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Diverse TCRs Recognize Murine CD1

Samuel M. Behar, T. A. Podrebarac, C. J. Roy, C. R. Wang, and M. B. Brenner

Human and murine T cells that specifically recognize CD1d and produce IL-4 and IFN-γ play a role in immunoregulation and tumor rejection. In the mouse, most CD1d1-reactive T cells described express an invariant Vα14-Jα281 TCR associated with TCR β-chains of limited diversity. Similarly, human CD1d-reactive T cells express a highly restricted TCR repertoire. Here we report the unexpected result that in mice immunized with CD1d1-bearing transfecchant cells, a diverse repertoire of TCRs was expressed by CD1d1-reactive T cell clones isolated by limiting dilution without preselection for NK1 expression. Only 3 of 10 CD1d1-reactive T cell clones expressed the invariant Vα14-Jα281 TCRα rearrangement. T cells expressing Vα10, -11, -15, and -17, and having non-germline-encoded nucleotides resulting in diverse V-J junctions were identified. Like CD1d1-reactive T cells expressing the invariant Vα14-Jα281 TCR α-chain, CD1d1-reactive clones with diverse TCRs produced both Type 1 (IFN-γ) and Type 2 (IL-4, IL-10) cytokines. This establishes the existence of significant diversity in the TCRs directly reactive to the CD1d1 protein. Our findings reveal that CD1d interacts with a broad array of TCRs, suggesting substantial redundancy and flexibility of the immune system in providing T cells serving the role(s) mediated by CD1d reactivity.


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The mouse genome encodes two CD1 genes (CD1D1 and CD1D2) likely to be the result of a gene duplication that are homologues of human CD1d (12–14). While the crystal structure of murine CD1d confirmed its overall similarity to MHC class I, the striking feature was a substantially larger Ag-binding groove that was lined almost exclusively with hydrophobic and nonpolar amino acids (3). These structural data, together with the identification of α-glycosylacylphosphatidylsugonosines as murine CD1 (mCD1)-restricted Ags for NK T cells (15), supports the idea that mouse CD1, as first proposed for human CD1, may have evolved to present lipid Ags to T cells (4, 6, 8).

T cell hybridomas generated from NK1.1+ thymocytes recognized CD1d expressed on thymocytes, as well as CD1-transfected cell lines, in the absence of any exogenous Ag (16). NK1.1+ T cells use an invariant TCR α-chain that results from the recombination of Vα14.1 (TCRAV14S1) with Jα281 (TCRJ15) and pairs with one of three Vβ-chain families: Vβ2, -7, or -8 (17, 18). NK1.1+ T cells release large amounts of IL-4 within hours of activation, and it has been proposed that this population of T cells may be the earliest source of IL-4 that could shift an immune responses toward a Th2 phenotype. However, it is now appreciated that NK1.1+ T cells have the capacity to produce IFN-γ, indicating that their function is not exclusively in Th2 T cell responses and that their potential for various cytokine-mediated roles needs further study (19–21).

Given the broad diversity of T cells directed against CD1a, -b, and -c in humans, we sought to examine further the diversity and function of T cells reactive to mCD1. Thus, rather than select for study only those T cells that expressed Vα14 TCRs or the NK1.1 marker, we stimulated murine splenic cells with CD1d-expressing transfectants and established a panel of T cell clones that recognized CD1d directly. Although several Vα14+ clones were obtained, a majority of the T cell clones derived used a diverse Vα and Vβ repertoire. These T cells were potent cytolytic T cells that also secreted large amounts of IL-10 and IFN-γ upon specific activation, suggesting their potential for diverse roles in immunoregulation and host defense.

Materials and Methods

Antibodies and antigens

The Abs M1/42, 3.9.8, HLK (anti-MHC I), M5/114, 15.2 (anti-MHC II), GK1.5 (anti-CD4), and 53-6.72 (anti-CD8α) were obtained from the American Type Culture Collection (Rockville, MD). Abs 53-5.8 (anti-CD8β), 3 Abbreviations used in this paper: mCD1, murine CD1; CDR3, complementarity-determining region 3.
H57-597 (anti-εTCR), GL3 (anti-γδ TCR), PK136 (anti-NK1.1), 5E6 (anti-Ly49C), IB1 (anti-mCD1 (22)) were obtained from Pharmingen (San Diego, CA). The hamster Abs 38-4.5 (IgG control), 3H3.23.2 and 5C6.4 (both anti-mCD1) were used as spent culture supernatants (46). The Abs (H1 and 3C11 (rat anti-mCD1) were generously provided by Steve Balk (Beth Israel Hospital, Boston, MA), and 10A7 (anti-NKR-P1A/B) was a kind gift of James Ryan (University of California at San Francisco, CA). The mCD1-restricted peptide, p99a (EHDFH-HIREWGNHK) (23), was synthesized by the Brigham and Women’s Hospital Biopolymer Laboratory.

**Cell culture**

RMA-S was grown in complete media consisting of RPMI 1640 media supplemented with 10 mM HEPES, 2 mM L-glutamine, 10 mM nonessential amino acids, 10 mM essential amino acids, 0.055 mM 2-ME (all from Life Technologies, Gaithersburg, MD), and 10% heat-inactivated FCS (HyClone Laboratories, Logan, UT). All T cells were grown in complete media supplemented with 1.5 mM recombinant human IL-2 (gift of Ajinomoto, Kawasaki, Japan). T cell cloning was done by plating T cells at limiting dilution in round bottom plates in the presence of 2 × 10^4 mitomycin C-inactivated RMA-S.CD1d1 cells.

**Transfections**

The RMA-S cell line was derived by mutagenesis from a C57BL/6 T cell lymphoma cell line (RBL-5) and contains a mutant TAP2 gene, making these cells defective in the MHC class I Ag-processing pathway (24). Because these cells also lack MHC class II, they were selected as an APC to study CD1-restricted T cells. RMA-S cells were transfected with the expression vector pSRα-NEO (25) containing the mCD1D1 cDNA (kindly provided by Dr. Steve Balk, Beth Israel Hospital, Boston, MA) by electroporation. Stably transfected cells were first selected for G418 resistance and then for high surface expression of mCD1 by sorting cells that stained brightly with the 3C11 and H11 Abs using the FACS Sort (Becton Dickinson, San Jose, CA). CD1^d1^ RMA-S (RMA-S.CD1d1) clones were subsequently established by limiting dilution. The CD1^d1^ L cell transfectant was provided by Dr. Steve Balk (Beth Israel Hospital, Boston, MA).

**Derivation of T cell lines and clones**

RMA-S.CD1d1 cells were loaded with the p99a peptide at 37°C for 4 h, washed, inactivated with mitomycin C, and injected i.m. in C57BL/6 mice (line 14). At the same time, p99a peptide in CFA (initial immunization) orIFA (subsequent immunizations) was injected s.c. in the contralateral thigh. This was repeated 10 days later. Alternately, mice were immunized by s.c. injection with p99a in CFA, in the absence of RMA-S.CD1d1 cells (line 24). After euthanasia by CO2 asphyxiation, spleen and lymph nodes were removed, inactivated with mitomycin C, and injected i.m. in C57BL/6 mice (23) or BALB/c mice. RMA-S cell lines were heterogeneous and contained predominantly CD8^+^ T cell lines.

**FACS**

Cells (2 × 10^5) were stained with 50 μl of ascertes (diluted 1:400) or purified Ab (5 μg/ml) in FACS buffer (5% bovine calf serum/0.01% azide) for 1 h at 4°C. The cells were washed, and 20 μl of FITC labeled F(ab')2 rabbit anti-hamster Ig or PE-labeled donkey anti-rat Ig (both from Jackson ImmunoResearch Laboratories, West Grove, PA) were added for 1 h at 4°C. After washing extensively with FACS buffer, the cells were counterstained with propidium iodide and analyzed using a FACSort (Becton Dickinson).

**Proliferation assay**

T cells were cultured in triplicate at a concentration of 5 × 10^4 cells/well in the presence of 10^5 mitomycin C (Sigma, St. Louis, MO)-treated CD1^d1^ RMA-S cells in a flat-bottom 96-well plate in a total volume of 200 μl/well. Abs were used where indicated at a final concentration of 25 μg/ml or a final dilution of 1:4 for spent culture supernatants. The cultures were incubated for a total of 48–72 h at 37°C in 5% CO2 and pulsed with 1 μCi of [H]thymidine (6.7 Ci/mmol, New England Nuclear, Boston, MA) per well for the last 6 h of culture, and [H] incorporation was measured by liquid scintillation counting to assess T cell proliferation. Cultures were harvested onto fiberglass filter mats (Wallac, Gaithersburg, MD) using an automated harvester (Tomtec, Orange, CT) and counted using a 1205 Beta plate liquid scintillation counter (Wallac). Specific lysis was calculated as [(sample cpm - spontaneous cpm)/(maximum cpm - spontaneous cpm)] × 100. In general, the SD of the triplicates was 5–10% of the mean.

**Cytotoxicity assay**

The RMA-S cell lines, stably transfected with mCD1D1 genes, or untransfected, were used as targets in a standard [3H]Thy release assay. Targets were labeled with 200 μCi of [3H]Thy for 2 h. Two thousand target cells were incubated with effector T cells for 4 h in triplicate, and the E:T ratio varied as indicated in the figures. Ab blocking studies were done by adding purified Ab at a final concentration of 25 μg/ml or supernatants at a final dilution of 1:3. Chromium release was assessed by spotting 25 μl of the supernatant onto fiberglass filter mats that were counted in a 1205 Beta plate liquid scintillation counter (Wallac). Specific lysis was calculated as [(sample cpm - spontaneous cpm)/(maximum cpm - spontaneous cpm)] × 100. In general, the SD of the triplicates was 5–10% of the mean.

**Cytokine assays**

A sandwich ELISA was used to determine the amount of IL-2, -4, -10, and IFN-γ produced by the T cell clones 24 and 48 h after specific Ag stimulation with RMA-S.CD1d1 cells compared with untransfected RMA-S cells. Abs specific for the cytokines tested were obtained from Pharmingen and used according to the manufacturer’s directions. The limits of detection were 78 pg/ml (IL-2, -4, -10, and IFN-γ).

**PCR and Nucleotide Sequencing**

Expression of the canonical Vα14-Jα281 variable region by the T cell clones was determined using nested PCR. RNA was extracted from the T cell clones or thymus using RNAzol (Tel Test, Friendswood, TX), and first strand cDNA synthesis was conducted using random hexamers. The first round of amplification used gene-specific oligonucleotide primers to the Vα14 and Cα gene segments (17, 26) and Taq polymerase. The PCR reactions were diluted 1:100, and a second round of amplification was conducted using internal primers specific for Vα14 and Jα281, and the products were visualized using ethidium bromide on a 2% agarose gel. The cloning of the TCRs cDNA from the Vα14-Jα281-negative T cell clones was conducted using inverse PCR (27). First and second strand cDNA synthesis was done using oligo(dT) and Superscript (both from Life Technologies). Circularization of the cDNA was performed using DNA ligase. First round PCR was done using Cα primers (28) oriented in opposite directions and KlenTaq (Clonetech). The PCR product was diluted 1:100 and reamplified using an internal nested primer set (28) that contained 5′ EcoRI sites to facilitate cloning. The nested PCR products were cloned into the pBluescript II sequencing vector (Stratagene) and sequence at the Brigham and Women’s Hospital automated DNA sequencing facility using a 3′ Co-specific primer (5′-CAGGATCTTATTAAACTGT GTAC-3′) and Vα-specific primers (Vα10: GGAAGTCCTGCTA GCTGT; Vα11: CCTCCCCATCTCCTTGTG; Vα15: GAGAGG TCAGAACATGAG; (17, 26).

Determination of the Vβ gene segment usage was done by RT-PCR using primers previously described (29), except for the Vβ5 and Cβ primers. Involving additional primers were used: Vβ5: AACAAGTTCAG CAGATTCTG; Vβ5: GGGTGTCCCAAGTCTCCAAGA; Vβ8: GAAA CAAGGGCAGTCAGATAGCAGTGGAGCCG; Cβ: CCCAGGGCTTCGCAGCTAGTGT (for PCR); Cβ: GATGG CTCAACAAAGG ACC (for sequencing).

**Results**

**Derivation of T cell lines**

T cells purified from the spleen and lymph nodes of C57BL/6 mice that had been immunized with RMA-S cells transfected with murine CD1D1 (RMA-S.CD1d1) cells and the p99a (23) peptide or immunized with the p99a peptide alone, were used to generate mCD1-restricted T cell lines. Although this foreign peptide was included in the immunization because of an earlier report showing its ability to prime CD1d1-restricted T cells in vivo (23), none of the T cell lines we derived was specific for the p99a peptide. However, a number of the T cell lines recognized CD1d1 directly without the addition of p99a. Direct recognition of CD1d1 by the T cell lines was specific since they proliferated in response to RMA-S.CD1d1 cells but not to untransfected RMA-S cells (data not shown), and the proliferation was blocked by Abs 3H3 and 5C6 to mCD1 but not Abs specific for MHC class I or II (Fig. 1). The T cell lines were heterogeneous and contained predominantly CD8^+^ T cells.
and CD4⁺ CD8⁻ T cells, and some CD4⁺ T cells, and the generation of sublines that were enriched for CD8⁺ or CD4⁻ CD8⁻ T cells indicated that both populations could recognize CD1d1.

**Recognition of CD1 by murine T cell clones**

To better understand the diversity of T cells that recognize CD1d1, and to examine their potential effector functions, we established T cell clones by limiting dilution from the CD1d1 specific T cell lines. Seven clones were obtained that expressed neither CD4 nor CD8 (CD4⁻ CD8⁻, or DN) (data not shown). Three T cell clones had heterogeneous expression of the CD8α chain and did not express the CD8β chain. This pattern of expression is typical of cells that express the CD8αα homodimer, such as γδ T cells, human NK cells, and activated CD4⁻ CD8⁻ αβ TCR⁺ T cells grown in vitro. All of the T cell clones expressed the TCR-αβ and the CD28-costimulatory molecule (data not shown).

Despite the fact that these T cell clones were derived from C57BL/6 mice, an NK1.1⁺ strain, neither the original T cell lines nor the T clones expressed the NK1.1 Ag that was recognized by PK136 mAb. The T cell clones did not express other NK markers such as the NKR-1PA or NKR-1PB Ags (recognized by the 10A7 mAb), nor the Ly-49C Ag, that are expressed by some NK T cells. These data suggested that these T cells were distinct from the NK T cell subset.

To establish that the T cell clones recognized CD1, a cytolytic assay was conducted using RMA-S-CD1d1 transfectants and RMA-S untransfected control cells as targets. Both the CD8⁺ and DN T cell clones specifically lysed the CD1-transfected target across a range of E:T ratios (Fig. 2A). For example, at E:T 100:1, T cell clone 14S.3 lysed nearly 100% of the CD1⁺ target, but only 25% of the untransfected targets (Fig. 2A). To rule out that the T cell clones were recognizing a clonal variation unrelated to CD1 expression between the RMA-S-CD1d1 and RMA-S NT, recognition of mouse L cell fibroblasts either untransfected or transfected with CD1d1 (14) was also examined. The 14S T cell clones specifically lysed the L.CD1 but not untransfected L cells confirming the specificity for CD1-transfected targets (Fig. 2B).

To further confirm the restriction of these CD1-restricted T cell clones, Ab blocking experiments were performed. Abs to MHC I, MHC II, CD4, and CD8 did not block the specific recognition of RMA-S-CD1d1. In contrast, three anti-mCD1 Abs significantly inhibited the specific lysis of the RMA-S-CD1d1 by the CD1-restricted T cell clones. For example, mAb 1B1 (rat anti-CD1), and mAbs 3H3 and 5C6 (both hamster anti-CD1), blocked lysis of the RMA-S-CD1d1 transfectant by 14S3 (DN) and 14S.10 (CD8αα⁻) T cell clones (Fig. 3).

**Cytokine production by CD1-restricted T cell clones**

Stimulation of the T cell lines with RMA-S-CD1d1 cells led to the production of >10,000 pg/10⁶ T cells of IL-4, IL-10, and IFN-γ; however, because these T cells were serially propagated in culture, they lost their ability to produce large amounts of IL-4 despite preservation of the ability to secrete >40,000 pg of IFN-γ/10⁶ T cells (data not shown). The T cell clones most consistently made large amounts of IFN-γ and IL-10 (Table I). For example, in one representative assay, the T cell clones 14S.3, 14S.4, 14S.7, 14S.10, and 24.7 all made >80,000 pg IFN-γ/10⁶ T cells. In the same assay, these T cell clones produced between 8,000 and 22,000 pg...
of IL-10/106 T cells (Table I). There was more variation among the T cell clones with respect to the production of IL-2 and IL-4. T cell clones 14S.3, 14S.4, and 14S.7 did not produce any detectable IL-2 while clones 14S.10, 24.7, and 24.8 all produced 3,800 to 17,000 pg/106 T cells (Table I). For comparison, the CD4+ T cell clone AE7 (30), which is specific for pigeon cytochrome c and is restricted by I-Ek, produced only IL-2 and IFN-γ. The production of both IL-10 and IFN-γ were sampled at 24 and 48 h poststimulation and stored at −20°C until cytokine quantitation was done by ELISA (IFN-γ, IL-2, IL-4, and IL-10). Results are shown as picograms per 106 T cells/milliliter. The patterns of cytokine secretion were similar for both time points. AE7, an I-Ak-restricted T cell clone specific for pigeon cytochrome c, was stimulated with irradiated splenocytes, in either the presence or the absence of pigeon cytochrome c. AE7 T cells and APCs did not secrete any detectable cytokines in the absence of Ag. In the presence of Ag, IL-2 (5,323 pg/ml) and IFN-γ (78 pg/ml). Similar results were obtained in two to four experiments.

**Table I. Cytokine production by T cell clones**

<table>
<thead>
<tr>
<th>Phenotype</th>
<th>Vβ</th>
<th>Dβ</th>
<th>Jβ</th>
<th>Va</th>
<th>Ja</th>
<th>Ia</th>
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<tr>
<td>14S.3</td>
<td>DN</td>
<td>BV8S2A1</td>
<td>—</td>
<td>2.5</td>
<td>AV22S1</td>
<td>TRAJ50 NEW.02</td>
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<tr>
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<td>—</td>
<td>2.5</td>
<td>AV22S1</td>
<td>TRAJ50 NEW.02</td>
</tr>
<tr>
<td>14S.7</td>
<td>DN</td>
<td>BV8S2A1</td>
<td>—</td>
<td>2.5</td>
<td>AV22S1</td>
<td>TRAJ50 NEW.02</td>
</tr>
<tr>
<td>14S.10</td>
<td>CD8αα</td>
<td>BV8S1</td>
<td>—</td>
<td>2.6</td>
<td>AV11S3</td>
<td>TRAJ4 NEW.15</td>
</tr>
<tr>
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<td>DN</td>
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<td>2.6</td>
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<td>TRAJ15 281</td>
</tr>
<tr>
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<td>DN</td>
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<td>2</td>
<td>2.5</td>
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<td>24.9</td>
<td>DN</td>
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<td>2</td>
<td>2.4</td>
<td>AV14S1</td>
<td>TRAJ15 281</td>
</tr>
<tr>
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<td>BV8S2A3</td>
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<td>TRAJ40 TA65</td>
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<td>2</td>
<td>2.1</td>
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<td>TRAJ32 TT11</td>
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</table>

The gene segments used to encode the TCRs of the CD1-reactive T cell clones was determined by comparing the sequences to the NCBI database using the BLAST algorithm. Nomenclature for Va and Vβ family members is based on the WHO-IUIS recommendations (43). Jβ nomenclature is from Ref. 44. The Ja nomenclature is from IMGT, the international ImMunoGeneTics database (45), and alternate nomenclature from the mouse TCR-αβ chain locus (GenBank accession number M64239). Note that in the IMGT nomenclature, Jα281 is named TRAJ18. Discrimination between the AV10S2 and AV10S9 family members was not possible using the Va sequence determined for T cell clone 14S15.

**The CD1-restricted T cell clones predominantly use Vβ8**

The T cell lines were polyclonal with respect to Vβ usage as determined by flow cytometry with Vβ-specific mAbs. In most of the T cell lines studied, Vβ8.1/2 was dominant, and varying proportions of Vβ2-, Vβ5.1/2-, Vβ7-, and Vβ8.3-expressing T cells were detected (data not shown). The Vβ usage of the T cell clones was determined by RT-PCR analysis followed by sequencing (Table II, Fig. 4). T cell clones 14S.3, 14S.4, and 14S.7 all expressed identical Vβ TCR rearrangements of Vβ8.2 (BV8S2A1) and Jβ2.5. The T cell clones 14S.10, 14S.11, 24.8, and 24.9 each expressed unique Vβ TCR rearrangements, but all used a member of the Vβ8 family (BV8S1, BV8S2A3, BV8S2A2, and BV8S3, respectively). Three other T cell clones used genes from the Vβ5, Vβ6, and Vβ14 families (Table II, Fig. 4).

Thus, despite the diverse array of Vα gene segments used by the CD1-reactive T cell clones, five of the eight unique Vα chains were paired with a member of the Vβ8 family. Moreover, five of the clones shared a three-aminoc acid motif in the complementarity-determining region 3 (CDR3) that consisted of a negatively charged residue followed by a bulky hydrophobic residue. Clones 24.8, 24.9, 14S11, 14S15, and 14S6 contained the amino acids DWG, while clone 14S3 has the amino acids EI-I in the Vβ CDR3 region (Fig. 4). This D/E-hydrophobic amino acid motif was independent of which Vβ or Jβ gene segments were used and was entirely encoded by the TCRβD2 (Dβ2) gene segment (Fig. 5). Although the TCRβD2 gene segment can be translated in all three reading frames, the third reading frame was selected to be used in all five TCR rearrangements.

**Discussion**

The paradigm of CD1 recognition by murine T cells has been dominated by the discovery that a major fraction of NK1.1+ T cells expressing a highly restricted TCR repertoire directly recognize CD1d1 and rapidly produce large amounts of IL-4 upon activation. NK1.1+ T cells comprise 20% of HSA<sup>+</sup> thymocytes in adult C57BL/6 mice, and significant numbers are also found in the bone marrow and the liver (31). The selection of NK1.1+ T cells during development requires mCD1, as mice with targeted mutations of β<sub>2</sub>-microglobulin or CD1 are largely devoid of the NK1.1+ subset of αβTCR<sup>+</sup> T cells (32–34). Characteristically, these unique T cells express a limited TCR repertoire, exemplified...
by the canonical rearrangement of Vα14-Jα281 which pairs with Vβ chains encoded by the Vβ2, Vβ7, or Vβ8 families (17, 18). However, as strongly indicated by the data presented in the current study, it is likely that recognition of mCD1 involves many other T cells beyond the Vα14-Jα281 NK T cell subset.

Cardell et al. provided the first evidence that mCD1 recognition was not limited to NK1.1+ T cells (35). Hybridomas made from splenic CD4+ T cells isolated from class II-deficient mice recognized mCD1, yet did not express the Vα14-Jα281 canonical TCR (35, 36). However, since MHC class II-deficient mice have markedly abnormal T cell development, it was unclear to what extent these findings reflected the situation in the normal immune system. Here, using an approach that allowed detection of unselected T cells that were CD1d1 reactive, we demonstrated that a wide diversity of TCR sequences for the T cell clones 14S.3, 14S.4, and 14S.7 were identical with each other; only one sequence is shown for comparison. The nucleic acid sequence data are available from GenBank under accession number AF093858-76.

![FIGURE 4.](image)

The deduced TCR Vα and Vβ CDR3 region amino acid sequence of the CD1-reactive T cell clones. The nucleic acid sequence of the CDR3 regions of the Vα and Vβ chains were determined as described in the methods. The Vβ DWG shared motif is boxed and a similar sequence, EI, is underlined. The invariant Vα14-Jα281 TCR α-chain is shown for comparison (V141281). *The TCR Vα and Vβ sequences for the T cell clones 14S.3, 14S.4, and 14S.7 were identical with each other; only one sequence is shown for comparison. The nucleic acid sequence data are available from GenBank under accession number AF093858-76.

![FIGURE 5.](image)

Nucleic acid sequence of the TCR Vβ CDR3 region of the CD1 reactive T cell clones. The underlined sequence is the junctional sequence between the end of the germline-encoded V segment and the beginning of the germline-encoded J segment and includes the D segment and the N nucleotide additions. The portion of the VDJ junctional sequence that is encoded by the germline-encoded TCRBD2 segment is shown in bold type. The nucleic acid sequence that encodes the shared motif is enclosed by a box. The germline sequence of the TCRBD2 segment is shown for comparison. *The TCR Vα and Vβ sequences for the T cell clones 14S.3, 14S.4, and 14S.7 were identical with each other; only one sequence is shown for comparison.
have been selected for reactivity with a serum Ag or an endogenous Ag present in RMA-S cells. Because neither the nature nor the diversity of endogenous Ags that CD1 presents is known, it is not possible from our analysis to ascertain the significance of this motif occurring in >60% of our Vβ chain sequences. However, the location of these three amino acids in the CDR3 loop suggests that they may play a role in the recognition of the CD1d1 backbone or some structural feature shared by self ligands for both Vα14+ and Vα14+ TCRs.

The derivation of T cell clones provided opportunities to characterize the function of CD1d1-reactive T cells in ways that are not possible when T cell hybridomas are studied. CD1-restricted NK1.1+ T cells produce both IL-4 and IFN-γ and have been implicated as important regulatory T cells (37). Although variable levels of IL-4 were produced by the T cell clones described herein, the non-Vα14+ T cells were most notable for their production of substantial amounts of IL-10 and IFN-γ upon activation. Although the functional impact of individual T cells producing both IL-10 and IFN-γ is not yet appreciated, a similar subset of human T cells specific for Borrelia burgdorferi have been recently identified in patients with chronic Lyme disease and may be relevant to the perpetuation of the disease (38). The capacity of CD1-reactive T cells to produce Th2-type cytokines may have important implications. Recent data from humans with type I diabetes mellitus correlated a marked reduction in the number of the Vα24-JoQ CD1d-reactive T cells and a diminution of IL-4 production by them with progression to disease (39). Paired siblings whose Vα24-JoQ T cells continued to produce IL-4 and who maintained high serum levels of IL-4 did not develop chronic diabetes despite having multiple risk factors. Systemic sclerosis and the lpr SLE mouse model have been shown to correlate with a selective reduction in the Vα24-JoQ T/Vα14 T cell subsets (40, 41). These results emphasize that CD1-reactive T cells are likely important regulatory and effector cells that may play a critical role in autoimmunity.

The CD1-specific T cell clones were efficient cytolytic T cells. Since these cells were CD4-CD8- or expressed variable cell surface levels of CD8αα homodimers, their cytotoxic function was independent of the expression of the CD8αβ coreceptor. Similar findings have been observed for several human CD4−CD8− T cell lines that are restricted by CD1 (42). The capacity of the murine T cell clones to produce large amounts of IFN-γ and specifically recognize and lyse CD1+ APC indicates a likely role in host defense or in hypersensitivity-mediated inflammatory reactions. These results demonstrate, that similar to the NK T cells, CD1-reactive T cells with diverse TCRs have the potential to be important regulatory and effector cells.

We suggest that the large repertoire of murine T cells capable of recognizing CD1 indicates that distinct endogenous Ags may be loaded by mCD1 which positively selects diverse T cells. In support of the latter hypothesis, the crystal structure determination of mCD1 suggests that the Ag-binding cavity of the molecule is not empty, but contains a single acyl chain (3). Recognition of α-galactosylacylphosphatidylcholine by Vα14+ NK T cells is restricted by mCD1 (15) and indicates that mCD1 has the ability to present foreign lipid Ags that are similar structurally to the Ags that have been shown to be presented by human CD1 (4, 6, 8, 9). The binding of α-galactosylacylphosphatidylcholine would occupy the dimensions of the CD1 binding pocket (15). In this context, the various TCRs competent to recognize mCD1 may reflect the potential for diverse lipid and glycolipid Ags to be presented. The direct recognition of mCD1 (in the absence of exogenous Ag) that has been reported, may in fact represent weak reactivity to endogenous Ags that has been augmented by the use of APCs that express greater than physiologic levels of mCD1 (e.g., mCD1 transfectant cell lines) and T cells that have a low threshold of activation (e.g., in vitro-cultured T cell clones and hybridomas). In support of this, the T cell clones described herein had a much weaker proliferative response to native APC such as splenocytes or thymocytes than to the CD1-transfected cell lines (S.M.B, unpublished observations).

The role of lipid Ags bound in the CD1d1 cavity in T cell recognition will have to await further characterization of the lipids presented by CD1d1. Our data show that the number and diversity of T cells reactive to CD1d1 are larger than previously expected, and the ability of these cells to function as potent cytotoxic T cells and to produce IFN-γ and IL-10 in addition to IL-4 emphasizes their likely importance in immune responses. The correlations of CD1-reactive T cell responses with murine autoimmune models and human diabetes point further toward their role in autoimmunity.

References


