Published in final edited form as:

Eur J Immunol. 2012 September ; 42(9): 2505–2510. doi:10.1002/eji.201242531.

The Majority of CD1d-Sulfatide Specific T Cells in Human Blood Use a Semi-Invariant V δ 1 TCR

Li Bai^{1,2,*}, Damien Picard^{1,*}, Brian Anderson³, Vinod Chaudhary³, Adrienne Luoma⁴, Bana Jabri⁵, Erin J. Adams⁴, Paul B. Savage³, and Albert Bendelac¹

¹Committee on Immunology and Department of Pathology, Howard Hughes Medical Institute, University of Chicago, Chicago IL 60637 USA

²Institute of Immunology, School of Life Sciences, University of Science and Technology of China, Hefei 230027, China

³Department of Chemistry and Biochemistry, Brigham Young University, Provo, UT 84602-5700, USA

⁴Committee on Immunology and Department of Biochemistry and Molecular Biology, University of Chicago, Chicago IL 60637 USA

⁵Committee on Immunology and Department of Medicine, University of Chicago, Chicago IL 60637 USA

SUMMARY

$\alpha\beta$ T-cell lines specific for sulfatide, an abundant myelin glycosphingolipid presented by various CD1 molecules, have been previously derived from PBMCs of patients with demyelinating diseases such as multiple sclerosis (MS) but also from healthy subjects. Using an unbiased tetramer-based MACS enrichment method to enrich for rare antigen-specific cells, we confirmed the presence of CD1d-sulfatide-specific T cells in all healthy individuals examined. Surprisingly, the great majority of fresh sulfatide-specific T cells belonged to the $\gamma\delta$ lineage. Furthermore, these cells used the V δ 1 TCR variable segment, which is uncommon in the blood but predominates in tissues such as the gut and specifically accumulates in MS lesions. Recombinant V δ 1 TCRs from different individuals were shown to bind recombinant CD1d-sulfatide complexes in a sulfatide-specific manner. These results provide the first direct demonstration of MHC-like-restricted, antigen-specific recognition by $\gamma\delta$ TCRs. Together with previous reports, they support the notion that human V δ 1 T cells are enriched in CD1-specific T cells and suggest that the V δ 1 T-cell population that accumulates in MS lesions might be enriched in CD1-sulfatide specific cells.

Keywords

gamma delta T cell; CD1; lipid; sulfatide

INTRODUCTION

$\alpha\beta$ T cells specific for sulfatide, a sulfated β -galactosylceramide that is particularly abundant in myelin, are commonly found in mouse and humans [1–5]. For example,

Corresponding author: Albert Bendelac: University of Chicago, 929 East 57th Street, GCIS Room W506, Chicago, IL 60637, abendela@bsd.uchicago.edu, tel 773 834 8646, fax 773 834 1999.

*L.B. and D.P. are co-first authors

Conflict of Interest. The authors declare no financial or commercial conflict of interest.

multiple cell lines and cloned T cells have been derived from the blood of healthy individuals upon stimulation with sulfatides and antigen-presenting cells expressing CD1a, b or c. Likewise, $\alpha\beta$ T cells specific for CD1d-sulfatide could be identified by tetramer staining in the mouse. Notably, their frequency was reported to increase in the absence of the enzyme CGT required for sulfatide synthesis, suggesting that these autoreactive cells were normally eliminated by exposure to self antigen [4]. The potential pathological relevance of sulfatide-specific T cells is suggested by their increased frequency in the blood of patients with multiple sclerosis and in the lesions of experimental allergic encephalomyelitis in mice [1, 4], as both conditions are associated with demyelination and subsequent release of sulfatides.

In an attempt to enumerate and characterize rare CD1-sulfatide-specific T cells in fresh human blood in an unbiased manner, without prior expansion in culture, we have used tetramers for direct enrichment prior to analysis *ex vivo*. Unexpectedly, we found that the main population of CD1d-sulfatide-specific T cells in the blood of healthy subjects was composed of $\gamma\delta$ T cells, which may have escaped previous identification because they grew poorly in conventional culture conditions compared with $\alpha\beta$ T cells. Nearly all these cells expressed V δ 1⁺ TCRs, rather than the more common V δ 2⁺ TCRs expressed by peripheral blood lymphocytes. Further, we showed using cell-free assays that recombinant V δ 1⁺ TCRs could directly bind recombinant CD1d molecules in a sulfatide-specific manner, providing the first direct demonstration of MHC-like restricted antigen-specific recognition by $\gamma\delta$ TCRs. These findings provide unexpected insights into the biology of antigen recognition by $\gamma\delta$ TCRs and, together with previous reports [6, 7], support the emerging notion that human V δ 1 T cells may be largely specialized in the recognition of lipids presented by CD1 family members. In addition, as V δ 1 T cells were previously shown to dominate the $\gamma\delta$ T-cell population found in the lesions and cerebrospinal fluid of MS patients [8–10], the sulfatide-specific V δ 1 T cells found in healthy subjects may become involved in the pathogenic process.

RESULTS AND DISCUSSION

Identification of CD1d-sulfatide-specific V δ 1⁺ T cells among fresh PBMC in healthy individuals

We first assessed the lipid loading conditions required to generate CD1d-sulfatide complexes. As shown by their altered migration on a native isoelectrofocusing gel, recombinant human CD1d molecules were efficiently loaded after one hour of incubation with a 10-fold molar excess of sulfatide in pH 7.2 HEPES-buffered saline, without requirement for lipid transfer protein (Fig. 1A). We used these loading conditions prior to adding streptavidin in order to generate sulfatide-loaded human CD1d tetramers. These tetramers were used in flow cytometry experiments to obtain an unbiased representation of the existing population of sulfatide-specific CD1d-restricted T cells in healthy individuals. The frequency of CD1d-sulfatide⁺ cells among CD3⁺ T cells was under 10^{−4} in the PBMC of all individuals examined (Fig. 1B). Because it is typically difficult to distinguish genuine staining from background at this low frequency, ~10⁸ PBMC were MACS-enriched using CD1d-sulfatide tetramers, a procedure that allowed the recovery of ~10⁵ cells, of which 0.6 to 5.7% were CD1d-sulfatide⁺ CD3⁺ T cells in different individuals examined (Fig. 1B–C). Surprisingly, these cells rarely expressed the $\alpha\beta$ TCR, but were mostly $\gamma\delta$ T cells that used the V δ 1 chain. On average, in 7/7 individuals examined, 83% of the tetramer-positive cells (range 48–100%) were $\gamma\delta$ T cells that expressed the same V δ 1 variable gene segment (Fig. 1C). In another individual (#4) for which V δ 1 staining was not available, RT-PCR detected V δ 1 TCR chain expression among 200 sorted tetramer⁺ cells. Most circulating $\gamma\delta$ T cells in humans express the V γ 9-V δ 2 TCR, representing 3% of adult blood T cells on average, whereas V δ 1 T cells represent only ~0.8% of T cells [11]. As shown in Fig. 1D, the CD1d-

sulfatide specific T cells represented less than 1% of these circulating V δ 1 T cells. Thus, the frequency of CD1d-sulfatide specific V δ 1⁺ T cells was generally very low, well under 10⁻⁴ in all the healthy subjects examined.

CD1d-sulfatide-specific V δ 1⁺ T cells are clonally expanded

When tetramer-positive cells were directly sorted as single cells and expanded as clones prior to TCR sequencing, 8/8 clones derived from individual #1 exhibited the same V δ 1-J δ 1 nucleotide sequence, indicating the presence of a clonally expanded population circulating in the peripheral blood (Fig. 2). A similar experiment with individual #2 yielded only one clone, which also expressed a V δ 1 variable chain recombined with the same J δ 1 segment as individual #1, but with differences at the D+N junction. In additional experiments, we FACS-sorted the CD1d-sulfatide tetramer-positive T cells from 2 other individuals and, after RT-PCR amplification, we sequenced their V δ 1 chains. Again, all the V δ 1 sequences recovered from a given individual were identical to each other at the nucleotide level, again suggesting that they originated from the same *in vivo* expanded clones. Fig. 2 shows the sequence alignment of the CDR3 δ sequences recovered from these 4 individuals. In addition, we RT-PCR amplified and sequenced the V γ -J γ chains used by clone DP10.7 from subject #1 and clone AB18.1 from subject #2. Different chains were used, including V γ 4-J γ 1 for clone DP10.7 and V γ 5-J γ 2 for clone AB18.1. Thus, while the CD1d-sulfatide-specific TCRs have similar V δ 1-J δ 1 chains, they can use variable V γ chains.

Antigen-specific recognition of CD1d molecules by V δ 1⁺ T cells

Clones DP10.7 demonstrated specificity for sulfatide because it was stained by purified bovine sulfatide (a mixture of sulfatides of varying acyl chain length and saturation dominated by acC24:0 and acC24:1) as well as by synthetic sulfatide acC26:0 and was not stained by CD1d- α GalCer or unloaded tetramers (Fig. 3A, top left panel). Furthermore, tetramer staining was specifically inhibited by preincubation with anti-TCR $\gamma\delta$ but not by isotype control antibody (Fig. 3A, top right panel). Tetramer staining did not decay significantly over an extended period of 6 hours both at ice-cold temperature and at 37°C, suggesting substantial affinity of the V δ 1 TCR for the CD1d-sulfatide complex (Fig. 3A, bottom panels). Clone AB18.1 was brightly stained by CD1d-sulfatide, but also bound unloaded CD1d tetramers, albeit at half the level of CD1d-sulfatide. Interestingly, CD1d- α GC tetramers exhibited even lower binding than unloaded CD1d, further supporting the antigen-specific recognition of CD1d-lipid complexes. While staining of AB18.1 was stable for 6 hours at ice-cold temperature, a faster decay was observed at 37°C (Fig. 3B). Thus, tetramer staining experiments are consistent with strong, antigen-specific recognition of CD1d-sulfatide by the V δ 1-J δ 1 TCRs.

TCR binding to CD1d-sulfatide complexes in a cell-free assay

The question of the antigenic specificity of $\gamma\delta$ T cells, in particular whether putative antigens are directly recognized or are presented by MHC-like molecules remains largely unresolved [12]. With the exception of the MHC class I-like ligand T10, whose direct recognition in the absence of associated peptide was demonstrated at the biochemical and structural level [13], other $\gamma\delta$ T cell antigens have not been fully characterized. Because of the cellular readout of the tetramer staining experiments, the apparent interaction between the the V δ 1 TCRs, CD1d and sulfatide might be indirect, involving some other cellular product. We generated recombinant DP10.7 and AB18.1 TCRs to probe direct binding with CD1d-sulfatide complexes in a cell-free system. After incubation for 15 min with equimolar amounts of CD1d-sulfatide complexes, both TCRs demonstrated direct binding as shown by the formation of complexes in native gel electrophoresis (Fig. 3C). Importantly, both TCRs required sulfatide loading for binding CD1d, providing direct proof of antigen-specific recognition in a cell-free system.

CONCLUDING REMARKS

Our findings underscore the value of unbiased studies of fresh antigen-specific T cells using MHC or CD1 tetramers in humans over *in vitro* expanded cell lines. While the origin and function of the V δ 1⁺ CD1d-sulfatide reactive T cells remain to be elucidated, especially in the context of demyelinating processes, the current findings, together with reported examples of blood or gut derived V δ 1 T cell clones with specificity for CD1 molecules [6, 7], support the emerging notion that a large fraction of this enigmatic $\gamma\delta$ T cell population may be specialized for recognition of lipids presented by CD1 family members. In addition, our biochemical studies constitute the first direct demonstration of antigen presentation by MHC-like molecules to human $\gamma\delta$ T cells, a longstanding hypothesis which has received surprisingly little support over the years.

MATERIALS AND METHODS

Human Subjects

Blood samples were obtained following a protocol approved by the Institutional Review Board. Written informed consent was received from all participants prior to inclusion in the study.

Lipids

Sulfatide purified from bovine brain (B sulfatide), a mixture containing predominant acC24:0 and ac24:1 species, was purchased from Matreya. Sulfatide acC26:0 was synthesized in our laboratory. All the lipid antigens were dissolved in DMSO at a concentration of 20 mg/ml and stored at 20 °C.

Flow cytometry

CD1d-lipid tetramers were produced by incubating 11 μ g of CD1d with 1.7 μ g of lipid antigens in 20 μ l PBS at 37 °C overnight. CD1d-lipid complexes were then incubated with streptavidin-APC (Invitrogen, CA) for 2h at room temperature in a 110 μ l reaction at 4:1 molar ratio.

Fluorochrome labeled monoclonal antibodies against human CD3 (HIT3a), TCR V δ 1 (TS8.2), TCR $\alpha\beta$ (IP26) and TCR $\gamma\delta$ (B1) were purchased from eBioscience, BD Biosciences and Immunotech. Samples were analyzed on an LSR II (BD Bioscience), or sorted on a FACS Aria (BD Bioscience) with doublet exclusion and DAPI staining of dead cells in most experiments. Data was analyzed using FlowJo (Tree Star).

For CD1d-Sulfatide⁺ cells enrichment, human PBMC were isolated from blood by centrifugation over Ficoll-Paque (GE healthcare, NJ). Cells stained with allophycocyanin (APC) conjugated CD1d-Sulfatide tetramer on ice for 1h, before enrichment with anti-APC beads and the autoMACS cell separator (Miltenyi Biotec).

Generation of CD1d-sulfatide tetramer⁺ clones

CD1d-sulfatide tetramer⁺ cells were enriched by MACS and sorted as single cells in 96 well plates with feeder cells consisting of irradiated (4500 rad) allogeneic PBMC (5×10^4 /well) and irradiated (10,000 rad) EBV transformed cells (5×10^3 /well) in RPMI1640 medium containing 8% FCS (Biowest), 2% AB human serum (Atlanta Biologicals), 55 μ M 2-ME, 2mM L-glutamine (Cellgro), 1mM sodium pyruvate (Invitrogen), 1mM nonessential amino acids (Invitrogen), 1 μ g/ml PHA and 100 U/ml human IL-2.

TCR sequencing

Total RNA was isolated from sorted CD1d-sulfatide⁺CD3⁺Vδ1⁺ cells using a combination of Trizol (Invitrogen) and the RNeasy Mini Kit (Qiagen) and was reverse-transcribed with Superscript III (Invitrogen) using oligo-d(T). TCR sequences were amplified by PCR with the following primers (14): 5'-ctgtcaactcaagaagcagcgaaa-3' (Vδ1), 5'-tgaggagatgacaatagcaggatc-3' (Cδ), 5'-cgcaaggacaaggacaacttgagatt-3' (Vγ2), 5'-ctatgacgtctccaccgcaagg-3' (Vγ3), 5'-cggaagcacaaggaaacttgagaat-3' (Vγ4), 5'-gggtggagctggatattgatactacga-3' (Vγ5), 5'-gcaagcacagggaagagccttaaattta-3' (Vγ8), 5'-tggtgaagtcatacagttctctgggtg-3' (Vγ9), and 5'-gaatcgtgttgctctcttttcttgcc-3' (Cγ). For sequencing, PCR products were subcloned using Topo TA Cloning Kit (Invitrogen, CA) and randomly selected clones were processed for sequencing (Applied Biosystems 3730XL). Sequences were analysed using the IMGT tools (<http://imgt.cines.fr/>).

CD1d production and sulfatide loading

Human CD1d was expressed by the baculovirus method in Hi5 insect cells. Purified CD1d at 1 mg/mL was incubated for 1 hour with a 10-fold molar excess of bovine brain sulfatides (Matreya) in HEPES-buffered saline, pH 7.2, at 37 °C. 4 µg of sulfatide-loaded CD1d, and 4 µg of unloaded control CD1d, were run on an pH 3–9 isoelectrofocusing gel (GE Healthcare) using the PhastGel system (GE Healthcare).

Recombinant TCRs and CD1d binding assay

The DP10.7 TCR γ and δ variable domains were fused to the TCR constant domains from β and α chains, respectively, and the hybrid ectodomains were cloned into the pET28a vector and expressed in BL21 *e. coli* cells. The resultant inclusion bodies were solubilized in 7 M guanidine HCl and refolded as a heterodimer by dilution with 50 mM Tris pH 8.0 in the presence of 10:1 reduced:oxidized glutathione (Sigma). The protein was purified by Ni-NTA chromatography (Qiagen) followed by Superdex 200 size exclusion chromatography (Amersham). For production by the baculovirus method in Hi5 insect cells, both the DP 10.7 TCR and AB18.1 γ and δ variable domains were fused to αβ TCR constant domains, as above, then cloned into the pACGP67a vector (PharMingen) and produced as a heterodimer by the baculovirus expression system in High FiveTM insect cells as described [13]. Human CD1d and β2M were cloned into the pACGP67a vector for baculovirus expression in High FiveTM cells, with CD1d containing a C-terminal 6x His tag, allowing for heterodimer purification over Ni-NTA resin. CD1d was purified by Superdex 200 size exclusion chromatography, loaded with a 10-fold molar excess of bovine brain sulfatides (Matreya) and then purified by MonoQ anion exchange chromatography (Amersham). For the native gels, equimolar amounts of the TCRs and either unloaded or sulfatide-loaded CD1d were combined at a total protein concentration of 1 mg/mL and incubated for 15 min at RT. 4 µl of the complexes were loaded on a 12.5% homogenous gel (GE Healthcare), along with the same amount of non-complexed TCRs and CD1d proteins as controls. The gels were run using the PhastGel system (Pharmacia Biotech) and stained with PhastGel system (Pharmacia Biotech) and stained with PhastGel Blue R (Amersham) at 0.02% in 3:1:6 methanol:acetic acid:water. Gels were destained with 3:1:6 methanol:acetic acid:water for imaging.

Acknowledgments

We thank C. Ciszewski for help with T cell cloning, the Animal Resource Center, Core Flow Cytometry Facility, DNA Sequencing Facility; the NIAID tetramer facility for CD1d tetramers. This work was supported by NIH grants AI038339 and AI053725 (to AB), AI073922 and Kinship Foundation Searle Scholars (EJA) DK67180 (JB), AI053725 (AB and PBS), and by the Digestive Disease Research Core Center P30 DK42086. A.B. is a Howard Hughes Medical Institute Investigator.

REFERENCE CITED

1. Shamshiev A, Donda A, Carena I, Mori L, Kappos L, De Libero G. Self glycolipids as T-cell autoantigens. *Eur J Immunol.* 1999; 29:1667–1675. [PubMed: 10359121]
2. Shamshiev A, Donda A, Prigozy TI, Mori L, Chigorno V, Benedict CA, Kappos L, et al. The alphabeta T cell response to self-glycolipids shows a novel mechanism of CD1b loading and a requirement for complex oligosaccharides. *Immunity.* 2000; 13:255–264. [PubMed: 10981968]
3. Blomqvist M, Rhost S, Teneberg S, Lofbom L, Osterbye T, Brigl M, Mansson JE, et al. Multiple tissue-specific isoforms of sulfatide activate CD1d-restricted type II NKT cells. *Eur J Immunol.* 2009; 39:1726–1735. [PubMed: 19582739]
4. Jahng A, Maricic I, Aguilera C, Cardell S, Halder RC, Kumar V. Prevention of autoimmunity by targeting a distinct, noninvariant Cd1d-reactive T cell population reactive to sulfatide. *J Exp Med.* 2004; 199:947–957. [PubMed: 15051763]
5. Shamshiev A, Gober HJ, Donda A, Mazorra Z, Mori L, De Libero G. Presentation of the same glycolipid by different Cd1 molecules. *J Exp Med.* 2002; 195:1013–1021. [PubMed: 11956292]
6. Spada FM, Grant EP, Peters PJ, Sugita M, Melian A, Leslie DS, Lee HK, et al. Self-recognition of Cd1 by gamma/delta T cells: implications for innate immunity [see comments]. *J Exp Med.* 2000; 191:937–948. [PubMed: 10727456]
7. Russano AM, Bassotti G, Agea E, Bistoni O, Mazzocchi A, Morelli A, Porcelli SA, et al. Cd1-restricted recognition of exogenous and self-lipid antigens by duodenal gammadelta+ T lymphocytes. *J Immunol.* 2007; 178:3620–3626. [PubMed: 17339459]
8. Wucherpfennig KW, Newcombe J, Li H, Keddy C, Cuzner ML, Hafler DA. Gamma delta T-cell receptor repertoire in acute multiple sclerosis lesions. *Proc Natl Acad Sci U S A.* 1992; 89:4588–4592. [PubMed: 1374907]
9. Shimonkevitz R, Colburn C, Burnham JA, Murray RS, Kotzin BL. Clonal expansions of activated gamma/delta T cells in recent-onset multiple sclerosis. *Proc Natl Acad Sci U S A.* 1993; 90:923–927. [PubMed: 8430106]
10. Nick S, Pileri P, Tongiani S, Uematsu Y, Kappos L, De Libero G. T cell receptor gamma delta repertoire is skewed in cerebrospinal fluid of multiple sclerosis patients: molecular and functional analyses of antigen-reactive gamma delta clones. *Eur J Immunol.* 1995; 25:355–363. [PubMed: 7875196]
11. Lee PT, Putnam A, Benlagha K, Teyton L, Gottlieb PA, Bendelac A. Testing the NKT cell hypothesis of human iddm pathogenesis. *J Clin Invest.* 2002; 110:793–800. [PubMed: 12235110]
12. Meyer C, Zeng X, Chien YH. Ligand recognition during thymic development and gammadelta T cell function specification. *Semin Immunol.* 2010; 22:207–213. [PubMed: 20430644]
13. 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. [PubMed: 15821084]
14. Ehl S, Schwarz K, Enders A, Duffner U, Pannicke U, Kuhr J, Mascart F, et al. A variant of SCID with specific immune responses and predominance of gamma delta T cells. *J Clin Invest.* 2005; 115:3140–3148. [PubMed: 16211094]

Fig. 1. CD1d-sulfatide tetramer staining and MACS enrichment of healthy PBMC
(A) Loading of human CD1d molecules with purified bovine sulfatide demonstrated by native IEF. Data are representative of two independent experiments. (B) CD3/tetramer staining before and after tetramer MACS enrichment in PBMCs from 3 healthy subjects. Starting population included $\sim 1 \times 10^8$ PBMCs, with $\sim 1 \times 10^5$ cells typically recovered after MACS enrichment. Gated CD3⁺ tetramer⁺ cells were further characterized for TCR $\alpha\beta$, TCR $\gamma\delta$ and V $\delta 1$ expression. Numbers indicate percentages in the gated population. Data are representative of 1 to 5 experiments per individual. (C) Frequency of CD1d-sulfatide tetramer⁺ CD3⁺ PBMCs in 8 healthy individuals; nd, not determined; for individual #4, presence of V $\delta 1$ T cells was inferred from the amplification of a V $\delta 1$ -J $\delta 1$ sequence by RT-PCR after CD1d-sulfatide tetramer MACS enrichment followed by FACS sorting of 200 tetramer⁺ cells. (D) Frequency of CD1d-sulfatide tetramer⁺ cells among gated CD3⁺ V $\delta 1$ ⁺ cells PBMCs. Values indicate average percentage $\pm$ SEM of 6 individuals examined.

	V δ 1	D+N	J δ 1
#1	TGTGCTCTTGGGGAAC	CCTCCTACTGGGGGTCCCCCGACGACCCGGG	TCATCTTT
Clone DP10.7	C A L G E	P S Y W G F P R T T R	V I F
#2	TGTGCTCTTGGGGA	CCAGATTCTGTACTGGGGTCTCAGCC	ACACCGATAAACTCATCTTT
Clone AB18.1	C A L G D	Q I L Y W G L S	H T D K L I F
#3	TGTGCTCTTGGGGA	TGGGATTCTCTATACTGGGGGATAACGGCCTCGT	ACACCGATAAACTCATCTTT
	C A L G D	G I P L Y W G I L T A S	Y T D K L I F
#4	TGTGCTCTTGGGAACT	AACGGTGGGGGCCGTTTC	ACACCGATAAACTCATCTTT
	C A L G E L	T V G A V	H T D K L I F
<hr/>			
	V γ 4	D+N	J γ 1
#1	TGTGCCACCTGGG	GTGAGAAG	TATTATAAGAACTCTTT
Clone DP10.7	C A T W	D E K	Y Y K K L F
<hr/>			
	V γ 5	D+N	J γ 2
#2	TGTGCCACCTGGGACAGG	AATA	ATAAGAACTCTTT
Clone AB18.1	C A T W D R	N	N K K L F

Fig. 2. TCR γ and δ sequences of CD1d-sulfatide-specific V δ 1⁺ T cells

Nucleotide and amino acid sequences of TCR γ and δ CDR3 regions in 4 different individuals. The top 2 sequences were obtained from clones derived from individuals #1 and #2, DP10.7 and AB18.1 respectively, and the bottom 2 sequences were obtained from freshly sorted CD1d-sulfatide tetramer⁺ cells of individuals #3 and #4.

Fig. 3. Antigen specificity of CD1d-sulfatide tetramer⁺ V δ 1⁺ T cell clones. (A) Clone DP10.7 stained with tetramers loaded with bovine sulfatide (B sulfatide), synthetic sulfatide acC26, α GalCer or unloaded as indicated (top left). Tetramer staining was inhibited by anti-TCR $\gamma\delta$ (top right). Decay of tetramer staining (MFI) at indicated time and temperature is shown in the bottom plots. Data are representative of two independent experiments. (B) Similar analysis as in (A) for clone AB18.1. Data are representative of two independent experiments. (C) Native gel electrophoresis of the DP10.7 TCR, AB18.1 TCR, and CD1d demonstrates a specific interaction between the TCRs and sulfatide-loaded CD1d. Equimolar amounts of the TCR (produced in insect cells) and CD1d proteins were mixed and incubated at room temperature

for 15 min, before running on a native gel along with control proteins as indicated. The binding of sulfatide-loaded, but not unloaded, CD1d by DP10.7 was observed with different preparations of TCR produced in bacteria or in insect cells.