Tissue-Specific Recognition of Mouse CD1 Molecules

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Tissue-Specific Recognition of Mouse CD1 Molecules

Se-Ho Park, Jessica H. Roark, and Albert Bendelac

Although there is evidence that some members of the CD1 gene family may present particular types of foreign Ags, such as mycobacterial lipid Ags or synthetic hydrophobic peptides, to αβ T cells, most CD1 isoforms share the unusual property of being recognized by a high frequency of naturally autoreactive αβ T cells. In the case of mouse CD1.1 and its human counterpart CD1d, a significant fraction of the autoreactive T cells express semi-invariant TCRs. CD1.1-specific T cells have a restricted tissue distribution and very promptly secrete a large panel of potent cytokines, including IL-4 and IFN-γ, upon primary activation through their TCR, suggesting that they might regulate some immune responses in these tissues. We show here that their autorecognition of mouse CD1.1 is highly dependent upon the cell type in which CD1.1 is expressed. For example, some of these T cells only respond to CD1.1 expressed by splenic dendritic cells, some respond preferentially to cortical thymocytes, and others respond to splenic B cells. Tissue specificity of CD1.1 recognition is also observed with various cell lines transfected with CD1.1 cDNA. These results show that different CD1-specific T cells are expressed in different tissues and can be specifically recognized by autoreactive T cells. They suggest that CD1.1 may be naturally associated with a variety of self ligands that overlap only partially in different cell types.


The CD1 locus encodes β,µ-associated proteins that are distant homologues of MHC molecules. Up to five distinct genes have been identified in humans, and interspecies sequence comparison, especially of the α1 and α2 domains, has revealed the existence of two conserved subfamilies (1, 2). The CD1d family is represented in humans, mice, rats, and rabbits, and the CD1a,b,c,e family is represented in humans and sheep, but not in mice or rats. The observation that CD1 and MHC map to two paralogous regions of the genome suggests that they arose as a result of a chromosomal duplication that included the entire ancestor locus (3, 4). In contrast to classical MHC molecules, however, CD1 proteins are nonpolymorphic and are conserved across species. Therefore, they are not targets of either allogeneic responses or transplantation rejection, nor do CD1 molecules appear to act as restricting elements for responses to conventional Ags (5).

There is evidence, however, that human CD1b can sample the endosomal compartment using a targeting sequence encoded in its cytoplasmic tail (6, 7) and present unconventional Ags, such as mycobacterial cell wall mycolic acid and lipoarabinomannans (5, 8, 9), to αβ T cells. Screening of a random peptide phage display library indicated that mouse CD1.1, the homologue of human CD1d, could bind synthetic peptides of 14 to 24 amino acids with a defined hydrophobic motif and present them to αβ T cells (10). The crystal structure of CD1.1 revealed an MHC-like fold with a large hydrophobic Ag binding groove, which appeared to be empty in the protein sample produced in insect cells (11). Structure analysis suggested that although CD1.1 may be stably expressed as an empty surface molecule (10, 12), it should be capable, in theory, of accommodating ligands such as small peptides or lipids.

Although recent evidence points to the potential role of CD1 in presenting foreign Ags to T cells, many CD1-restricted T cells appear to be autoreactive (5, 13, 14). Whether this unusual finding reflects biases in culture conditions or some inherent property of the CD1 system is unclear and the answer may vary according to particular CD1 isoforms. However, recent studies in the mouse have uncovered an entire subset of CD1-autoreactive T cells (15), a large fraction of which express TCRs made of an invariant Vα14-Jα281 α-chain and polyclonal β-chains belonging mainly to the Vβ8 family (16–18). A similar subset using Vβ4-JαQ and Vβ11 exists in humans (16, 19–22). Such cells do not seem to require exposure to pathogens or foreign Ags to expand, as they are found in normal numbers in germfree animals and can be generated in fetal thymic organ culture or in fetal liver cell suspension culture (14, 15, 23). Both the mouse and human subsets coexpress receptors of the NK lineage and have a CD4-positive or CD4/CD8 double-negative (CD8αβ- in humans) phenotype (14). They are absent in mice with targeted inactivation of CD1 (24–26) (S.-H. Park, J. H. Roark, and A. Bendelac, manuscript in preparation). Other CD1-autoreactive T cells that do not have restriction of TCR α- or β-chain usage and may not coexpress NK receptors have also been found among the residual CD4 T cells of MHC class II KO mice (27). The frequency of CD1 autoreactivity in this population was also high, reaching 8%, compared with the 1% MHC class II autoreactive cells in mainstream CD4 T cells. Overall, the size of the CD1.1-autoreactive T cell population is quite significant, ranging from 0.5 to 2% of the lymphocyte population in the spleen to 20 to 40% in the liver or bone marrow (14). It is likely therefore that CD1 recognition by naturally autoreactive cells, which are numerous and secrete large amounts of influential cytokines, is bound to regulate the outcome of some important immune responses.

In this report we set out to investigate the natural CD1 ligands recognized by CD1-autoreactive mouse T cells. Combined results

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3 Abbreviations used in this paper: KO, knockout; NOD, nonobese diabetic; HSA, heat-stable Ag; DC, dendritic cells; PE, phycoerythrin; high, high level; low, low level.
from two previous studies had suggested that CD1 autorecognition by different T cells depended upon the type and the genetic background of the CD1-expressing cell (15, 27). However, it was not clear whether differences in T cell recognition were related to the expression of modified versions of the same protein, such as, for example, CD1/glycolipid complexes, or to a range of other possible factors. These included the expression of different membrane levels of CD1 by various cell types, as Abs were not available to monitor surface expression; CD1 gene polymorphism, as CD1 sequences were not available in the relevant mouse strains; and differential expression and recognition of CD1 isotypes, as the mouse CD1 locus encodes two genes, CD1.1 and CD1.2, with different patterns of mRNA expression (28). Although CD1.1 and CD1.2 are 95% identical, their products can nevertheless be distinguished by T cells (S.-H. Park and A. Bendelac, manuscript in preparation).

To address these confounding factors, we first generated CD1-specific mAbs (52) to define the levels of CD1 surface expression by different cell types using flow cytometry. We also took advantage of the existence of a natural CD1.2 mutant mouse strain, C57BL/6, in which we identified a mutation that predicts the absence of surface expression, thus considerably reducing the complexity of the system. Second, we derived a panel of CD1-autoreactive T hybridomas from splenic or thymic cells to study their CD1.1 recognition on various freshly purified CD1.1-presenting cell types as well as on a panel of CD1.1-transfected tumor lines. To our surprise, we found that a significant fraction of CD1-autoactive hybridomas, whether using a semi-invariant Vα14-Jα281/Vβ8 TCR or other diverse TCRs, displayed individual and unique patterns of CD1.1 reactivity. These results demonstrate that different CD1 ligands are recognized by individual T cell clones and may suggest that CD1.1 is naturally associated with a set of self ligands that varies considerably from one tissue to the other.

Materials and Methods

Mice

C57BL/6, B6 II−/− (29) (backcrossed 10 times to B6), C57BL/6.MHC II−/− (30, 31) (I-A−, b−/−; backcrossed eight times to B6), and C57BL/6.TAP1−/− (32) (backcrossed four times to B6), were maintained under specific pathogen-free conditions in our barrier facility. Other mouse strains were purchased from The Jackson Laboratory (Bar Harbor, ME), except NOD, which was obtained from Taconic Farms (Germantown, NY). CD1-congenic mice, referred to as B10.NOD-CD1 and NOD.B6-CD1 here, were gifts from Drs. Linda Wicker and Larry Peterson (Merck Research Laboratories, Rahway, NJ) and Charles Russell (Biofluids, Rockville, MD) enriched with 10% heat-inactivated FCS, glutamine, antibiotics, and streptomycin.

CD1.1 and CD1.2 cloning and sequencing

CD1.1 and CD1.2 cDNAs were PCR amplified using the following set of specific primers: CD1.1-5′, AAGCAGAAAGTGGGCAGGGCC; CD1.1-3′, GCAAGTACGCATTTCCAGTTG; CD1.2-5′, AGCAGAGTAAAGGAGGCCGG; and CD1.2-3′, CCTCACTGCCATCATGATG. Amplified products were subcloned into the pCR3 expression vector (Invitrogen, San Diego, CA), and sequences from multiple subclones were determined using the Sequenase 2.0 kit (Amersham, Arlington Heights, IL). Sequencing was also performed on total PCR products using the dSNA cycling sequencing system (Life Technologies, Gaithersburg, MD) and on genomic DNA.

Anti-CD1 mAbs

A large panel of anti-CD1 mAbs was generated and is described in detail elsewhere (52). Briefly, the mAb-secreting hybridomas were generated by fusion with the Sp2/0 myeloma of spleen cells from rats repeatedly immunized with various CD1.1-transfected cell lines or with mouse thymocytes. CD1 specificity was established by the appropriate pattern of staining of transfected cell lines, by the lack of staining of cells from CD1 KO mice, and by immunoprecipitation. All mAbs, except 15F7, cross-reacted with CD1.2, as judged by staining of RBL cells transfected with a B6 CD1.2 cDNA in which the frameshift mutation had been corrected. The mAbs 19G11 and 15F7 used in this study are rat IgG2bs that were purified over protein G-Sepharose and conjugated with biotin or were used pure in cell culture assays at a final concentration of 2 μg/ml.

CD1.1 transfected lines

The human C1R B cell (34), the rat RBL basophil leukemia (35), and the B6 mouse C57SV fibroblast (36) lines were transfected with 10 to 30 μg of linearized CD1.1 vector by the electroporation method using a Gene Pulser (Bio-Rad, Hercules, CA), and stable transfectants were selected by G418 treatment and cell sorting. By flow cytometry, transfected cells were found to express approximately 10 times more CD1.1 per cell than thymocytes.

T cell hybridoma generation

A new panel of T cell hybridomas was generated by fusion of cells stimulated for 4 days by anti-CD3 with BW5147Ab, as previously described (16). To enrich for CD1 specificity, the cell populations were obtained from C57BL/6.I-Aβ-deficient mice and depleted of HSA-positive and CD8-positive thymocytes with Ab and complement, and B220-positive and CD8-positive splenocytes by panning on Ab-coated plates, as previously described (37). Hypoxanthine-aminopterin-thymidine-resistant hybridomas were screened for IL-2 production upon stimulation with a 1/1 mixture of B6 spleen and thymus cells, and positive hybridomas were subclassed. CD1.1 specificity was determined by blocking IL-2 production with an anti-CD1 mAb. Hybridomas expressing the canonical invariant Vα14-Jα281 TCR α-chain were identified by PCR with primers for Vα14 and Jo281 as previously described (16).

Cell preparations and FACS analysis/sorting

Cells were stained for three-color FACS analysis with directly conjugated Abs obtained from PharmMingen (San Diego, CA); RA4–5 anti-CD4, 53.6.7 anti-CD8, RA3–6B2 anti-B220, M1/69 anti-HSA, and 2.4G2 anti-Fc receptor or made in the laboratory (33D1 anti-dendritic cell (DC)-specific surface marker). CD1 was surface stained with 19G11-biotin followed by streptavidin-PE (Caltag, San Francisco, CA). FACS analyses were performed on a FACScan (Becton Dickinson, Mountain View, CA). FACS sorting was performed using a Vantage (Becton Dickinson, Lincoln Park, NJ) equipped with dual (argon and dye) lasers.

Cell subset enrichment

For DC enrichment, spleen cell suspensions (2 × 10^7 cells/ml in culture medium, as described below) were allowed to adhere on plastic culture dishes (Falcon 3025, Becton Dickinson Labware) for 2 h at 37°C in a 5% CO2 incubator. Nonadherent cells were then removed with several washes and vigorous pipetting, and adherent cells were reincubated overnight. Cells that detached during the second culture period were recovered after centrifugation over a 50% Percoll gradient and contained >95% DC identified by morphology, expression of 33D1, and lack of staining with 2.4G2 (38). Splenic B cells, T cells, and thymocyte CD4^+ CD8^−, CD4^− CD8^+, and CD8^+ CD4^+ subsets were obtained by cell sorting after staining spleen cells with anti-B220-FITC and CD8-PE or staining thymocytes with anti-CD4-FITC and CD8-PE. HSA^bright and HSA^dim single-positive thymocytes were sorted after staining thymocyte suspensions with anti-CD4-PE, CD8-APC, and HSA-FITC. B cell-depleted spleen cells were obtained after panning (1 spleen equivalent in 10 ml of PBS-0.1% BSA) for 30 min at 4°C over plates (Falcon 1013) coated with goat anti-μ Ab (Southern Biotechnology Associates, Birmingham, AL) and were <5% B220 positive.

Ag stimulation of T cell hybridomas

Unirradiated whole thymocytes or splenocytes, purified cellular fractions (5 × 10^5 cells, except DC used at 3 × 10^5 cells, per well), or CD1.1-transfected cell lines (5 × 10^5 cells) were incubated in flat-bottom micro-wells in the presence of 5 × 10^5 hybridoma T cells in a final volume of 0.2 ml in a mixture of Click’s medium and RPMI (1:1) and corrected medium and RPMI (1:1) enriched with 10% heat-inactivated FCS, glutamine, antibiotics, and 5 × 10^{-5} M 2-ME. The IL-2 content of supernatants collected after an incubation period of 20 h was measured using the CTLL bioassay as previously described (39).

Results

CD1.1 expression by C57BL/6 thymocytes and splenocytes

In earlier studies, we found that three hybridomas derived from normal thymic NK1 T cells reacted to B6 thymocytes but only moderately to splenocytes (15, 39). Independently, Cardell et al.
derived seven CD1-specific hybridomas from the residual splenic CD4 cells of MHC II KO mice, and these reacted well to spleen cells, although no analysis of their reactivity to thymocytes was presented (27). Interestingly, two out of seven hybridomas did not recognize spleen cells from μMT KO (B cell-less) mice, and five of seven failed to respond to spleen cells from at least one genetically different strain of mouse. Although these observations could be explained by differential expression of CD1.1 vs CD1.2 in different cell types and/or by some degree of polymorphism, a more intriguing possibility raised by Cardell et al. was that such autoreactive T cells recognized modified CD1 molecules, for example associated with as yet undefined, tissue-specific coligands.

We set out to distinguish between these possibilities and determine whether CD1-autoreactive αβ TCRs may indeed recognize modified CD1 molecules. First, we sequenced CD1.1 and CD1.2 in some relevant mouse strains and discovered that the B6 strain harbors a frameshift mutation at the beginning of the fourth exon encoding the α3 domain of CD1.2, which is predicted to abolish surface expression (Fig. 1).

Next, we produced a panel of anti-CD1 mAbs. By flow cytometry, we then quantitatively analyzed the level of CD1.1 expressed by B6 thymic and splenic cells. Surprisingly, we found that a majority of spleen cells expressed a level of CD1.1 that was similar to or higher than that observed on thymocytes (Fig. 2). More detailed studies defined a hierarchy of CD1.1 expression with DC > B cells = double-positive thymocytes > single-positive thymocytes > mature peripheral T cells (see Footnote 4).

These results suggested that the poor recognition of spleen CD1.1 by our first series of NK1 T cell-derived hybridomas was not due to a lower level of surface expression on spleen, and therefore, that these T cells must discriminate qualitatively between CD1.1 expressed by B6 thymocytes and splenocytes.

Tissue-specific recognition of CD1.1

To examine this possibility, we derived a new series of hybridomas by fusing both thymic and splenic CD8-negative T cells obtained from B6.MHC II−/− mice with BW5147. Hypoxanthine-aminopterin-thymidine-resistant hybrids were screened for IL-2 secretion upon stimulation with a 1/1 mixture of thymocytes and splenocytes, and CD1-specific clones were identified by blocking their response with anti-CD1 mAbs. Nine of these new clones and three previously described ones (DN32.D3, DN32.F3, and 431.A11, derived from B6 thymic NK1 T cells) (15, 16) were used for further studies. Although some of the T cell hybridomas used the same invariant Vα14-Jα1a2 TR α-chain (Table I, left column), they differed in their TCR β-chain usage (15, 16) (data not shown).

When stimulated with thymocytes or splenocytes, the hybridomas displayed three main patterns (Table I). Four of 12 (DN32.D3, DN32.F3, TA.H1, and 431.A11) responded mainly to thymocytes (producing 7–36 times more IL-2 than with splenocytes), one (IC8.DC1) responded mainly to splenocytes (producing 16 times more IL-2 than with splenocytes), and seven responded to both thymocytes and splenocytes (IL-2 values within a threefold range). Thus, the results obtained with the extended T hybridoma panel demonstrated that spleen cells could be as good or even better CD1.1-presenting cells than thymocytes for some CD1.1-autoreactive T cells and confirmed that for other CD1.1-autoreactive T cells, thymocytes were the best stimulators, implying the existence of qualitative differences in CD1.1 presentation by different cell types. We confirmed that CD1.2 recognition is not involved in the B6 system by showing that autoreactive T cells were inhibited to respond to B6 thymocytes or splenocytes by blocking with 15F7 (Table I), a CD1.1-specific mAb (see Footnote 4).

Since the panel of T hybridomas included cells derived from thymus cells and others derived from spleen cells, it was possible to examine whether the tissue specificity of recognition correlated with the tissue of origin. Although a large proportion (58%) of hybrids reacted to both splenic and thymic cells, it is striking to note that all four thymocyte-specific clones (DN32.D3, DN32.F3, TA.H1, and 431.A11) were derived from the thymus, and that IC8.DC1, the single spleen cell-specific clone, was derived from the spleen. In other words, no spleen-specific hybrid was found among thymus-derived CD1.1-autoreactive cells, and conversely, no thymus-specific hybrid was found in the spleen cell-derived population. Although the dataset is relatively limited, it is strengthened by the fact that the CD1.1-autoreactive hybrids used in this study were selected with an unbiased screening assay testing for responsiveness to either thymic or splenic cells. The results suggest, therefore, that CD1.1-autoreactive cells that reside in a particular tissue may have accumulated or expanded in response to local CD1.1 presentation.

Fine tissue specificity of CD1.1-responding hybridomas

To investigate the basis of this tissue specificity, we purified some of the major cell types in thymic and splenic populations and compared their ability to stimulate eight of the hybridomas. To our surprise, the level of specificity increased further, in that six patterns now emerged (Table II). One hybridoma, IC8.DC1, which

FIGURE 1. Map of the CD1 locus with the position of the frameshift mutation in CD1.2. In the B6 strain, a single nucleotide (G) deletion at position 690 from the translation start site causes a frameshift near the 5′ end of the α3 exon of CD1.2. The sequences of B6 and 129 are aligned for comparison. E, EcoRI site.

FIGURE 2. CD1.1 expression by B6 thymocytes and splenocytes. Arrows point to the average level of expression of CD1.1 by indicated cell subsets (identified by multiparameter analysis). The level of CD1 expression by splenic DC was determined on purified DC recovered after overnight culture (see Materials and Methods).
Table I. Tissue specificity of CD1.1 recognition by T cell hybridomas

<table>
<thead>
<tr>
<th>IL-2 (U/ml)</th>
<th>Thymic Stimulators</th>
<th>Sclerotic Stimulators</th>
<th>TH/SPL stim ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>B6</td>
<td>B6.TAP</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Va14 No Ab 15F7</td>
<td>B6.TAP</td>
<td></td>
</tr>
<tr>
<td>Thymus-derived hybrids</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>DN32.D3</td>
<td>+</td>
<td>36 &lt;1 33</td>
<td>&lt;1</td>
</tr>
<tr>
<td>DN32.F3</td>
<td>+</td>
<td>19 &lt;1 20</td>
<td>&lt;1</td>
</tr>
<tr>
<td>TB.B2</td>
<td>+</td>
<td>4 &lt;1 3</td>
<td>&lt;1</td>
</tr>
<tr>
<td>TA.H1</td>
<td>+</td>
<td>7 &lt;1 11</td>
<td>&lt;1</td>
</tr>
<tr>
<td>TB.H1</td>
<td>+</td>
<td>11 &lt;1 8</td>
<td>&lt;1</td>
</tr>
<tr>
<td>431.A11</td>
<td>−</td>
<td>19 &lt;1 15</td>
<td>4</td>
</tr>
<tr>
<td>TB.A7</td>
<td>−</td>
<td>46 2 37</td>
<td>2</td>
</tr>
<tr>
<td>TB.D7</td>
<td>−</td>
<td>22 &lt;1 18</td>
<td>&lt;1</td>
</tr>
<tr>
<td>TC.B11</td>
<td>−</td>
<td>13 &lt;1 23</td>
<td>&lt;1</td>
</tr>
<tr>
<td>Spleen-derived hybrids</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1C8,DC1</td>
<td>−</td>
<td>14 &lt;1 11</td>
<td>2</td>
</tr>
<tr>
<td>SB.C12</td>
<td>−</td>
<td>6 &lt;1 6</td>
<td>&lt;1</td>
</tr>
<tr>
<td>SA.A5</td>
<td>−</td>
<td>3 &lt;1 6</td>
<td>&lt;1</td>
</tr>
</tbody>
</table>

* T hybridomas are designated + or − according to their utilization of Va14-Jα281. The calculated ratio of IL-2 released in the presence of thymocytes vs splenocytes is shown in the rightmost column and is representative of 3 to 5 experiments (bold values highlight the tissue-specific hybridomas). 15F7 is a CD1.1-specific rat IgG2b mAb used at 2 μg/ml. It specifically blocked CD1 recognition when compared with control rat IgG2b mAbs, and, conversely, did not impair activation of control non-CD1-specific T hybridomas (data not shown).

DN32.D3 predominantly responded to spleen cells, reacted only to purified DC, secreting up to 1500 U of IL-2, and was blind to other cell types, including splenic B cells or T cells, or to cortical or medullary thymocytes. DN32.D3 responded to cortical thymocytes, and although no reactivity was initially detected against whole spleen cells, a minimal response was obtained after removal of B cells, and exposure to purified splenic DC revealed a good response, equivalent to that against cortical thymocytes. 431.A11 responded to cortical thymocytes, but also exhibited some variable degree of reactivity to SP thymocytes. Purified immature (HSAhigh) SP thymocytes, which constitute 20 to 80% of SP cells and have not yet modulated CD1.1 expression (see Footnote 4), appeared to stimulate 431.A11 as efficiently as the DP cells, whereas they failed to stimulate DN32.D3 (Table III). Again, despite the lack of a strong response to whole spleen cells, reactivity of 431.A11 to DC could be revealed after exposure to purified DC (Table II). A fourth pattern of specificity was revealed for TB.H1 and TC.B11, which responded strongly to B cells and DC and poorly to cortical thymocytes (Table II). TB.A7 and TB.D7 also had a unique pattern of specificity, including DC, B cells, and cortical thymocytes. TA.H1, like 431.A11, responded to DC and cortical thymocytes, but poorly to B cells (Table II); however, unlike 431.A11, its recognition of CD1.1 was independent of the mouse genetic background (data not shown and see Fig. 3), implying that TA.H1 recognizes a sixth distinct CD1.1 ligand. Testing of these hybridomas against a panel of mouse, rat, and human cell lines transfected with CD1.1 confirmed the high level of tissue specificity, in that TA.H1 reacted to none, 431.A11 only responded to mouse fibroblast line C57SV, DN32.D3 responded to C57SV and also to the rat basophil leukemia cell RBL, and 1C8, DC1, TB.A7, TB.D7, and TC.B11 responded to the human C1R B cell line in addition to C57SV and RBL (Table IV). In summary, we obtained evidence for up to six distinct CD1.1 Ags by simply testing eight CD1.1-specific T cell hybridomas against a limited sample of CD1.1-presentation cells types.

Most hybridomas responded to thymocytes or splenocytes from a variety of mouse strains (not shown). However, one of them, 431.A11, only recognized thymocytes from the genetically related strains C57BL/6, C57BL/10, C58, C57BR, and C57L, but not from BALB/c, SJL, C3H, AKR, DBA/2, 129, New Zealand White, New Zealand Black, or NOD (Fig. 3). Again, this difference was not related to polymorphism of the CD1 genes themselves. We took advantage of the existence of reciprocal B6/TAP and NOD congenics for the Idd10 locus, which encompasses CD1 (33). Figure 3 shows that 431.A11 recognized thymocytes with the B10, but not the NOD, genetic background regardless of the origin of CD1.

Table II. Fine cell type specificity of CD1.1 recognition by autoreactive T cell hybridomas

<table>
<thead>
<tr>
<th>IL-2 (U/ml)</th>
<th>Thymus</th>
<th>Spleen</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Whole</td>
<td></td>
</tr>
<tr>
<td></td>
<td>DP</td>
<td>SP</td>
</tr>
<tr>
<td></td>
<td>B cell</td>
<td>depleted</td>
</tr>
<tr>
<td>Whole</td>
<td>1</td>
<td>0.5</td>
</tr>
<tr>
<td>B cell</td>
<td>48</td>
<td>159</td>
</tr>
<tr>
<td>DC</td>
<td>11</td>
<td>1000</td>
</tr>
<tr>
<td>1C8, DC1</td>
<td>54</td>
<td>45</td>
</tr>
<tr>
<td>DN32.D3</td>
<td>36</td>
<td>24</td>
</tr>
<tr>
<td>431.A11</td>
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<td>2</td>
</tr>
<tr>
<td>TB.H1</td>
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<td>2</td>
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</tr>
<tr>
<td>TB.A7</td>
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<td>9</td>
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<td>TB.D7</td>
<td>15</td>
<td>6</td>
</tr>
<tr>
<td>TA.H1</td>
<td>12</td>
<td>9</td>
</tr>
</tbody>
</table>

* DP, double-positive; SP, single-positive; DC, dendritic cells. Results are representative of 2 to 5 experiments.

Table III. Stage-specific recognition of thymic CD1.1 by T cell hybridomas

<table>
<thead>
<tr>
<th>IL-2 (U/ml)</th>
<th>Thymocytes</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>White</td>
</tr>
<tr>
<td></td>
<td>DP</td>
</tr>
<tr>
<td>DN32.D3</td>
<td>20</td>
</tr>
<tr>
<td>431.A11</td>
<td>13</td>
</tr>
</tbody>
</table>
TAP-independent recognition of CD1.1

The results described above strongly suggested that most CD1.1 molecules expressed at the cell membrane are recognized in association with a set of coligands. The results shown in Table I demonstrate, however, that all 12 CD1-specific hybridomas, whether using the invariant Vα14 TCR α-chain or other TCRs, recognized CD1.1 expressed by fresh thymocytes or splenocytes equivalently in the presence or the absence of TAP. This correlates with the fact that surface expression of CD1.1 by TAP-deficient cells is equivalent to that of TAP-sufficient cells (12, 27, 40, 41). By comparison, recognition of β2m-deficient cells, which express low to undetectable CD1.1, was completely abolished. The results confirm and extend previous observations (12, 27, 40, 41), clearly indicating that the peptide pool contributed by TAP does not influence T cell recognition of CD1.1, either because CD1.1 is protected from binding peptides in the endoplasmic reticulum or because TAP does not transport peptides with the size or sequence required to bind CD1.1 efficiently. Conversely, the fact that TAP-deficient cells, which express much less classical MHC class I molecules but similar amounts of CD1.1 on their surface, did not induce a higher level of activation of the CD1.1 autoautoreactive hybrids is noteworthy. It suggests that the CD1.1-autoreactive hybridomas, including those derived from NK1 T cells that express Ly49 receptors, are not susceptible to MHC class I-mediated inhibition of activation. This finding correlates with our observation that Ly49 receptor expression is lost upon fusion of NK1 T cells with BW5147 αβ⁺ (data not shown).

Discussion

Among the most intriguing aspects of the mouse CD1 and human CD1d systems are the high frequency of autoautoreactive T cells that respond to CD1d, the expression of semi-invariant TCRs and NK receptors by many of these cells, and their unique cytokine secretion properties. These properties are linked developmentally, since transgenic expression of the Vα14 TCR α-chain by mainstream developing thymocytes is sufficient to impart the NK1 phenotype and the IL-4 secretion potential (42). The expression of NK receptors appears to be a late, separate event in the thymic maturation process that requires expression of the common cytokine receptor γ-chain (43). In mice deficient for the common γ-chain, a typical IL-4 producer Vα14/Vβ8 population is found in the thymus, but is arrested at a pre-NK1 stage where it does not express NK receptors such as NK1.1 and Ly49. It is not found in the periphery, possibly because the lack of inhibitory NK receptors, which may be necessary to keep the TCR autoreactivity in check, prevents escape from negative selection. The control of NK1 T cell activation may therefore be a complex phenomenon that results from the balance between positive signals such as those mediated by TCR autorecognition of CD1 and possibly also by NK receptors such as NK1.1 (44) and by negative signals such as those transduced by members of the Ly49 family upon binding to MHC class I molecules. Thresholds and balance between these signaling pathways may be set in the thymic environment or, as suggested for true NK cells, may be flexible and calibrated in different tissue environments (45).

By showing that CD1.1 is constitutively expressed by peripheral APCs, notably splenic B cells and DC, and that the level of expression is stimulatory to a large fraction of CD1-specific T cell hybridomas, the present results suggest that a significant population of T cells residing in peripheral tissues may be constitutively triggered through their CD1-autoreactive TCRs. This is supported by the fact that all resident CD1.1-autoreactive cells were found to respond prominently to their own tissue of origin, whereas in some cases they did not respond to CD1.1-expressing cells of other tissues. Thus, resident CD1.1-autoreactive cells may have expanded or accumulated in response to previous local stimuli. Autoautoreactive responses may be down-regulated through the MHC class I-specific, inhibitory NK receptors expressed by many of these CD1-specific hybridomas, since engagement of MHC-specific inhibitory receptors can inhibit TCR-mediated activation (46–48). In addition, we have found that none of the T hybridomas used in this study expressed such NK receptors as NK1.1, Ly49A, C, or I (not shown) even though many originated from the fusion of T cells that did express these receptors. A similar observation was recently reported by Shimamura et al. (18). Together, these results suggest that the latent autoreactivity of NK1 T cells may be revealed in hybridomas, not because of inherent differences in activation thresholds, but because they have lost the suppressor arm of the activation control.

Table IV. Cell-type specificity of recognition of CD1.1-transfected lines

<table>
<thead>
<tr>
<th>IL-2 (U/ml)</th>
<th>C57SV-CD1.1</th>
<th>C1R-CD1.1</th>
</tr>
</thead>
<tbody>
<tr>
<td>RBL-CD1.1</td>
<td>7</td>
<td>32</td>
</tr>
<tr>
<td>DN32.D3</td>
<td>75</td>
<td>30</td>
</tr>
<tr>
<td>431.A11</td>
<td>&lt;1</td>
<td>14</td>
</tr>
<tr>
<td>TB.H1</td>
<td>9</td>
<td>ND</td>
</tr>
<tr>
<td>TCB11</td>
<td>18</td>
<td>44</td>
</tr>
<tr>
<td>TB.A7</td>
<td>57</td>
<td>40</td>
</tr>
<tr>
<td>TB.D7</td>
<td>10</td>
<td>39</td>
</tr>
<tr>
<td>TA.H1</td>
<td>&lt;1</td>
<td>&lt;1</td>
</tr>
</tbody>
</table>

* Similar results were obtained in 2 to 5 experiments. Untransfected cell lines did not stimulate T cell hybridomas (data not shown). ND, not determined.
Key to understanding the biology of CD1 is the question of the nature of the CD1 ligands recognized by T cells. There is evidence that CD1.1 may be expressed on the membrane as a stable empty molecule and also that it may bind hydrophobic peptides with a putative anchoring motif (10–12). In addition, sequence analysis has revealed two remarkable features that may govern crucial aspects of intracellular trafficking and Ag presentation. First, the intracytoplasmic tail of CD1.1 displays an endosomal targeting signal similar to that of human CD1b (6), and second, the CD1.1 leader peptide itself (14) displays the canonical CD1.1 binding motif elucidated by Castano et al. (10). These observations have led us to propose a model where the head (leader peptide) and the tail of CD1.1 play a role that is similar in essence to that of the C- and the N-termini of the invariant chain involved in the MHC class II pathway, protecting the groove from Ag loading in the endoplasmic reticulum and driving CD1.1 to sample Ags in the endosomal compartment (14). The model is compatible with the idea that CD1.1 may be expressed and recognized in association with a variety of self as well as foreign hydrophobic ligands of peptideic or lipidic nature.

The results presented in this paper support the existence of such ligands. Indeed, cell type specificity of antigenic recognition by T cells usually reflects the recognition of tissue-specific peptides (49), but might also be related to the presence of distinct lipid Ags (5, 7–9). Another possibility is the existence of variably glycosylated residues in CD1 that would alter the interaction with some TCRs, although it seems unlikely that they could generate such a high level of diversity. Costimulatory ligands differentially expressed on various cell types could also account for tissue specificity of activation, but they are unlikely to account for the present results for several reasons. Firstly, T hybridomas are notoriously less dependent than fresh cells on costimulation for activation (50); secondly, the level of diversity observed would require the existence and differential expression of several costimulatory ligands/receptors by both CD1.1-presenting cells and T hybrids; finally, since known costimulatory ligands and receptors are conserved and sometimes even work across species (51), they would not account for the fact that the 431.A11 hybrid only responds to CD1.1-expressing cells of a particular C57/C58 genetic background. The differential expression of MHC-specific inhibitory receptors by T hybridomas is also unlikely, since they do not seem to express most receptors encoded in the NK complex, and many of the cell types used in this study have the same MHC genotype. In addition TAP-deficient CD1.1-expressing cells stimulate T hybrids to the same extent as wild-type cells.

Thus, despite the fact that no successful elution of putative CD1-bound ligands has been reported to date, the explanation for the phenomenon of tissue specificity reported in this paper may well reside in the demonstrated ability of CD1.1 to bind synthetic peptides or in its putative ability to bind lipids. Furthermore, the fact that tissue specificity of recognition is also observed with T hybridomas using the invariant Vα281 rearrangement of NK1.1 T cells suggests that fine specificity differences can be imparted solely by the TCR β-chain.

Although there is no indication at the moment as to the nature of a putative, enigmatic set of natural peptides or lipids, the cross-species conservation of the CD1d isotype and of semi-invariant CD1-autoreactive TCRs suggests that these ligands share at least some conserved features. In addition, the high level of tissue specificity demonstrated by our experiments suggests that the ligands expressed in some cell types only partially overlap. Tissue specificity may be more apparent in those cell types that express relatively lower amounts of CD1.1, because ligands would compete for CD1.1 binding, whereas DC that express higher levels of CD1.1 also seem to express a fuller set of CD1 ligands.

It remains unclear whether tissue-specific CD1 ligands elicit distinct functional subsets of autoreactive T cells, or whether their diversity has little functional significance but is merely a reflection of the fact that the self Ags associated with CD1.1 only partially overlap in different tissues. In any case, the results presented in this paper suggest that CD1.1 is naturally associated with self ligands and therefore imply that it might also present foreign ligands. Future studies aiming at identifying these putative self and foreign ligands associated with CD1.1 should provide major insights into the biology of the CD1 system and the pathway of immune regulation by CD1-autoreactive T cells.

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