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The CD3 Conformational Change in the γδ T Cell Receptor Is Not Triggered by Antigens but Can Be Enforced to Enhance Tumor Killing

Elaine P. Dopfer,[1,2] Frederike A. Hartl,[1,2] Hans-Heinrich Oberg,[3] Gabrielle M. Siegers,[1,2,4] O. Sascha Yousefi,[1,2,5] Sylvia Kock,[6] Gina J. Fiala,[1,2,5] Beatriz Garcillán,[7] Andrew Sandstrom,[8] Balbino Alarcón,[9] Jose R. Regueiro,[7] Dieter Kabelitz,[3] Erin J. Adams,[8] Susana Minguet,[1,2] Daniela Wesch,[3] Paul Fisch,[6] and Wolfgang W.A. Schamel[1,2,*]

[1]Department of Molecular Immunology, Faculty of Biology, BIOSS Centre for Biological Signaling Studies and Centre of Chronic Immunodeficiency (CCI), University of Freiburg, Schänzlestrasse 18, 79108 Freiburg, Germany
[2]Max Planck Institute of Immunobiology and Epigenetics, Stübeweg 51, 79104 Freiburg, Germany
[3]Institute of Immunology, Christian-Albrechts University of Kiel, 24105 Kiel, Germany
[4]Robarts Research Institute, Department of Anatomy and Cell Biology, Robarts Research Institute, Western University, London, ON N6A 5B7, Canada
[5]Spemann Graduate School of Biology and Medicine (SGBM), Albert Ludwigs University Freiburg, 79104 Freiburg, Germany
[6]Institute of Pathology, University Medical Center Freiburg, 79106 Freiburg, Germany
[7]Faculty of Medicine, Universidad Complutense, 28040 Madrid, Spain
[8]Department of Biochemistry and Molecular Biology, University of Chicago, IL 60637, USA
[9]Centro de Biología Molecular Severo Ochoa, Consejo Superior de Investigaciones Científicas, Universidad Autonoma de Madrid, 28049 Madrid, Spain
*Correspondence: wolfgang.schamel@biologie.uni-freiburg.de
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SUMMARY

Activation of the T cell receptor (TCR) by antigen is the key step in adaptive immunity. In the αβTCR, antigen induces a conformational change at the CD3 subunits (CD3 CC) that is absolutely required for αβTCR activation. Here, we demonstrate that the CD3 CC is not induced by antigen stimulation of the mouse G8 or the human Vγ9Vδ2 γδTCR. We find that there is a fundamental difference between the activation mechanisms of the αβTCR and γδTCR that map to the constant regions of the TCRαβ/γδ heterodimers. Enforced induction of CD3 CC with a less commonly used monoclonal anti-CD3 promoted proximal γδTCR signaling but inhibited cytokine secretion. Utilizing this knowledge, we could dramatically improve in vitro tumor cell lysis by activated human γδ T cells. Thus, manipulation of the CD3 CC might be exploited to improve clinical γδ T cell-based immunotherapies.

INTRODUCTION

αβ T cells use their αβ T cell antigen receptor (αβTCR) to recognize an almost infinite number of peptide antigens presented by major histocompatibility complex molecules (pMHC) on antigen-presenting cells (APCs). In contrast, γδ T cells have a rather limited germline-encoded receptor repertoire. Their γδTCRs in part recognize stress-induced self-antigens, lipids, or pyrophosphates that are secreted by some microbes or are overproduced in tumor cells (Bonneville et al., 2010; Chien and Konigshofer, 2007; Vantourout and Hayday, 2013). γδTCRs can also deliver ligand-independent signals for γδ T cell development in the thymus (Jensen et al., 2008).

In mice, 1% of the γδ T cells recognize the nonclassical MHC class I molecule T22, which is expressed on activated cells, such as lipopolysaccharide (LPS)-stimulated B cells (Crowley et al., 2000; Matis et al., 1987). One example is the G8 γδTCR that uses its CDR3δ loop to bind with high affinity to T22 (Adams et al., 2005; Crowley et al., 2000; Weintraub et al., 1994).

In human blood, the main subset of γδ T cells is Vγ9Vδ2 that accounts for 2%–10% of all T cells. The Vγ9Vδ2 TCR recognizes self and foreign nonpeptidic phosphorylated small organic compounds, collectively termed phosphoantigens (Bukowski et al., 1995, 1998; Constant et al., 1994; Espinosa et al., 2001; Tanaka et al., 1995). Vγ9Vδ2 T cells are also stimulated by tumor cells, such as the Daudi B cell lymphoma (Fisch et al., 1997), that likely express high levels of phosphoantigens (Gober et al., 2003). Antigen recognition and tumor cell killing by Vγ9Vδ2 T cells can be enhanced with aminobisphosphonates, such as zoledronate (Roelofs et al., 2009), which increase accumulation of endogenous phosphoantigen.

TCRs consist of a clonotypic TCRαβ or TCRγδ heterodimer, two CD3 dimers (CD3δε, CD3γε), and a ζζ dimer. TCRαβ and TCRγδ chains contain variable (V) immunoglobulin domains that bind to the antigen, and constant (C) domains that associate with CD3. CD3 and ζ contain tyrosines in their cytoplasmic tails that are phosphorylated upon antigen binding to TCRαβ or TCRγδ. In this report, "TCRαβ" or "TCRγδ" denote the TCRαβ or TCRγδ heterodimers, and "αβTCR" or "γδTCR" the complete TCRs including the CD3 and ζ chains. Although similar in domain structure, the architecture of γδTCRs differs from that of αβTCRs (see the Discussion).

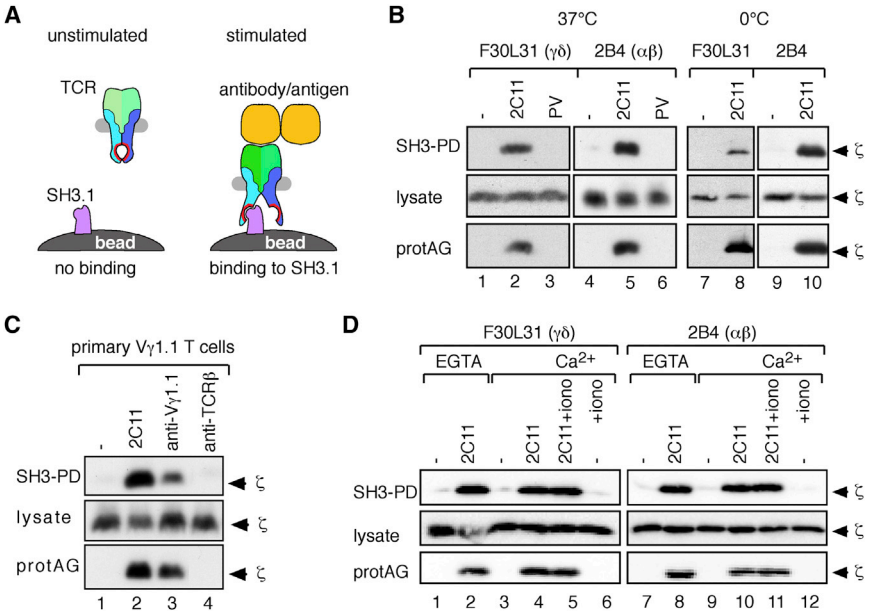

Figure 1. CD3 Conformational Change Induction at Murine γδTCRs

(A) Schematic of the SH3-PD assay.

(B) The murine γδ T cell hybridoma F30L31 and αβ T cell hybridoma 2B4 were left untreated (–) and stimulated for 5 min at 37°C or 0°C with 5 μg/ml anti-CD3 mAb 2C11 or for 5 min with pervanadate (PV). After lysis, one aliquot of lysates was incubated with SH3 beads and another with protein A- and protein G beads. Lysates and bead-purified proteins were analyzed by anti-ζ WB (n > 3).

(C) Pooled thymocytes and splenocytes of TCRβ$^{-/-}$Vγ1.1tg mice were stimulated at 37°C with anti-CD3 (2C11), anti-Vγ1.1, or anti-TCRβ (H57-597) antibodies. The samples were treated as in (B) (n = 3).

(D) F30L31 and 2B4 cells were left untreated (–) or stimulated with 2C11 in the presence of 4 mM EGTA. Additionally, cells were left untreated and stimulated with 2C11, with 2C11 plus 1 μg/ml ionomycin or with ionomycin alone in the presence of 0.9 mM Ca^{2+}. After lysis, one aliquot of lysates was incubated with SH3 beads and another with protein A and protein G beads. In the lysis and washing buffers, EGTA or Ca^{2+} were present as indicated. Lysates and bead-purified proteins were analyzed by anti-ζ WB (n = 3).

Stimulation of the αβTCR and the γδTCR initiates intracellular signaling cascades, such as Ca^{2+} influx, PI3K/AKT, Ras/Erk, and NFκB pathways that are extensively studied. However, how antigen binding to TCRαβ or TCRγδ is communicated to the cytosolic tails of CD3 and ζ is less well understood (Kuhns and Davis, 2012). It has been suggested that the αβTCR exists in two conformations. In the closed conformation, adopted by the unstimulated αβTCR, the cytosolic tails of CD3 and ζ might be shielded from phosphorylation (Minguet and Schamel, 2008). In the open conformation, induced by productive antigen or antibody binding, CD3 and ζ phosphorylation might be promoted by an unknown mechanism.

The experimental assay to measure this CD3 conformational change (CD3 CC) makes use of the increased accessibility of a proline-rich sequence (PRS) in the CD3ε cytoplasmic tail. In the closed conformation, the PRS cannot bind to the first SH3 domain of the adaptor protein Nck. In contrast, in the open conformation the PRS is accessible and thus binds to this SH3 domain (Borroto et al., 2013, 2014; de la Cruz et al., 2011; Gil et al., 2002, 2005; Martínez-Martín et al., 2009; Minguet et al., 2007). In fact, PRS exposure is correlated with an overall rearrangement in the structure of the CD3 and ζ cytoplasmic tails (Risueño et al., 2008).

The CD3 CC, as measured by PRS exposure, precedes CD3 phosphorylation (Gil et al., 2002) and is required for αβ T cell activation (Minguet et al., 2007), in that engineered ligands that could not induce the CD3 CC did not result in αβTCR phosphorylation and downstream signaling. Likewise, point mutations in the extracellular part of CD3ε that do not allow the outside-in transmission of the CD3 CC, such as CD3εK76T or CD3εC80G, inhibit αβTCR signaling in vitro and in vivo (Martínez-Martín et al., 2009). Thus, without the CD3 CC, an αβTCR cannot be activated.

CD3ε also contains a cytosolic basic-rich sequence that has been proposed to interact with the acidic lipids of the inner membrane leaflet, shielding the cytoplasmic CD3ε tyrosines from phosphorylation in the unstimulated αβTCR (Deford-Watts et al., 2009; Xu et al., 2008). Signaling by the αβTCR leads to Ca^{2+}-influx neutralizing the negative lipid head groups and thus, freeing the CD3ε cytosolic domain from the membrane and promoting sustained signaling after the initial αβTCR trigger (Shi et al., 2013). Whether Ca^{2+} ions can influence the exposure of the PRS is currently unknown.

To date, studies exploring the induction of the CD3 CC in γδTCRs are lacking. Here, we tested if the CD3 CC can be induced in the mouse and human γδTCR and whether it regulates γδ T cell activation.

RESULTS

Murine γδTCRs Undergo a Conformational Change at CD3ε upon Antibody Stimulation

To assess whether the γδTCR undergoes the CD3 CC upon stimulation, we used the murine γδ T cell line F30L31 expressing a Vγ1.1 TCR, and as a control the mouse αβ T cell line 2B4. We carried out an SH3 pull-down (PD) assay using the first SH3 domain of Nck (Figure 1A). Anti-ζ western blotting showed that resting, unstimulated TCRs did not bind to the SH3-coupled beads (Figure 1B, lanes 1 and 4), whereas stimulation with the anti-CD3 monoclonal antibody (mAb) 145-2C11 (2C11) at 37°C induced binding of the γδTCR and the αβTCR to SH3 beads (lanes 2 and 5). Stimulation with the phosphatase inhibitor pervanadate (PV) did not trigger binding of the TCRs to SH3 (lanes 3 and 6). As a further control, we incubated the lysates with protein A- and G-coupled beads to immune-precipitate the TCRs via the bound anti-CD3 mAb (lower panels). In addition, anti-TCRγδ antibody stimulation also induced γδTCR binding to SH3 (Figure S1A). A hallmark of the CD3 CC is its independence from any metabolic process (Gil et al., 2002; Minguet et al.,