Group 1 CD1 Genes in Rabbit

Sandra M. Hayes and Katherine L. Knight

*J Immunol* 2001; 166:403-410; doi: 10.4049/jimmunol.166.1.403

http://www.jimmunol.org/content/166/1/403

---

**References**
This article cites 45 articles, 19 of which you can access for free at:
http://www.jimmunol.org/content/166/1/403.full#ref-list-1

**Subscription**
Information about subscribing to *The Journal of Immunology* is online at:
http://jimmunol.org/subscription

**Permissions**
Submit copyright permission requests at:
http://www.aai.org/About/Publications/JI/copyright.html

**Email Alerts**
Receive free email-alerts when new articles cite this article. Sign up at:
http://jimmunol.org/alerts
Group 1 CD1 Genes in Rabbit

Sandra M. Hayes and Katherine L. Knight

CD1 is an Ag-presenting molecule that can present lipids and glycolipids to T cells. The CD1 genes were first identified in the human, and since then, homologs have been identified in every mammalian species examined to date. Over a decade ago, CD1B and CD1D homologs were identified in the rabbit. We have extended this earlier study by identifying additional CD1 genes with the goal of developing the rabbit as an animal model to study the function of CD1 proteins. We constructed a thymocyte cDNA library and screened the library with CD1-specific probes. Based on nucleotide sequence analyses of the CD1+ cDNA clones obtained from the library, we have identified two CD1A genes and one CD1E gene as well as determined the complete sequence of the previously identified CD1B gene. The CD1E+ cDNA clones lacked the transmembrane and cytoplasmic domains and, if translated, would encode for a soluble or secreted CD1E protein. In addition, expression studies demonstrated that the CD1 genes were expressed in peripheral lymphoid tissues as well as in skin, gut, and lung. Of interest is the finding that CD1A2, CD1B, and CD1E genes were found to be expressed by rabbit B cell populations. The rabbit, with a complex CD1 locus composed of at least two CD1A genes, one CD1B gene, one CD1D gene, and one CD1E gene, is an excellent candidate as an animal model to study CD1 proteins. The Journal of Immunology, 2001, 166: 403–410.

The immune system has evolved to cope with a diverse array of Ags. The presentation of peptide Ags by class I and II MHC molecules to CD8+ and CD4+ T cells, respectively, has been the paradigm for an Ag presentation system (1–3). However, not all Ags are peptides. Glycolipids and lipids can also serve as Ags. CD1 is an Ag-presenting molecule that presents such nonpeptide Ags.

The CD1 family represents a third lineage of Ag-presenting molecules that is distinct from the class I and class II MHC families (4). Unlike MHC molecules that display allelic polymorphism, CD1 molecules display little or no polymorphism between individuals of an outbred population (5). This conservation in CD1 structure suggests that CD1 has evolved to present Ags of limited structural variability (4).

The human CD1 locus is composed of five genes, designated CD1A, CD1B, CD1C, CD1D, and CD1E (6–8). The CD1 locus, which encompasses 190 kb, is located on chromosome 6 (9–11). Proteins for all the human CD1 genes, except the CD1E gene, have been identified (7, 8, 12). Like the MHC proteins, CD1 proteins have three extracellular domains, with the first and second domains binding Ag and the third domain binding to 2-microglobulin (6, 8, 12). The CD1 proteins can be divided into two groups on the basis of shared sequence homology (8). The group 1 CD1 proteins are CD1a, CD1b, and CD1c, whereas the group 2 CD1 protein is CD1d. The deduced amino acid sequence of the CD1E gene places it in an intermediate position between the two groups (8).

Even though group 1 and group 2 CD1 proteins differ in their amino acid sequences, these two groups share a similar tissue distribution and cellular expression. Human CD1 proteins are primarily expressed by cortical thymocytes, certain thymic leukemias, and APC (reviewed in Ref. 4). However, differential expression of the CD1 proteins is observed on some cell populations. For instance, CD1c and CD1d are the only CD1 proteins found on B cells (13, 14), CD1a is the predominant CD1 protein found on the Langerhans’ cells in the skin (15), and CD1d is the only CD1 protein found on intestinal epithelium (16).

Structural similarities with the class I and class II molecules along with expression on professional APC led Porcelli et al. (17) to propose that CD1 is an Ag-presenting molecule for human T cells. The first reported CD1-restricted αβ and γδ T cell clones recognized group 1 CD1 Ags on the MOLT-4 thymic leukemia cell line and on transfected cell lines (17). Human T cell clones that recognize CD1d on CD1d-transfected cell lines have also been identified (18). Subsequent studies demonstrated that group 1 CD1 molecules could present mycobacterial Ags to αβ T cells (19). The chemical nature of these mycobacterial Ags differed from that of the mycobacterial peptide Ags in that they were resistant to proteases. Fractionation of the mycobacterial extract and subsequent purification of the fractions showed that mycolic acid and lipoparabinomannan were two mycobacterial Ags presented by CD1b and CD1c to T cells (20–22). To date, no presentation of mycobacterial lipid or glycolipid Ags by human CD1d has been shown. However, human CD1d has been shown to present a synthetic glycolipid, α-galactosylceramide, to T cells (23, 24).

Because in vivo studies cannot be performed in humans, animal models are needed to study the function of CD1-restricted T cells. Mice are the only established animal model used to study the function of CD1-restricted T cells. Because mice lack group 1 CD1 genes (25), mice can only be used to study T cells restricted to the group 2 protein and not to the group 1 proteins. Therefore, another animal is needed as a model to study group 1 CD1-restricted T cells. CD1 genes or proteins have been found in sheep, guinea pig, pig, cattle, and rabbit (26–32). However, there are differences between humans and these animal species in the size and complexity of the CD1 genes. We have shown that the rabbit is an excellent animal model to study CD1 proteins.
of their CD1 loci. Sheep, with approximately seven CD1 genes, have had three CD1B homologs and one CD1D homolog cloned and sequenced (26, 27). In addition, a CD1E homolog has been identified by NH$_2$-terminal sequencing of a protein immunoprecipitated with a putative anti-CD1 mAb (27). The guinea pig, even with ~10 CD1 genes, does not display as much complexity as the human, in that the majority of cloned and sequenced CD1 genes are CD1B and CD1C homologs (28). The rabbit, with approximately eight CD1 genes, as estimated by Southern blot analysis, has had one CD1B and one CD1D homolog cloned and partially sequenced (32). Therefore, the rabbit is similar to the human in that it has both group 1 and group 2 CD1 genes and, by having multiple CD1 genes, has a potentially complex CD1 locus.

Because of these data, we chose to develop the rabbit as an animal model to study the role of group 1 CD1 molecules in protective immunity. To accomplish this, we first cloned and sequenced rabbit group 1 CD1 genes by constructing and screening a thymocyte cDNA library. Here we report the identification of two CD1A genes, one CD1B gene, and one CD1E gene. The identification of these three homologs combined with the previous identification of a CD1D homolog make the rabbit the only animal species in which four homologs of the five human CD1 genes have been identified.

Materials and Methods

CD1 probes

Human CD1A and CD1C probes were amplified by RT-PCR from human neonatal thymocyte RNA using the primer sets whose sequences are listed in Table I. The products were gel purified and radiolabeled with [32P]dCTP using a random hexamer primer system (33). A full-length rabbit CD1B probe was PCR-amplified from rabbit thymocyte cDNA using degenerate PCR primers (see Table I). The resulting PCR product was cloned into pGEMT Easy (Promega, Madison, WI), and its nucleotide sequence was determined using a random hexamer primer system (33). A full-length rabbit CD1B probe was PCR-amplified from rabbit thymocyte cDNA using degenerate PCR primers (see Table I). The resulting PCR product was cloned into pGEMT Easy (Promega, Madison, WI), and its nucleotide sequence was determined to confirm that it was rabbit CD1B. An EcoRI digest of the clone containing the full-length rabbit CD1B gene resulted in a 308-bp product containing the exons encoding the leader and α1 domains (radio-labeled and used to screen library) and a 694-bp product containing the exons encoding the α2, α3, transmembrane, and cytoplasmic domains (radio-labeled and used as a pan CD1 probe for Southern blot analysis of rabbit CD1 PCR products).

Construction and screening of DNA libraries

For the thymocyte cDNA library, poly(A) mRNA was purified from freshly isolated rabbit thymocytes using the QuickPrep Micro mRNA Purification Kit (Amersham Pharmacia Biotech, Piscataway, NJ). Synthesis of double-stranded cDNA and directional cloning of the double-stranded cDNA products were performed using the Superscript Plasmid System for cDNA synthesis and plasmid cloning (Life Technologies, Gaithersburg, MD). The resulting cDNA library was transformed into electrocompetent DH10B Escherichia coli cells and plated at a density of 7000 – 8000 colonies/150 mm plate for a total of ~80,000 colonies. The library was transferred to nitrocellulose membrane filters and screened with each of the three probes. The library filters were hybridized overnight with radiolabeled probe at 60°C in 6× SSC, 0.5% SDS, 5× Denhardt’s reagent, 10% dextran sulfate, and 100 μg/ml salmon sperm DNA. The nucleotide sequences of DNA from colony-purified CD1 clones were determined using primers specific for the flanking T7 and SP6 polymerase sites in the pSPORT vector. All sequencing was performed on the automated sequencer, ABI PRISM 310 Genetic Analyzer (Applied Biosystems, Foster City, CA).

An amplified rabbit partial MboI-digested genomic library in the phage EMBL4, previously constructed in our laboratory, was used in this study. The library (1 × 10$^8$ PFU) was plated with Escherichia coli (KHB03) cells and was screened as described for the thymocyte cDNA library. Plaque-purified CD1 probe clones were restriction mapped, fragments containing the CD1 gene or portions thereof were subcloned into pUC19, and the nucleotide sequence was determined.

Generation of rabbit CD1$^+$ transfectants

The cDNA for rabbit CD1B was subcloned into the expression vector pSFFV-gst, while those of rabbit CD1A1 and CD1A2 were subcloned into the expression vector pRES-neo (Clontech, Palo Alto, CA). Mouse myeloma SP2/0 cells were transfected by electroporation and selected with 1 μg/ml mycophenolic acid (Sigma, St. Louis, MO), pSFFV-CD1B (or with 1 mg/ml G418 (Calbiochem, San Diego, CA), pRES-CD1A1 and -CD1A2). Cells that survived selection were then screened with the panel of anti-CD1 mAbs to detect surface expression of rabbit CD1B proteins.

mAbs and flow cytometric analysis

The mAbs used in this study were anti-human CD1b (clone SN13/K5-1B; Ancell, Bayport, MN), anti-human CD1c (clone M241, Ancell; clone L161, Serotec, Raleigh, NC; clones F10/2A3.1 and F10/21A3, provided by Dr. Steven Porcelli, Albert Einstein School of Medicine, New York, NY), anti-human CD1a (clone NA1/34-HLK, Serotec; clone OKT6, provided by Dr. Steven Porcelli), anti-rabbit thymocyte (clones LAT-1, LAT-2, and LAT-3, provided by Dr. Stewart Sell, Albany Medical College, Albany, NY), anti-rabbit IgM (clone 367), and anti-rabbit MHC class II (clone 2C4). Polyclonal goat anti-mouse IgG Abs were affinity purified on a mouse IgG column, adsorbed against a rabbit IgG column, and FITC-conjugated for flow cytometric analysis or biotinylated for immunohistochemical analysis. One- and two-color flow cytometric analyses were performed as previously described (34). The cells were analyzed on a FACS Calibur (Becton Dickinson, Mountain View, CA) using CellQuest software (Becton Dickinson).

Immunohistochemical analysis

Ppopliteal lymph nodes from adult rabbits were embedded in OCT compound (Tissue-Tek, Torrance, CA), snap-frozen in liquid nitrogen, and stored at ~80°C. Frozen lymph node samples were sectioned (5–7 μm) and fixed in acetone for 3 min. The fixed sections were stained with the anti-rabbit CD1b mAb (LAT-3) and developed with biotinylated polyclonal goat anti-mouse IgG Abs, and avidin-biotin conjugates were conjugated with alkaline phosphatase (Vector, Burlingame, CA) and visualized with the alkaline phosphatase substrate, Vector Red (Vector). The sections were then counterstained with hematoxylin to visualize individual nuclei.

RT-PCR and Southern blot analyses

RNA was isolated from several adult rabbit lymphoid and nonlymphoid tissues, adherent and nonadherent splenocytes, and FACS-sorted IgM$^+$ lymph node cells using TRIzol (Life Technologies), according to the manufacturer’s directions, and amplified by RT-PCR with the rabbit CD1 gene-specific primer sets listed in Table I. The PCR conditions were as follows: 94°C for 30 s, 57°C for 30 s, and 72°C for 60 s for 28 cycles (for tissues) or 35 cycles (for cell populations). PCR products were separated by PAGE

<table>
<thead>
<tr>
<th>Gene</th>
<th>Sense Primer (5’→3’)</th>
<th>Antisense Primer (5’→3’)</th>
<th>$t_{an}$ a</th>
</tr>
</thead>
<tbody>
<tr>
<td>Human CD1A</td>
<td>AAAACAAAATCTGGTCTCGAGTT</td>
<td>CTTGTCACCTGATCCTCAAAGGAAGA</td>
<td>60</td>
</tr>
<tr>
<td>Human CD1C</td>
<td>TGGGCCAGCGTCCAGGGCTCA</td>
<td>TTACCTGATCTCAGGATAT</td>
<td>60</td>
</tr>
<tr>
<td>Rabbit CD1B</td>
<td>ATGCCTGCTTCTTGCCATTT</td>
<td>TCAT/CA/AGGAT/AGT/CCTGATAGA</td>
<td>54</td>
</tr>
<tr>
<td>Rabbit CD1A</td>
<td>ATGCTCGTCTGCTTTCTCC</td>
<td>CCATGCGGAGGATATGTCATG</td>
<td>57</td>
</tr>
<tr>
<td>Rabbit CD1A</td>
<td>ATGCTCGTCTGCTTTCTCC</td>
<td>TCACCTGAAAGGGGAAGGGAGGTA</td>
<td>57</td>
</tr>
<tr>
<td>Rabbit CD1E</td>
<td>ATGCTCGTCTGCTTTCC</td>
<td>CACCTGCAAGGACGAGTCCATG</td>
<td>57</td>
</tr>
<tr>
<td>Rabbit GAPDH</td>
<td>ATCAGAGTGGGATGTCTCTGTG</td>
<td>CTGCAGTCACCACTCTTCTGAG</td>
<td>64</td>
</tr>
</tbody>
</table>

a Annealing temperature used for primer set in PCR.

b Primer set sequences are based on previously reported nucleotide sequences: human CD1A (GenBank, M28825), human CD1C (GenBank, M28827), human CD1B (GenBank, M28826), and rabbit CD1B (GenBank, M26248).

Downloaded from http://www.jimmunol.org/ by guest on April 11, 2017
and transferred to nitrocellulose membranes (0.2-μm pore size; Schleicher & Schuell, Keene, NH). The blots were hybridized overnight at 56°C in 0.1 M SSC, 0.5% SDS, 5% Denhardt’s reagent, and 100 μg/ml salmon sperm DNA with a rabbit CD1B probe containing exon 4 (α3 domain). The blots were then washed twice at 56°C in 2× SSC and 0.1% SDS and once at 56°C in 1× SSC and 0.1% SDS. After the final wash, blots were air-dried and exposed to film. A GAPDH PCR (for primer sequences see Table I; 94°C for 30 s, 64°C for 30 s, and 72°C for 60 s for 25 cycles) was also performed to demonstrate that equivalent amounts of RNA were used for all tissues and cell populations.

**Results**

**Cloning and sequencing of rabbit group 1 CD1 genes from a thymocyte cDNA library**

Mammalian homologs of all human group 1 CD1 genes have been reported (26–29, 32). Comparison of the nucleotide sequences of these mammalian CD1 genes with those of human CD1 genes shows that there is more homology for a given CD1 isoform among all species than among all CD1 genes for a given species (reviewed in Ref. 4). Because of this conservation in the sequence of each CD1 isoform, we developed a cloning strategy to identify CD1A, CD1B, and CD1C homologs in the rabbit by constructing a thymocyte cDNA library and screening it with probes specific for each group 1 CD1 isoform.

To identify rabbit CD1A homolog(s), we used a human CD1A probe specific for the α1 domain, the most isoform-specific region of the CD1 molecule (8), to screen the thymocyte cDNA library. Using this probe, we identified two rabbit CD1A genes, which we termed rabbit CD1A1 and CD1A2. Comparison of the nucleotide sequences of these two CD1A genes with those of all human CD1 genes showed that these were indeed rabbit CD1A homologs (GenBank accession no. AF276977 and AF276978). In Fig. 1A the deduced amino acid sequences of rabbit CD1A1, rabbit CD1A2, and human CD1A are shown. There is 67% amino acid identity between CD1A1 and human CD1A, 70% amino acid identity between CD1A2 and human CD1A, and 71% amino acid identity between the two rabbit homologs. The major difference between the rabbit and human CD1A homologs is in the cytoplasmic domain (Fig. 1A). The cytoplasmic domain of human CD1A is composed of three amino acids, whereas the cytoplasmic domains of rabbit CD1A1 and rabbit CD1A2 are much longer, each with 14 amino acids. Recently, a CD1A homolog was identified in the pig, and analysis of the predicted amino acid sequence of this gene also displayed a cytoplasmic tail composed of 14 amino acids (29).

Within the cytoplasmic domain of pig CD1A is a serine-containing motif similar to one found in the cytoplasmic tail of MHC class I molecules (SD/EXSL; where X is any amino acid) (35, 36). A similar serine-containing motif is found in rabbit CD1A2, but not in CD1A1. This motif, SPNSL, is shown boxed in Fig. 1A. Therefore, rabbit CD1A1 and CD1A2 share structural similarities with pig CD1A.

**FIGURE 1.** Comparison of deduced amino acid sequences of rabbit and human CD1 genes. Dots depict amino acid identity, and spaces have been introduced to maximize homology. Arrows mark the boundaries of the structural domains. A, CD1A. The boxed area represents a serine-containing motif that is similar to one (SD/EXSL; where X is any amino acid) found in the cytoplasmic tails of MHC class I molecules. B, CD1B. The boxed areas represent a tyrosine-containing motif (YXXZ, where X is any amino acid and Z is a hydrophobic amino acid) found in human CD1B, CD1C, and CD1D molecules that is required for trafficking to the endosome. C, CD1E. The GenBank accession numbers for the rabbit CD1 genes are CD1A1 (AF276977), CD1A2 (AF276978), CD1B (AF276979), and CD1E (AF276980).
of exon 4, the exon that encodes for the cytoplasmic domains. Sequence analysis showed that the coding regions of these cDNA clones were terminated shortly after the end of exon 4, the exon that encodes for the α3 domain (Fig. 1C). We do not know whether this is a splice variant of a full-length CD1E gene or whether this is a CD1E gene encoding for a soluble product.

In summary, our cloning strategy led to the identification of three new rabbit CD1 genes, CD1A1, CD1A2, and CD1E, as well as the complete sequence of the previously identified CD1B gene.

Identification of rabbit CD1 genes from a genomic phage library

In addition to screening a thymocyte cDNA library, we screened a genomic phage library to obtain CD1 genes that may be expressed at low levels in the thymus, such as a putative CD1C gene. We screened the genomic phage library with a mixture of the CD1A-, CD1B-, and CD1C-specific probes. Using this approach, we isolated four CD1+ phage clones. Restriction map analysis of these phage clones revealed that they were nonoverlapping and contained distinct genes (Fig. 2). Southern hybridization of the restriction digests with the group 1 CD1-specific probes (5′ region) and the pan rabbit CD1 probe (3′ region) allowed us to locate and orient the CD1-coding regions on the restriction map (Fig. 2). The smallest restriction fragments that hybridized to the probes were then subcloned and sequenced to determine their identities. Nucleotide sequence analysis of the 1.5-kb HindIII fragment of phage 20B (Fig. 2A) demonstrated that it was identical with the rabbit CD1A1 gene identified from the thymocyte cDNA library (data not shown). Phages 18 and 19 (Fig. 2, B and C), even though their restriction maps differ, both contain CD1B genes with identical nucleotide sequences for the regions sequenced (5′ untranslated region, exon 1, intron 1, exon 2, exon 6, and 3′ untranslated region; data not shown). Furthermore, the nucleotide sequences of these CD1B genes are identical with the sequence of the CD1B gene isolated from the thymocyte cDNA library. The existence of these two clones may represent a gene duplication event that took place within the rabbit CD1 locus, an allelic polymorphism of the rabbit CD1B gene, or a library cloning artifact. They have been designated CD1B1 (phage 18) and CD1B2 (phage 19) in Fig. 2. Because we cannot identify which CD1B gene gave rise to the CD1B cDNA identified in the thymocyte cDNA library, we will refer to the cDNA species simply as CD1B. We determined by partial nucleotide sequence analysis of the 4.3- and 2.0-kb HindIII fragments that the fourth clone, phage 12 (Fig. 2D), contained the CD1E gene (data not shown). However, in the total 2 kb of DNA sequenced we were unable to locate exons 1 and 5 and, consequently, do not know whether the CD1E gene encodes for a soluble or transmembrane protein. Therefore, screening of the genomic library resulted in the identification of one CD1A gene (CD1A1), two CD1B genes (CD1B1 and CD1B2), and one CD1E gene.

Tissue distribution and cellular expression of rabbit CD1 homologs

Because of the relatively high degree of amino acid sequence identity between rabbit and human CD1 homologs (65–75%), we tested whether any anti-human CD1 mAbs cross-reacted with rabbit CD1 proteins to identify reagents that can be used to examine CD1 protein expression in rabbit tissues and cell populations. In addition, we tested a panel of anti-rabbit thymocyte mAbs to determine whether any of them recognized rabbit CD1 molecules. By screening these mAbs on rabbit thymocytes and CD1-transfected cell lines, we determined both cross-reactivity and specificity.

From the panel of anti-human CD1 mAbs tested (listed in Materials and Methods), only the anti-human CD1b mAb, K5-1B8, stained rabbit thymocytes and CD1B-transfected SP2/0 cells (Fig. 3, A and C). An anti-rabbit thymocyte mAb, LAT-3, also reacted

---

**FIGURE 2.** Partial restriction maps of four CD1+ phage clones obtained from a rabbit genomic phage library. A. Phage 20B, containing the CD1A1 gene. B. Phage 18, containing the CD1B1 gene. Detailed restriction mapping of the 3.7-kb BamH1XbaI fragment is shown in the inset. C. Phage 19, containing the CD1B2 gene. Detailed restriction mapping of the 3.7-kb BamH1XbaI fragment is shown in the inset. D. Phage 12, containing the CD1E gene. Mapping of the EcoR I sites in phase 12 is incomplete. Solid boxes represent the CD1 genes, and arrows represent orientation of the genes, shown here 5′ to 3′. Enzyme abbreviations are: R, EcoRI; B, BamHI; H, HindIII; S, SalI; SI, SacI; SII, SacII; X, XbaI; K, KpnI.

Even though a CD1B homolog has been identified in the rabbit, only the leader and α1 domains were sequenced (32). Therefore, we screened the cDNA library with a rabbit CD1B α1 domain probe to obtain the full-length cDNA of this rabbit CD1B gene and to identify any new rabbit CD1B genes. Because multiple CD1B homologs have been identified in both guinea pig (28) and sheep (26), we expected to identify more than one CD1B homolog in the rabbit. When we screened the thymocyte cDNA library with the rabbit CD1B probe, we identified seven CD1B+ cDNA clones. Sequence analysis of these clones demonstrated that they were all derived from the same CD1B gene. Four cDNA clones contained intron 1, two clones were mis-spliced to nucleotides >30 bp downstream from the beginning of exon 2, and one clone was the putative full-length cDNA (Fig. 1B and data not shown). Comparison of the deduced amino acid sequences of the full-length rabbit and human CD1B genes showed 76% amino acid identity between the two CD1B genes (Fig. 1B). A structural component of the human CD1b molecule that plays a role in intracellular trafficking is a tyrosine motif found in the cytoplasmic tail (37). This motif, YXXX (where X is any amino acid and Z is a hydrophobic amino acid), targets the CD1b molecule to the endosome, thereby allowing CD1b to sample exogenous lipids and glycolipid Ags (38). This motif is also conserved in rabbit CD1B and is shown boxed in Fig. 1B. Therefore, the rabbit CD1B homolog that we have identified is structurally similar to human CD1B.

To identify a CD1C homolog in the rabbit, we screened the thymocyte cDNA library with a human CD1C probe specific for the α1 domain. However, we identified not a rabbit CD1C homolog but, instead, a rabbit CD1E homolog. Comparison of the deduced amino acid sequences for rabbit and human CD1e is shown in Fig. 1C. There is 65% amino acid identity between rabbit and human CD1e. It is important to note that none of the three isolated cDNA clones encoded for the transmembrane and cytoplasmic domains. Sequence analysis showed that the coding regions of these cDNA clones were terminated shortly after the end of exon 4, the exon that encodes for the α3 domain (Fig. 1C).
ences in the activation or differentiation state of these B cells.

Peripheral blood and those in lymphoid tissues may reflect differences in CD1b expression between B cells in lymphoid tissues, such as Peyer's patch and mesenteric lymph nodes (data not shown). Therefore, B cells located in lymph node (Fig. 4A) as well as bright staining on large cells in the paracortex or T cell area (Fig. 4B). Because of their large size, location in the T cell area, and MHC class II expression, we believe these cells to be interdigitating dendritic cells.

To examine the tissue distribution and cellular expression of the other rabbit CD1 genes, we employed RT-PCR and Southern blot analyses. We isolated RNA from various lymphoid and nonlymphoid tissues and from purified cell populations and performed RT-PCR using rabbit CD1 gene-specific primers (Table I). The resulting PCR products were separated by PAGE, transferred to nitrocellulose membranes, and probed by Southern hybridization with a pan CD1 probe. Transcripts of all rabbit CD1 homologs were detected at high levels in all lymphoid tissues, especially the thymus (Fig. 5A). In addition, CD1A1 transcripts were detected in skin and gut, whereas CD1A2 and CD1E transcripts were detected in skin, lung, and gut. Of the all the genes, only CD1A2 was found in significant amounts in all tissues examined, including liver and kidney (Fig. 5A). Two different m.w. transcripts were observed for both CD1A genes. The higher m.w. transcript, when sequenced, represented the full-length cDNA, whereas the lower m.w. transcript represented splice variants in which the upstream region of exon 2 is missing (data not shown).

To determine which cell populations in the lymphoid tissues expressed the rabbit CD1A and CD1E genes, we performed RT-PCR and Southern blot analyses on purified cell populations. First, we fractionated splenocytes into adherent and nonadherent cell populations to examine whether the CD1 genes were expressed by myelomonocytic lineage cells (predominantly found in adherent fraction) and by lymphocytes (predominantly found in nonadherent fraction). In addition, we FACS-sorted IgM+ B cells, to determine whether any of the homologs were expressed by B cells. At this time, we are unable to examine the levels of CD1 transcripts in T cells, because the anti-T cell mAbs that are available also stain small numbers of other cell types. The results of this experiment are shown in Fig. 5B. All three CD1 homologs are transcribed in adherent and nonadherent splenocytes, albeit at different levels. This is not the case for expression in B cells, where CD1E is the predominant transcript detected (Fig. 5B). For the two CD1A genes, we detected the higher m.w. transcript of CD1A2, but neither species of CD1A1, in IgM+ B cells. Taken together, these findings suggest that, like CD1B, CD1A2 and CD1E genes are expressed by B cell populations in peripheral lymphoid tissues. However, not until specific mAbs are available will we know....
whether rabbit CD1A and CD1E transcripts are translated into their respective proteins.

**Phylogenetic analysis of mammalian CD1 genes**

In this study we identified four CD1 genes: two CD1A genes, one CD1B gene, and one CD1E gene. This means that, to date, CD1A, CD1B, CD1D, and CD1E homologs have been identified in the rabbit. To find out how the rabbit homologs compare with human CD1 proteins and most of the known mammalian CD1 homologs, we used the multiple alignment analysis program, CLUSTALX, to generate a phylogenetic tree (Fig. 6). The dendogram shows that indeed there is more homology for each CD1 homolog among different species than among all CD1 homologs for each species. This conservation in protein structure for each CD1 homolog may reflect conservation in both the type of lipids it presents to T cells and the mechanism by which it traffics through the cell to sample lipid Ags.

**Discussion**

In this study our goal was to identify group 1 CD1 genes in rabbit to develop this animal as a model to study the function of group 1 CD1 proteins. We chose the rabbit as a candidate for an animal model because previous studies have demonstrated that the rabbit has a complex CD1 locus, with approximately eight CD1 genes and has both group 1 (CD1B) and group 2 (CD1D) homologs (32). To identify additional group 1 CD1 genes, we screened a rabbit thymocyte cDNA library with probes specific for group 1 CD1 genes. As a result, we identified two CD1A genes and one CD1E gene and determined the full-length sequence of the previously reported CD1B gene.

Rabbit and pig are the only two mammals in which homologs of human CD1A have been identified (Ref. 29 and this study). Rabbit CD1a and pig CD1a have longer cytoplasmic tails (14 aa) than human CD1a (3 aa; Fig. 1A) (29). Within this longer cytoplasmic tail, there is a motif that is very similar to a serine-containing motif, SD/EXSL (where X is any amino acid), that is found in the cytoplasmic tail of MHC class I molecules (35, 36). Vega and Strominger (36) have shown that this motif is required for endocytosis and is similar to one found in other molecules that undergo endocytosis. In pig CD1a, the motif is CDPSS, where the first serine residue is replaced by cysteine, and the leucine residue is replaced by serine. We also found a similar motif in rabbit CD1a2, but not in CD1a1. This motif, SPNSL, shows a substitution of the D/E residue with proline (Fig. 1A). Therefore, rabbit CD1a2 and pig CD1a have extracellular domains similar to human CD1a molecules and intracellular domains similar to MHC class I molecules.

The significance of this motif is presently unknown, but one can speculate that it plays a role in recycling of the mammalian CD1a molecule, in marking the molecule for degradation, and/or in targeting the molecule to endosomes for sampling of lipid and glycolipid Ags.

Even though multiple CD1B homologs have been identified in sheep (26) and guinea pig (28), we were only able to identify one rabbit CD1B homolog from the thymocyte cDNA library. Interestingly, when we screened a genomic phage library, we isolated two phage clones containing CD1B genes. Restriction map analysis of these two phage clones demonstrated that they were indeed distinct genes (Fig. 2, B and C). However, nucleotide sequence analysis showed that they were identical for all regions sequenced. Because rabbits are maintained as outbred animals, these two genes may be allelic polymorphisms of the CD1B gene.
polymorphisms have been found for all human CD1 genes, and the polymorphic differences are usually located in exon 2 (39). Because we did not observe differences in the sequences of the genes but in the regions flanking the genes, the two phage clones may contain two CD1B genes and not two CD1B alleles. Due to their complete identity in the sequences of their introns and exons, these clones may represent a gene duplication event that occurred within the rabbit CD1 locus. Another possibility is that a cloning artifact occurred during the construction of the library, resulting in the addition of a fragment of DNA upstream of the CD1B gene in phage 19 (Fig. 2, B and C). Not until a physical map of the rabbit CD1 locus is generated will we know whether these CD1B+ phage clones represent allelic polymorphisms, distinct genes, or a cloning artifact.

We have successfully used CD1A- and CD1B-specific probes to identify rabbit CD1A and CD1B genes, respectively. However, when we used a CD1C-specific probe to screen the thymocyte cDNA library, we identified a rabbit CD1E gene rather than a CD1C gene. There is ~76% homology in the first 100 bp of exon 1 (α1 domain) of human CD1C and rabbit CD1E, which may explain why we isolated CD1E cDNA clones with the human CD1c α1 probe. A CD1E gene has also been identified in guinea pig (28), and it appears to be similar to the one in the human, in that it is transcribed in the thymus but is not translated into a protein. However, recent data in sheep demonstrate that a CD1E gene can encode for a functional protein (27). Rhind et al. (27) performed NH2-terminal sequencing on a CD1 protein that was purified using an anti-sheep CD1 mAb. Comparison of this amino acid sequence with those of human CD1 proteins resulted in the identification of a sheep CD1E homolog. Although we can identify rabbit CD1E transcripts in a variety of tissues and cell populations, future experiments are required to determine whether the rabbit CD1E gene encodes for functional protein.

Based on the deduced amino acid sequence of the CD1E+ cDNA clones, we predict that they encode for soluble and/or secreted forms of rabbit CD1e. Transcripts encoding for soluble molecules have been found at relatively high frequencies for human CD1A, CD1C, and CD1E genes (40). Using isoform-specific mAbs, Woolfson and Milstein (41) have shown that truncated CD1A and CD1C transcripts are indeedtranslated, and their protein products are detected in the culture supernatants of CD1A and CD1C transfectants. Even though secreted forms of HLA class I (41), class II (42), and HLA-G (43) molecules have been detected, little is known about their role in T cell development and function. Recent data suggest that these secreted Ag-presenting molecules have immunomodulatory roles, as demonstrated by the ability of soluble HLA class I molecules to induce apoptosis in alloreactive CTL (44) and mitogen-stimulated CD8+ cells (45). Future studies are required to determine the role, if any, that soluble CD1 molecules play in the development and function of CD1-restricted T cells.

Despite the conservation in the nucleotide sequences of human and rabbit group 1 CD1 isoforms, we observed a significant difference between human and rabbit in the expression of these CD1 isoforms in peripheral B cell populations. In the human, CD1c is the only group 1 CD1 protein expressed in peripheral B cell populations (14). However, in the rabbit we detected the CD1b protein on a subset of IgMlow B cells and a subset of IgMhigh B cells in secondary lymphoid tissues (Fig. 3E and data not shown). In addition, we detected CD1A2 and CD1E transcripts in FACS-sorted IgM+ lymph node cells (Fig. 5B). These data suggest that many of the rabbit CD1 isoforms are expressed by peripheral B cells. However, the rabbit is not the only mammal in which CD1 isoforms other than CD1c are expressed by B cells. Both pig CD1a (29) and sheep CD1e (27) are expressed by B cell populations. This expression of multiple CD1 proteins by rabbit B cells may be due to differences between the human and rabbit in the evolutionary pressures that shaped their CD1 loci. The amino acid differences in both the α1 and α2 domains and the cytoplasmic tails allow the human CD1 proteins to traffic to different cellular compartments and to bind different lipid and glycolipid Ags. Therefore, the expression of group 1 CD1 isoforms in rabbit B cell populations may ensure that different lipid and glycolipid Ags are being sampled from a variety of cellular compartments and then presented to T cells.

The majority of lipid and glycolipid Ags presented by group 1 CD1 proteins are derived from the cell wall of mycobacteria. Consequently, T cells that recognize these foreign lipid Ags may play a role in protective immunity to mycobacterial infection. Siegel et al. (46) have shown that there is a correlation between a strong cell-mediated response to M. leprae and the expression of group 1 CD1 proteins by dendritic cells in the dermis of leprosy patients. In lepromatous skin lesions, where there is a weak cell-mediated response, little or no group 1 CD1 expression was detected in the dermis. However, in tuberculoid skin lesions, where there is strong cell-mediated response, group 1 CD1 proteins were expressed at high levels in the dermis. These correlative data suggest that the expression of group 1 CD1 proteins by dermal dendritic cells is linked to protective cell-mediated immunity (46). The rabbit has been used as an animal model to study tuberculosis for almost 50 yr (47). In addition, rabbits are similar to humans in that they are relatively resistant to tuberculosis, and they develop both liquefied caseous and cavitary tuberculous lesions (48, 49). However, these lung lesions do not occur in mice and rarely occur in guinea pigs (48, 49). Therefore, by cloning and characterizing the rabbit group 1 CD1 genes, we have provided the foundation on which to study the regulation of CD1 gene expression and the role of group 1 CD1 proteins in mycobacterial infection.

Acknowledgments

We thank Ann Mathies and Marcia Credo for their assistance with the analysis of the genomic phage clones.

References


logy 100:37.


