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Structural biology of γδ T cell receptors

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T cell receptor (TCR) diversity underpins cellular immunity. While αβ TCRs have been extensively studied in the context of major histocompatibility complex (MHC)-restricted antigen recognition, the γδ TCR system remains underexplored. Unlike their αβ counterparts, γδ TCRs display versatile, often MHC-independent recognition modes, engaging diverse ligands ranging from butyrophilins (BTNs) and other disparate molecules. Recent advances in cryo-electron microscopy (cryo-EM) paired with crystallographic data have illuminated critical aspects of γδ TCR -ligand interactions, the CD3 complex architecture, and the inherent flexibility underpinning their varied recognition modes. In this review, we compare the classical αβ TCR-MHC paradigm against the backdrop of emerging γδ TCR structures, highlighting the latest cryo-EM findings and their implications for immunobiology.

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Introduction

The human adaptive immune system, comprised of B, αβ, and γδ T cells is defined by their expression of somatically recombined cellular surface receptors that afford protection against diverse pathogens. αβ and γδ T cells, which provide cellular immunity, are defined by the T cell receptor (TCR) that they express, αβ TCRs and γδ TCRs, respectively [1]. Antigen recognition by TCRs serves to regulate T cell activation and their participation within the immune response. T cells, upon recognition of their cognate ligand, can perform diverse effector functions that include the direct lysis infected or cancerous cells, and cytokine production, driving the recruitment of other immune cells to resolve the immune challenge.

In concert with cellular immunology, structural-based techniques have afforded fundamental insights into the mechanisms underpinning αβ TCR and activation. However, our understanding of γδ TCR binding is less clear than that of their αβ TCR counterparts. Recent work highlighted here, specifically utilizing cryo-electron microscopy (cryo-EM), has made major inroads toward understanding the principles of γδTCR recognition.

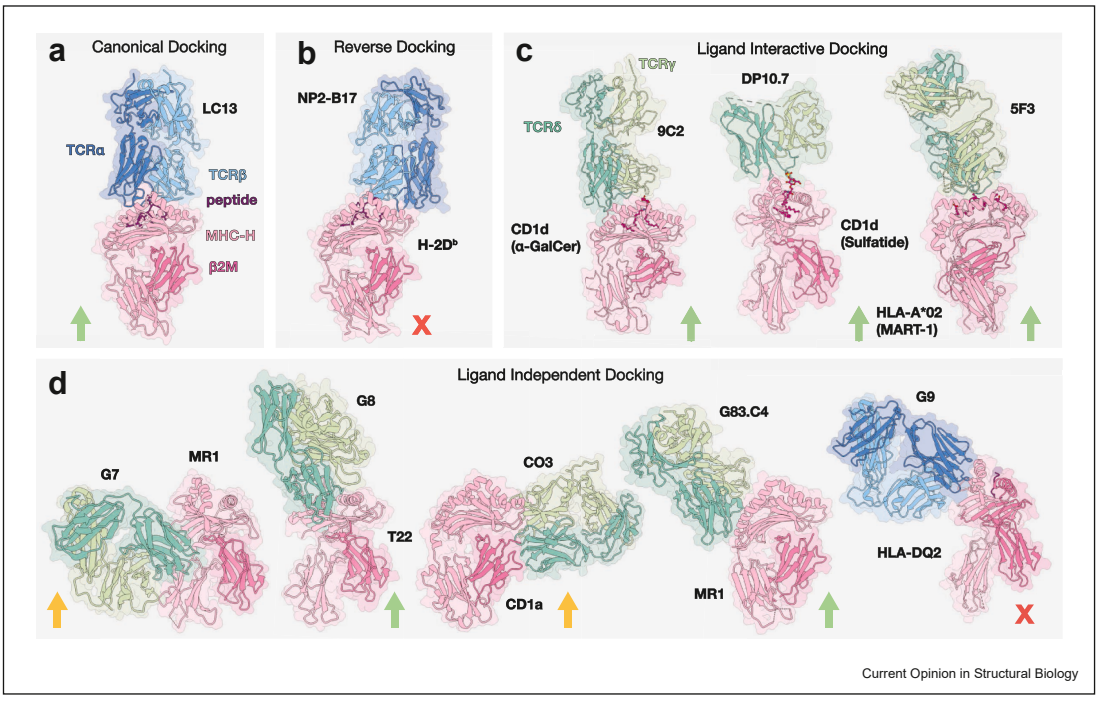
The αβ T cell receptor-major histocompatibility complex paradigm: a structural benchmark

A central tenet of conventional αβ T cell activation is the simultaneous recognition of the MHC and the presented peptide via the TCR [2]. Recognition models, unearthed via decades of crystallographic studies, depict a conserved docking of the αβ TCR atop the MHC-peptide antigen complex. For example, the TCR α-chain and β-chains are positioned over α2 and α1-helices of MHC-I, respectively (Figure 1a). Changes to this canonical TCR docking polarity have been rarely observed, whereby the TCR docks atop peptide-MHC at a 180° orientation, termed 'reverse docking' (Figure 1b) [3]. This altered docking topology perturbs cellular activation due to spatial changes that preventing co-receptor engagement and subsequent recruitment of cellular kinases; and, this altered signaling profile inhibits the recruitment of 'reverse docking' TCRs into the functional immune response [3]. This stringency depicts αβ TCR canonical docking as a key regulator of T cell activation, yet this governing principle seemingly does not extend to γδ TCRs.

γδ T cell receptors: Unconventional recognition by Vδ2$^-$ cells

Since their identification, γδ TCRs have been postulated to employ antigen recognition strategies distinct from αβ TCRs as a consequence of their elongated

Figure 1



X-ray crystallography-determined TCR-ligand binding modalities. a. The canonical αβTCR-pMHC docking, represented by LC13 TCR in complex with HLAB8-EBV peptide complex (PDB: 1MI5). **b.** Reverse-polarity αβTCR-pMHC docking, represented by NP2-B17 in complex with H2Db-NP (PDB: 5SWS). **C.** Ligand-interactive docking of γδ TCR toward MHC-I-like complexes (PDB: 4LHU, 4MNG, 6D7G). **D.** Unconventional docking of TCRs to MHC-I-like molecules (PDB: 6MWR, 1YPZ, 7RYO, 7LLI, 9EJG). Green arrow indicates proven T-cell activation, orange arrow indicates moderate T-cell activation, red 'x' indicates no signaling competency. TCR α/β and γ/δ chains are shown in light/dark variants of blue and green, respectively, MHC/MHC-I-like molecules shown in light pink, β_2M is shown in dark pink. HLA, human leukocyte antigen; MHC, major histocompatibility complex; TCR, T cell receptor.

CDR3δ loops being reminiscent of immunoglobulins [4]. Hindering our understanding has been a lack of bona fide γδ T cell antigens, although confirmed ligands have been noted for their diversity and include stress-induced ligands, MHC-I-like molecules, soluble antigens, and recently, haptens [5]. The γδ T cell ligands have been reviewed in Refs. [6,7].

Given the diversity and complexity of γδ T cell ligands, γδ T cells are generally characterized by their variable (V) chain usage into $V\delta2^+$ or $V\delta2^-$ T cell subsets. $V\delta1^+$ and $V\delta3^+$ T cells have roles implicated in the response to cellular stress and tumor surveillance as well as protection from viruses [8–15]. These cells are predominantly located in peripheral tissue, enriched in the skin, gut, and lung [16,17].

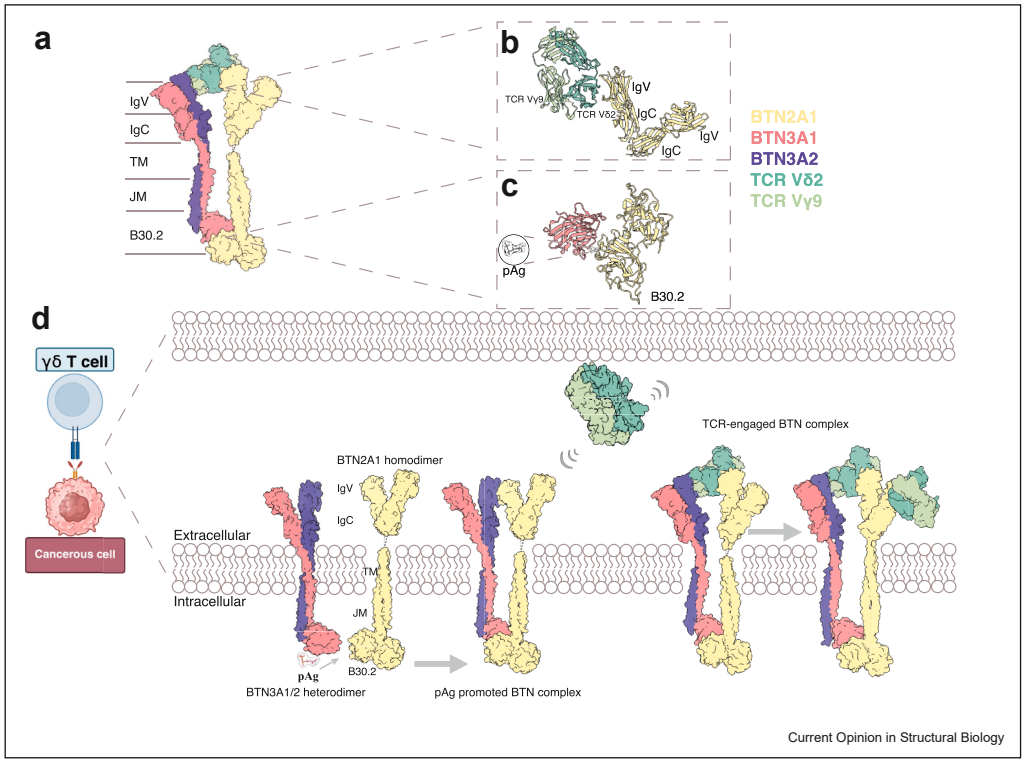
X-ray crystallography has proven to be a powerful tool in illuminating how γδ TCRs bind to MHC-I-like ligands as occurs in conditions such as cellular stress. These studies have revealed two distinct recognition modalities, ligand-interactive or ligand-independent (Figure 1c and d). Two CD1d-restricted and one human leukocyte antigen (HLA)-restricted γδ TCRs have been determined to co-interact with the presented lipid and peptide antigen, respectively, reminiscent of αβ TCR-ligand interactions [18–20]. In contrast, other ligated γδ TCR structures to date do not invoke co-recognition and can employ antigen recognition strategies not yet identified in αβ T cells (Figure 1d) [21–24]. Although αβ TCRs that divert from the co-recognition paradigm have been identified, recognizing the MHC-like ligands, CD1a and CD1c [7]. Additionally, we have recently demonstrated that both αβ and γδ T cells can recognize the soluble protein phycoerythin and that an αβ TCR can bind HLA-II in a peptide-independent manner, thereby blurring the boundaries of αβ and γδ TCR recognition [25,26]. The peptide-independent recognition of HLA-II by an αβ TCR was incapable of triggering cellular activation, whereas similar ligand-independent recognition by γδ TCRs display sustained triggering potential. Thus, αβ T cell activation requires stringent docking parameters, while γδ T cell activation appears lenient toward architectural differences.

Structural insights into γδ T cell receptor-butyrophilin recognition: From crystal to cryo-electron microscopy

Vγ9Vδ2 T cells are a unique subset of γδ T cells that recognize intracellular accumulation of phosphoantigens (pAgs), which arise from dysregulated isoprenoid metabolism during infection or tumorigenesis [27,28]. Unlike other T cell subsets, $V\delta2^+$ T cells do not rely on MHC or MHC-like antigen presentation; instead, pAg sensing is mediated by the butyrophilin family (Figure 2) (BTN3A1, BTN3A2/3, and BTN2A1) [29–31]. $V\delta2^+$ T cell activation by the butyrophilins (BTNs) is dependent on BTN2A1/3A1 engaging pAgs via their intracellular B30.2 domain as BTN over-expression alone is insufficient for $V\delta2^+$ activation (Figure 2c) [32]. In parallel, Karunakaran et al. showed that BTN2A1 recognition of the Vγ9 chain occurs independently of pAg binding, and proposed a composite ligand model in which the γδ TCR simultaneously engages BTN2A1 and an additional pAg-induced ligand in a CDR3-dependent manner [33]. Earlier biophysical and biochemical studies demonstrated that pAg binding induces conformational rearrangements within the BTN3A1 B30.2 domain and the adjacent juxtamembrane (JM) region, providing a mechanistic basis for signal transmission across the membrane [34–37]. Building on this foundation, Karunakaran et al. further showed that the BTN3A JM region regulates both the trafficking and conformational organization of homomeric and heteromeric BTN complexes, thereby defining the topological requirements for productive Vγ9Vδ2 T cell activation [38]. Despite these advancements in our understanding of pAg-mediated activation, the precise molecular details of this process remained elusive.

The first structural breakthrough of BTN-TCR engagement came from the crystal structure of a Vγ9Vδ2 TCR bound to the BTN2A1 IgV ectodomain (ECD) [39], which showed an unusual lateral binding mode where the BTN2A1 IgV domain primarily contacted germline-encoded regions of the Vγ9 chain rather than the hypervariable CDR3 loops that dominate αβ TCR interactions (Figure 2b). Prior to this, Willcox et al. employed Nuclear Magnetic Resonance (NMR) spectroscopy and molecular modeling to demonstrate that BTN2A1 and BTN3A1 ectodomains can associate *in cis*, forming a distinctive W-shaped

Figure 2



Structural basis of Vγ9Vδ2 TCR recognition of BTN2A1 and BTN3A complexes triggered by phosphoantigen sensing. a. Structural overview of the Vγ9Vδ2 TCR bound to BTN2A1, BTN3A1, BTN3A2 complexes determined via cryo-EM (PDB ID: 9JQR). **b.** Crystal structure of Vγ9Vδ2-TCR clone G115 bound to the BTN2A1 IgV domain (PDB ID: 8DFW). **c.** Crystal structure of the B30.2 domains of BTN3A1 and 2A1 (PDB ID: 8HJT) **d.** Overview of Vγ9Vδ2 TCR recognizing pAg-BTN complexes. pAgs promote the assembly of BTN2A1 and BTN3A1/2 complexes. These assembled BTN complexes are recognized by Vγ9Vδ2 TCRs, which are accompanied by conformational changes within the BTN complex (PDB IDs:9J5J, 9JQR, 9JQQ). Vγ/Vδ are shown shades of light/dark green, BTN2A1 in yellow, BTN3A1/2 in pink and purple, respectively. TM denotes transmembrane domain, JM indicates juxtamembrane domain. BTNs, butyrophilins; EM, electron microscopy; TCR, T cell receptor.

configuration that is incompatible with TCR engagement [40]. This arrangement was subsequently corroborated by cryo-EM analyses, reinforcing the conclusion that dynamic reorganization of BTN complexes is required to permit productive TCR binding.

Building on these findings, cryo-EM has recently demonstrated how intracellular phosphoantigen binding induces conformational changes in BTN3A1 that stabilize the BTN2A1-BTN3A1 heterodimer [41]. Their structure revealed that pAg engagement induces an 'inside-out' rearrangement of BTN3A1, stabilizing the BTN2A1-BTN3A1 heterodimer and promoting receptor dimerization (Figure 2d). This induced dimerization provides a mechanistic explanation for how intracellular metabolic signals can be translated into extracellular TCR recognition, a signaling strategy distinct from the fixed peptide—MHC display used by αβ T cells.

Further structural insights were provided [42], who used cryo-EM to resolve a multimeric BTN complex engaging Vγ9Vδ2 TCR. Their study uncovered a previously unappreciated 2:1 stoichiometry, where a single TCR bridges two BTN heterodimers in a 'plier-like' conformation (Figure 2d). This arrangement implies that BTN clustering could serve not only as a ligand platform but also as a spatial regulatory mechanism for TCR activation, reminiscent of innate immune receptor arrays. The structure also captured key interactions involving both CDR3δ and germline-encoded framework regions, supporting the idea that γδ TCRs exploit multiple contact surfaces to recognize flexible BTN assemblies.

Distinct by design; T cell receptor-CD3 architectures

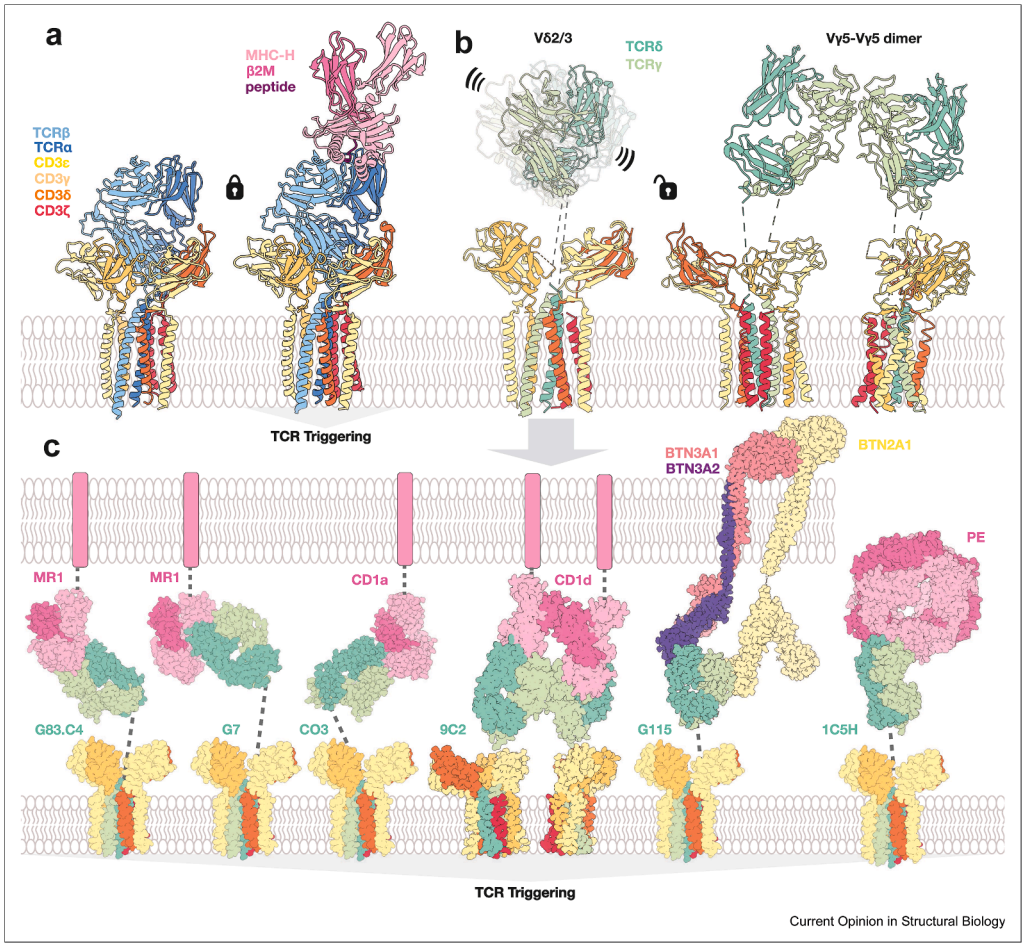
Although γδ and αβ TCR differ in their protein sequences, both TCRs associate with the co-receptor CD3 molecules to form the TCR-CD3 signaling complex [43—46]. Both full-length TCR heterodimers (α/β and γ/δ) present on the cell-surface accompanied by dimers of CD3 proteins CD3-εδ, CD3-εγ, and CD3-ζ_2. The stoichiometry of such signaling complexes is equivalent in both human γδ and αβ T cells. Extensive structural characterization through complementary nuclear magnetic resonance and X-ray crystallography techniques has unearthed the structures of the CD3 dimers [47—51]. Yet, until recently, questions persisted on the architecture of the TCR-CD3 complexes and if this architecture was maintained across TCR systems given the differences in TCR sequences, TCR ligand binding modes and the impact of these differences on TCR triggering.

In 2019, the first high-resolution structure of the long-awaited fully assembled αβ TCR-CD3 complex was resolved via cryo-EM to a global resolution of 3.7 Å [52], improved to 3.2 Å in a subsequent study (Figure 3a) [53]. Extracellularly, the immunoglobulin-like domains of both CD3 heterodimers asymmetrically cup the constant domains of the TCRα and TCRβ chains (Figure 3a). This clasp-like architecture stabilizes the transmembrane α-helical core of the receptor complex. This tightly packed bundle of individual α-helices (one from each present chain) is held together by an intricate network of salt bridges from precisely positioned charged residues that drive complex formation [54]. Later structures incorporating pMHC revealed that the complex remains structurally unaltered during antigen engagement (Figure 3a) [55,56]. Together, these structures reveal top-to-bottom conserved charged interactions resulting in a rigid and highly ordered αβ TCR-CD3 complex, which undergoes minimal structural rearrangement during ligand engagement. Rather than relying on large-scale restructuring, the αβ TCR-CD3 complex appears optimized for stable assembly and to be an efficient signaler of TCR-antigen recognition.

Five years after the resolution of the αβ TCR-CD3 complex, a flurry of γδ TCR-CD3 complexes were reported, marking a turning point in our understanding of these enigmatic receptors. Xin et al. reported a 3.4 Å Vγ9Vδ2 TCR-CD3 complex and a Vγ5Vδ1 TCR-CD3 receptor-obligate dimer [57]; Gully et al. resolved a Vγ8Vδ3 TCR-CD3 complex to 3.01 Å, adding further diversity to the structural repertoire [58]; and Hoque et al. confirmed the findings of Xin et al., reporting the very same TCR-CD3 architectural features [59]. These studies solidified a very distinct paradigm for γδ TCR-CD3 receptors; unlike the rigid nature of αβ TCR-CD3 architecture, γδ T-cells exhibit inherent flexibility within their TCR extracellular domains (Figure 3b). The highly ordered transmembrane helical bundle between TCR systems remains conserved, with key charged interacting residues being maintained, alongside co-ordinated cholesterol molecules.

In contrast with that of the αβ TCR-CD3, the clasp-like architecture of extracellular CD3 domains is surprisingly untethered to the ECD of either γ or δ chain (Figure 3b). In both Vγ9Vδ2 and Vγ8Vδ3 complexes, volume corresponding to the TCR soluble domains could not be resolved with respect to other components of the complex, meaning it does not adopt one single arrangement. Both Vγ5Vδ1 TCR-CD3 complexes revealed dimerization mediated by Vγ5 domains, highlighting the structural plasticity of the fully assembled γδ T-cell receptor (Figure 3b). The spatial adaptability of the γδ TCR may be critical in conserving CD3 co-receptor association throughout versatile antigen engagement (Figure 3c), something that the locked architecture of the αβ TCR-CD3 complex does not allow for. This adaptability clarifies how TCR triggering and subsequent activation can be maintained across recognition of MHC-like family,

Figure 3



Structural plasticity of the γδTCR-CD3 complex allows for diverse triggering mechanisms. a. Rigid structure of the αβTCR-CD3 complex (PDB: 7FJE) and αβTCR-CD3-pMHC complex (PDB: 7PHR). **b.** Structure of the γδTCR-CD3 complex, representative of both Vδ2 and Vδ3 structures, flexible TCR ECD domain is modeled (PDB: 9CIA), structure of the obligate Vγ5 γδTCR-CD3 dimer (PDB: 8JCB). **c.** Modeled immune synapse of diverse γδTCR-CD3-ligand recognition events (PDB: 9CI8, 7LLI, 6MWR, 7RYO, 8JCB, 4LHU, 9JQR, 9O62). MHC-I-like/PE-β molecules are shown in light pink, β_2M/PE-α is shown in dark pink. TCR α/β and γ/δ chains are shown in light/dark variants of green and blue, respectively, CD3ε in yellow, CD3γ in light orange, CD3δ in light orange, and CD3ζ in red. BTN2A1 is shown in yellow, BTN3A1 and BTN3A2 are shown in pink and dark blue, respectively. BTNs, butyrophilins; ECD, ectodomain; MHC, major histocompatibility complex; TCR, T cell receptor.

B7-like family and other obscure antigens within the same membrane-bound complex (Figure 3c).

Conclusions

Recent examples of cryo-EM applied to the field of T-cell structural immunology have provided much-needed clarity surrounding perceived abnormalities of γδ T cell biology. The application of cryo-EM has marked a major methodological breakthrough in the study of γδ TCR–butyrophilin recognition. Unlike earlier crystallographic approaches limited to partial ectodomains, cryo-EM has enabled the visualization of full-length BTN complexes in multivalent assemblies with the Vγ9Vδ2 TCR. These findings are consistent with earlier NMR studies combined with modeling, which contributed to defining the *in cis* BTN2A1–BTN3A1 IgV–IgV interaction now considered to represent the physiological arrangement. Cryo-EM has further revealed the unexpected discovery of a 2:1 BTN:TCR stoichiometry and 2:2 dynamic 'plier-like' conformations, offering unprecedented insight into the spatial organization of the immune synapse. Notably, the discrepancy between docking modality and immune activation of γδ and αβ T cell lineages is now better understood through the lens of contrasting TCR spatial constraints and architectural flexibility. By leveraging the strengths of cryo-EM in resolving membrane protein complexes, these recent findings have broadened our knowledge toward fundamental mechanistic understandings of γδ TCR recognition of antigen at the immune synapse. To our knowledge, no ligand

engaged γδ TCR-CD3 complex has been determined. It would be interesting whether a liganded γδ TCR-CD3 complex undergoes a conformational change given the dynamic γδ TCR flexibility or if it mirrors the unaltered liganded αβ TCR-CD3 complex. Ultimately, these advances underscore the unique capacity of cryo-EM to resolve flexible, heterogeneous signaling transmembrane complexes that are often inaccessible to traditional structural techniques.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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References

Papers of particular interest, published within the period of review, have been highlighted as:

* of special interest
** of outstanding interest

1. Morath A, Schamel WW: **Alphabeta and gammadelta T cell receptors: similar but different**. *J Leukoc Biol* 2020, **107**: 1045–1055.
2. Garcia KC, Degano M, Stanfield RL, Brunmark A, Jackson MR, Peterson PA, Teyton L, Wilson IA: **An alphabeta T cell receptor structure at 2.5 A and its orientation in the TCR-MHC complex**. *Science* 1996, **274**:209–219.
3. Zareie P, Szeto C, Farenc C, Gunasinghe SD, Kolawole EM, Nguyen A, Blyth C, Sng XYX, Li J, Jones CM, *et al.*: **Canonical T cell receptor docking on peptide-MHC is essential for T cell signaling**. *Science* 2021, **372**:eabe9124.
4. Rock EP, Sibbald PR, Davis MM, Chien YH: **CDR3 length in antigen-specific immune receptors**. *J Exp Med* 1994, **179**: 323–328.
5. Guo J, Chowdhury RR, Mallajosyula V, Xie J, Dubey M, Liu Y, Li J, Wei YL, Palanski BA, Wang C, *et al.*: **Gammadelta T cell antigen receptor polyspecificity enables T cell responses to a broad range of immune challenges**. *Proc Natl Acad Sci U S A* 2024, **121**, e2315592121.
6. Willcox BE, Willcox CR: **Gammadelta TCR ligands: the quest to solve a 500-million-year-old mystery**. *Nat Immunol* 2019, **20**:121–128.
7. Sok CL, Rossjohn J, Gully BS: **The evolving portrait of gammadelta TCR recognition determinants**. *J Immunol* 2024, **213**:543–552.
8. Halary F, Pitard V, Dlubek D, Krzysiek R, de la Salle H, Merville P, Dromer C, Emilie D, Moreau JF, Dechanet-Merville J: **Shared reactivity of Vdelta2(neg) gammadelta T cells against cytomegalovirus-infected cells and tumor intestinal epithelial cells**. *J Exp Med* 2005, **201**:1567–1578.
9. Fujishima N, Hirokawa M, Fujishima M, Yamashita J, Saitoh H, Ichikawa Y, Horiuchi T, Kawabata Y, Sawada KI: **Skewed T cell receptor repertoire of Vdelta1(+) gammadelta T lymphocytes after human allogeneic haematopoietic stem cell transplantation and the potential role for Epstein-Barr virus-infected B cells in clonal restriction**. *Clin Exp Immunol* 2007, **149**:70–79.
10. Groh V, Steinle A, Bauer S, Spies T: **Recognition of stress-induced MHC molecules by intestinal epithelial gammadelta T cells**. *Science* 1998, **279**:1737–1740.
11. Ravens S, Schultze-Florey C, Raha S, Sandrock I, Drenker M, Oberdorfer L, Reinhardt A, Ravens I, Beck M, Geffers R, *et al.*: **Human gammadelta T cells are quickly reconstituted after stem-cell transplantation and show adaptive clonal expansion in response to viral infection**. *Nat Immunol* 2017, **18**: 393–401.
12. Lien SC, Ly D, Yang SYC, Wang BX, Clouthier DL, St Paul M, Gadalla R, Noamani B, Garcia-Batres CR, Boross-Harmer S, *et al.*: **Tumor reactive gammadelta T cells contribute to a complete response to PD-1 blockade in a Merkel cell carcinoma patient**. *Nat Commun* 2024, **15**:1094.
13. Wu Y, Biswas D, Usaite I, Angelova M, Boeing S, Karasaki T, Veeriah S, Czyzewska-Khan J, Morton C, Joseph M, *et al.*: **A local human Vdelta1 T cell population is associated with survival in nonsmall-cell lung cancer**. *Nat Cancer* 2022, **3**: 696–709.
14. Mangan BA, Dunne MR, O'Reilly VP, Dunne PJ, Exley MA, O'Shea D, Scotet E, Hogan AE, Doherty DG: **Cutting edge: CD1d restriction and Th1/Th2/Th17 cytokine secretion by human Vdelta3 T cells**. *J Immunol* 2013, **191**:30–34.
15. Mensurado S, Blanco-Dominguez R, Silva-Santos B: **The emerging roles of gammadelta T cells in cancer immunotherapy**. *Nat Rev Clin Oncol* 2023, **20**:178–191.
16. Carding SR, Egan PJ: **Gammadelta T cells: functional plasticity and heterogeneity**. *Nat Rev Immunol* 2002, **2**:336–345.
17. Ribot JC, Lopes N, Silva-Santos B: **Gammadelta T cells in tissue physiology and surveillance**. *Nat Rev Immunol* 2021, **21**:221–232.
18. Luoma AM, Castro CD, Mayassi T, Bembinster LA, Bai L, Picard D, Anderson B, Scharf L, Kung JE, Sibener LV, *et al.*: **Crystal structure of Vdelta1 T cell receptor in complex with CD1d-sulfatide shows MHC-like recognition of a self-lipid by human gammadelta T cells**. *Immunity* 2013, **39**: 1032–1042.
19. Benveniste PM, Roy S, Nakatsugawa M, Chen ELY, Nguyen L, Millar DG, Ohashi PS, Hirano N, Adams EJ, Zuniga-Pflucker JC: **Generation and molecular recognition of melanoma-associated antigen-specific human gammadelta T cells**. *Sci Immunol* 2018, **3**:eaav4036.
20. Uldrich AP, Le Nours J, Pellicci DG, Gherardin NA, McPherson KG, Lim RT, Patel O, Beddoe T, Gras S, Rossjohn J, *et al.*: **CD1d-lipid antigen recognition by the gammadelta TCR**. *Nat Immunol* 2013, **14**:1137–1145.
21. Le Nours J, Gherardin NA, Ramarathinam SH, Awad W, Wiede F, Gully BS, Khandokar Y, Praveena T, Wubben JM, Sandow JJ, *et al.*: **A class of gammadelta T cell receptors recognize the underside of the antigen-presenting molecule MR1**. *Science* 2019, **366**:1522–1527.
22. Rice MT, von Borstel A, Chevour P, Awad W, Howson LJ, Littler DR, Gherardin NA, Le Nours J, Giles EM, Berry R, *et al.*: **Recognition of the antigen-presenting molecule MR1 by a Vdelta3(+) gammadelta T cell receptor**. *Proc Natl Acad Sci U S A* 2021, **118**, e2110288118.
23. Wegrecki M, Ocampo TA, Gunasinghe SD, von Borstel A, Tin SY, Reijneveld JF, Cao TP, Gully BS, Le Nours J, Moody DB, *et al.*: **Atypical sideways recognition of CD1a by autoreactive gammadelta T cell receptors**. *Nat Commun* 2022, **13**:3872.
24. Adams EJ, Chien YH, Garcia KC: **Structure of a gammadelta T cell receptor in complex with the nonclassical MHC T22**. *Science* 2005, **308**:227–231.
25. Rashleigh L, Venugopal H, Rice MT, Gunasinghe SD, Sok CL, Gherardin NA, Almeida CF, Van Rhijn I, Moody DB, Godfrey DI, *et al.*: **Antibody-like recognition of a gammadelta T cell receptor toward a foreign antigen**. *Structure* 2025, **33**: 1649–1662 e1645.
26. Lim JJ, Jones CM, Loh TJ, Dao HT, Tran MT, Tye-Din JA, La Gruta NL, Rossjohn J: **A naturally selected alphabeta T cell**

receptor binds HLA-DQ2 molecules without co-contacting the presented peptide. *Nat Commun* 2025, **16**:3330.

27. Chen S, Li Z, Huang W, Wang Y, Fan S: **Prognostic and therapeutic significance of BTN3A proteins in tumors**. *J Cancer* 2021, **12**:4505–4512.

28. Constant P, Davodeau F, Peyrat MA, Poquet Y, Puzo G, Bonneville M, Fournie JJ: **Stimulation of human gamma delta T cells by nonpeptidic mycobacterial ligands**. *Science* 1994, **264**:267–270.

29. Yuan L, Ma X, Yang Y, Qu Y, Li X, Zhu X, Ma W, Duan J, Xue J, Yang H, *et al.*: **Phosphoantigens glue butyrophilin 3A1 and 2A1 to activate Vgamma9Vdelta2 T cells**. *Nature* 2023, **621**: 840–848.

30. Sandstrom A, Peigne CM, Leger A, Crooks JE, Konczak F, Gesnel MC, Breathnach R, Bonneville M, Scotet E, Adams EJ: **The intracellular B30.2 domain of butyrophilin 3A1 binds phosphoantigens to mediate activation of human Vgamma9Vdelta2 T cells**. *Immunity* 2014, **40**:490–500.

31. Yang Y, Li L, Yuan L, Zhou X, Duan J, Xiao H, Cai N, Han S, Ma X, Liu W, *et al.*: **A structural change in Butyrophilin upon phosphoantigen binding underlies phosphoantigen-mediated Vgamma9Vdelta2 T cell activation**. *Immunity* 2019, **50**:1043–1053 e1045.

32. Rigau M, Ostrouska S, Fulford TS, Johnson DN, Woods K, Ruan Z, McWilliam HEG, Hudson C, Tutuka C, Wheatley AK, *et al.*: **Butyrophilin 2A1 is essential for phosphoantigen reactivity by gammadelta T cells**. *Science* 2020, **367**:eaay5516.

33. Karunakaran MM, Willcox CR, Salim M, Paletta D, Fichtner AS, Noll A, Starick L, Nohren A, Begley CR, Berwick KA, *et al.*: **Butyrophilin-2A1 directly binds germline-encoded regions of the Vgamma9Vdelta2 TCR and is essential for phosphoantigen sensing**. *Immunity* 2020, **52**:487–498 e486.

34. Hsiao CH, Lin X, Barney RJ, Shippy RR, Li J, Vinogradova O, Wiemer DF, Wiemer AJ: **Synthesis of a phosphoantigen prodrug that potently activates Vgamma9Vdelta2 T-lymphocytes**. *Chem Biol* 2014, **21**:945–954.

35. Nguyen K, Li J, Puthenveetil R, Lin X, Poe MM, Hsiao CC, Vinogradova O, Wiemer AJ: **The butyrophilin 3A1 intracellular domain undergoes a conformational change involving the juxtamembrane region**. *FASEB J* 2017, **31**:4697–4706.

36. Salim M, Knowles TJ, Baker AT, Davey MS, Jeeves M, Sridhar P, Wilkie J, Willcox CR, Kadri H, Taher TE, *et al.*: **BTN3A1 discriminates gammadelta T cell phosphoantigens from nonantigenic small molecules via a conformational sensor in its B30.2 domain**. *ACS Chem Biol* 2017, **12**:2631–2643.

37. Gu S, Sachleben JR, Boughter CT, Nawrocka WI, Borowska MT, Tarrasch JT, Skiniotis G, Roux B, Adams EJ: **Phosphoantigen-induced conformational change of butyrophilin 3A1 (BTN3A1) and its implication on Vgamma9Vdelta2 T cell activation**. *Proc Natl Acad Sci U S A* 2017, **114**:E7311–E7320.

38. Karunakaran MM, Subramanian H, Jin Y, Mohammed F, Kimmel B, Juraske C, Starick L, Nohren A, Lander N, Willcox CR, *et al.*: **A distinct topology of BTN3A IgV and B30.2 domains controlled by juxtamembrane regions favors optimal human gammadelta T cell phosphoantigen sensing**. *Nat Commun* 2023, **14**:7617.

39. * Fulford TS, Soliman C, Castle RG, Rigau M, Ruan Z, Dolezal O, Seneviratna R, Brown HG, Hanssen E, Hammet A, *et al.*: **Vgamma9Vdelta2 T cells recognize butyrophilin 2A1 and 3A1 heteromers**. *Nat Immunol* 2024, **25**:1355–1366.

This study provided the first structural insights into how the BTN2A1 IgV domain interacts with the Vγ9Vδ2 TCR and reveals an unconventional, antigen-driven mechanism that underpins pAg-mediated γδ T cell immunity.

40. Willcox CR, Salim M, Begley CR, Karunakaran MM, Easton EJ, von Klopotek C, Berwick KA, Herrmann T, Mohammed F, Jeeves M, *et al.*: **Phosphoantigen sensing combines TCR-dependent recognition of the BTN3A IgV domain and germline interaction with BTN2A1**. *Cell Rep* 2023, **42**, 112321.

41. ** Zhu Y, Gao W, Zheng J, Bai Y, Tian X, Huang T, Lu Z, Dong D, Zhang A, Guo C, *et al.*: **Phosphoantigen-induced inside-out stabilization of butyrophilin receptor complexes drives dimerization-dependent gammadelta TCR activation**. *Immunity* 2025, **58**:1646–1659 e1645.

This publication through cryo-EM provides a structural basis for 'inside-out' pAg mediated T cell activation. The authors demonstrate that pAg's drive BTN2A1-BTN3A1-BTN3A2 complex formation via their B30.2 domains, enabling Vγ9Vδ2 TCR engagement and enhancing cellular activation.

42. ** Zhang M, Wang Y, Cai N, Qu Y, Ma X, Xue J, Chen X, Zhang X, Xiao J, Zhang Y: **Structures of butyrophilin multimers reveal a plier-like mechanism for Vgamma9Vdelta2 T cell receptor activation**. *Immunity* 2025, **58**:1660–1669 e1667.

The authors reveal that BTNs cooperate to sense phosphoantigens and engage the Vγ9Vδ2 TCR through a 'plier-like' gripping mechanism that drives γδ T cell activation. Additionally, through targeted TCR mutagensis the authors validate the observed BTN3A2-TCR engagement. These insights provide a structural foundation for the γδ T cell pAg recognition mechanism, distinct from conventional MHC-dependent αβ and other non-pAg restricted γδ T cells.

43. Krangel MS, Band H, Hata S, McLean J, Brenner MB: **Structurally divergent human T cell receptor gamma proteins encoded by distinct C gamma genes**. *Science* 1987, **237**: 64–67.

44. Krangel MS, Bierer BE, Devlin P, Clabby M, Strominger JL, McLean J, Brenner MB: **T3 glycoprotein is functional although structurally distinct on human T-cell receptor gamma T lymphocytes**. *Proc Natl Acad Sci U S A* 1987, **84**: 3817–3821.

45. Brenner MB, McLean J, Dialynas DP, Strominger JL, Smith JA, Owen FL, Seidman JG, Ip S, Rosen F, Krangel MS: **Identification of a putative second T-cell receptor**. *Nature* 1986, **322**: 145–149.

46. Lefranc MP, Rabbitts TH: **Two tandemly organized human genes encoding the T-cell gamma constant-region sequences show multiple rearrangement in different T-cell types**. *Nature* 1985, **316**:464–466.

47. Call ME, Schnell JR, Xu C, Lutz RA, Chou JJ, Wucherpfennig KW: **The structure of the zetazeta transmembrane dimer reveals features essential for its assembly with the T cell receptor**. *Cell* 2006, **127**:355–368.

48. Kjer-Nielsen L, Dunstone MA, Kostenko L, Ely LK, Beddoe T, Mifsud NA, Purcell AW, Brooks AG, McCluskey J, Rossjohn J: **Crystal structure of the human T cell receptor CD3 epsilon gamma heterodimer complexed to the therapeutic mAb OKT3**. *Proc Natl Acad Sci U S A* 2004, **101**:7675–7680.

49. Arnett KL, Harrison SC, Wiley DC: **Crystal structure of a human CD3-epsilon/delta dimer in complex with a UCHT1 single-chain antibody fragment**. *Proc Natl Acad Sci U S A* 2004, **101**: 16268–16273.

50. Sun ZJ, Kim KS, Wagner G, Reinherz EL: **Mechanisms contributing to T cell receptor signaling and assembly revealed by the solution structure of an ectodomain fragment of the CD3 epsilon gamma heterodimer**. *Cell* 2001, **105**:913–923.

51. Sun ZY, Kim ST, Kim IC, Fahmy A, Reinherz EL, Wagner G: **Solution structure of the CD3epsilondelta ectodomain and comparison with CD3epsilongamma as a basis for modeling T cell receptor topology and signaling**. *Proc Natl Acad Sci U S A* 2004, **101**:16867–16872.

52. Dong D, Zheng L, Lin J, Zhang B, Zhu Y, Li N, Xie S, Wang Y, Gao N, Huang Z: **Structural basis of assembly of the human T cell receptor-CD3 complex**. *Nature* 2019, **573**:546–552.

53. Chen Y, Zhu Y, Li X, Gao W, Zhen Z, Dong D, Huang B, Ma Z, Zhang A, Song X, *et al.*: **Cholesterol inhibits TCR signaling by directly restricting TCR-CD3 core tunnel motility**. *Mol Cell* 2022, **82**:1278–1287 e1275.

54. Call ME, Pyrdol J, Wiedmann M, Wucherpfennig KW: **The organizing principle in the formation of the T cell receptor-CD3 complex**. *Cell* 2002, **111**:967–979.

55. Susac L, Vuong MT, Thomas C, von Bulow S, O'Brien-Ball C, Santos AM, Fernandes RA, Hummer G, Tampe R, Davis SJ: **Structure of a fully assembled tumor-specific T cell receptor ligated by pMHC**. *Cell* 2022, **185**:3201–3213 e3219.

56. Saotome K, Dudgeon D, Colotti K, Moore MJ, Jones J, Zhou Y, Rafique A, Yancopoulos GD, Murphy AJ, Lin JC, *et al*.: **Structural analysis of cancer-relevant TCR-CD3 and peptide-MHC complexes by cryoEM**. *Nat Commun* 2023, **14**:2401.

57. ** Xin W, Huang B, Chi X, Liu Y, Xu M, Zhang Y, Li X, Su Q, Zhou Q: **Structures of human gammadelta T cell receptor-CD3 complex**. *Nature* 2024, **630**:222–229.
This publication was the first to report the innate flexibility displayed by the Vδ2Vγ9 TCR-CD3 complex, it also highlights the dynamism of the receptor by reporting Vδ1Vγ5 dimer complexes. Importantly it further delves into the intricacies of both by presenting flexibility as a consequence of connecting-peptide length and Vγ5 dimerization being intrinsically linked to T-cell activation.

58. * Gully BS, Ferreira Fernandes J, Gunasinghe SD, Vuong MT, Lui Y, Rice MT, Rashleigh L, Lay CS, Littler DR, Sharma S, *et al*.: **Structure of a fully assembled gammadelta T cell antigen receptor**. *Nature* 2024, **634**:729–736.
This publication corroborates the idea of γδ TCR flexibility at the membrane by expanding the repertoire to a Vδ3Vγ8 TCR-CD3 complex and confirming that the length of connecting peptides is causative of such flexibility.

59. * Hoque M, Grigg JB, Ramlall T, Jones J, McGoldrick LL, Lin JC, Olson WC, Smith E, Franklin MC, Zhang T, *et al*.: **Structural characterization of two gammadelta TCR/CD3 complexes**. *Nat Commun* 2025, **16**:318.
This publication cements previous understanding by reporting the same findings for both Vδ2Vγ9 and Vδ1Vγ5 TCR-CD3 complexes discussed in Xin et al. Using a γδ TCR antibody, binding is reported at a site that would be occluded in a 'rigid' complex.