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A chimeric T cell receptor with super-signaling properties

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Abstract

A key question yet to be resolved concerns the structure and function relationship of the TCR complex. How does antigen recognition by the TCR-αβ chains result in the activation of distinct signal transduction pathways by the CD3-γδε/ζ complex? To investigate which part of the TCR-β chain is involved in TCR signaling, we exchanged different domains of the constant regions of the TCR-β chain with the corresponding TCR-γ chain domains. We show here that hybridoma cells expressing a chimeric TCR-β chain (βIII) containing intracellular and transmembrane TCR-γ amino acids, together with a wild-type TCR-α (αwt) chain, were 10 times more sensitive to antigenic stimulation compared to cells expressing TCR-αwt/βwt chains. This super-signaling phenotype of the βIII chain was observed in two different TCRs. One specific for an alloantigen (I-A^{bm12}) and one for an autoantigen (I-A^{b}/$MOG_{35–55}$). We found that this chimeric αwt/βIII TCR had normal association with CD3-γδε and ζ chains. To investigate the effect of the chimeric βIII chain in transgenic T cells, we made $MOG_{35–55}$-specific TCR transgenic mice expressing either the αwt/βwt or chimeric αwt/βIII TCR. Similar to what was observed in hybridoma cells, transgenic αwt/βIII T cells showed a super-signaling phenotype upon antigenic stimulation. Further studies may help us understand the effect of increased TCR signaling on autoimmunity and may lead to the identification of signaling molecules that can be targeted to stop the progression of autoimmune disorders such as multiple sclerosis.

Introduction

Although the structure and function relationship of the T cell receptor (TCR) has been investigated for more than two decades, it remains unknown which of the extracellular, transmembrane or intracellular domains of the constant region are responsible for interactions with the different components of the TCR complex. More importantly, it is also not known which of these domains are responsible for TCR-induced proliferation, apoptosis and cytokine production.

T cells express a TCR consisting of clonotypic αβ- or γδ-heterodimers associated with the CD3-γδε and ζ chain homodimers. The αβ and γδ TCRs show sequence homology but may still have different structural properties. For example, a CD3-ε epitope detected with the mAb WT31 in the αβ TCR complex is masked in T cells expressing the γδ TCR (1,2). In addition, unlike the αβ TCR, the γδ TCR is predominantly expressed without CD3-δ (3), and CD3-δ deficient mice lack αβ- but not γδ-expressing T cells (4). These examples indicate that the γδ TCR can be expressed at the cell surface in the absence of the CD3-δ chain, and that the structural requirement for cell surface expression may differ between αβ- and γδ-expressing T-cell subsets.

While the variable region of the TCR-αβ and -γδ chains is responsible for antigen recognition, intracellular immunoreceptor tyrosine-based activation motifs (ITAMs) in the CD3-γδε and ζ chains are essential for TCR signaling. However, it is still not certain which domains in the constant regions of the TCR-αβ chains are involved in signal transmission, or how the TCR links antigen recognition to ITAM phosphorylation and downstream signaling. Interestingly, structural differences between αβ and γδ T cells impact on receptor signaling and αβ/γδ lineage determination (5). Some evidence suggests that γδ T cells may induce different

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downstream signaling events to αβ T cells. For example, γδ-expressing T cells do not depend on CD4 or CD8 for activation, and have different requirements for PLC-γ1 and protein tyrosine kinase activation of the guanine nucleotide-exchange factor Vav (6–9).

To better understand the signaling capabilities of the TCR, it is important to study domains in the TCR-αβ that are not directly involved in antigen recognition, but may influence the association with the CD3-γδε and ζ chains. To do this, we took advantage of the similarities and differences between the αβ and γδ TCR constant regions by making chimeric α and β chains containing different lengths of TCR-δ and γ chain amino acids, respectively. By studying some of these chimeric TCR chains, we have previously identified a motif in the connecting peptide domain of the TCR-α chain (αCPM), and shown that it is involved in antigen-signaling and association with the CD3-δ chain (10,11). Interestingly, disruption of αCPM affects positive but not negative selection of thymocytes (10,11), indicating that the different domains of the TCR carry out specialized functions. Furthermore, TCR-β connecting peptide domain is also involved in signaling, since hybridoma cells possessing mutations within this domain are unresponsive to superantigen stimulation. In spite of the pronounced signaling defect found, the interactions of the chimeric TCR-β chain with the CD3-γδε and ζ chains are preserved (11,12).

The T- and B-cell receptors contain a conserved antigen receptor transmembrane (CART) motif, suggesting an important role for this domain in antigen receptor assembly and function (13). To study the role of the TCR-β chain transmembrane domain on TCR structure and function, we utilized a previously generated chimeric TCR-β chain (βIII) which contains TCR-γ amino acids downstream of the conserved transmembrane Y_{145} residue (11). We demonstrate that T hybridoma and transgenic T cells expressing the chimeric TCR-βIII chain show super-signaling properties upon stimulation through the TCR with antigen, but not upon CD3 cross-linking. As the association with the CD3-γδε and ζ chains is intact, the super-signaling TCR-βIII complex appears to have normal structure but altered function.

Methods

Cell lines

All cell lines were grown in IMDM supplemented with 5% FCS, 2 mM glutamine, 100 U/ml penicillin G, 100 μg/ml streptomycin sulphate and 5×10^{-5} M 2-mercaptoethanol (all from Gibco BRL, Auckland, New Zealand), referred to as complete medium unless otherwise stated. The human B cell line LG-2 (14) expressing MHC class II DR1 molecule was utilized to present the superantigen staphylococcal enterotoxin B (SEB) to T hybridoma cells. The 58α$^-$β$^-$ and 58hCD4α$^-$β$^-$ have been previously described (11). The 58α$^-$β$^-$ and 58hCD4 are T hybridoma cell lines which do not express TCR-αβ, and in addition 58hCD4 has been transfected with the human CD4 protein. The Phoenix Eco packaging cell line (a gift from Garry Nolan, Stanford University School of Medicine, CA) was cultured in complete medium containing 10% FCS. The indicator cell line HT-2 (15) was grown in complete medium with the addition of 20 U/ml IL-2.

Generation of DNA constructs and myelin oligodendrocyte glycoprotein (MOG) peptide 35–55-specific transgenic mice

The Vα2.1 and Vβ8.1 TCR cDNAs were isolated from the T-cell hybridoma 3BBM74 as previously described (11). They confer reactivity to the I-A^{bm12} alloantigen and the SEB superantigen. Cloning of the $MOG_{35–55}$-specific T-cell receptor 2D2 (Vα3.2 and Vβ11) has previously been described (16). For construction of wild-type and chimeric TCR tg mice, the VJ segments of the 2D2 α and VDJ of the β chain were amplified by PCR using 2D2 cDNA as template and the following primers: Vα-5′: GATCGAATTCGTCGACATGCTCCTGGCGCTCC, Vα-3′: GTTCTGGGTTCTGGATGTTGGGCTTGATAGATAACTTG, Vβ-5′: GATCGAATTCGTCGACATGGCCCCCAGGCTCCTT, Vβ-3′: GTCACATTTCTCAGATCCTCTACAACTGTGAGTCTGG. The constant α and β chains were amplified by PCR from LXSN-Vα2.1, LXSP-Vβ8.1 and LXSP-Vβ8.1-βIII (11) as templates for the constant α, constant β and constant βIII (chimeric β/γ) chains respectively. The following primers were used: Cα-5′: CAAGTTATCTATCAAGCCCAACATCCAGAACCCAGAAC, Cβ-5′: CCAGACTCACAGTTGTAGAGGATCTGAGAAATGTGAC, LXSN/P-3′: GGGCGGGACTATGGTT. The variable and constant chain PCR products were sewn together in a PCR using either the Vα or Vβ upstream primer with LXSN/P-3′ and the resulting PCR products were TA cloned in pCR2.1 (Invitrogen, Carlsbad, CA) in accordance with the manufacturer's recommendations. The cloned PCR products were sequenced, excised from the cloning vector with *Sal*I/*Bam*HI, and cloned into the *Sal*I/*Bam*HI sites of the pHSE3′ vector (17). For construction of transgenic mice, the DNA fragment coding for the TCR chains were excised from pHSE3′ with *Xho*I, gel purified, and microinjected into C57BL/6J oocytes as previously described (16).

Retroviral infection of 58hCD4 cells

The generation of virus-containing supernatants by transfection of Phoenix cells and retroviral infection of hybridoma cells was done as previously described (11). Briefly, 2.5×10^5 Phoenix cells/well were seeded in 6-well plates and transfected with 2 μg of retroviral vector the day after using 6 μl of Fugene6 (Roche Diagnostics, Auckland, NZ) according to the manufacturer's recommendations. The supernatant, 2 ml, containing the retroviral particles was supplemented with 10 μg/ml of polybrene (Sigma, Global Science, Auckland, New Zealand) and added to 2.5×10^5 hybridoma cells. For selection of positive cells, LXSP-infected cells were added 3 μg/ml of Puromycin (Sigma), and LXSN infected cells 1 mg/ml G418 (Merck, Palmerston North, New Zealand). The selective drug was added to the cell culture 24 h after infection. Bulk-infected cells were sorted by flow cytometry for equal expression of the TCRs and human CD4 chains and then used in indicated experiments.

Electroporation of 58α$^-$β$^-$ T hybridoma cells

For expression of 2D2 TCR and murine CD4 in 58α$^-$β$^-$ T hybridoma cells, pcDNA3/neo (for G418 selection) and pcDNA/L3T4 (murine CD4) were linearized with *Pvu*I and *Sca*I respectively, while pHSE3′/2D2αwt, pHSE3′/2D2βwt and pHSE3′/2D2βIII were linearized with *Xho*I. The linearized DNA was precipitated and dissolved in PBS. For electroporation,

5×10^6 58$\alpha^-\beta^-$ T hybridoma cells were electroporated in 200 μl PBS containing 2 μg pcDNA3/neo, 4 μg pcDNA3/L3T4, 10 μg pHSE3′/2D2αwt and 10 μg of either pHSE3′/2D2βWT or pHSE3′/2D2βIII at 960 μF and 0.25 kV using a Bio-Rad Gene Pulser (Bio-Rad, San Diego, CA). Surviving cells were cultured for 24 h in complete medium after which they were seeded at 5×10^3 cells/well in 96-well plates in complete medium containing 1 mg/ml of G418. Surviving clones were screened for Vα3.2, Vβ11 and mCD4 expression using flow cytometry. One 2D2 wt and one 2D2 βIII expressing clone was found to respond to $MOG_{35–55}$ and therefore selected for this study. These clones were stained with anti CD4/PE and anti TCR-β/FITC and sorted for equal expression of TCR and mCD4, using a FACSVantage Flow Cytometry System (Becton Dickinson).

Antibodies, peptides and superantigen

The $MOG_{35–55}$ peptide was synthesized by Mimotopes (Clayton, Australia) and was more than 90% pure, confirmed by HPLC. The peptide sequence for mouse $MOG_{35–55}$ is MEVGWYRSPFSRVVHLYRNGK. SEB was purchased from Toxin Technology, Inc. (Sarasota, FL). The antibodies B20.1 (anti Vα2.1), RR3-16 (anti Vα3.2), H28–710 (anti TCR-α constant region), F23.1 (anti Vβ8), RR3-15 (anti Vβ11), H57–597 (anti TCR-β constant region), 145–2c11 (anti CD3-ε), H129.19 (anti mouse CD4), RPA-T4 (anti human CD4), or H146–968A (anti ζ) were used. All mAbs were either purchased from BD PharMingen (San Diego, CA) or purified from culture supernatants. Goat anti-rabbit-HRP (Southern Biotechnology Associates, AL) and goat anti-Armenian hamster-HRP (Jackson ImmunoResearch Laboratories, Inc.) were used as secondary antibodies for their respective primary antibodies.

Hybridoma stimulation assay

For hybridoma stimulation assays, 5×10^4/well hybridoma cells were seeded in 96-well plates. Hybridoma cells were stimulation with indicated concentration of the $MOG_{35–55}$ peptide and 5×10^5/well irradiated (2000 Rad) C57BL/6J splenocytes. For stimulation with SEB, 2×10^4/well LG-2 cells and SEB at indicated concentrations were added. The cultures were incubated for 24 h, after which culture supernatants were harvested. To quantify the concentration of IL-2 in the culture supernatants, 5×10^3 HT-2 cells in 100 μl were cultured with 100 μl culture supernatant for 24 h and 0.5 μCi/ml of [^{3}H]thymidine added for the final 6 h of incubation. The amount of IL-2 was expressed as the HT-2 stimulation index (SI) (proliferation of HT-2 cells with culture supernatant from stimulated cells/proliferation of HT-2 cells with culture supernatant from unstimulated cells). Alternatively, recombinant IL-2 was added as a standard to calculate total units of IL-2.

T-cell stimulation and proliferation assay

Splenocytes, 4×10^6 cells/well in 24-well plates, from wild-type and βIII 2D2 mice were stimulated with 10 μg/ml $MOG_{35–55}$, and IL-2 (50 U/ml) added on day 2, 5 and 8. On day 9, cultures were washed twice and 3×10^4 T cells/96-well mixed with 5×10^5 irradiated splenocytes and antigen. The T-cell cultures were incubated for 72 h and 0.5 μCi/ml of [^{3}H]thymidine added for the final 9 h of incubation.

Results

Exchanging transmembrane and intracellular domains of TCR-β for TCR-γ generates a super-signaling TCR

To identify domains in the constant region of the TCR-β chain important for TCR signal transduction, we have previously made a number of chimeric TCR-β chains by replacing domains of the β-chain constant region with homologous domains of the TCR-γ chain. We have previously found that T cells expressing chimeric TCR-β chains containing TCR-γ-derived amino acids in the TCR-β connecting peptide domain are defective in response to superantigen. Point mutations revealed that a single amino acid residue, Q_{136}, located within the connecting peptide domain of the TCR-β chain controls the ability of the αβ TCR to transmit a full signal (12). In addition, we have shown that the short intracellular domain of the TCR-α chain, but not the β-chain, is important for TCR downregulation but not antigen signaling (18). In this paper, we study the chimeric TCR-β/γ chain, βIII, containing TCR-β-derived sequences up to and including the conserved transmembrane tyrosine at position 145, followed by the TCR-γ sequence encoding the remaining transmembrane and intracellular domains (Fig. 1) (11). The chimeric βIII chain was first introduced in the 3BBM74 TCR, which confers reactivity to the I-A^{bm12} alloantigen and SEB (19). Cells were sorted to achieve comparable TCR expression level of hybridoma cells possessing wild-type or chimeric βIII chains. Sorted wild-type and chimeric βIII chain expressing cells stained with TCR-α, TCR-β or CD3-ε specific mAbs showed similar levels of cell surface expression (data not shown). Upon stimulation with SEB, T-cell hybridoma cells expressing TCR-αwt with chimeric TCR-βIII responded to almost 10-fold lower concentrations of SEB compared to hybridoma cells expressing TCR-αwt/βwt (Fig. 2A). Interestingly, upon cross-linking the TCR with plate-bound anti-CD3 mAb, cells expressing the βwt or chimeric βIII chains responded similarly (Fig. 2B). Thus, hybridoma cells with the chimeric TCR-βIII chain are hyper-responsive to antigenic but not cross-linking signals, indicating that these stimuli activate distinct signaling pathways.

To further investigate whether this chimeric TCR also shows super-signaling properties to an autoantigen, we introduced the chimeric βIII chain into the TCR-β chain of the 2D2 TCR (Vα3.2/Vβ11), which specifically recognizes the CNS-derived $MOG_{35–55}$ peptide (16). We then electroporated 58$\alpha^-\beta^-$ T hybridoma cells with CD4, 2D2 αwt/βwt or 2D2 αwt/βIII TCRs and selected for drug-resistant clones. We sorted the hybridoma cells for equal numbers of surface-expressed 2D2 αwt/βwt or 2D2 αwt/βIII TCR and CD4 (Fig. 3A, and data not shown). When these cells were tested for responsiveness to $MOG_{35–55}$, hybridoma cells expressing the chimeric βIII TCR responded better to antigenic challenge, and thus showed a super-signaling phenotype compared to hybridoma cells expressing 2D2 αwt/βwt TCR (Fig. 3B).

Similar structure of αwt/βwt and αwt/βIII TCRs

We have previously shown that the αwt/βIII TCR associates normally with the CD3-γδε and ζ chains at the cell surface, using biotinylation and immunoprecipitation techniques (11). Furthermore, we immunoprecipitated the 2D2 αwt/βwt or αwt/βIII TCR with an antibody specific for TCR-β and subsequently

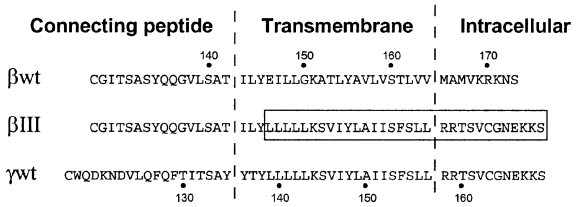

Fig. 1. Amino acid sequence of TCR-β, TCR-γ or chimeric TCR-β/γ (βIII) chains. The C-terminal sequences of the TCR-β, TCR-βIII and TCR-γ chains are shown using the single-letter amino acid code. Only the sequence of the connecting peptide, transmembrane, and intracellular domains are shown. The box indicates the sequence derived from TCR-γ. For more information see (11).

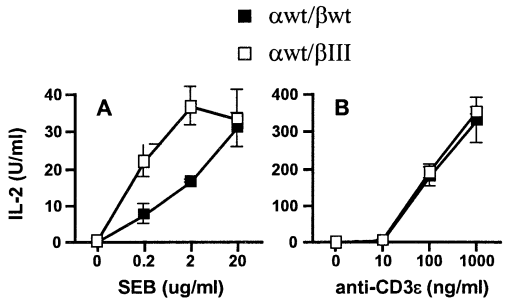

Fig. 2. Response of αwt/βwt or αwt/βIII I-A^{bm12}-specific TCR to SEB and anti CD3. The 58hCD4 hybridoma cell line was infected with I-A^{bm12}-specific αwt/βwt or αwt/βIII TCRs, sorted for similar TCR and CD4 expression, and then stimulated either with LG-2 cells and SEB (A), or plate-bound anti CD3ε mAb (B). Supernatants from quadruplicate cultures of each antigen concentration were assayed for IL-2 concentration using the HT-2 bioassay. One representative experiment out of four is shown.

probed western blots with antibodies specific for the TCR-α, CD3-γ, -δ, -ε, or ζ chains and found no differences in the assembly of TCR-αβ chains with the TCR complex (data not shown).

Super-signaling MOG$_{35–55}$-specific TCR transgenic T cells

In order to evaluate the super-signaling chimeric βIII TCR in a more physiological system, we generated TCR transgenic mice expressing either the βwt or chimeric βIII chain together with the wild-type 2D2 Vα3.2 chain. We stimulated splenocytes from 2D2 TCR transgenic mice with MOG$_{35–55}$ and then expanded the T cells with IL-2 for 9 days. Naive splenocytes from both 2D2 αwt/βwt and αwt/βIII mice, and cells cultured for 9 days with IL-2, expressed similar levels of the Vα3.2, Vβ11, CD3ε and CD4 molecules at the cell surface (Fig. 4A, and data not shown). These short-term T-cell lines were then tested for proliferative responses to antigen and superantigen stimulation. We found that the T cells expressing the αwt/βIII TCR proliferated better in response to MOG$_{35–55}$ (Fig. 4B) and SEB (Fig. 4C), compared to T cells carrying the αwt/βwt TCR. These results indicate that the chimeric βIII chain renders T cells hyper-responsive to antigenic signals delivered through the TCR-αβ chains, and that the phenotype is not due to increased TCR expression.

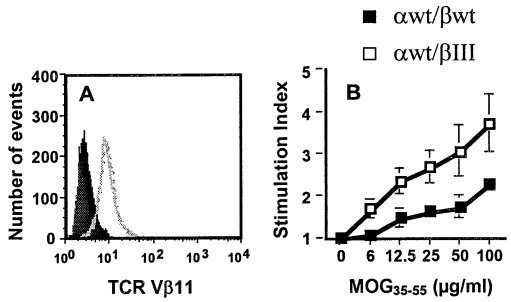

Fig. 3. Response of αwt/βwt or αwt/βIII chimeric MOG$_{35–55}$-specific TCR to MOG$_{35–55}$. The 58α$^-$β$^-$ hybridoma cell line was electroporated with mouse CD4, 2D2-αwt/βwt or αwt/βIII TCRs. Antigen-specific clones, one of each, were selected and sorted by flow cytometry. TCR expression levels of 2D2 αwt/βwt (thick line) or αwt/βIII (broken line) cells, or no antibody (solid area) were determined using an antibody specific for TCR-Vβ11 (A). Clones were stimulated with irradiated splenocytes and MOG$_{35–55}$. Supernatants from quadruplicate cultures of each antigen concentration were assayed for IL-2 concentration using the HT-2 bioassay (B). The *y*-axis represents the stimulation index of HT-2 cells (CPM with antigen/CPM with no antigen). One representative experiment out of three is shown.

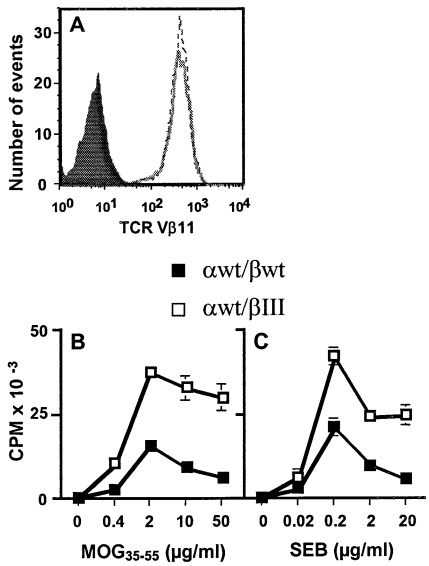

Fig. 4. Proliferative response of 2D2 transgenic T cells to MOG$_{35–55}$ and SEB. Splenocytes from 2D2 αwt/βwt or αwt/βIII TCR transgenic mice were stimulated with MOG$_{35–55}$ and expanded with IL-2 for 9 days. TCR-Vβ11 expression of 2D2 αwt/βwt (thick line) or αwt/βIII (broken line) cells, or no antibody (solid area) after 9 days is displayed (A). Similar results were found using anti-TCR-Vα3.2 or CD3-ε mAbs (data not shown). Proliferation of 2D2 αwt/βwt or αwt/βIII T cells in response to MOG$_{35–55}$ (B) and SEB (C) is depicted. One representative experiment out of three is shown.

Discussion

The fact that antigen stimulation through the same TCR-αβ chains can be translated into a number of different effector functions makes the TCR complex a fascinating cell surface receptor to study. To determine whether the different domains

of the TCR chain constant regions may be involved in different effector functions, we have replaced part of the extracellular connecting peptide, transmembrane and intracellular domains of the TCR-αβ with the corresponding domains of the TCR-γδ chains. In this way, we have previously found that the connecting peptide domains of the TCR-α and -β chains control antigen responsiveness (11,12).

Here, we further analyze the role of the chimeric TCR-βIII chain, which contains TCR-γ amino acids downstream of the conserved transmembrane Y_{145} residue. Introducing the chimeric βIII TCR together with the wild-type TCR-α chain renders hybridoma cells and transgenic T cells ~10-fold more sensitive to antigen stimulation ($MOG_{35–55}$ and SEB), but not to stimulation by anti-CD3 cross-linking. We have previously shown that the αwt/βIII TCR associates with the CD3-γδε and ζ chains at the cell surface, using biotinylation and immunoprecipitation techniques [(11) and data not shown]. Thus, the super-signaling phenotype of the chimeric βIII TCR is not due to a change in the assembly of the TCR with CD3-γδε or ζ chains or higher surface expression of the chimeric TCR (Figs 3A and 4A). The phenotype of the chimeric βIII chain is most likely mediated by changes in the transmembrane domain of the TCR-β chain, since we have previously demonstrated that the short intracellular domain of the TCR-β chain is dispensable for TCR signaling (18).

The transmembrane domain of the TCR-β chain has previously been implicated to be involved in TCR signaling. By introducing point mutations, it has been revealed that TCR-β transmembrane residues contribute towards both T-cell activation and apoptosis (20,21). Mutating one or both of the two conserved tyrosines (Y_{145} and/or Y_{155}) to phenylalanine in the transmembrane region of the β chain causes a marked reduction in antigen responsiveness in mouse hybridoma cells. Likewise, mutating the conserved transmembrane C-terminal tyrosine (Y_{155}) to leucine in Jurkat cells affects the association of the ζ chain with the TCR complex. Interestingly, these Jurkat cells show normal IL-2 secretion, but reduced apoptosis and FasL upregulation in response to anti-CD3 stimulation (22–24). However, the super-signaling phenotype of the chimeric TCR-βIII might be due to a different mechanism, since both the TCR-β and TCR-γ chains possess the transmembrane Y_{145} and Y_{155} residues (11). In addition, a chimeric TCR-β chain, βII, containing TCR-γ-derived amino acids from the conserved transmembrane residue K_{151} and downstream paired with the wild-type TCR-α chain does not show any super-signaling properties [(11) and data not shown]. The only difference between these two chimeric TCR-β chains is between positions 146–150 in the transmembrane domain, where βII encodes the TCR-β-derived amino acids EILLG, and βIII the TCR-γ derived amino acids LLLLL. Thus, the super-signaling phenotype is most likely attributed to the replacement of the conserved E_{146} and G_{150} amino acids in the TCR-β chain to L_{146} and L_{150}.

Transmembrane domains often possess an α-helix structure with every three/four amino acids ending up in the same vertical plane (13). The spacing between E_{146} and G_{150} is four amino acids and they are therefore likely to face the same side. Bäckström *et al.* (11) have previously reported that, whereas single point mutations within the αCPM do not affect TCR signaling, substituting three amino acid residues within the same motif renders the resulting TCR non-functional. This indicates that small changes are tolerated without affecting the function of the TCR. It is possible that the E_{146} and G_{150} residues of the TCR-β chain are part of an important TCR-β chain transmembrane motif and interact directly with the CD3/ζ complex and that the interface is made up by a number of additional amino acids, all of which contribute to the interaction. Substituting E_{146} and G_{150} with the corresponding TCR-γ leucine residues in the βIII chimera could therefore be structurally tolerated, but with altered TCR signaling. On the other hand, the corresponding TCR-γ leucine residues, in conjunction with residues found further downstream, might be part of an important motif in the γδ TCR complex which makes the βIII chain interact more efficiently in comparison to βII (or the wild-type TCR-β chain) and thus renders this TCR hyperactive. Another possibility is that the TCR-γ chain leucine residues are less efficient, in comparison to the wild-type TCR-β chain, in interacting with a transmembrane-resident molecule involved in TCR-induced inhibition, and therefore cells expressing the βIII TCR show a fictional hyperproliferative response.

In summary, the βIII chain generates a TCR with an apparent super-signaling phenotype which is not limited to one TCR, since both alloantigen ($I\text{-}A^{bm12}$) and autoantigen-specific ($MOG_{35–55}$) TCRs expressing this chimeric βIII chain are hyper-responsive upon antigen stimulation (Figs 2 and 3). We are currently in the process of investigating how the chimeric βIII chain affects downstream signaling pathways. In addition, the super-signaling $MOG_{35–55}$-specific C57BL/6J TCR transgenic mice may represent a novel model for studying how changes in TCR signaling affect the development of an autoimmune disorder, using the experimental autoimmune encephalomyelitis model for human multiple sclerosis.

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Abbreviations

CART	conserved antigen receptor transmembrane
CPM	connecting peptide motif
ITAM	immunoreceptor tyrosine-based activation motif
MOG	myelin oligodendrocyte glycoprotein
SEB	Staphylococcal enterotoxin B
TCR	T-cell receptor

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