A Naive-Like Population of Human CD1d-Restricted T Cells Expressing Intermediate Levels of Promyelocytic Leukemia Zinc Finger

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*J Immunol* published online 1 June 2011

http://www.jimmunol.org/content/early/2011/05/29/jimmunol.1100761
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Rare CD1d-α-galactosylceramide–specific T cells that do not express the invariant Vα24 chain of human NKT cells were recently identified after expansion in vitro with the lipid Ag, but their phenotype and frequency in vivo and lineage relationship with NKT cells could not be elucidated. By using a CD1d tetramer-based method to enrich these cells from fresh peripheral blood, we demonstrated their naive-like CD62LloCD45RO−CD4+ phenotype and relatively high frequency of ∼10−5 in several healthy individuals. Notably, these cells expressed the NKT lineage-specific transcription promyelocytic leukemia zinc finger (PLZF), indicating a developmental relationship with NKT cells and ruling out the possibility that they were conventional MHC-restricted T cells cross-reacting against CD1d-α-galactosylceramide. Although PLZF is known to direct the effector program of NKT cells, we show in this study that the naive-like cells expressed it at a significantly lower amount than NKT cells. Further, we present mouse studies demonstrating a sharp PLZF expression threshold requirement for induction of the effector phenotype. These findings directly demonstrate in vivo the existence of naive-like CD1d-restricted human T cells marked by intermediate levels of PLZF. The Journal of Immunology, 2011, 187: 000–000.
CD45RO−CD45RA−CCR7−CD161−CD4+ cell-surface phenotype for these Vα24-negative CD1d-αGalCer–specific T cells, apparently undistinguishable from conventional naive MHC class II-restricted T cells. Surprisingly, however, these cells expressed PLZF, the NKT lineage-specific transcription factor, indicating a lineage relationship with NKT cells and ruling out the possibility that they were merely conventional MHC-restricted T cells with a CD1d-αGalCer cross-reactive TCR. Interestingly, these naive-like CD1d-restricted T cells expressed significantly lower amounts of PLZF than NKT cells. Further studies in the mouse system established that a high threshold of PLZF expression was required for effector differentiation.

Thus, our studies establish the existence in human peripheral blood of naive-like CD1d-restricted T cells expressing intermediate levels of PLZF and provide strong support to the notion that CD1 molecules can select both naive and effector T cells during thymic development.

Materials and Methods

Human peripheral blood

Blood was drawn from 22–54-y-old healthy volunteers in accordance with a protocol approved by the University of Chicago’s Institutional Review Board.

Mice

PLZF−/− mice carrying an induced deletion of exon 2 of the Zbth16 gene (6) also in the B6 background were a gift from Dr. P.P. Pandolfi (Beth Israel Deaconess Cancer Center, Boston, MA). The previously described B6. PLZF-transgenic line #1797 (driven by the CD4 promoter) (4) was produced in the same injection series as the other founder lines #1960 and #2161. PLZF−/− mice carrying an induced deletion of exon 2 of the PLZF gene were also in the B6 background were a gift from Dr. P.P. Pandolfi (Beth Israel Deaconess Medical Center, Boston, MA)

Cell isolation and flow cytometry

CD1d-αGalCer tetramers were prepared as described previously (7, 8) using the αGalCer analog PBS-57 (8). Fluorochrome-labeled mAbs (clone indicated in parentheses) against human CD3 (HIT3A or UCHT1), CD4 (RPA-T4 or OKT4), CD8a (RPA-T8), CD45RA (HI100), CD62L (DREG-56), CD161 (DX12 or PH-3G10), CD197 (TG8/CRC7), Vβ2 (MP2D5), Vβ11 (C21), and Vα24 (Cl5) were purchased from BD Biosciences, Beckman Coulter, BioLegend, eBioscience, or Pierce Thermo Scientific.

A lineage relationship with NKT cells and ruling out the possibility that they were merely conventional MHC-restricted T cells with a CD1d-αGalCer cross-reactive TCR. Interestingly, these naive-like CD1d-restricted T cells expressed significantly lower amounts of PLZF than NKT cells. Further studies in the mouse system established that a high threshold of PLZF expression was required for effector differentiation.

Thus, our studies establish the existence in human peripheral blood of naive-like CD1d-restricted T cells expressing intermediate levels of PLZF and provide strong support to the notion that CD1 molecules can select both naive and effector T cells during thymic development.

In vitro expansion of CD1d-αGalCer–specific PBMC

All in vitro experiments were performed using RPMI 1640 medium containing 2 mM L-glutamine (Cellgro), 1 mM sodium pyruvate (Invitrogen), 1 mM nonessential amino acids (Invitrogen), 55 μM 2-ME, 8% FCS (Biowest), 2% AB human serum (Atlanta Biologicals), and penicillin-streptomycin (Sigma-Aldrich). A total of 2 × 10^6 freshly isolated PBMC were plated in 24-well plates at a density of 1 × 10^6 cells/ml and cultured in the presence of 100 ng/ml αGalCer analog PBS-57 (8) and 100 U/ml human IL-2. Cultures were split when necessary and fed every 3 to 4 d with fresh medium containing IL-2.

Generation of PBMC clones

CD3+CD1d-tetramer positive clones were single-cell sorted from PBMC of three healthy donors using a FACS Aria (BD Biosciences) or MoFlo (DakoCytomation) and plated in 96-well plates. Sorted single cells were stimulated with feeder cells consisting of irradiated (2500 rad) allogenic PBMC (50,000/well) and irradiated (5000 rad) EBV-transformed cells (5000/well) in medium containing 1 μg/ml PMA and 100 U/ml human IL-2. Proliferating clones were maintained by restimulation every 3 wk, and reactivity to CD1d-tetramer was verified by tetramer staining.

TCR sequencing

Total RNA was extracted from PBMC clones with a combination of TRIzol (Invitrogen) and the RNaseasy Micro Kit (Qiagen) and reverse-transcribed with Superscript III reverse transcriptase (Invitrogen) and oligo(dT).

PCR sequences were amplified with primers to CD3 mRNA using Platinum Taq DNA polymerase (Invitrogen), the αGalCer V region primer paired with a V region primer other than oVα24, as described previously (10). TCRβ sequences of Vβ11+ clones were amplified with the CB C region primer and a V region primer other than Vβ11, whereas the TCRγ sequences of Vγ11+ clones were amplified with the C region primer and Vβ11 (11). Following amplification, PCR products were gel purified and subcloned using the TOPO TA Cloning Kit (Invitrogen). Single colonies were inoculated, DNA-prepared, and sequenced by the University of Chicago DNA Sequencing Facility using a 3730xl DNA Analyzer (Applied Biosystems).

Sequencing results were aligned to known V and J genes using the IMGT VbaseTics V-QUEST program (http://www.imgt.org/IMGT_vquest/share/textes/) (12).

Quantitative real-time PCR

Total RNA was isolated from resting PBMC clones with a combination of TRIzol (Invitrogen) and the RNaseasy Micro Kit (Qiagen) and reverse-transcribed with random primers using the AffinityScript qPCR cDNA Synthesis Kit (Stratagene). Transcripts for human PLZF were quantified with primers spanning intron 3 (forward primer: 5′-TAGTTTGGGGAGGAGAATGC-3′; reverse primer: 5′-ACCGCAGTGCAGCAACAGAAAAGG-3′) and normalized to hypoxanthine phosphoribosyltransferase (forward primer: 5′-GAAAGGGTGGTTAATCTCTCATGG-3′; reverse primer: 5′-CTCTCCATGCTCTCTCTCTCTCTC-3′). Quantitative PCR was performed on an Mx3005p system (Stratagene) using Brilliant SYBR Green qPCR Master Mix (Stratagene).

Microarray

Total RNA was isolated from either resting (18 d following stimulation) or stimulated (3 d following stimulation) PBMC clones with a combination of TRIzol (Invitrogen) and the RNaseasy Micro Kit (Qiagen) and reverse-transcribed with random primers using the AffinityScript qPCR cDNA Synthesis Kit (Stratagene). Transcripts for human PLZF were quantified with primers spanning intron 3 (forward primer: 5′-TAGTTTGGGGAGGAGAATGC-3′; reverse primer: 5′-ACCGCAGTGCAGCAACAGAAAAGG-3′) and normalized to hypoxanthine phosphoribosyltransferase (forward primer: 5′-GAAAGGGTGGTTAATCTCTCATGG-3′; reverse primer: 5′-CTCTCCATGCTCTCTCTCTCTCTC-3′). Quantitative PCR was performed on an Mx3005p system (Stratagene) using Brilliant SYBR Green qPCR Master Mix (Stratagene).

Statistical analysis

The unpaired, two-tailed t test was used for all statistical calculations and performed with Prism (GraphPad).

Results

In vitro expansion of Vo24+ CD1d-αGalCer+ PBMC

Fig. 1 shows that, as previously reported (13, 14), a rare population of Vo24+ CD1d-αGalCer+ T cells, which is barely seen in whole PBMC, can preferentially expand upon stimulation with αGalCer in vitro and typically express a weaker tetramer staining than canonical Vo24+ NKT cells. Studies of 18 clones derived from PBMC clones with a combination of TRIzol (Invitrogen) and the RNaseasy Micro Kit (Qiagen) and reverse-transcribed with random primers using the AffinityScript qPCR cDNA Synthesis Kit (Stratagene). Transcripts for human PLZF were quantified with primers spanning intron 3 (forward primer: 5′-TAGTTTGGGGAGGAGAATGC-3′; reverse primer: 5′-ACCGCAGTGCAGCAACAGAAAAGG-3′) and normalized to hypoxanthine phosphoribosyltransferase (forward primer: 5′-GAAAGGGTGGTTAATCTCTCATGG-3′; reverse primer: 5′-CTCTCCATGCTCTCTCTCTCTCTC-3′). Quantitative PCR was performed on an Mx3005p system (Stratagene) using Brilliant SYBR Green qPCR Master Mix (Stratagene).

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NAIVE-LIKE CD1-RESTRICTED LYMPHOCYTES

FlowJo (Tree Star).

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from three healthy individuals (#1, #2, and #4) are presented in Table I as specific background for the fresh precursor characterization presented below. The findings confirmed the expression of a diverse set of Vα-chains, the overrepresentation of other components of the canonical NKT TCR, including the Jε18 segment and the Vβ11 chain, and the predominant CD4 phenotype.

MACS enrichment and characterization of fresh Vα24−CD1d-αGalCer+ PBMC

To directly examine the fresh precursors of these CD1d-αGalCer−specific cells in vivo, we used a method of MACS enrichment based on tetramer staining to identify the rare cells that bound the tetramers, yet lacked Vα24 (Fig. 2A). In four individuals (#1, #2, #3, and #4) examined, these cells could be sufficiently enriched from PBMC for meaningful analyses. They represented a variable proportion of the total tetramer-positive population, but were present at a relatively stable frequency between 4 × 10−5 and 1 × 10−3 among total CD3+ PBMC in repeat examinations over a time period ranging from 1 mo to 2 y (Table II). In contrast, as previously reported (15), the frequency of Vα24+CD1d-αGalCer+ NKT cells in circulating blood varied widely among these individuals, ranging between 1 × 10−4 and 5 × 10−3, although it remained stable in each individual over time.

In agreement with studies of cultured cells (Table I) (13, 14), the fresh Vα24+CD1d-αGalCer+ cells exhibited an overrepresentation of Vβ11, reaching 47−63% on average, unambiguously confirming that they were the bona fide counterparts of the Vα24-negative clones (Fig. 2A, Table II). In marked contrast with the Vα24+CD1d-αGalCer+ NKT cells, the majority of Vα24+CD1d-αGalCer+ expressed a CD45RA−CCR7−CD62Lhigh phenotype characteristic of naive lymph node-seeking T cells, and they conspicuously lacked the CD161 (NKR-P1A) receptor. Thus, although only a limited number of key surface markers could be examined due to the rarity of these cells, their predominantly naive phenotype stood in stark contrast with the effector phenotype of NKT cells. The Vα24-negative cells mainly expressed CD4, as previously reported for their in vitro-derived clones, although in some individuals, a substantial fraction was found to express CD8α (Table II).

PLZF expression in fresh Vα24−CD1d-αGalCer+ PBMC

The expression of the transcription factor PLZF in Vα24−CD1d-αGalCer+ cells was directly examined by intranuclear FACS staining. Because of the cell losses associated with the permeabilization/fixation procedure in samples containing few cells, the human Vα24−CD1d-αGalCer+ cells were FACs-sorted after MACS enrichment and mixed with Jε18−/− mouse fitter cells prior to intranuclear staining of PLZF. In three out of three individuals examined, PLZF expression was detected well above the background level of control CD4 cells (Fig. 2B, 2C). Notably, however, the amount of PLZF was significantly reduced by ~50% when compared side by side with the Vα24-positive NKT cells from the same individuals in the same experiments.

PLZF expression in cultured Vα24−CD1d-αGalCer+ PBMC

PLZF mRNA was quantitated in clones simultaneously derived from Vα24−CD1d-αGalCer+ (n = 16), Vα24+CD1d-αGalCer+ (n = 12), and control CD4 (n = 15) PBMC from the three individuals shown in Table I. Fig. 3A shows PLZF levels measured by quantitative RT-PCR in resting clones harvested 18 d after stimulation with PHA and IL-2. All CD1d-αGalCer+ cell clones, whether Vα24− or Vα24+, expressed high PLZF mRNA, whereas 13 out of 15 control CD4 cell clones derived from the same three healthy donors did not. As in the fresh cell experiments, the Vα24−CD1d-αGalCer+ clones tended to express less PLZF than the Vα24+CD1d-αGalCer+, although the 37% average difference did not quite reach statistical significance (p = 0.084). As shown below, this may be due to the natural variations of PLZF levels in cultured cells over time.

To test the stability of PLZF expression in culture, we examined a randomly selected set of clones 3 d after PHA and IL-2 stimulation when the cells were blasting or 18 d after when they were resting. In these experiments, the differences between the Vα24−CD1d-αGalCer+ and the Vα24+CD1d-αGalCer+ clones reached statistical significance in both the resting and stimulated states. Note, however, that the clones showed a uniform tendency to decrease their levels of PLZF by 2-fold on average after stimulation, as shown by the significant deviation from the diagonal in Fig. 3B, indicating that PLZF expression fluctuated according to the rested or stimulated status of cells in culture. These experiments were performed on clones derived immediately after the first round of PBMC stimulation with αGalCer in vitro, and it is not known whether the cultured cells would maintain PLZF expression after multiple rounds of stimulation.

![FIGURE 1. In vitro expansion of CD1d-αGC−specific PBMC.](http://www.jimmunol.org/DownloadedFrom/network.jpg)
Altough, these findings establish the existence of a T cell population with a naive-like phenotype and a diverse TCR repertoire specific for CD1d associated with the foreign synthetic Ag αGalCer. These cells also expressed PLZF, the master regulator of the effector phenotype in the innate-like NKT cell lineage, albeit at a reduced level.

Manipulation of PLZF levels in mouse T cells

Although seemingly modest, the 2-fold reduction of PLZF protein might explain the lack of effector phenotype in the Vα24−CD1d−αGalCer− cells by comparison with their Vα24+ counterparts. Indeed, in the mouse system, we observed haploinsufficiency for PLZF as mice heterozygous for exon 2 deletion (+/−) exhibited a ~5-fold reduction of NKT cell frequency compared with wild-type (WT) (Fig. 5A). Thus, <1% of transgenic CD4 cells expressed the naive CD62LhighCD44low phenotype compared with 80% of WT. In line 1963, where heterozygous expression of PLZF reached 25% on average of the level expressed in the heterozygous 1797 line, the CD62L/CD44 phenotype was comparable to WT. Interestingly, homozygous expression of the PLZF transgene in the same line, which induced a mean fluorescence intensity (MFI) equivalent to 34% of the level expressed in heterozygous 1797 line on average, brought about a very modest downregulation of CD62L and upregulation of CD44. In heterozygous 1960 mice, expression showed a variegate phenotype. One fraction expressed 62% of the PLZF levels of the heterozygous 1797 on average and displayed full conversion to a naive phenotype.

Table II. Frequency and phenotype of CD1d-αGalC+Vα24+ cells in vivo

<table>
<thead>
<tr>
<th>Individual No.</th>
<th>Population</th>
<th>CD3+ (%)</th>
<th>CD4+ (%)</th>
<th>CD6α+ (%)</th>
<th>CD45RA+ (%)</th>
<th>CD62L+ (%)</th>
<th>CCR7+ (%)</th>
<th>CD161+ (%)</th>
<th>Vj11+ (%)</th>
<th>Vj2+ (%)</th>
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</thead>
<tbody>
<tr>
<td>1</td>
<td>Tetr+Vα24+</td>
<td>0.012 ± 0.0038</td>
<td>ND</td>
<td>43</td>
<td>13 ± 3.4</td>
<td>43 ± 7.2</td>
<td>ND</td>
<td>60 ± 15</td>
<td>97 ± 2</td>
<td>0.093 ± 0.13</td>
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<tr>
<td></td>
<td>Tetr+Vα24+</td>
<td>0.0011 ± 0.00051</td>
<td>ND</td>
<td>26</td>
<td>73 ± 20</td>
<td>86 ± 5.2</td>
<td>ND</td>
<td>5.1 ± 2.6</td>
<td>63 ± 18</td>
<td>7.4 ± 3.7</td>
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<tr>
<td></td>
<td>Tetr</td>
<td>0.10 ± 0.0041</td>
<td>ND</td>
<td>41</td>
<td>72 ± 9.2</td>
<td>65 ± 11</td>
<td>ND</td>
<td>9.7 ± 6.6</td>
<td>5.1 ± 5.6</td>
<td>8.3 ± 0.08</td>
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<tr>
<td>2</td>
<td>Tetr+Vα24+</td>
<td>0.046 ± 0.018</td>
<td>ND</td>
<td>12</td>
<td>41 ± 4.5</td>
<td>6.8 ± 4.5</td>
<td>13</td>
<td>91 ± 3.1</td>
<td>97 ± 2.8</td>
<td>0.044 ± 0.068</td>
</tr>
<tr>
<td></td>
<td>Tetr</td>
<td>0.00011 ± 0.000011</td>
<td>ND</td>
<td>71 ± 5.9</td>
<td>16 ± 6.7</td>
<td>62 ± 16</td>
<td>61 ± 13</td>
<td>28 ± 3.4</td>
<td>59 ± 23</td>
<td>3.6 ± 1.5</td>
</tr>
<tr>
<td>3</td>
<td>Tetr+Vα24+</td>
<td>0.016 ± 0.0032</td>
<td>38 ± 3.3</td>
<td>31</td>
<td>14</td>
<td>35 ± 17</td>
<td>21</td>
<td>73 ± 2</td>
<td>100 ± 0.085</td>
<td>0.78</td>
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<tr>
<td></td>
<td>Tetr</td>
<td>0.00043 ± 0.000009</td>
<td>72 ± 4.8</td>
<td>28</td>
<td>64</td>
<td>79 ± 11</td>
<td>67</td>
<td>18 ± 17</td>
<td>63 ± 9.7</td>
<td>0</td>
</tr>
<tr>
<td>4</td>
<td>Tetr+Vα24+</td>
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<td>66 ± 1.5</td>
<td>32</td>
<td>58</td>
<td>66 ± 17</td>
<td>80</td>
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<td></td>
<td>Tetr</td>
<td>0.00033 ± 0.00033</td>
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<td>100</td>
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<td>86</td>
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<td>82</td>
<td>ND</td>
<td>ND</td>
<td>7.6</td>
<td>6.2</td>
<td></td>
</tr>
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</table>

The frequency of CD1d-αGalC+Vα24+ cells within CD3+ PBMC is shown for different individuals. Their phenotype is compared with those of CD1d-αGalC+Vα24+ and CD1d−αGalC− cells. Data shown as mean ± SD of two to four independent blood samples for each individual, except individual 4, who was examined only once.

ND, not determined; Tetr, tetramer.
FIGURE 3. CD1d-αGC<sup>−/−</sup> T cell clones express intermediate amounts of PLZF. PLZF mRNA expression was quantitated by quantitative RT-PCR (A) and microarray (B) experiments. A, Each data point represents the mean of triplicate PCR values of an individual resting clone harvested 18 d after stimulation with PHA and IL-2. Control (tetramer<sup>−</sup>) CD<sup>4</sup><sup>+</sup> clones, tetramer<sup>+</sup>Vo<sup>24</sup> clones, and tetramer<sup>−</sup>Vo<sup>24</sup> clones were derived from each of three individuals, and a total of 15, 16 and 12 clones of each respective phenotype are represented in the figure. B, Four to five randomly selected clones of each category were further analyzed by microarray 18 d poststimulation (resting) or 3 d after PHA and IL-2 (stimulated). The normalized expression levels of the 205883_at PLZF probe set from individual experiments were analyzed by microarray 18 d poststimulation (resting) or 3 d after PHA and IL-2 (stimulated). The differences in PLZF expression between tetramer<sup>−</sup>Vo<sup>24</sup> clones and tetramer<sup>+</sup>Vo<sup>24</sup> clones were statistically significant (p < 0.05) whether examined at the resting or activated state. Note that most of the dots fell below the diagonal: a linear regression of all data points had a slope of 0.503, significantly different from 1 (p < 0.0001), indicating downregulation of PLZF upon activation.

Discussion

We have used a tetramer-based MACS enrichment strategy to identify, enumerate, and characterize the rare Vo<sup>24</sup> CD1d-αGC<sup>−/−</sup> T cells circulating in human peripheral blood. The identification of these cells in fresh blood was unambiguously validated by their characteristic overuse of V<sub>B</sub>11, similar to their Ag-stimulated progeny recently described by others (13, 14).

Our study revealed novel features of profound significance for our understanding of immune responses to lipid Ags. First, these cells predominantly expressed a naive CD45RA<sup>+</sup>CCR7<sup>−</sup>/CD62L<sup>−</sup> phenotype that predicted recirculation properties of lymph node-seeking T cells. Such properties are a prerequisite for the initiation of adaptive-like immune responses in lymph node draining sites of microbial invasion and are consistent with studies documenting the adaptive-like dynamics of CD1-restricted mycobacterial lipid-specific responses in mice and guinea pigs (17, 18). The functional properties of these naive-like cells could not be assessed in this study because the tetramer-MACS method used for enrichment impaired the survival and expansion of cells in culture. Our Vo<sup>24</sup>-negative T cell clones expanded from cultures stimulated with αGalCer produced IL-4 and IFN-γ upon stimulation, similar to previous reports, but a similar profile was observed in control clones derived from CD1d-αGalCer-negative CD4 T cells or from Vo<sup>24</sup> NKT cells (not shown), consistent with the ability of human T cells to acquire a broad range of cytokines after in vitro culture. Thus, the cytokine properties of the naive-like cells remain to be determined.

Secondly, the naive-like cells expressed PLZF, the signature transcription factor that induces NKT cell development and effector differentiation. Our study revealed novel features of profound significance for our understanding of immune responses to lipid Ags. First, these cells predominantly expressed a naive CD45RA<sup>+</sup>CCR7<sup>−</sup>/CD62L<sup>−</sup> phenotype that predicted recirculation properties of lymph node-seeking T cells. Such properties are a prerequisite for the initiation of adaptive-like immune responses in lymph node draining sites of microbial invasion and are consistent with studies documenting the adaptive-like dynamics of CD1-restricted mycobacterial lipid-specific responses in mice and guinea pigs (17, 18). The functional properties of these naive-like cells could not be assessed in this study because the tetramer-MACS method used for enrichment impaired the survival and expansion of cells in culture. Our Vo<sup>24</sup>-negative T cell clones expanded from cultures stimulated with αGalCer produced IL-4 and IFN-γ upon stimulation, similar to previous reports, but a similar profile was observed in control clones derived from CD1d-αGalCer-negative CD4 T cells or from Vo<sup>24</sup> NKT cells (not shown), consistent with the ability of human T cells to acquire a broad range of cytokines after in vitro culture. Thus, the cytokine properties of the naive-like cells remain to be determined.

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demonstrated that substantial amounts of PLZF protein, estimated between 34 and 64% of the maximal levels observed in NKT cells, were required for effector conversion. Collectively, these findings suggest that, although PLZF may mark T cell lineages selected by ligands expressed on thymocytes, different levels can be induced with substantial consequences on the naive versus effector type of differentiation imparted.

The mechanisms governing the induction of different levels of PLZF remain unclear at present. It is conceivable that the levels may depend on the avidity of TCR–ligand interactions during thymic development. Although they lack Vα24 but often express Jα18 and Vβ11, the other components of the NKT TCR, the Vα24–CD1d-αGalCer+ thymocytes may have lower avidity than NKT cells for a common set of self-lipid Ags involved in their thymic selection. For example, they did not respond to iGb3 (14), a self-Ag recognized as a weak agonist by most human NKT cells (21).

The frequency of the naive recirculating CD1d-αGalCer–specific T cells was on the order of $1 \times 10^{-5}$ and was stable over repeated samplings in the individuals studied. This surprisingly high frequency, which is similar to or superior to estimates for MHC-peptide–specific T cells (22), may be explained by the existence of two germline gene segments, Jα18 and Vβ11, with intrinsic recognition properties for αGalCer and CD1d, respectively (23). As cells expressing PLZF represent a very small percentage of the naive human T cell repertoire, it is possible that the size of the naive CD1-restricted T cell population is very small compared with that of the MHC-restricted T cells. Alternatively, many CD1-restricted cells may express little or no PLZF, perhaps because they follow distinct developmental rules or because they are primarily selected by MHC ligands and cross-react against CD1.

Recent reports have begun to assess the frequency and phenotype of fresh CD1-restricted T cells based on their ability to exhibit self-reactivity against CD1-transfectants in vitro (24, 25), and one report further demonstrated their presence among PBMC with a naive CD45RA+RO− phenotype (25). Although these studies did not attempt to assess the expression of PLZF in fresh cells, studies of T cell lines and clones, however, may help evaluate the frequency of PLZF expression among CD1-restricted T cells, although it is unknown at present whether PLZF expression is a stable marker in long-term cultures. Mouse models transgenically expressing the human CD1 isotypes that are missing in mice may also prove particularly valuable to elucidate this important issue.

In conclusion, this study provides direct physical evidence for the existence of a sizable population of naive-like CD1d-restricted T cells that express a diverse TCR repertoire and recirculate in human peripheral blood. It suggests that at least a fraction of these cells may express PLZF at low or intermediate levels and therefore belong to a lineage distinct from most MHC-restricted T cells but related to NKT cells. The results also reveal the critical importance of a high threshold of PLZF expression for induction of the effector phenotype in T cells and raise the fundamental issue of the mechanisms regulating PLZF expression during T cell development.

Acknowledgments

We thank the members of the Bendelac laboratory for discussions, advice, and help at various stages of the study, the National Institute of Allergy and Infectious Diseases tetramer facility for CD1d tetramers, the University of Chicago Animal Resource Center, Core Flow Cytometry Facility, and DNA Sequencing Facility.
Disclosures
The authors have no financial conflicts of interest.

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