *C*. Strikingly, the amino acids at this position in the β chain are always polar or charged. In contrast, γ chains always contain hydrophobic amino acids at this position (Fig. 5 *C* and reference 23).

Discussion

We took advantage of the differences between α/β and γ/δ TCRs to generate signaling-defective receptor mutants. Since the TCR-β and -γ constant regions are structurally homologous, we could replace domains of Cβ with similar domains of Cγ. Most of the resulting chimeric chains paired with a wt α chain and were expressed on the cell surface (Fig. 1 *B*). Hybridomas expressing αwt/βVI TCRs are 100-fold less sensitive to antigenic signals than hybridomas expressing wt receptors (Fig. 2, *A* and *B*). The signaling defect seems specific for antigenic ligands, since αwt/βVI TCRs respond fully to mAbs capable of receptor cross-linking (Fig. 2 *C* and data not shown). What distinguishes the functional βV chimera from the defective βVI chimera are the amino acids present from position 132 to 136 that are derived from Cβ and Cγ, respectively (Fig. 1 *A*). Site-directed mutagenesis defined Gln_{136} as a critical residue in this region. This position has been conserved in the β chains of all known species from the skate to humans (23) and is either polar or charged (Fig. 5 *C*). In contrast, the analogous residue in the γ chain has been conserved as a hydrophobic amino acid (Fig. 5 *C*). This conservation is

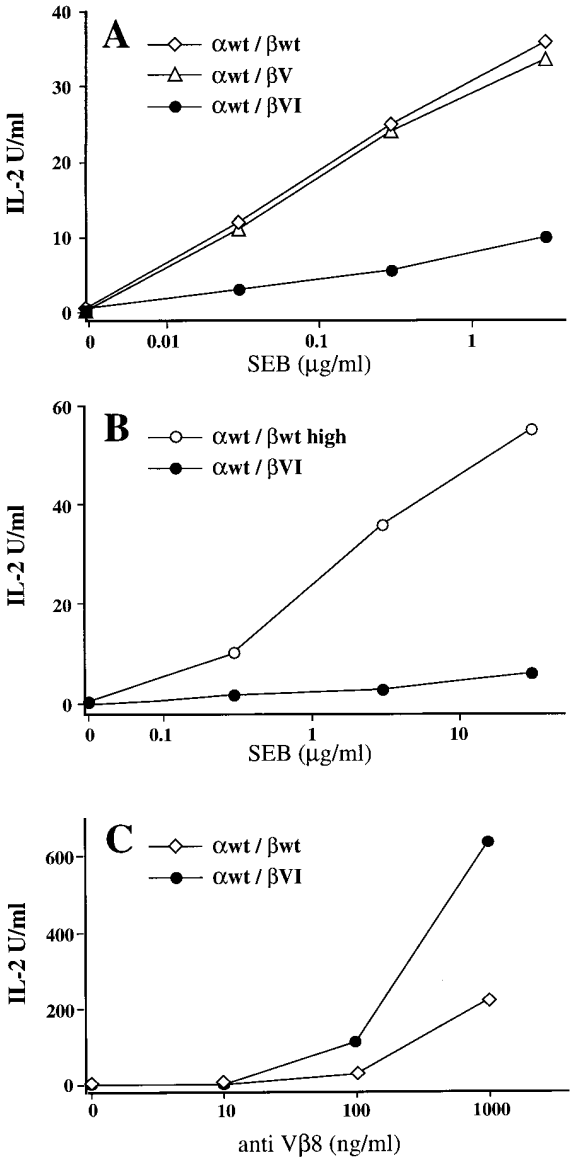


Figure 2. Response of chimeric TCRs to SEB and anti-TCR mAbs. In each experiment, 5×10^4 58hCD4 cells/well expressing wt or chimeric TCRs were stimulated with 2×10^4 DAP.3-DR1 cells and increasing doses of SEB (*A* and *B*) or plate-bound anti-Vβ8 (*C*), and the culture supernatants were assayed for IL-2. Similar results were obtained using plate-bound anti–CD3-ϵ and anti-Vα2 mAbs (data not shown). Results shown are representative of two or more experiments.

functionally significant since changing this residue from a hydrophilic to a hydrophobic amino acid is accompanied by a significant loss in signaling efficiency (Figs. 2 and 5).

There is evidence that the α/β and γ/δ heterodimers do not interact with the CD3 complex in the same way. For example, γ/δ TCRs can be expressed in the absence of CD3-δ (11). Furthermore, a CD3-ϵ epitope, contained within human α/β receptors and recognized by the mAb WT31 is masked in γ/δ receptors (9, 10, 24). Many of these differences might be attributed to the CP (membrane proximal) domains of the different TCR chains. We have

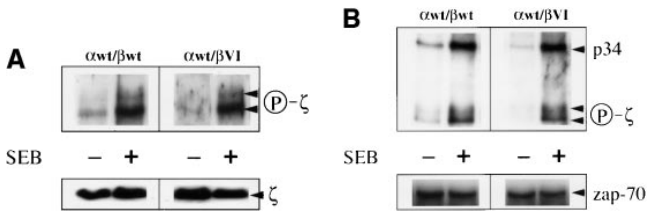


Figure 3. ζ chain phosphorylation of SEB-stimulated hybridomas expressing chimeric TCRs. Hybridomas (58hCD4) expressing wt or chimeric TCRs were stimulated using .221 cells with (10 μg/ml) or without SEB. Cells were lysed in 1% Triton X-100, and the relevant chains were immunoprecipitated with an anti-ζ mAb (*A*) or with an anti–ZAP-70 antiserum (*B*). After Western blotting, the presence of tyrosine phosphorylated proteins was detected with the antiphosphotyrosine mAb 4G10 as described in Materials and Methods. The positions of phosphorylated ζ chains, p34, unphosphorylated ζ chains, and ZAP-70 are indicated.

previously identified a motif within the α CP domain which is strikingly absent from TCR-δ chains. This motif controls the proper association of CD3-δ and -ζ to the TCR complex and is required for antigen-driven signal transduction (15).

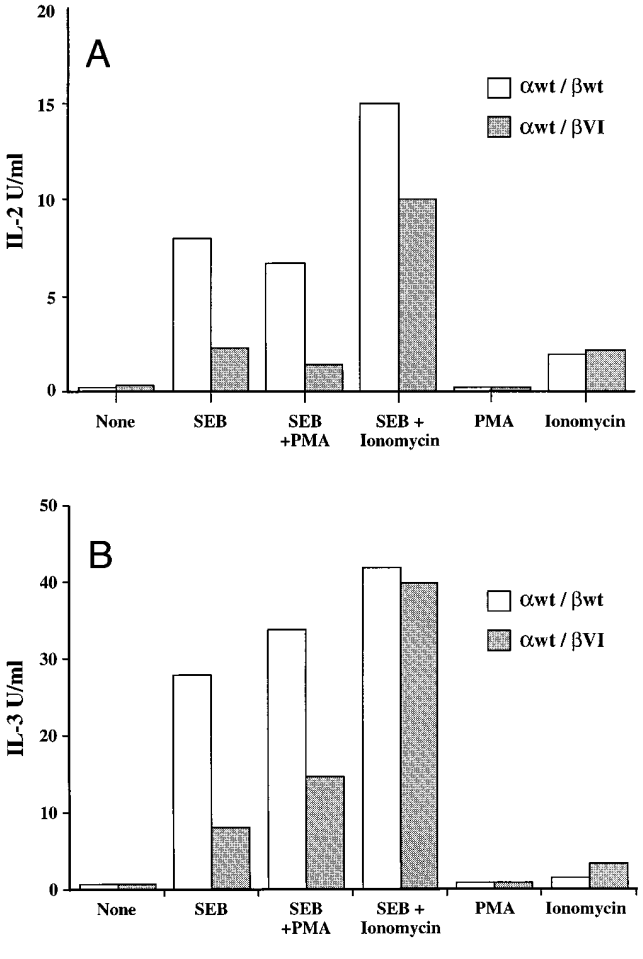
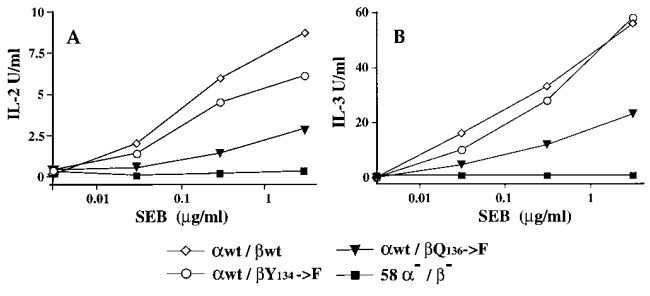


Figure 4. Effects of calcium ionophore and PMA on superantigen stimulation. The 58hCD4 hybridoma (5×10^4 cells/well) expressing wt or chimeric TCRs was stimulated with 2×10^4 DAP.3-DR1 cells and the indicated combinations of 3 μg/ml SEB, 100 ng/ml ionomycin, or 30 ng/ml PMA. Culture supernatants were assayed for IL-2 (*A*) or IL-3 (*B*).



C

Cβ CP

	136
Skate Cβ	CGLSKEAKI**Q**TMET
Trout Cβ	GEITTDYYV**K**STQT
Axolotl Cβ1	CGVTEDSFR**S**SAKI
Axolotl Cβ2	CGLTADSFR**T**SANT
Frog Cβ	CGVSPDELK**R**IVNN
Chicken Cβ	CDIFKENYQ**R**SATA
Bovine Cβ	CGVTSASYQ**Q**GVLS
Chimpanzee Cβ	CGFTSVSYH**Q**GVLS
Dog Cβ	CGFTSVSYH**Q**GVLS
Human Cβ	CGITSASYQ**Q**GVLS
Monkey Cβ	CGFTSESYQ**Q**GVLS
Mouse Cβ1	CGITSASYQ**Q**GVLS
Mouse Cβ2	CGFTSASYQ**Q**GVLS
Porcine Cβ	CGFSSASYQ**Q**GVLS
Rabbit Cβ1	CGFTSASYQ**Q**GVLS
Rabbit Cβ2	CGISSASYQ**Q**GVLS
Rat Cβ	CGITSASYQ**Q**GVLS

Cγ CP

Skate Cγ	CPPSVQELQENS EEEIP**V**SSLQV
Bovine Cγ1	CL EGESSTLQLQ**L**MNTSA
Cγ2	CL EGKSSTLQLQ**L**MNTSA
Cγ3	CL KDKNNTLQLH**L**MNTS
Human Cγ1	CS KDANDTLLLQ**L**TNTS
Cγ2	WS KDANDTLLLQ**L**TNTS
Mouse Cγ1	CW QDKNDVLQFQ**F**TSTS
Cγ2	CW QDKNDVLQLQ**F**TITS
Cγ3	CW QDKNDVLQLQ**F**MSTS
Cγ4	CM KGRKDMLQLQ**V**TTTY
Porcine Cγ1	CQ KDESNSLQLQ**L**ENTS
Cγ2	CL KDESNTLQLQ**L**ENTS
Sheep Cγ1	CL FDESNTLQLQ**L**MNTS
Cγ2	CQ EGESSALRLQ**L**TTTS
Cγ3	CL KDENNTLQLQ**L**MSTS
Cγ4	CL KDENNTVELQ**L**AYNS
Chicken Cγ	CG TQSGNSTVVNRDH**M**THKA

Figure 5. Point mutation of Gln_{136} affects antigen responsiveness. Hybridomas expressing the wt TCR-α chain and wt or mutant β chains were stimulated with DAP.3-DR1–presenting cells and SEB as described in Fig. 2. IL-2 and IL-3 responses are shown in *A* and *B*, respectively. Sequences of the CP domains from different species (22, 23) are shown in *C*, using the single letter amino acid codes. Sequences were aligned using the Lasergene Navigator MegAlign Software program (Clustal alignment method with the PAM250 residue weight table). The conserved amino acids present in the TCR-β and γ chain CP domains are indicated in boldface.

Examining the CP domain of the β chain, we have found a region within Cβ which controls the efficacy of antigen-driven signal transduction. Although alteration of this region does not lead to changes in the subunit composition of the TCR complex (data not shown), a clear biological defect was observed (Fig. 2). Mutation of this region had no observable effect on the amount of ζ chain phosphorylation nor on the recruitment of ZAP-70 to the ζ homodimer (Fig. 3). Thus, the signaling defect in this mutant is likely downstream from ZAP-70 recruitment. The signaling defect can be corrected or at least compensated in the presence of a calcium ionophore (Fig. 4), suggesting that the mutation within the β chain may lead to the inefficient induction of Ca^{2+} mobilization.

There is little structural information available for the CP domains since this region has been truncated from the TCR heterodimers used in crystallographic analyses (25, 26). Nevertheless, the CP domains of the α and β chains may function as a unit since amino acids within these domains have been conserved in α/β TCRs during the last 500 million yr (15, 22, 23). The length of the CP domains has been conserved as well. Considering the region between the interchain Cys and the transmembrane domain (defined by the beginning of the CART motif [22]), all known β chains contain 13 amino acids (22, 23). On the other hand, α chains contain from 17 to 20 amino acids in this region. Thus, in α/β TCRs, a length disparity between the α and β chain CP domains has been conserved throughout vertebrate evolution. In contrast, the lengths of the TCR-γ and -δ chain CP domains (between the interchain Cys and the transmembrane domain) are comparable in each species examined thus far (22, 23). Therefore, a length similarity between the δ and γ chains has been conserved in this region in γ/δ receptors during a similar period of vertebrate evolution.

Functionally, the CP domains may mediate an interaction with the ζ homodimer. In this regard, α/β TCRs compete more avidly for the ζ chain compared to a γ/δ TCR (27). Furthermore, mutation of the α-CPM leads to a TCR complex that is unstably associated with the ζ chains (15). Nevertheless, why the CP domains have diverged between α/β and γ/δ TCRs is puzzling. It is conceivable that the CP domains in the α/β TCR have a specialized function such as interacting with the CD4 and CD8 coreceptors or that they have evolved to optimize the efficiency of signal transduction from MHC encoded ligands.

The authors thank Drs. Luca Bolliger, Thomas Göbel, Wayne Hein, Michel Record, and Sabine Stotz for careful reading of the manuscript, Beatrice Pfeiffer for photography, Dr. L. Samelson for the rabbit anti–ZAP-70 antiserum, and O. Acuto for the 58hCD4 cell line.

The Basel Institute for Immunology was founded and is supported by F. Hoffmann-La Roche LTD, Basel, Switzerland.

Address correspondence to Dr. Ed Palmer, Basel Institute for Immunology, Grenzacherstrasse 487, CH-4005 Basel, Switzerland. Phone: 41-61-605-1277; FAX: 41-61-605-1364; E-mail: Palmer@bii.ch B. Thomas Bäckström's current address is Malaghan Institute of Medical Research, PO Box 7060, Wellington South, New Zealand.

Received for publication 16 July 1997 and in revised form 26 September 1997.

References

1. Weiss, A., and D.R. Littman. 1994. Signal transduction by lymphocyte antigen receptors. *Cell.* 76:263–274.
2. Ashwell, J.D., and R.D. Klausner. 1990. Genetic and mutational analysis of the T-cell antigen receptor. *Annu. Rev. Immunol.* 8:139–167.
3. Malissen, B., and A.M. Schmitt-Verhulst. 1993. Transmembrane signalling through the T-cell-receptor–CD3 complex. *Curr. Opin. Immunol.* 5:324–333.
4. Reth, M. 1989. Antigen receptor tail clue. *Nature.* 338:383–384.
5. Chan, A.C., B.A. Irving, J.D. Fraser, and A. Weiss. 1991. The zeta chain is associated with a tyrosine kinase and upon T-cell antigen receptor stimulation associates with ZAP-70, a 70-kDa tyrosine phosphoprotein. *Proc. Natl. Acad. Sci. USA.* 88:9166–9170.
6. Hatada, M.H., X. Lu, E.R. Laird, J. Green, J.P. Morgenstern, M. Lou, C.S. Marr, T.B. Phillips, M.K. Ram, K. Theriault, et al. 1995. Molecular basis for interaction of the protein tyrosine kinase ZAP-70 with the T-cell receptor. *Nature.* 376: 32–38.
7. Wange, R.L., and L.E. Samelson. 1996. Complex complexes: signaling at the TCR. *Immunity.* 5:197–205.
8. Sciammas, R., Y. Tatsumi, A.I. Sperling, K. Arunan, and J.A. Bluestone. 1994. TCR γδ cells: mysterious cells of the immune system. *Immunol. Res.* 13:268–279.
9. van de Griend, R.J., J. Borst, W.J. Tax, and R.L. Bolhuis. 1988. Functional reactivity of WT31 monoclonal antibody with T cell receptor-γ expressing $CD3^+4^-8^-$ T cells. *J. Immunol.* 140:1107–1110.
10. Salmeron, A., F. Sanchez-Madrid, M.A. Ursa, M. Fresno, and B. Alarcon. 1991. A conformational epitope expressed upon association of CD3-ϵ with either CD3-δ or CD3-γ is the main target for recognition by anti-CD3 monoclonal antibodies. *J. Immunol.* 147:3047–3052.
11. Dave, V.P., Z. Cao, C. Browne, B. Alarcon, G. Fernandex-Miguel, J. Lafaille, A. de la Hera, S. Tonegawa, and D.J. Kappes. 1997. CD3δ deficiency arrests development of the αβ but not the γδ T cell lineage. *EMBO (Eur. Mol. Biol. Organ.) J.* 16:1360–1370.
12. Correa, I., M. Bix, N.S. Liao, M. Zijlstra, R. Jaenisch, and D. Raulet. 1992. Most γδ T cells develop normally in β 2-microglobulin–deficient mice. *Proc. Natl. Acad. Sci. USA.* 89:653–657.
13. Bigby, M., J.S. Markowitz, P.A. Bleicher, M.J. Grusby, S. Simha, M. Siebrecht, M. Wagner, C. Nagler-Anderson, and L.H. Glimcher. 1993. Most γδ T cells develop normally in the absence of MHC class II molecules. *J. Immunol.* 151: 4465–4475.
14. Schweighoffer, E., and B.J. Fowlkes. 1996. Positive selection is not required for thymic maturation of transgenic γδ T cells. *J. Exp. Med.* 183:2033–2041.
15. Bäckström, B.T., E. Milia, A. Peter, B. Jaureguiberry, C.T. Baldari, and E. Palmer. 1996. A motif within the T cell receptor α chain constant region connecting peptide domain controls antigen responsiveness. *Immunity.* 5:437–447.
16. DiGiusto, D.L., and E. Palmer. 1994. An analysis of sequence variation in the β chain framework and complementarity determining regions of an allo-reactive T cell receptor. *Mol. Immunol.* 31:693-699.
17. Letourneur, F., and B. Malissen. 1989. Derivation of a T cell hybridoma variant deprived of functional T cell receptor α and β chain transcripts reveals a nonfunctional α-mRNA of BW5147 origin. *Eur. J. Immunol.* 19:2269–2274.
18. Lechler, R.I., V. Bal, J.B. Rothbard, R.N. Germain, R. Sekaly, E.O. Long, and J. Lamb. 1988. Structural and functional studies of HLA-DR restricted antigen recognition by human helper T lymphocyte clones by using transfected murine cell lines. *J. Immunol.* 141:3003–3009.
19. Shimizu, Y., and R. DeMars. 1989. Production of human cells expressing individual transferred HLA-A,-B,-C genes using an HLA-A,-B,-C null human cell line. *J. Immunol.* 142: 3320–3328.
20. Bäckström, B.T., B. Rubin, A. Peter, G. Tiefenthaler, and E. Palmer. 1997. T cell receptor α-chain tail is required for protein kinase C–mediated down-regulation, but not for signaling. *Eur. J. Immunol.* 27:1433–1441.
21. Rozdzial, M.M., R.T. Kubo, S.L. Turner, and T.H. Finkel. 1994. Developmental regulation of the TCR ζ-chain. Differential expression and tyrosine phosphorylation of the TCR ζ-chain in resting immature and mature T lymphocytes. *J. Immunol.* 153:1563–1580.
22. Campbell, K.S., B.T. Bäckström, G. Tiefenthaler, and E. Palmer. 1994. CART: a conserved antigen receptor transmembrane motif. *Semin. Immunol.* 6:393–410.
23. Rast, J.P., M.K. Anderson, S.J. Strong, C. Luer, R.T. Litman, and G.W. Litman. 1997. α, β, γ, and δ T cell antigen receptor genes arose early in vertebrate phylogeny. *Immunity.* 6:1–11.
24. Van Neerven, J., J.E. Coligan, and F. Koning. 1990. Structural comparison of α/β and γ/δ T cell receptor-CD3 complexes reveals identical subunit interactions but distinct cross-linking patterns of T cell receptor chains. *Eur. J. Immunol.* 20: 2105–2111.
25. Garboczi, D.N., P. Ghosh, U. Utz, Q.R. Fan, W.E. Biddison, and D.C. Wiley. 1996. Structure of the complex between human T-cell receptor, viral peptide and HLA-A2 [comment]. *Nature.* 384:134–141.
26. Garcia, K.C., M. Degano, R.L. Stanfield, A. Brunmark, M.R. Jackson, P.A. Peterson, L. Teyton, and I.A. Wilson. 1996. An αβ T cell receptor structure at 2.5 A and its orientation in the TCR-MHC complex. *Science.* 274:209–219.
27. Kuhlmann, J., S. Caspar-Bauguil, C. Geisler, and B. Rubin. 1993. Characterization of T cell receptor assembly and expression in a Ti γδ–positive cell line. *Eur. J. Immunol.* 23: 487–493.