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Crystal Structure of Vδ1 T Cell Receptor in Complex with CD1d-Sulfatide Shows MHC-like Recognition of a Self-Lipid by Human γδ T Cells

Adrienne M. Luoma,[1,2] Caitlin D. Castro,[2] Toufic Mayassi,[1,3] Leslie A. Bembinster,[2] Li Bai,[4,5,6] Damien Picard,[5,6] Brian Anderson,[7] Louise Scharf,[2] Jennifer E. Kung,[2] Leah V. Sibener,[2] Paul B. Savage,[7] Bana Jabri,[1,3] Albert Bendelac,[1,5,6] and Erin J. Adams[1,2,*]

[1]Committee on Immunology, University of Chicago, Chicago, IL 60637, USA
[2]Department of Biochemistry and Molecular Biology, University of Chicago, Chicago, IL 60637, USA
[3]Department of Medicine, University of Chicago, Chicago, IL 60637, USA
[4]Institute of Immunology, School of Life Sciences, University of Science and Technology of China, Hefei 230027, China
[5]Department of Pathology, University of Chicago, Chicago, IL 60637, USA
[6]Howard Hughes Medical Institute
[7]Department of Chemistry and Biochemistry, Brigham Young University, Provo, UT 84602, USA
*Correspondence: ejadams@uchicago.edu
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SUMMARY

The nature of the antigens recognized by γδ T cells and their potential recognition of major histocompatibility complex (MHC)-like molecules has remained unclear. Members of the CD1 family of lipid-presenting molecules are suggested ligands for Vδ1 TCR-expressing γδ T cells, the major γδ lymphocyte population in epithelial tissues. We crystallized a Vδ1 TCR in complex with CD1d and the self-lipid sulfatide, revealing the unusual recognition of CD1d by germline Vδ1 residues spanning all complementarity-determining region (CDR) loops, as well as sulfatide recognition separately encoded by non-germline CDR3δ residues. Binding and functional analysis showed that CD1d presenting self-lipids, including sulfatide, was widely recognized by gut Vδ1+ γδ T cells. These findings provide structural demonstration of MHC-like recognition of a self-lipid by γδ T cells and reveal the prevalence of lipid recognition by innate-like T cell populations.

INTRODUCTION

There are two major lineages of T lymphocytes, αβ and γδ T cells, which are defined by their T cell receptor (TCR) gene-segment usage. Although the αβ subset is the prominent T cell population devoted to the orchestration of the adaptive immune response in the circulation and lymph nodes, γδ T cells are particularly abundant in peripheral tissues, most notably the skin and intestinal epithelium, where they monitor early signs of tissue infection or stress (Vantourout and Hayday, 2013). The coexistence of two main T cell lineages has been conserved throughout vertebrate evolution, highlighting how each plays an important, nonredundant role in host defense and survival.

In humans there are two main populations of γδ T cells, Vδ1 and Vδ2 T cells, which predominate in the epithelium and circulation, respectively (McVay and Carding, 1999). It is hypothesized that the TCR dependence of tissue localization might be linked to recognition of a conserved tissue-specific self-ligand, highlighting the importance of γδ TCR ligand characterization for our understanding of γδ T cell biology. However, these ligands remain poorly characterized in both the murine and human systems, and those that have been described exhibit strikingly little overlap between species (Vantourout and Hayday, 2013). Most identified ligands are self-derived and stress induced, in accord with an important role for γδ T cells in the early detection of tissue damage. Such ligands include nonclassical MHC-like molecules, such as murine T22, which has previously been the only ligand for which structural studies have revealed the mode of TCR recognition (Adams et al., 2005; Bonneville et al., 1989; Spada et al., 2000; Willcox et al., 2012). Other proposed ligands bear no resemblance to MHC molecules (Constant et al., 1994; Scotet et al., 2005; Zeng et al., 2012). In humans, intestinal epithelial Vδ1$^+$ cells have been shown to respond to the stress-induced MHC-like molecules MHC class I-related chain A (MICA) and MICB, though the role of the γδ TCR in this response has been controversial because of the very low affinity of the interaction and because of competitive MICA recognition by other surface receptors, such as natural killer group 2, member D (NKG2D) (Bauer et al., 1999; Groh et al., 1998; Xu et al., 2011).

One of the few reported human γδ TCR ligands verified in several independent studies is the lipid-presenting MHC-like molecule CD1d. CD1d is a well-characterized TCR ligand for natural killer T (NKT) cells of the αβ T cell lineage, specializing in the presentation of both self-derived and foreign lipids (Rossjohn et al., 2012). However, NKT cells are more than 10-fold less abundant in humans than in mice, and although the full extent of CD1d recognition by γδ T cells has yet to be determined, γδ T cells appear to be a substantial complement to NKT cells in the surveillance of lipid antigens (Bendelac et al., 2007). CD1d-mediated lipid-antigen recognition has been described for γδ T cells in both the circulation and the intestinal epithelium (Agea et al., 2005; Bai et al., 2012; Mangan et al., 2013; Russano et al., 2007). These cells predominantly use the Vδ1 TCR gene

segment; however, recent studies have also identified CD1d-specific cells among the less prevalent Vδ3$^+$ T cell population (Mangan et al., 2013). Lipid-specific Vδ1$^+$ T cells exhibit heterogeneous phenotypes, which seem to vary according to tissue residence. Circulating phospholipid-specific γδ T cells have a Th2-polarized phenotype, whereas those of intestinal origin can mediate Th1-polarized or regulatory cytokine secretion (Agea et al., 2005; Russano et al., 2006; Russano et al., 2007). The lipid repertoire surveyed by Vδ1$^+$ T cells comprises both exogenous pollen-derived phospholipids and self-lipids, including the glycolipid sulfatide, which is enriched in neuronal, kidney, and intestinal epithelial tissue (Bai et al., 2012; Breimer et al., 2012; Russano et al., 2006; Takahashi and Suzuki, 2012). The detection of self-ligands coincides with the roles of tissue-resident γδ T cells in tissue homeostasis and repair yet, conversely, might explain the accumulation of these T cells in tissues in the context of autoimmune pathology (Komano et al., 1995; Selmaj et al., 1991; Toulon et al., 2009; Wucherpfennig et al., 1992).

Despite these studies implicating γδ T cells as an important lipid-reactive T cell population with diverse functional roles, the structural basis of CD1d-lipid recognition is unknown, as it is for all human γδ TCRs. In stark contrast, abundant structural studies have revealed the details of the αβ TCR interaction with multiple ligand classes (Borg et al., 2007; Garcia et al., 1996; López-Sagaseta et al., 2013). Here we provide the structural basis of human γδ TCR recognition and show how γδ TCRs detect ligands in the context of an antigen-presenting molecule, revealing a new paradigm for the antigen specificity of Vδ1$^+$ T cells.

RESULTS

Vδ1$^+$ T Cells Recognize CD1d Directly through the γδ TCR

We have previously described two peripheral-blood-derived CD1d-sulfatide-reactive human Vδ1$^+$ clones, the Vγ4Vδ1 DP10.7 and Vγ5Vδ1 AB18.1 TCRs (Figure 1A) (Bai et al., 2012). To determine whether this interaction is specifically mediated by the Vδ1$^+$ γδ TCR, we used biochemical methods to measure the interaction between soluble, recombinant CD1d loaded with sulfatide and Vδ1$^+$ γδ TCRs. CD1d loaded with endogenous, insect-cell-derived lipids (unloaded) failed to bind to the DP10.7 TCR (the equilibrium dissociation constant, K_d, was not measurable), whereas it bound to the AB18.1 with moderate affinity (K_d = 20.6 μM) (Figures 1B and 1C). Loading with mixed bovine brain sulfatides (predominantly 24:0 and 24:1 acyl chains) dramatically enhanced CD1d binding to the DP10.7 TCR (K_d = 5.5 μM) and moderately affected binding to the AB18.1 TCR (K_d = 8.9 μM). Both clones bound to CD1d loaded with 24:1 sulfatide (acyl chain 24:1) at affinities comparable to those found with mixed sulfatides (DP10.7 K_d = 5.6 μM; AB18.1 K_d = 13.9 μM). The inability of the DP10.7 clone to bind to 24:1 βGalCer-loaded CD1d (K_d > 100 μM) showed that the sulfate moiety was essential for recognition given that βGalCer lacks the 3′-galactose sulfate group characteristic of sulfatide lipids. In line with sulfatide's having a secondary role in recognition by the AB18.1 TCR, the absence of the 3′ sulfate group only moderately affected AB18.1 binding (K_d = 16.8 μM). Thus, the DP10.7 TCR makes energetically important contacts with the sulfatide antigen, whereas the AB18.1 TCR can recognize CD1d molecules largely indiscriminately of lipid antigens. Thus, it is likely that variability in CDR3δ loop or γ chain sequences modulates Vδ1$^+$ TCR engagement of CD1d-lipid surfaces (Figure 1A).

The reactivity of Vδ1$^+$ T cells toward CD1d molecules loaded with endogenous membrane lipids has been demonstrated previously (Russano et al., 2007), corroborating our biochemical characterization of endogenous CD1d-presented-lipid recognition by the AB18.1 TCR. Beause the DP10.7 TCR exhibits exquisite sulfatide-specific reactivity, we confirmed the functional basis of this interaction by TCR transduction of Jurkat J.RT3-T3.5 T cells. Clone JR.2, a control Vδ1 TCR reported to recognize CD1c molecules (Spada et al., 2000), did not respond to plate-bound CD1d molecules with or without sulfatide loading. However, transduction of the DP10.7 TCR conferred a sulfatide-specific response correlating to the amount of CD1d immobilization, as measured by CD69 upregulation (Figure 1D). The higher basal expression of CD69 by DP10.7 TCR-transduced cells in the presence of sulfatide most likely stems from the expression of CD1d on Jurkat cells themselves (Metelitsa et al., 2001). Together, these results demonstrate that Vδ1$^+$ T cells recognize and respond to CD1d-sulfatide directly through the γδ TCR.

Functional Characterization of CD1d-Sulfatide-Specific Vδ1$^+$ T Cells

To understand markers associated with CD1d-sulfatide-specific T cells, we performed phenotypic studies on Vδ1$^+$ T cells isolated from the blood by CD1d-sulfatide tetramer MACS enrichment and FACS sorting (Bai et al., 2012). Like many γδ T cells, they expressed CD8αβ or CD8αα or were CD8 negative (Figure S1A in the Supplemental Data available with this article online). In agreement with the sequencing results demonstrating that these cells were the progeny of expanded T cell clones, the fresh cells exhibited a CD45RA$^+$CD62L$^-$CCR7$^-$CD28$^-$ effector phenotype. Notably, given the preeminence of Vδ1$^+$ T cells in the human gut, these sulfatide-specific T cells isolated from the blood did not express CD103, a marker for cells of intestinal origin. However, they expressed the myeloid markers CD16, CD11b, and CD11c in the two individuals examined (Figure S1A).

The rarity of CD1d-sulfatide-specific T cells in the blood precluded an analysis of their functional properties, such as cytokine secretion or cytolytic functions, directly ex vivo. Nevertheless, experiments carried out with clones DP10.7 and AB18.1 demonstrated predominant secretion of TNF-α and IFN-γ but little IL-4, IL-13, or IL-10, thus indicating a Th1 phenotype, upon stimulation with ionomycin and PMA (Figure S1B).

The Structural Basis for Vδ1$^+$ γδ TCR Recognition of CD1d-Sulfatide

To understand the molecular basis for Vδ1 TCR recognition of CD1d-sulfatide, we sought to crystallize the TCR-CD1d-sulfatide ternary complex. We developed a fusion human-mouse CD1d molecule (CD1dmα3) in which the native human ligand-binding α1 and α2 domains were fused with the murine α3 domain and heterodimerized with murine β2-microgobulin (β2m). We undertook these efforts because of the many published murine CD1d structures and the hypothesis that murine CD1d molecules might crystallize more readily (Rossjohn et al., 2012). This substitution did not affect DP10.7 TCR recognition

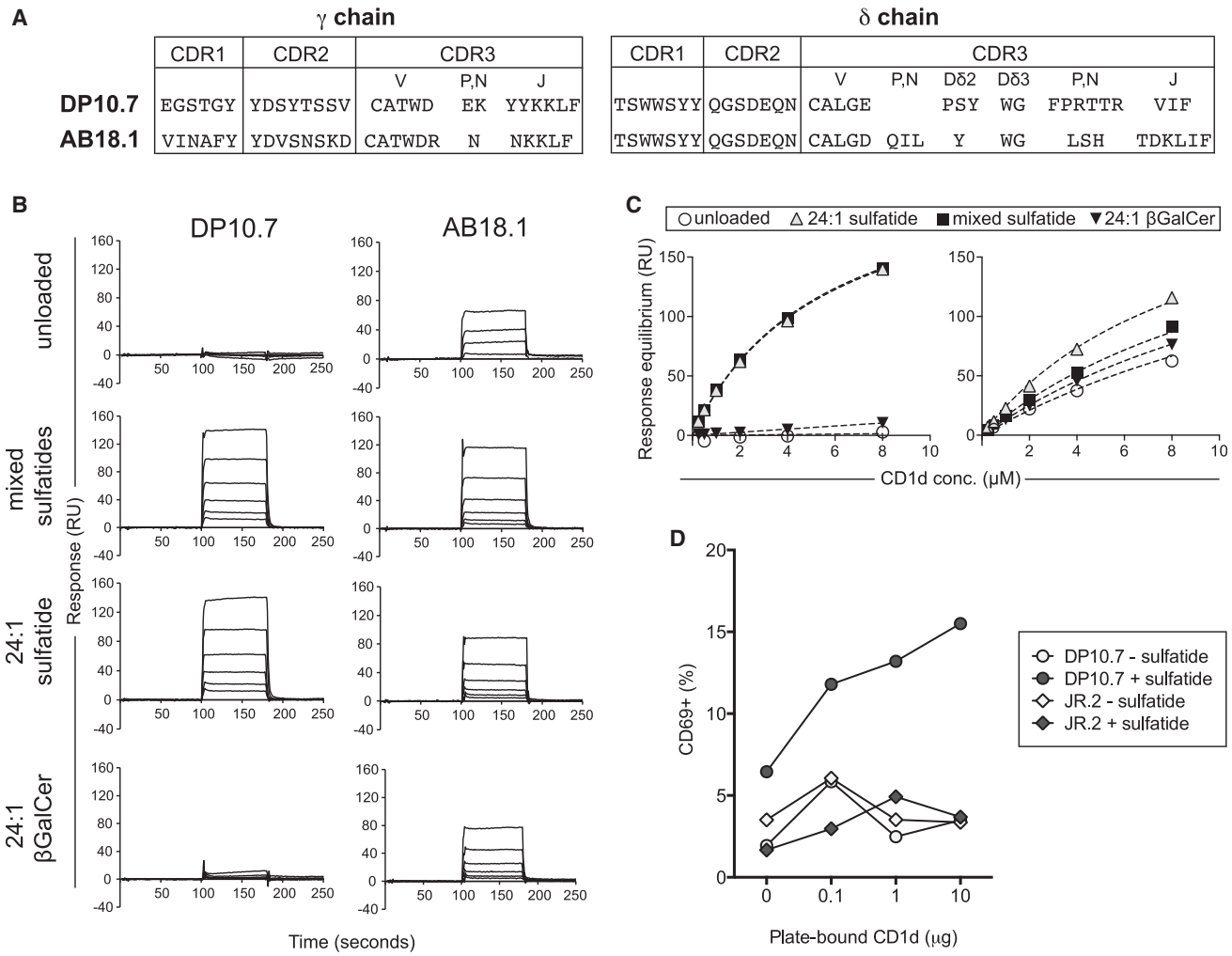

Figure 1. CD1d-Sulfatide Recognition by Human Vδ1$^+$ T Cells is TCR Dependent

(A) CDR-loop amino acid sequences of two CD1d-sulfatide-specific Vδ1 clones (DP10.7 and AB18.1).

(B) Surface plasmon resonance (SPR) binding responses of CD1d purified with endogenous lipids (unloaded) or loaded with indicated sulfatide variants to C-terminally biotinylated DP10.7 and AB18.1 TCRs immobilized on a streptavidin Biacore chip. Response units of CD1d binding (0.258 μM) after reference channel subtraction are shown.

(C) Equilibrium affinity analysis of DP10.7 and AB18.1 TCR binding to indicated CD1d forms.

(D) Percent CD69 upregulation on DP10.7 TCR-transduced Jurkat J.RT3-T3.5 T cells stimulated by plate-bound CD1d purified with endogenous lipids (white circles) or loaded with sulfatide (gray circles). A control JR.2 TCR, which is CD1c specific, was similarly transduced and stimulated with CD1d presenting endogenous lipids (white diamonds) or CD1d-sulfatide (gray diamonds) (see also Figure S1).

and resulted in the successful crystallization of the unliganded CD1dmα3-sulfatide complex (Figures S2A and S2B and Table S1). These results confirmed that the structure of our fusion was identical to the native human CD1d structure, particularly within the human α1 and α2 domains (Figure S2B). The electron density for sulfatide was unambiguous, revealing a stabilizing network of hydrogen bonds with CD1d as described for murine structures (Figures S2C and S2D) (Zajonc et al., 2005). In our structure, the sulfatide head-group was displaced by >4 Å in comparison with the murine CD1d-sulfatide structure because a tryptophan residue, rather than a glycine residue, was present in the human form (Figure S2E). This provides direct evidence that human and mouse CD1d molecules can differ in their presentation of the same lipid.

To crystalize the ternary complex of the DP10.7 TCR, human CD1d, and sulfatide, we utilized the single-chain (sc) TCR version, in which the variable domains are expressed as a fusion protein. This strategy has been used previously for both αβ and γδ TCR crystallization (Maynard et al., 2005; Xu et al., 2011). We succeeded in crystallizing the ternary scDP10.7 TCR-CD1dmα3-24:1 sulfatide complex and determined its structure to 3.0 Å resolution (Table S1).

Exclusive δ-Chain-Mediated Recognition of CD1d-Sulfatide

The DP10.7 TCR docks at a tilted angle, such that all CDR loops from the δ chain contact the CD1d-sulfatide complex and there is no contribution from the γ chain (Figure 2A). In fact, there

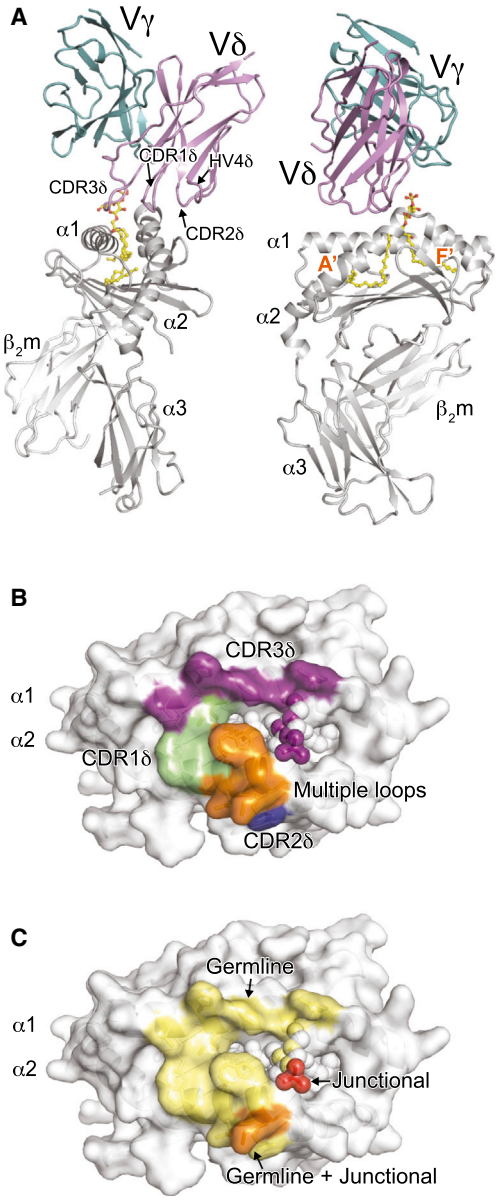

Figure 2. Complex Structure of DP10.7 TCR and CD1d-Sulfatide Reveals Exclusive δ Chain Dependence

Overview of DP10.7 TCR-CD1d-sulfatide complex and contacts.

(A) CD1d heterodimer, light gray; sulfatide, yellow; and DP10.7 TCR γ-chain and δ-chain, light teal and violet, respectively. The side view (left) of the complex shows a tilted TCR docking angle, which restricts CD1d-sulfatide contacts to the δ chain. The front view (right) shows the orientation of TCR over the CD1d A′ pocket.

(B) The surface of CD1d-sulfatide is shown in white, and residues that contact the TCR are colored according to CDR loop (residues ≤ 4.0 Å from TCR). CD1d residues contacted by CDR1δ, CDR2δ, and CDR3δ are shown in green, marine blue, and purple, respectively. CD1d residues contacted by multiple CDR and HV4 loop residues are shown in orange. Atoms of sulfatide contacted by CDR3δ shown as purple spheres.

(C) The surface of CD1d-sulfatide is shown in white, and residues (CD1d) or atoms (sulfatide) that contact the TCR are colored according to germline origin, nongermline origin, or both in light yellow, red, and light orange, respectively (see also Figure S2 and Table S1).

are no γ chain residues within 5 Å of the CD1d-sulfatide surface. This dominance of the δ chain is consistent with our mutagenesis, discussed below, and the exclusive use of the Vδ1 domain in γδ T cells that respond to CD1d (Agea et al., 2005; Bai et al., 2012; Russano et al., 2007). The δ chain docks above the CD1d A′ pocket, which binds the acyl 24:1 chain of sulfatide (Figure 2A). The Vδ1-encoded residues of the CDR1, CDR2, and HV4δ loops make contacts nearly exclusively with the α2 helix of CD1d (Figure 2B). The CDR3δ loop largely contacts the α1 helix of CD1d and the sulfatide head group but also shares some contacts with those of the other CDR and HV loops. The sulfatide antigen is contacted solely by junctional CDR3δ loop residues (Figure 2C), suggesting that other CD1d-restricted Vδ1 T cells might share this common docking footprint and utilize recombined CDR3δ loops to detect different lipid antigens.

The buried surface area (BSA) of the DP10.7 TCR-CD1d-sulfatide interface is ~730 Å^2, with 600 Å^2 from the TCR-CD1d interface and 130 Å^2 from the TCR-sulfatide interface. This interface is the smallest reported among TCR-ligand structures; it is slightly less than that of iNKT TCR-CD1d-αGalCer complexes (Pellicci et al., 2009) and considerably smaller than that of the murine type II NKT TCR-CD1d-sulfatide complex, (~1010 Å^2), although the affinity is about three times higher (Patel et al., 2012). Numerous ionic interactions in the DP10.7 TCR-CD1d-sulfatide interface most likely compensate for the smaller interface area to facilitate a higher-affinity interaction.

Comparisons with other TCR-ligand complex structures reveal that the DP10.7 TCR engages CD1d-sulfatide with a unique docking footprint. The murine G8-T22 complex is the only other γδ TCR structure with bound ligand, and it is similarly dominated by δ chain interactions (Adams et al., 2005). However, the DP10.7 footprint is more evenly divided among all δ chain loops, rather than nearly exclusively relying on the CDR3δ loop as in G8-T22. (Figures 3A and 3B). The DP10.7 TCR footprint is also distinct from the recognition mode of CD1d-restricted type I and II NKT TCRs. The DP10.7 TCR contacts CD1d only through the δ chain; this bias in CD1d docking contrasts with the distribution of CD1d contacts across both the α and β chains in NKT cell recognition. However, the DP10.7 TCR docks above the CD1d A′ pocket in an orientation that is overall similar to that of the type II NKT TCR (Figures 3A and 3D) (Girardi et al., 2012; Patel et al., 2012). Similarly, both the DP10.7 and type II NKT TCRs contact lipid antigens with their recombined CDR3 loops, in contrast to the invariant germline CDR1α loop-mediated lipid contacts made by type I NKT TCRs (Figures 3A, 3C and 3D) (Borg et al., 2007; Rossjohn et al., 2012). Because CDR3δ loops have the most potential sequence diversity among all recombined immunoreceptors (Davis and Bjorkman, 1988), the central orientation of the CDR3δ over the CD1d antigen-binding pocket suggests the potential of Vδ1 TCRs to engage a diverse array of lipid antigens.

TCR Contacts with CD1d Are Germline Encoded, Whereas CDR3δ Junctional Residues Contact Sulfatide

Electron density for all DP10.7 CDR loop residues and the sulfatide ligand were unambiguous, permitting detailed analysis of the TCR-CD1d-sulfatide interface (Figure S2 and Table S2). We also performed TCR alanine mutagenesis and entire CDR loop-swapping experiments via a tetramer-binding assay to

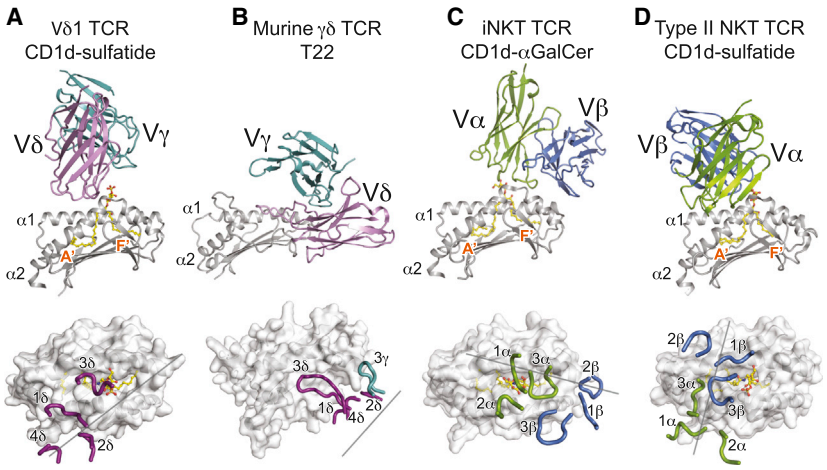

Figure 3. The Vδ1 DP10.7 TCR Employs a Unique Docking Mode

(A) View of TCR variable domains (top) in complex with α1, α2 domains of ligands and CDR loop footprint and docking orientation (bottom) of TCR-ligand complexes.
(B) Murine G8 γδ TCR-T22 complex (Protein Data Bank accession code 1YPZ).
(C and D) Human iNKT TCR-CD1d-αGalCer complex (Protein Data Bank accession code 2PO6) (C) and murine type II NKT TCR-CD1d-sulfatide complex (Protein Data Bank accession code 4El5) (D).
The surface of ligand α1, α2 domains is shown in white; lipid ligands, if present, are shown in yellow. The TCR γ chain is shown in light teal, and the δ chain is shown in violet (A and B); the TCR-α chain is shown in green, and the β chain is shown in blue (C and D). Grey lines represent vector connecting the conserved disulfide bond in each V domain (see also Table S2).

complement our structural analyses (Table S3). The CDR1δ accounts for 32% of the total BSA, most of which is contributed by Trp30 (Figure 4A). This residue makes extensive nonpolar contacts, most prominently a π-stacking interaction with Trp160. Additional hydrogen bonds are formed by the CDR1δ Trp30 and Ser31 with CD1d Thr157 and Glu156, respectively (Figure 4A). The importance of this loop is validated by the observed reduction in binding when the unrelated Vδ2-encoded CDR1δ is grafted in its place. (Figure 4D; see also Table S3). Alanine mutagenesis revealed that several residues other than the key Trp30 and Ser31 were important for binding, and these residues probably affect the overall loop conformation (Figure 4E). Although the CDR2δ loop is docked toward the outside of the CD1d α2 helix and contributes only 11% toward the total BSA, it forms many polar and ionic contacts with CD1d. Asp54 is at the center of a network of salt bridges that make these contacts with Lys152 and Arg155 (Figure 4B). Ser53 is positioned to engage in several hydrogen bonds with Glu156 and Lys152. Alanine mutagenesis validates the contribution of these key CDR2δ residues (Figure 4E). Lys69 of the TCR HV4δ loop also contributes to this network of polar contacts by forming a salt bridge with Glu156. The numerous specific Vδ1 interactions with CD1d highlight the strongly biased usage of this gene segment in described CD1d-reactive γδ TCRs (Agea et al., 2005; Bai et al., 2012; Russano et al., 2007), whereas CD1d-sulfatide reactive type II NKT TCRs can use several different Vα and Vβ genes for CD1d recognition (Park et al., 2001).

In contrast, the extended CDR3δ loop forms fewer polar contacts with CD1d, although overall it plays a key role in CD1d-sulfatide recognition, as shown in our structure and by a marked reduction in binding upon substitution of this loop with an unrelated CDR3δ loop sequence (Figures 4C and 4D; see also Table S3). The tip of the CDR3δ is composed of Dδ segment-encoded residues Tyr98 and Trp99, which make several VdW interactions with CD1d residues concentrated in the α1 helix (Figure 4C). Tyr98 contributes a main-chain hydrogen bond to Trp153 of the α2 helix via its backbone carbonyl, but otherwise no polar contacts are made by the five Dδ2 and Dδ3 residues of the CDR3δ loop. As such, alanine mutagenesis of Dδ-encoded residues minimally affected binding to CD1d-sulfatide (Figures 4D and 4E). All polar contacts with the sulfatide antigen are mediated by junctionally encoded residues Phe101 and Arg103, which form a main-chain hydrogen bond and salt bridge, respectively, with the sulfate moiety (Figure 4C). Alanine mutagenesis of these residues shows an individually modest contribution, whereas dual Phe101 and Arg103 mutagenesis reduces CD1d binding nearly as much as swapping the entire CDR3δ loop (Figures 4D and 4E). This corroborates the key role of sulfatide interactions demonstrated by SPR measurements (Figures 1B and 1C). Overall, there were no residues that were absolutely required for CD1d-sulfatide recognition, in contrast to the conserved "hot-spot" residues characteristic of T22-reactive TCRs (Adams et al., 2008; Sandstrom et al., 2012). Our structure shows an interwoven network of contacts, and it is likely that compensatory interactions can substitute for single alanine mutations. Furthermore, our tetramer-based detection has an avidity affect, so smaller differences in binding might be partially masked. However, the observed binding reductions, such as for alanine substitution of Asp54 and Arg103, were reproducible and correlate to their importance in our complex structure.

In accordance with structural studies, swapping entire γ chain CDR loops with unrelated sequences has no effect on CD1d-sulfatide binding (Figure 4D; see also Table S3). Although the γ chain lacks energetic contributions here, it remains possible that γ chain CDR loops could mediate contacts of bulkier CD1d-presented lipid antigens, such as the gangliosides common in nervous tissue (Haig et al., 2011; Tettamanti et al., 1973).

Minimal Conformational Change by the DP10.7 TCR upon CD1d-Sulfatide Ligation

Conformational change during binding can play a large role in αβ TCR recognition of peptide-MHC, particularly in the CDR3 loops that reorganize to optimally contact peptide antigens (Garcia and Adams, 2005); however, a lack of structural data makes it unclear whether γδ TCRs require the same flexibility in recognition of their ligand. To address this question, we solved the unliganded structure of the DP10.7 TCR, resolved to 3.3 Å resolution. Despite the moderate resolution limit, we were able to model the backbones for all δ chain CDR loops and most side chains (Table S3 and Figure S4A). Despite participating in crystal contacts, the CDR loop structures were essentially

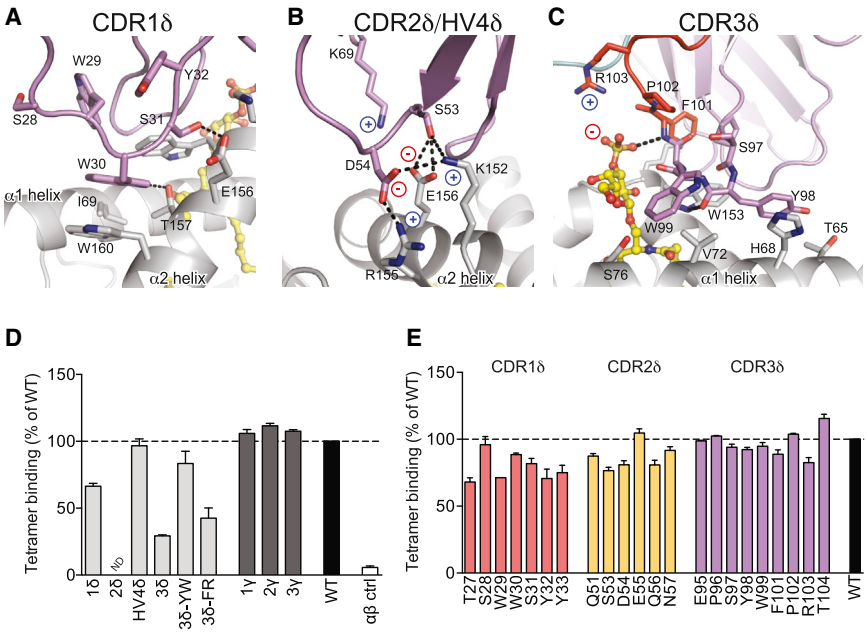

Figure 4. Analysis of DP10.7 TCR-CD1d-Sulfatide Contacts

(A–C) Polar contacts are shown in dashed black lines; charges are indicated so that salt-bridge contacts are emphasized. CD1d shown in light gray, sulfatide is shown in yellow, TCR germline residues are shown in violet, and nontemplated residues in red for CDR1δ (A); CDR2δ and HV4 (B); and CDR3δ (C) contacts.

(D and E) TCR mutagenesis and binding measurements were determined by a plate-binding assay. ELISA plate wells were coated with WT or mutant single-chain (sc) TCRs, and binding to CD1d-sulfatide tetramers labeled with HRP was measured by colorimetric readout (A450). Nonspecific binding to BSA was subtracted, and binding was calculated as a percentage of WT binding. Shown are the mean and SEM of at least two independent experiments. (D) Entire CDR loops were substituted with unrelated CDR loop sequences, and binding was compared with that of the WT DP10.7 TCR. Double-alanine mutations of indicted CDR3δ-loop residues were also analyzed. (E) Single alanine point mutations of the DP10.7 TCR δ chain were made, and binding versus that of WT TCR was measured as above (see also Figure S3 and Table S3).

superimposable with those of the TCR in complex, indicating that they adopt similar rigid conformations (Figure 5A). Side chain conformations did vary, most notably for the CDR2δ residue D54, which flipped about 180° in order to form salt bridges with CD1d α2 helix residues Arg155 and Lys152 in the complex structure (Figure 5B). Within the CDR3δ loop, the side chain of Arg103 moves ~1Å closer to the sulfatide head group upon binding in order to fall within salt-bridge distance (Figure 5C). Otherwise, residues in the unliganded DP10.7 TCR are positioned to optimally form contacts as found in the complex structure, indicative of a more rigid-body type interaction.

We also solved the structure of the AB18.1 TCR in the unliganded form. This structure provides additional insight into how Vδ1 TCRs might be preoriented for CD1d recognition (Table S1). Superimposition of this structure with the liganded DP10.7 again reveals nearly identical conformation of the CDR1,2 and HV4δ loops (Figure S4B). However, the AB18.1 TCR CDR3δ loop contains an intervening residue before the conserved YWG motif (Figure 1A), and thus the loop is in a somewhat different conformation (Figure S4C). Accommodation of the extra residue in the CDR3δ loop would require conformational changes upon CD1d-sulfatide binding if this TCR were to dock in a similar manner. However, the key salt bridge mediated by Arg103 in the DP10.7 structure would be lost if AB18.1 were docked in a similar fashion because, despite His104's potentially yielding a positive charge, its position is too far from the sulfate to form a salt bridge. This is in line with the AB18.1 TCR's recognizing CD1d-sulfatide with only a slightly higher affinity than it does the "unloaded" CD1d molecule. The potentially closer proximity of several CDR3δ residues with the α1 and α2 helices of CD1d might explain the ability of the AB18.1 TCR to bind CD1d independently of sulfatide loading, although a substantial shift in docking and γ chain contacts cannot be ruled out given that it is unknown whether the DP10.7 docking mode is conserved among this and other Vδ1 TCRs. Overall, the conformations of the germline Vδ1 loops from two different unbound TCRs match up nearly identically with that of the TCR in complex, despite different crystal packing, highlighting how these loops are preoriented for CD1d recognition.

Broad Recognition of CD1d among Vδ1 Clones

The exclusive Vδ1-residue-mediated recognition of CD1d revealed by our complex structure led us to investigate the prevalence of CD1d restriction among other human Vδ1$^+$ T cells. Vδ1$^+$ T cells comprise a prominent proportion of MS plaque-infiltrating γδ T cells and are found at high percentages within healthy intestinal epithelial tissues. To test whether these T cells can recognize CD1d-sulfatide, we produced TCRs derived from published Vδ1 sequences of multiple sclerosis (MS) plaque-infiltrating and intestinal biopsy-derived γδ lymphocytes and measured their binding to CD1d-sulfatide tetramers (Figure S4A) (Chowers et al., 1994; Wucherpfennig et al., 1992). Because only δ chain sequences were provided, we paired these with the DP10.7 TCR γ chain, which we showed to be irrelevant in CD1d recognition. Thus, use of the DP10.7 TCR γ chain allowed an analysis of δ-chain-exclusive interactions. This analysis does not, however, rule out contributions from the natively paired γ chains (advantageous or deleterious). Our analysis of two MS plaque clones demonstrated moderate to low binding to CD1d sulfatide; although this is most likely reflective of a low binding affinity, it establishes a basal recognition that might be enhanced by γ chain contributions, providing a possible link between Vδ1$^+$ T cell infiltration of MS plaques, CD1d expression, and the particular enrichment of sulfatide lipids in neuronal tissues (Takahashi and Suzuki, 2012).

In contrast to the moderate binding of TCRs from MS plaques, there was a striking prevalence of robust CD1d-sulfatide

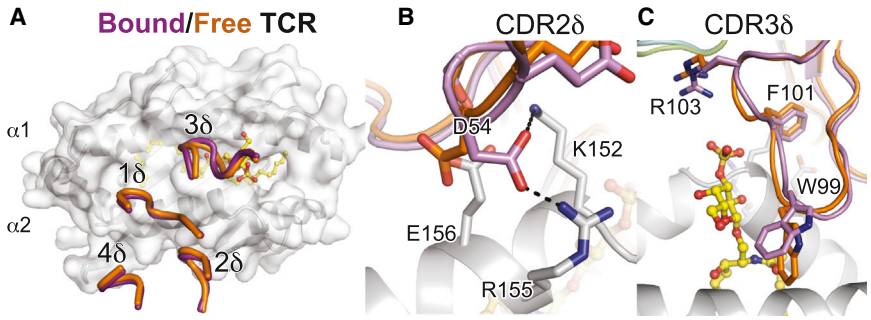

Figure 5. Minimal Conformation Change Required for CD1d-Sulfatide Recognition

(A) Free TCR (chain B, orange) was aligned to bound TCR (purple) shown with CD1d (light gray)-sulfatide (yellow) complex. δ chain CDR loops of free and bound TCR are shown over the CD1d surface.

(B and C) CDR2-δ- and CDR3-δ-loop side chains that make rearrangements upon ligation are highlighted (see also Figure S4).

interactions among TCRs from intestinally derived clones; some of these TCRs were bound as strongly as the DP10.7 TCR. Overall, those with shorter and more positively charged loops bound most strongly (Figure 6A; see also Figure S5A). We used biolayer interferometry (BLI) to measure a 25.8 μM CD1d-sulfatide affinity for the most highly binding clone, OJ33, and found that it was well within the 1–50 μM range of agonist TCR-ligand affinities (Figure S5B and S5C) (van der Merwe and Davis, 2003). This clone also bound unloaded CD1d with a moderate affinity (41.5 μM, Figures S5B and S5C). Despite our small sample size in this initial characterization, these results clearly show a potential for CD1d restriction among intestinal Vδ1 clones and specifically for CD1d recognition of sulfatide lipid antigens.

We then examined the potential for CD1d recognition among clones (paired δ and γ chains) previously characterized as recognizing other nonclassical MHC molecules. The clones IDP2 and δ1A/B-3 have been described as being specific for CD1c and MHC-class-I-related chain A (MICA), respectively (Brenner et al., 1986; Groh et al., 1998; Porcelli et al., 1989), although measurements of TCR interactions are either lacking or very weak (Xu et al., 2011). The δ1A/B-3 clone is the most well studied among a population of intraepithelial lymphocytes (IELs) that are reactive to MICA (Groh et al., 1998). Using the above tetramer-based assay, we found that IDP2 bound negligibly to CD1d-sulfatide, but surprisingly, the δ1A/B-3 clones bound in a similar range as the DP10.7 TCR (Figure 6A). We validated this interaction by BLI affinity measurements and calculated a K_d of 33.9 μM (Figures 6B and 6C). The δ1A/B-3 TCR affinity for unloaded CD1d was almost 10-fold less (K_d = 241.3 μM), indicating that this TCR makes energetically important sulfatide-specific contacts (Figures 6B and 6C). Alignment with the complexed DP10.7 TCR demonstrates that δ1A/B-3 sulfatide specificity is probably conferred by the CDR3δ residue Arg99, which is essentially superimposable with that of the key DP10.7 sulfatide contact Arg103 and could readily form a salt bridge with the sulfate moiety upon adoption of a different rotamer (Figure S5D). Validating published results showing that the δ1A/B-3 TCR binds MICA with very low affinity (K_d = 100-900 μM) (Xu et al., 2011), we observed very weak binding to MICA and were unable to accurately calculate an equilibrium affinity at the TCR concentrations used (Figures 6B and 6C). These results highlight the germline-encoded specificity of Vδ1 TCRs for CD1d molecules and demonstrate that this recognition is broadly distributed across blood- and gut-resident Vδ1 T cells.

Gut-Derived Vδ1$^+$ T Cells Are Stimulated by CD1d and Produce TNF-α

To further explore the recognition of CD1d in Vδ1 T cells in the gut, we purified IELs from jejunal biopsies (Jabri et al., 2000) and sorted them on the basis of expression of the Vδ1 TCR. We expanded these cells in an unbiased, CD1d-nonspecific manner by using irradiated feeder cells (Jabri et al., 2002) to generate enough cells for functional characterization. The expanded polyclonal cells uniformly expressed γδ TCRs containing a Vδ1 chain (Figure 7A). When stimulated with increasing concentrations of an anti-γδ TCR antibody, approximately one-fifth of the Vδ1 population produced TNF-α in response (Figure 7B), consistent with the TNF-α secretion noted in the characterization of blood Vδ1 by PMA-ionomycin treatment (Figure S1B). The effector activity of the remaining 80% of the Vδ1 polyclonal population is unknown but suggests a diverse effector response to TCR triggering of this population.

Using CD1d-transfected C1R cells as targets, we sought to determine whether CD1d could stimulate TNF-α production from this Vδ1 polyclonal population. To differentiate the Vδ1 response to CD1d presenting endogenous phospholipids (as documented in Russano et al. [Russano et al., 2007]) from that of CD1d presenting sulfatide on the C1R cells, we first established the loading efficiency of sulfatide in the C1R-CD1d transfectants used for these experiments. To do so, we took advantage of the exquisite specificity of the DP10.7 TCR to CD1d-sulfatide by generating tetramers of this TCR for use as a CD1d-sulfatide-specific staining reagent. Staining of unloaded C1R-CD1d transfectants expressing endogenous antigens with the DP10.7 TCR tetramer showed no surface staining, consistent with our SPR experiments presented in Figure 1, showing no detectable binding between the DP10.7 TCR and CD1d presenting endogenous lipid ("unloaded"). In contrast, nearly 100% of C1R-CD1d transfectants loaded with sulfatide stained strongly positive for the DP10.7 TCR tetramer (Figure 7C). This suggests that our protocol for loading C1R-CD1d transfectants with sulfatide effectively saturates the cell-surface CD1d with sulfatide, thus allowing us to discriminate the Vδ1 response against CD1d-sulfatide from that of CD1d presenting endogenous lipids.

We then measured the TNF-α response of the polyclonal Vδ1 cells to CD1d, either loaded with sulfatide or presenting endogenous lipids ("unloaded"). After 16 hr, approximately 4% of Vδ1 T cells were producing TNF-α in response to C1R cells transfected with CD1d presenting endogenous lipids, as measured by intracellular cytokine staining (Figure 7D), consistent with the reactivity noted by Spinozzi and colleagues

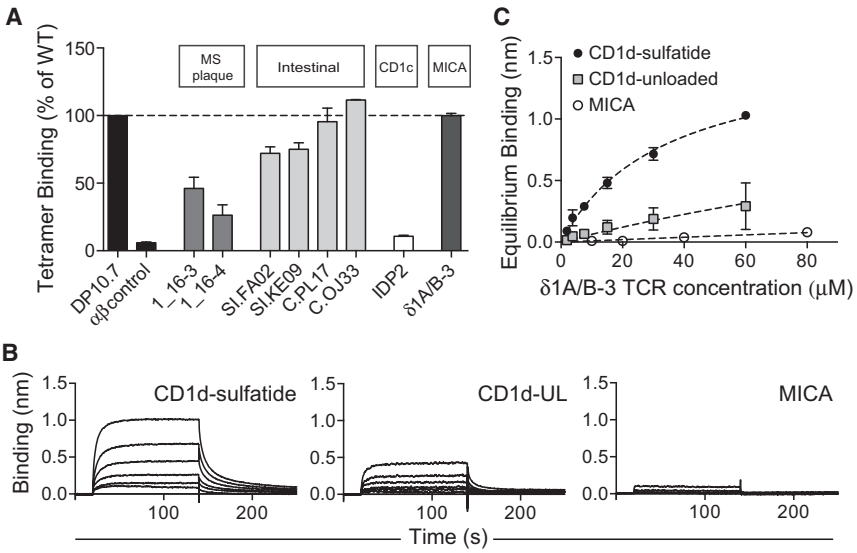

Figure 6. Widespread Recognition of CD1d-Sulfatide by Vδ1$^+$ T Cells

(A) Assay of CD1d-sulfatide tetramer binding to plate-bound scTCRs. CD1d-sulfatide tetramers were labeled with HRP for colorimetric readout. Nonspecific binding to BSA was subtracted, and binding was calculated as a percentage of that to DP10.7 TCR. Shown are the mean and SEM of at least two independent experiments.

(B) BLI analysis of δ1A/B-3 scTCR binding to immobilized CD1d-sulfatide, unloaded CD1d (CD1d-UL), or MICA. TCR concentrations ranged from 1.88–60 μM (CD1d-sulfatide and CD1d-UL) or from 10–80 μM (MICA). Shown are representative data from one of two experiments.

(C) Equilibrium affinity analysis of (B). Shown are the equilibrium binding mean and standard deviation from two experiments, as well as fits used for calculation of K_d values (see also Figure S5).

(Russano et al., 2007). In response to C1R cells transfected with CD1d and loaded with sulfatide, approximately 2% of these cells stained positive for TNF-α production. Considering that ~20% of this polyclonal Vδ1 population produces TNF-α in response to TCR triggering and correcting for the background 1% of TNF-α detected in mock-treated cells (Figure 7D, top), we can approximate that ~15% of TNF-α producing Vδ1 cells in this population respond to CD1d presenting endogenous lipids and ~5% respond to CD1d presenting sulfatide. After 24 hr the measured amount of TNF-α secreted in the supernatant of Vδ1 cells responding to the CD1d-endogenous versus CD1d-sulfatide samples were following a trend similar to that found with intracellular cytokine staining (Figure 7E). Together, these results demonstrate that a population of Vδ1 T cells in the gut respond to CD1d presenting endogenous antigens, including the lipid sulfatide.

DISCUSSION

One of the prevalent ideas about γδ T cell recognition is that it is antibody-like in nature, in contrast with peptide-MHC-specific αβ TCRs, which share an evolutionarily conserved, germline-encoded footprint upon the MHC surface (Born et al., 2013; Garcia and Adams, 2005; Marrack et al., 2008). This observation about γδ TCR recognition has arisen from the diverse structural nature of previously described γδ TCR ligands, many of which have been ascribed to singular TCR clones (Vantourout and Hayday, 2013). However, given the limited number of V gene segments available for γδ TCR recombination, it is plausible that a few major ligands dominate among the potential repertoire of recognizable antigens, such as T22 for the murine T22-reactive population (Adams et al., 2005; Shin et al., 2005) and MICA and phosphorylated small molecules for human Vδ1 and Vδ2 TCRs, respectively. However, a clear γδ V segment-directed recognition of an antigen-presenting molecule has yet to be explicitly demonstrated. Our structure reveals the molecular basis for this association and shows how the recombined CDR3δ loop mediates fine antigen specificity in a manner akin to that of classical αβ TCRs.

γδ T cells are particularly abundant among intraepithelial T cells of the small intestine and colon. Numerous studies have demonstrated that this population exhibits a crucial role in the preservation of the epithelial barrier via production of both tissue repair and inflammatory antimicrobial factors (Smith and Garrett, 2011). Given that the human γδ IEL population exhibits a substantial Vδ1 TCR cell bias, an important question remains as to whether these functions are controlled through TCR recognition of specific antigens. We examined a set of previously described human intestinal clones and found not only that these TCRs were CD1d specific but also that they recognized the self-lipid sulfatide. In addition, we demonstrate that a sizable percent of jejunally derived Vδ1 T cells producing TNF-α (~15%) are responsive to CD1d presenting endogenous lipids and that ~5% respond to CD1d presenting sulfatide. We cannot definitively conclude that these cells are specific to sulfatide because it is clear that some Vδ1 T cells (such as the AB18.1 T cell clone isolated from the blood) can recognize CD1d independently of lipids, yet it is unlikely that all of these CD1d-sulfatide-reactive T cells are lipid independent. Furthermore, we have only assessed one effector output (TNF-α production); it is possible that other cytokines are being produced by some of the remaining 80% of Vδ1 T cells in this population in response to CD1d stimulation.

Among the TCRs that we identified as CD1d specific, the most striking was the IEL clone δ1A/B-3 (Groh et al., 1998). This and other Vδ1$^+$ IEL clones have been described as being MICA reactive, and MICA is a widely acknowledged Vδ1 TCR ligand (Vantourout and Hayday, 2013). Our results showed that the δ1A/B-3 TCR binds with appreciable affinity to CD1d-sulfatide, suggesting this could be an alternative stimulatory TCR ligand for MICA-reactive T cells. Because the MICA-specific costimulatory receptor NKG2D is ubiquitously expressed among Vδ1$^+$ γδ T cells and binds MICA with high affinity, it would effectively out compete the low binding affinity of the δ1A/B-3 TCR for MICA recognition (Groh et al., 2001; Wu et al., 2002; Xu et al., 2011). MICA cell-surface expression and NKG2D engagement occurs only during cellular stress

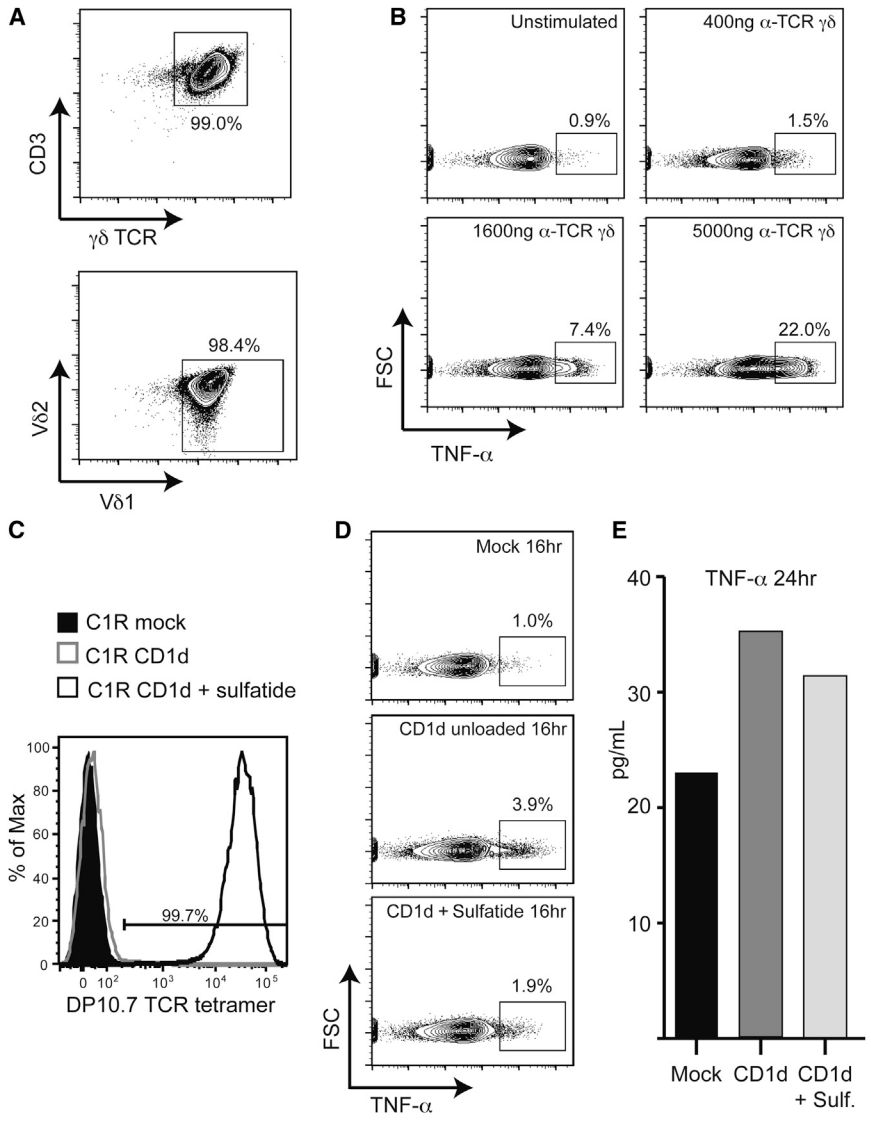

Figure 7. Reactivity of Gut Vδ1⁺ T Cells to CD1d Presenting Endogenous Lipids, Including Sulfatide

(A) Flow-cytometry contour plots showing CD3 and γδ TCR expression of a polyclonal Vδ1 IEL cell line (top) and Vδ1 versus Vδ2 TCR expression (bottom).

(B) Flow-cytometry contour plots of TNF-α intracellular-cytokine staining of a polyclonal Vδ1 IEL cell line stimulated with increasing concentrations of anti-γδ TCR antibody. The concentration of antibody is indicated at the top of each plot, and the displayed percentages indicate cells gated for TNF-α expression.

(C) Flow-cytometry histogram plot of DP10.7 TCR tetramer staining of C1R mock transfectant (shaded black), unloaded C1R-CD1d transfectant (gray line), or C1R-CD1d transfectant treated with 30 μg/ml sulfatide (black line). The percentage of tetramer-positive cells is indicated above the drawn gate.

(D) Flow-cytometry contour plots of intracellular TNF-α staining after 16 hr of coculture of a polyclonal Vδ1 IEL cell line with C1R mock transfectants (top), unloaded C1R-CD1d transfectants (middle), and C1R-CD1d transfectants loaded with sulfatide (bottom).

(E) Bar graph showing TNF-α levels measured in the culture supernatant by ELISA after 24 hr of coculture of a polyclonal Vδ1 IEL cell line with C1R-mock, unloaded C1R-CD1d, and C1R-CD1d sulfatide transfectants.

(Groh et al., 1999; Meresse et al., 2004); therefore, this signaling pathway might only be relevant during disease conditions. However the constitutive expression of CD1d in human intestinal epithelial cells (IECs) (Dougan et al., 2007; Perera et al., 2007), between which IEL stably reside, and the enrichment of endogenous lipids such as sulfatide in these tissues, (Breimer et al., 2012) might provide a range of signals to Vδ1⁺ IELs; these signals might be homeostatic in healthy tissue and inflammatory when paired with MICA-NKG2D in a disease setting.

CD1 molecules were among the first described ligands for γδ T cells, yet the field of CD1-lipid recognition largely diverted to the study of αβ T cells with the discovery of NKT cells (Bendelac et al., 1995; Porcelli et al., 1989). IELs within the intestine are one of the largest T cell populations in humans, and of these IELs, γδ T cells comprise almost 40% (Cheroutre, 2005; Deusch et al., 1991). Our results and those from others have shown that human Vδ1⁺ T cells and, as shown more recently, Vδ3⁺ T cells can recognize CD1d-lipid antigens, implicating γδ T cells as the largest lipid-reactive population in humans (Agea et al., 2005; Bai et al., 2012; Mangan et al., 2013; Russano et al., 2007; Spada et al., 2000). Although we have demonstrated sulfatide recognition by several Vδ1 clones, the potential for reactivity toward lipids with diverse chemical natures is clearly suggested by our structure, in which the recombined CDR3δ loop is positioned over the central lipid portal. Our results suggest that the prevalence of lipid recognition among all Vδ1⁺ T cell populations should be re-examined.

EXPERIMENTAL PROCEDURES

CD1d, MICA, and TCR Cloning, Expression, and Purification

These protocols are described in detail in the Supplemental Experimental Procedures.

Surface Plasmon Resonance

All SPR measurements were conducted with a Biacore 2000 instrument operated at 25°C. Eight hundred response units (RUs) of each TCR were immobilized on a streptavidin sensor chip; one flow cell was left blank for reference subtraction. All flow cells were then blocked with 1 μM biotin. Serial dilutions of CD1d (unloaded or loaded with indicated lipids) from 8 to 0.25 μM were flowed as analyte. Equilibrium dissociation constants were determined by nonlinear regression with GraphPad (Prism) software and a shared Bmax because identical immobilizations of the TCRs were used.

Jurkat Transductions and Plate-Stimulation Assays

The full-length δ and γ chains of the DP10.7 TCR and a control Vδ1 TCR JR.2 were cloned into pMSCV vectors (a gift of M. Kuhns) and retrovirally transduced into TCRβ-Jurkat J.RT3-T3.5 cells. To measure CD1d-sulfatide-specific stimulation, we coated 96-well flat-bottomed plates with purified WT human CD1d in the indicated amounts (0–10 μg/well) at 37°C for 2 hr. Wells were washed, and bovine brain sulfatides (Maytreya, 100 μg/ml) or DMSO control was added for 3 hr at 37°C. Wells were washed and 5 × 10^4 DP10.7 or JR.2 TCR J.RT3-T3.5 transductants were added for a 12 hr incubation. Activation was measured by CD69 expression (FN50, Biolegend).

Crystallization, Structure Determination, Refinement, and Analysis

These protocols are described in detail in the Supplemental Methods.

CD1d Tetramer Production, Plate Binding, and BLI Measurements of TCRs

These protocols are described in detail in the Supplemental Experimental Procedures.

Vδ1 IEL Cell Line Characterization and Stimulation

Intraepithelial lymphocytes were purified from a jejunal biopsy as described (Jabri et al., 2000) (details are available in the Supplemental Experimental Procedures).

ACCESSION NUMBERS

The coordinates and structure factors for the CD1d-sulfatide complex, svDP10.7 TCR-CD1d-sulfatide complex, DP10.7 TCR, and AB18.1 TCR have been deposited in the Protein Data Bank under the accession codes 4MQ7, 4MNG, 4MNH, and 4NDM, respectively.

SUPPLEMENTAL INFORMATION

Supplemental Information includes Supplemental Experimental Procedures, five figures, and three tables and can be found with this article online at http://dx.doi.org/10.1016/j.immuni.2013.11.001.

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REFERENCES

Adams, E.J., Chien, Y.H., and Garcia, K.C. (2005). Structure of a gammadelta T cell receptor in complex with the nonclassical MHC T22. Science *308*, 227–231.

Adams, E.J., Strop, P., Shin, S., Chien, Y.H., and Garcia, K.C. (2008). An autonomous CDR3delta is sufficient for recognition of the nonclassical MHC class I molecules T10 and T22 by gammadelta T cells. Nat. Immunol. *9*, 777–784.

Agea, E., Russano, A., Bistoni, O., Mannucci, R., Nicoletti, I., Corazzi, L., Postle, A.D., De Libero, G., Porcelli, S.A., and Spinozzi, F. (2005). Human CD1-restricted T cell recognition of lipids from pollens. J. Exp. Med. *202*, 295–308.

Bai, L., Picard, D., Anderson, B., Chaudhary, V., Luoma, A., Jabri, B., Adams, E.J., Savage, P.B., and Bendelac, A. (2012). The majority of CD1d-sulfatide-specific T cells in human blood use a semiinvariant Vδ1 TCR. Eur. J. Immunol. *42*, 2505–2510.

Bauer, S., Groh, V., Wu, J., Steinle, A., Phillips, J.H., Lanier, L.L., and Spies, T. (1999). Activation of NK cells and T cells by NKG2D, a receptor for stress-inducible MICA. Science *285*, 727–729.

Bendelac, A., Lantz, O., Quimby, M.E., Yewdell, J.W., Bennink, J.R., and Brutkiewicz, R.R. (1995). CD1 recognition by mouse NK1+ T lymphocytes. Science *268*, 863–865.

Bendelac, A., Savage, P.B., and Teyton, L. (2007). The biology of NKT cells. Annu. Rev. Immunol. *25*, 297–336.

Bonneville, M., Ito, K., Krecko, E.G., Itohara, S., Kappes, D., Ishida, I., Kanagawa, O., Janeway, C.A., Murphy, D.B., and Tonegawa, S. (1989). Recognition of a self major histocompatibility complex TL region product by gamma delta T-cell receptors. Proc. Natl. Acad. Sci. USA *86*, 5928–5932.

Borg, N.A., Wun, K.S., Kjer-Nielsen, L., Wilce, M.C., Pellicci, D.G., Koh, R., Besra, G.S., Bharadwaj, M., Godfrey, D.I., McCluskey, J., and Rossjohn, J. (2007). CD1d-lipid-antigen recognition by the semi-invariant NKT T-cell receptor. Nature *448*, 44–49.

Born, W.K., Kemal Aydintug, M., and O'Brien, R.L. (2013). Diversity of γδ T-cell antigens. Cell. Mol. Immunol. *10*, 13–20.

Breimer, M.E., Hansson, G.C., Karlsson, K.A., Larson, G., and Leffler, H. (2012). Glycosphingolipid composition of epithelial cells isolated along the villus axis of small intestine of a single human individual. Glycobiology *22*, 1721–1730.

Brenner, M.B., McLean, J., Dialynas, D.P., Strominger, J.L., Smith, J.A., Owen, F.L., Seidman, J.G., Ip, S., Rosen, F., and Krangel, M.S. (1986). Identification of a putative second T-cell receptor. Nature *322*, 145–149.

Cheroutre, H. (2005). IELs: Enforcing law and order in the court of the intestinal epithelium. Immunol. Rev. *206*, 114–131.

Chowers, Y., Holtmeier, W., Harwood, J., Morzycka-Wroblewska, E., and Kagnoff, M.F. (1994). The V delta 1 T cell receptor repertoire in human small intestine and colon. J. Exp. Med. *180*, 183–190.

Constant, P., Davodeau, F., Peyrat, M.A., Poquet, Y., Puzo, G., Bonneville, M., and Fournié, J.J. (1994). Stimulation of human gamma delta T cells by nonpeptidic mycobacterial ligands. Science *264*, 267–270.

Davis, M.M., and Bjorkman, P.J. (1988). T-cell antigen receptor genes and T-cell recognition. Nature *334*, 395–402.

Deusch, K., Lüling, F., Reich, K., Classen, M., Wagner, H., and Pfeffer, K. (1991). A major fraction of human intraepithelial lymphocytes simultaneously expresses the gamma/delta T cell receptor, the CD8 accessory molecule and preferentially uses the V delta 1 gene segment. Eur. J. Immunol. *21*, 1053–1059.

Dougan, S.K., Kaser, A., and Blumberg, R.S. (2007). CD1 expression on antigen-presenting cells. Curr. Top. Microbiol. Immunol. *314*, 113–141.

Garcia, K.C., and Adams, E.J. (2005). How the T cell receptor sees antigen—a structural view. Cell *122*, 333–336.

Garcia, K.C., Degano, M., Stanfield, R.L., Brunmark, A., Jackson, M.R., Peterson, P.A., Teyton, L., and Wilson, I.A. (1996). An alphabeta T cell receptor structure at 2.5 A and its orientation in the TCR-MHC complex. Science *274*, 209–219.

Girardi, E., Maricic, I., Wang, J., Mac, T.T., Iyer, P., Kumar, V., and Zajonc, D.M. (2012). Type II natural killer T cells use features of both innate-like and conventional T cells to recognize sulfatide self antigens. Nat. Immunol. *13*, 851–856.

Groh, V., Steinle, A., Bauer, S., and Spies, T. (1998). Recognition of stress-induced MHC molecules by intestinal epithelial gammadelta T cells. Science *279*, 1737–1740.

Groh, V., Rhinehart, R., Secrist, H., Bauer, S., Grabstein, K.H., and Spies, T. (1999). Broad tumor-associated expression and recognition by tumor-derived gamma delta T cells of MICA and MICB. Proc. Natl. Acad. Sci. USA *96*, 6879–6884.

Groh, V., Rhinehart, R., Randolph-Habecker, J., Topp, M.S., Riddell, S.R., and Spies, T. (2001). Costimulation of CD8alphabeta T cells by NKG2D via engagement by MIC induced on virus-infected cells. Nat. Immunol. *2*, 255–260.

Haig, N.A., Guan, Z., Li, D., McMichael, A., Raetz, C.R., and Xu, X.N. (2011). Identification of self-lipids presented by CD1c and CD1d proteins. J. Biol. Chem. *286*, 37692–37701.

Jabri, B., de Serre, N.P., Cellier, C., Evans, K., Gache, C., Carvalho, C., Mougenot, J.F., Allez, M., Jian, R., Desreumaux, P., et al. (2000). Selective expansion of intraepithelial lymphocytes expressing the HLA-E-specific natural killer receptor CD94 in celiac disease. Gastroenterology *118*, 867–879.

Jabri, B., Selby, J.M., Negulescu, H., Lee, L., Roberts, A.I., Beavis, A., Lopez-Botet, M., Ebert, E.C., and Winchester, R.J. (2002). TCR specificity dictates CD94/NKG2A expression by human CTL. Immunity *17*, 487–499.

Komano, H., Fujiura, Y., Kawaguchi, M., Matsumoto, S., Hashimoto, Y., Obana, S., Mombaerts, P., Tonegawa, S., Yamamoto, H., Itohara, S., et al. (1995). Homeostatic regulation of intestinal epithelia by intraepithelial gamma delta T cells. Proc. Natl. Acad. Sci. USA *92*, 6147–6151.

López-Sagaseta, J., Dulberger, C.L., Crooks, J.E., Parks, C.D., Luoma, A.M., McFedries, A., Van Rhijn, I., Saghatelian, A., and Adams, E.J. (2013). The molecular basis for Mucosal-Associated Invariant T cell recognition of MR1 proteins. Proc. Natl. Acad. Sci. USA *110*, E1771–E1778.

Mangan, B.A., Dunne, M.R., O'Reilly, V.P., Dunne, P.J., Exley, M.A., O'Shea, D., Scotet, E., Hogan, A.E., and Doherty, D.G. (2013). Cutting edge: CD1d restriction and Th1/Th2/Th17 cytokine secretion by human Vδ3 T cells. J. Immunol. *191*, 30–34.

Marrack, P., Scott-Browne, J.P., Dai, S., Gapin, L., and Kappler, J.W. (2008). Evolutionarily conserved amino acids that control TCR-MHC interaction. Annu. Rev. Immunol. *26*, 171–203.

Maynard, J., Petersson, K., Wilson, D.H., Adams, E.J., Blondelle, S.E., Boulanger, M.J., Wilson, D.B., and Garcia, K.C. (2005). Structure of an autoimmune T cell receptor complexed with class II peptide-MHC: insights into MHC bias and antigen specificity. Immunity *22*, 81–92.

McVay, L.D., and Carding, S.R. (1999). Generation of human gammadelta T-cell repertoires. Crit. Rev. Immunol. *19*, 431–460.

Meresse, B., Chen, Z., Ciszewski, C., Tretiakova, M., Bhagat, G., Krausz, T.N., Raulet, D.H., Lanier, L.L., Groh, V., Spies, T., et al. (2004). Coordinated induction by IL15 of a TCR-independent NKG2D signaling pathway converts CTL into lymphokine-activated killer cells in celiac disease. Immunity *21*, 357–366.

Metelitsa, L.S., Naidenko, O.V., Kant, A., Wu, H.W., Loza, M.J., Perussia, B., Kronenberg, M., and Seeger, R.C. (2001). Human NKT cells mediate antitumor cytotoxicity directly by recognizing target cell CD1d with bound ligand or indirectly by producing IL-2 to activate NK cells. J. Immunol. *167*, 3114–3122.

Park, S.H., Weiss, A., Benlagha, K., Kyin, T., Teyton, L., and Bendelac, A. (2001). The mouse CD1d-restricted repertoire is dominated by a few autoreactive T cell receptor families. J. Exp. Med. *193*, 893–904.

Patel, O., Pellicci, D.G., Gras, S., Sandoval-Romero, M.L., Uldrich, A.P., Mallevaey, T., Clarke, A.J., Le Nours, J., Theodossis, A., Cardell, S.L., et al. (2012). Recognition of CD1d-sulfatide mediated by a type II natural killer T cell antigen receptor. Nat. Immunol. *13*, 857–863.

Pellicci, D.G., Patel, O., Kjer-Nielsen, L., Pang, S.S., Sullivan, L.C., Kyparissoudis, K., Brooks, A.G., Reid, H.H., Gras, S., Lucet, I.S., et al. (2009). Differential recognition of CD1d-alpha-galactosyl ceramide by the V beta 8.2 and V beta 7 semi-invariant NKT T cell receptors. Immunity *31*, 47–59.

Perera, L., Shao, L., Patel, A., Evans, K., Meresse, B., Blumberg, R., Geraghty, D., Groh, V., Spies, T., Jabri, B., and Mayer, L. (2007). Expression of nonclassical class I molecules by intestinal epithelial cells. Inflamm. Bowel Dis. *13*, 298–307.

Porcelli, S., Brenner, M.B., Greenstein, J.L., Balk, S.P., Terhorst, C., and Bleicher, P.A. (1989). Recognition of cluster of differentiation 1 antigens by human CD4-CD8-cytolytic T lymphocytes. Nature *341*, 447–450.

Rossjohn, J., Pellicci, D.G., Patel, O., Gapin, L., and Godfrey, D.I. (2012). Recognition of CD1d-restricted antigens by natural killer T cells. Nat. Rev. Immunol. *12*, 845–857.

Russano, A.M., Agea, E., Corazzi, L., Postle, A.D., De Libero, G., Porcelli, S., de Benedictis, F.M., and Spinozzi, F. (2006). Recognition of pollen-derived phosphatidyl-ethanolamine by human CD1d-restricted gamma delta T cells. J. Allergy Clin. Immunol. *117*, 1178–1184.

Russano, A.M., Bassotti, G., Agea, E., Bistoni, O., Mazzocchi, A., Morelli, A., Porcelli, S.A., and Spinozzi, F. (2007). CD1-restricted recognition of exogenous and self-lipid antigens by duodenal gammadelta+ T lymphocytes. J. Immunol. *178*, 3620–3626.

Sandstrom, A., Scharf, L., McRae, G., Hawk, A.J., Meredith, S.C., and Adams, E.J. (2012). γδ T cell receptors recognize the non-classical major histocompatibility complex (MHC) molecule T22 via conserved anchor residues in a MHC peptide-like fashion. J. Biol. Chem. *287*, 6035–6043.

Scotet, E., Martinez, L.O., Grant, E., Barbaras, R., Jenö, P., Guiraud, M., Monsarrat, B., Saulquin, X., Maillet, S., Estève, J.P., et al. (2005). Tumor recognition following Vgamma9Vdelta2 T cell receptor interactions with a surface F1-ATPase-related structure and apolipoprotein A-I. Immunity *22*, 71–80.

Selmaj, K., Brosnan, C.F., and Raine, C.S. (1991). Colocalization of lymphocytes bearing gamma delta T-cell receptor and heat shock protein hsp65+ oligodendrocytes in multiple sclerosis. Proc. Natl. Acad. Sci. USA *88*, 6452–6456.

Shin, S., El-Diwany, R., Schaffert, S., Adams, E.J., Garcia, K.C., Pereira, P., and Chien, Y.H. (2005). Antigen recognition determinants of gammadelta T cell receptors. Science *308*, 252–255.

Smith, P.M., and Garrett, W.S. (2011). The gut microbiota and mucosal T cells. Front Microbiol *2*, 111.

Spada, F.M., Grant, E.P., Peters, P.J., Sugita, M., Melián, A., Leslie, D.S., Lee, H.K., van Donselaar, E., Hanson, D.A., Krensky, A.M., et al. (2000). Self-recognition of CD1 by gamma/delta T cells: implications for innate immunity. J. Exp. Med. *191*, 937–948.

Takahashi, T., and Suzuki, T. (2012). Role of sulfatide in normal and pathological cells and tissues. J. Lipid Res. *53*, 1437–1450.

Tettamanti, G., Bonali, F., Marchesini, S., and Zambotti, V. (1973). A new procedure for the extraction, purification and fractionation of brain gangliosides. Biochim. Biophys. Acta *296*, 160–170.

Toulon, A., Breton, L., Taylor, K.R., Tenenhaus, M., Bhavsar, D., Lanigan, C., Rudolph, R., Jameson, J., and Havran, W.L. (2009). A role for human skin-resident T cells in wound healing. J. Exp. Med. *206*, 743–750.

van der Merwe, P.A., and Davis, S.J. (2003). Molecular interactions mediating T cell antigen recognition. Annu. Rev. Immunol. *21*, 659–684.

Vantourout, P., and Hayday, A. (2013). Six-of-the-best: unique contributions of γδ T cells to immunology. Nat. Rev. Immunol. *13*, 88–100.

Willcox, C.R., Pitard, V., Netzer, S., Couzi, L., Salim, M., Silberzahn, T., Moreau, J.F., Hayday, A.C., Willcox, B.E., and Déchanet-Merville, J. (2012). Cytomegalovirus and tumor stress surveillance by binding of a human γδ T cell antigen receptor to endothelial protein C receptor. Nat. Immunol. *13*, 872–879.

Wu, J., Groh, V., and Spies, T. (2002). T cell antigen receptor engagement and specificity in the recognition of stress-inducible MHC class I-related chains by human epithelial gamma delta T cells. J. Immunol. *169*, 1236–1240.

Wucherpfennig, K.W., Newcombe, J., Li, H., Keddy, C., Cuzner, M.L., and Hafler, D.A. (1992). Gamma delta T-cell receptor repertoire in acute multiple sclerosis lesions. Proc. Natl. Acad. Sci. USA *89*, 4588–4592.

Xu, B., Pizarro, J.C., Holmes, M.A., McBeth, C., Groh, V., Spies, T., and Strong, R.K. (2011). Crystal structure of a gammadelta T-cell receptor specific for the human MHC class I homolog MICA. Proc. Natl. Acad. Sci. USA *108*, 2414–2419.

Zajonc, D.M., Maricic, I., Wu, D., Halder, R., Roy, K., Wong, C.H., Kumar, V., and Wilson, I.A. (2005). Structural basis for CD1d presentation of a sulfatide derived from myelin and its implications for autoimmunity. J. Exp. Med. *202*, 1517–1526.

Zeng, X., Wei, Y.L., Huang, J., Newell, E.W., Yu, H., Kidd, B.A., Kuhns, M.S., Waters, R.W., Davis, M.M., Weaver, C.T., and Chien, Y.H. (2012). γδ T cells recognize a microbial encoded B cell antigen to initiate a rapid antigen-specific interleukin-17 response. Immunity *37*, 524–534.