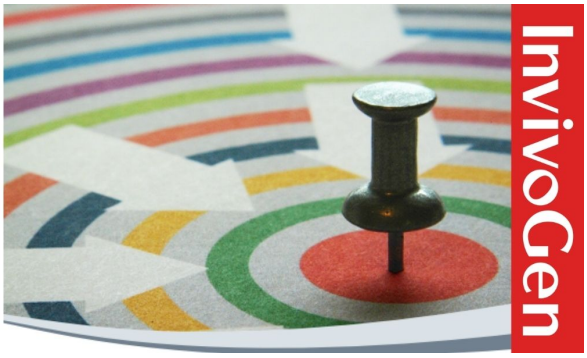

The Journal of Immunology
RESEARCH ARTICLE | JANUARY 15 1991

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S P Balk; ... et. al

J Immunol (1991) 146 (2): 768–774.
https://doi.org/10.4049/jimmunol.146.2.768

0022-1767/91/1462-0768$02.00/0
THE JOURNAL OF IMMUNOLOGY

Vol. 146, 768–774, No. 2, January 15, 1991
Printed in U.S.A.

ISOLATION AND EXPRESSION OF cDNA ENCODING THE MURINE HOMOLOGUES OF CD1[1]

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The cDNA encoding the murine CD1.1 and CD1.2 gene products were isolated and their complete nucleotide sequence was determined. The nucleotide sequence and genomic organization of these molecules were similar to human CD1. The sequences in the α1–α3 domains were almost identical to previously reported genomic clones from a different strain, indicating limited polymorphism amoung these molecules. The predicted amino acid sequence in the transmembrane region and in the cytoplasmic tail was identical for CD1.1 and CD1.2. The two cDNA were also homologous in the 5′ untranslated region but diverged in the 3′ untranslated region. In contrast to human CD1, which is expressed at high levels in thymus, the expression of CD1 message in murine thymus was not detected in either thymus leukemia Ag positive or negative strains. Cells expressing murine CD1.1 were generated after transfer of the CD1.1 cDNA into murine cell lines. Immunoprecipitation with a rat anti-mouse CD1.1 mAb showed that the transfected CD1 was expressed on the cell surface as a β2-microglobulin-linked heterodimer. These results demonstrate that the murine and human CD1 genes, although encoding homologous transmembrane glycoproteins, are expressed in distinct tissues and may serve different functions.

The human *CD1* locus encodes a family of cell surface glycoproteins that are structurally related to MHC class I molecules, but are only distantly related by sequence (1–16). Three human CD1 molecules have been identified by mAb, CD1a, b and c (1–6). The cDNA for a fourth molecule, CD1d, has been cloned (16), but no protein products for CD1d or for a fifth CD1 gene (13) have been identified. The CD1a-c molecules are expressed predominantly on immature cortical thymocytes (1–3). Outside the thymus, CD1a expression is limited to epidermal Langerhans cells (17), which also express lesser amounts of CD1c. CD1c is also expressed on a subpopulation of B cells (18, 19) and dermal dendritic cells (20, 21). The function of CD1 during thymocyte maturation or outside the thymus is unknown, but the recent identification of T cell clones specific for CD1 (22, 23) suggests that CD1 may serve as a ligand for some subpopulations of T cells.

CD1 was initially thought to be the human homologue of murine TL,[3] based on the similar MHC class I-like structure of these molecules and their expression on immature cortical thymocytes. Sequence analysis demonstrates, however, that CD1 is no more related to TL than to other MHC or MHC-like molecules (12, 13, 16). Two genes encoding the murine homologues of CD1 (termed CD1.1 and CD1.2) have been isolated and the exons corresponding to the α1-3 domains have been identified (24). The two murine CD1 genes are highly conserved and the exon encoding the α3 domain is conserved in the murine and human CD1 genes. The sequences of the exons encoding the α1 and α2 domains are less conserved and in these exons the murine CD1 genes are most similar to the human CD1d molecule (25). Transcription of the two murine CD1 genes has been detected in thymus, spleen, and liver (24), indicating a distinct tissue distribution compared with the human CD1a, b, and c molecules.

To further study the structure and function of murine CD1, the cDNA corresponding to the murine CD1.1 and CD1.2 genes were isolated. The complete nucleotide sequence of these cDNA and the genomic organization of the transmembrane region and cytoplasmic tail are presented here. Cells expressing the CD1.1 molecule were generated after transfer of the CD1.1 cDNA into murine cells. The transfected CD1.1 cDNA is shown by immunoprecipitation to be expressed on the cell surface in association with β2-microglobulin. The relationship between human and murine CD1 and the possible functions of these molecules are discussed.

MATERIALS AND METHODS

Library screening. A C57 × CBA F1 thymocyte cDNA library constructed in the λZAP vector was obtained from Stratagene (La Jolla, CA). The library was screened with a random oligonucleotide primed probe derived from the α2 and α3 exons of CD1d (16). Hybridizations were performed in a buffer containing 50% formamide/5× SSC at 42°C. The filters were washed in 0.2× SSC at 55°C. Positive phage plaques were purified by repeat screenings. The cDNA inserts in the pBluescript vector were isolated by helper phage rescue as described by the manufacturer (Stratagene). Sequencing was carried out with a modified T7 DNA polymerase (Sequenase, USB), using pBluescript sequencing primers and internal CD1 oligonucleotide primers.

Received for publication August 3, 1990.
Accepted for publication October 19, 1990.
The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked *advertisement* in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.
[1] This work was supported by Grant IM-289 from the American Cancer Society. S.P.B. was supported by a Physician Scientist Award CA-01310 from the National Institutes of Health. P.A.B. was supported by Grant AR07098 from the National Institutes of Health.
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[3] Abbreviation used in this paper: TL, thymus leukemia Ag; PCR, polymerase chain reaction.

Screening for CD1.2 cDNA by PCR amplification. Bacteriophage plaques were screened by PCR amplification (26) to identify CD1.2$^+$ clones. Plaques that were positive after primary screening were placed into 1 ml of phage dilution medium and 1 μl was amplified with primers specific for CD1.1 and CD1.2. The 5′ sense primer was from the 5′ end of the α1 domain (AATTACACCTTCCGCTGCCT) and the 3′ antisense primer was from the 3′ end of the α3 domain (CAGTAGAGGATGATATCCTG). Each 0.05-ml reaction mixture contained phage, 200 ng of each primer, 1.25 U of Taq 1 polymerase, 0.2 mM deoxynucleotide triphosphates and buffer supplied by the manufacturer (GeneAmp, Cetus Corp., Emeryville, CA). Five initial cycles were carried out at 94°C for 60 s, 55°C for 30 s, and 72°C for 90 s. The melting time was then shifted to 20 s for an additional 30 cycles and the polymerization time at 72°C was increased to 7 min in the final cycle. The PCR products were digested with *Acc*I, which cuts in the α2 domain of CD1.2 (nucleotides 647–652; see Fig. 2) but not CD1.1, and were analyzed by agarose gel electrophoresis to identify plaques containing CD1.2 cDNA.

Isolation of the 5′ end of CD1.2. A single-sided PCR amplification method was used to clone the 5′ end of CD1.2. One microliter of the λZAP thymocyte cDNA library was amplified with a CD1 antisense primer located in the α3 domain (CAGTAGAGGATGATATCCTG) and with sense primers overlapping the M13 sequencing primer (GACGTTGTAAAACGACGGCCAGT) or the reverse primer (CAGGAAACAGCTATGACCATGA). Each 0.05-ml reaction mixture contained 100 ng of the CD1 primer and the M13 or reverse primer and polymerase, nucleotides, and buffer as described above. The first five cycles were 94°C for 60 s, 55°C for 30 s, and 72°C for 4 min followed by 30 cycles at 94°C for only 20 s. One μl of the PCR product was then reamplified with an internal CD1 antisense primer located in the α2 domain (GCACTTTGATGGGCAAGT) and the M13 or reverse primer as described above except that all of the cycles were heated to 94°C for just 20 s. The polymerization step was extended to 7 min for the last cycle in all amplifications. The PCR products were digested with *Hin*dIII (which cuts in the α2 domain of CD1.1 and 1.2, nucleotides 686–691 in Fig. 2) or with *Acc*I (which cuts in the α2 domain of CD1.2 only at nucleotides 647–652, Fig. 2) and with *Eco*R1 and cloned into pUC 19. Positives were identified by colony hybridization using an internal oligonucleotide probe and were sequenced as above.

Cloning the 3′ end of the CD1.1 gene. One μg of C57BL6/J genomic DNA was amplified with a CD1 sense primer located in the α1 domain (AATTACACCTTCCGCTGCCT) and an antisense primer located in the 3′ untranslated region containing an added 5′ *Sal*1 site (CTGTCGACCTCTCCTTCATCCCCAGA). The 0.05-ml reaction mixture contained DNA, 200 ng of each primer and polymerase, nucleotides, and buffer as described above. Each cycle was 94°C for 60 s, 55°C for 30 s, and 72°C for 5 min for 25 cycles followed by an additional 7 min at 72°C. The PCR product was digested with *Hin*dIII (which cuts in the α2 domain, see above) and *Sal*1 and cloned into pUC 19. Positives were identified by colony hybridization with an internal oligonucleotide probe and were sequenced as above.

RNA analysis. RNA was isolated from thymocytes or whole thymus by extraction and precipitation in 3 M LiCl/6M urea (27). The RNA was separated on 1.2% agarose/formaldehyde gels and transferred to nylon membranes (Biotrans, ICN K & K Laboratories Inc., Plainview, NY). The blots were hybridized with a full length random oligonucleotide primed CD1.1 probe in a buffer containing 50% formamide/5× SSC at 42°C and were washed in 0.2× SSC/0.1% SDS at 65°C.

Generation of stable transfectants expressing CD1.1. The full length mt18 CD1.1 cDNA was excised from pBluescript using restriction sites in the polylinker (a 5′ *Xho*1 site and a 3′ *Bam*H1 site) and was cloned into the *Xho*1 and *Bam*H1 sites of the pSRα-neo expression vector (28). L cells were transfected using calcium phosphate and were selected in 0.5 mg/ml of G418. Northern blots of G418 resistant clones were analyzed to identify clones expressing high levels of CD1 message. BY155.16 cells (29) were transfected by electroporation and were selected in 4 mg/ml G418.

Antibodies and immunoprecipitation. The CD1.1 transfected cells were used to immunize rats and screen for rat anti-mouse CD1 hybridomas; the details of the mAb production and characterization are presented in a separate report by Bleicher et al.[4] One of the two antibodies produced, 1H1, was used here for immunoprecipitation and Western blotting. CD1.1 expressing and control BY155.16 cells were surface labeled with ^{125}I and lactoperoxidase. The cells were lysed in an 0.5% NonidetP-40 buffer. The lysates were precleared with normal rat serum bound to protein A-Sepharose beads and then immunoprecipitated with the 1H1 antibody bound to protein A-Sepharose. The immunoprecipitates were analyzed by SDS-PAGE on a 12.5% gel under nonreducing conditions.

Western blotting. An unlabeled lysate from CD1.1 transfected BY155.16 cells was precleared with protein A-Sepharose beads and then immunoprecipitated with a murine anti-β2-microglobulin mAb (NEI-026, NEN (30), directed against one of the two murine β2-microglobulin alleles). The immunoprecipitate was electrophoresed, transferred to nitrocellulose, and the membrane was blocked with 5% non-fat dry milk/PBS. The blot was then incubated with the 1H1 antibody (1/100 dilution of ascites in 5% milk/PBS), washed extensively, and incubated with ^{125}I-protein A (0.5 μCi/ml in 5% non-fat dry milk/PBS, Amersham Corp., Arlington Heights, IL). The blot was again washed extensively and exposed.

RESULTS

cDNA isolation. Murine thymocyte cDNA libraries were screened with a probe containing the conserved α3 domain of the human CD1d cDNA (see *Materials and Methods*). A thymocyte cDNA library constructed from a C57 × CBA mouse in the λZAP vector (Stratagene) yielded eleven independent positive clones. Partial sequencing showed that all of these corresponded to the murine CD1.1 gene (24). The complete nucleotide sequence and predicted amino acid sequence of the longest clone (mt18) was determined (Fig. 1).

The length of the mt18 clone, excluding the poly A tail, was 1838 bases. This cDNA contained a 91-bp 5′ untranslated region followed by the predicted initiation ATG. The nucleotide sequence of the leader peptide and the exons encoding the α1–α3 domains (through base 982) were identical to the genomic CD1.1 sequence reported previously from BALB/cJ (24). The open reading frame continued from nucleotide 983 to 1099 and encoded the last four amino acids of the α3 domain (DARQ), a hydrophobic transmembrane region and a 10 amino acid cytoplasmic tail (Fig. 1). This sequence indicates that murine CD1.1 is a transmembrane protein with a short cytoplasmic tail, similar to the human CD1 molecules.

The 3′ untranslated region was 736 bp long with a polyadenylation signal at position 1818. One significant feature of the 3′ untranslated region was a 30 bp GT repeat (1583–1612) followed by a 10 bp CA repeat (1613–1622), which may form a hairpin loop structure. There was sequence homolgy between CD1.1 and human CD1 in the 5′ untranslated region (see below) but not in the 3′ untranslated region.

None of the cDNA clones isolated initially corresponded to the murine CD1.2 gene. The cDNA library was therefore rescreened and PCR amplification was used to identify CD1.2$^+$ clones (see *Materials and Methods*). A single-sided PCR amplification method was also used to isolate the 5′ end of the CD1.2 cDNA (see *Materials and Methods*). These cDNA were used to derive the complete nucleotide sequence of CD1.2 (Fig. 2).

The murine CD1.2 contained a 5′ untranslated region of 293 bp followed by the predicted initiation ATG at nucleotide 294. The nucleotide sequences of the leader peptide through the exon encoding the α3 domain (ending at nucleotide 1184) were identical to the previously reported BALB/cJ genomic sequence (24), except for nucleotide 484 (C in this cDNA resulting in a threonine instead of an isoleucine). The open reading frame continued from nucleotide 1185 to 1301 and encoded the last four amino acids of the α3 domain, the transmembrane domain and a 10 amino acid cytoplasmic tail. The nucleotide sequence in this region (1185 to 1301) was iden-

[4] **Bleicher, P. A., S. P. Balk, S. J. Hagen, R. S. Blumberg, T. J. Flotte, and C. Terhorst.** 1990. Expression of murine CD1 on gastrointestinal epithelium. *Science 250:679.*

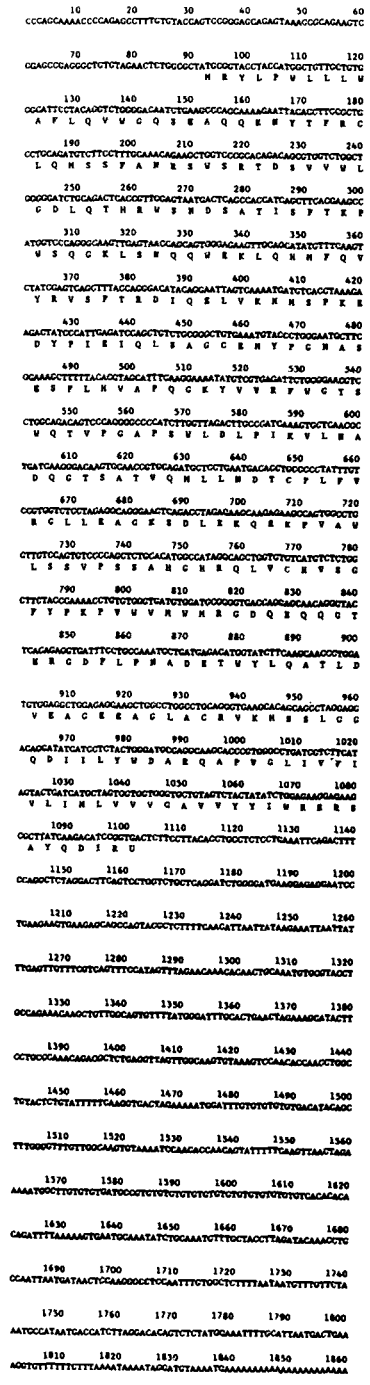

Figure 1. Nucleotide sequence and deduced amino acid sequence of the murine CD1.1 cDNA.

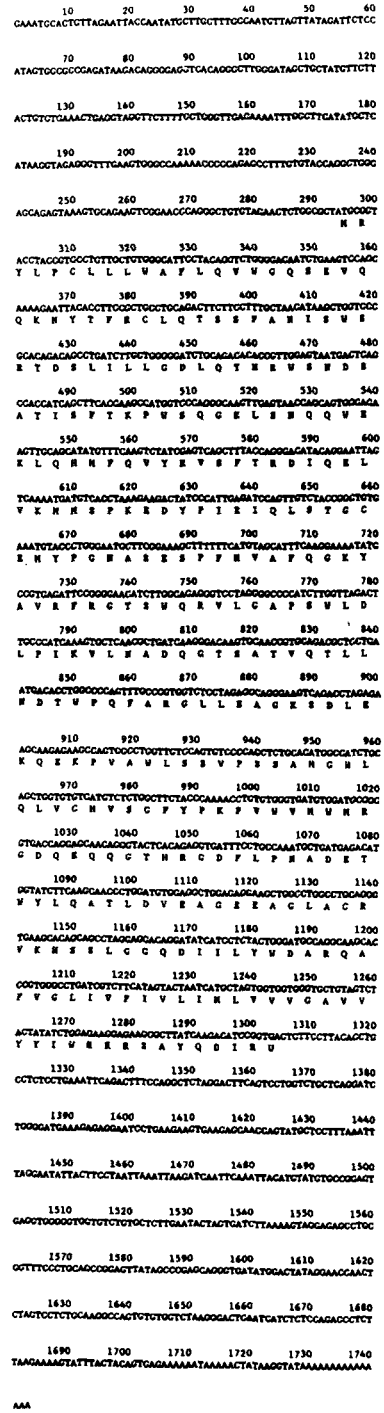

Figure 2. Nucleotide sequence and deduced amino acid sequence of the murine CD1.2 cDNA.

tical between CD1.1 and CD1.2, with the exception of a silent nucleotide difference at position 1229 (G to A). The homology between CD1.1 and CD1.2 extended to the 5′ untranslated region (see below) and the first 120 bases of the 3′ untranslated region, but the sequences in the 3′ untranslated region then diverged. A polyadenylation signal was identified at nucleotide 1709.

Genomic organization. Previously isolated genomic clones were used to identify the exons encoding the α1–α3 domains of murine CD1 (24). To determine the complete intron/exon organization of the murine CD1.1 gene, a genomic CD1.1 clone encoding the α3, transmembrane, and cytoplasmic domains was isolated. Oligonucleotide primers and the polymerase chain reaction were used to amplify and clone this region from C57BL/6J genomic DNA (see *Materials and Methods*). The last four amino acids of the α3 domain (AspAlaArgGln), the transmembrane region and the first four amino acids of the cytoplasmic tail (ArgArgArgSer) were encoded on one exon (Table I). The remainder of the cytoplasmic tail was encoded by a sixth exon.

Sequence homology with human CD1d. It was shown previously that the human CD1d molecule diverged from CD1a, b, and c in the α1 and α2 domain exons and in the 5′ untranslated region (16). A comparison between the

TABLE I

Genomic organization of the exons encoding α3, transmembrane (TM) and cytoplasmic (C) domains of the murine CD1.1 molecule

Intron	Exon		Intron
		Leu Tyr Trp A	
	α3	CTC TAC TGG G	gtaagaaa
	sp Ala Arg Gln	Arg Arg Arg Se	
caaaccag	AT GCC AGG CAA	α3/TM/C AGA AGG AGA AG	gtaagtct
	r Ala Tyr Gln		
tcttccag	C GCT TAT CAA	C/3′UT	

human and murine CD1 genes in the α1 and α2 domains demonstrated that the murine CD1 were most homologous with human CD1d (25). Figure 3 shows that the sequence homology between murine CD1 and CD1d extends to the 5′ untranslated region.

Thymic expression of CD1. The TL antigens were previously considered to be the murine homologues of CD1 based on their MHC class I-like structure and their expression on immature thymocytes in some strains of mice. TL is expressed on thymocytes from BALB/cJ mice and this strain has been shown, by RNase protection analysis, to express only low levels of CD1 RNA in thymus (24). To determine whether the thymic expression of CD1 differed in TL^+ or TL^- strains, Northern blot analysis was carried out on thymocytes from a series of strains. CD1 message was not detectable by this method in thymocyte RNA from the TL^- strain, C57BL/6 (Fig. 4) nor in any other strain tested (data not shown).

Murine CD1 is expressed on cell surface as β2-microglobulin linked heterodimer. The murine CD1.1 cDNA was cloned into the pSRα-neo expression vector (28) and stable transfectants were established. Immunoprecipitations were carried out to determine whether the CD1.1 molecule was expressed on the cell surface in association with β2-microglobulin. A rat anti-mouse CD1.1 mAb, 1H1 (see *Materials and Methods*) immunoprecipitated a heavy chain of about 48 kDa and a L chain of about 12 kDa from the cell surface of CD1.1 transfected cells (Fig. 5*A*). This molecular mass was consistant with a 36-kDa polypeptide backbone predicted from the nucleotide sequence and five potential sites for N-linked glycosylation (Fig. 1).

To confirm that the CD1.1 associated light chain was β2-microglobulin, a murine anti-β2-microglobulin antibody was used to immunoprecipitate β2-microglobulin-associated proteins from the CD1 transfected cell (see *Materials and Methods*). The immunoprecipitate was then Western blotted with the anti-CD1.1 antibody, 1H1. This antibody detected CD1.1 in the anti-β2-microglobulin immunoprecipitate (Fig. 5*B*), confirming that the L chain associated with CD1.1 was β2-microglobulin.

DISCUSSION

The complete nucleotide sequence of the cDNA encoding the murine CD1.1 and CD1.2 genes were determined (Figs. 1 and 2). The deduced amino acid sequences indicated that the murine CD1 molecules were transmembrane proteins with short cytoplasmic tails, similar to the human CD1 molecules. The sequences in the α1–α3 domain exons were almost identical to previously reported genomic sequences from another strain (24), indicating limited polymorphism in these molecules. The amino acid sequence of the transmembrane region and the cytoplasmic tail were identical for CD1.1 and CD1.2. The last four amino acids of α3 domain, the transmembrane region and part of the cytoplasmic tail were encoded on a fifth exon and the last six amino acids of the cytoplasmic tail were encoded by a sixth exon (Table I). This genomic organization is similar to the human CD1 genes (14–16).

A sequence comparison between the human and murine CD1 genes in the α1–α3 domains was reported previously (24) and indicated that the murine genes were most related to the human CD1d gene (16). This report demonstrates further sequence homology between the cytoplasmic tails of murine CD1 and human CD1b, c, and d, but not CD1a. A Tyr and Gln were conserved in all five molecules and a consensus sequence, Ser/Ala-Tyr-Gln-X-Ile/Val, was present. This region presumably interacts with other molecules on the cytoplasmic face of the plasma membrane, but the nature of these interactions or whether the cytoplasmic tail is phosphorylated under any circumstances is unknown. It is noteworthy that this cytoplasmic tail is not conserved in CD1a, which suggests that CD1a may interact with a distinct set of cytoplasmic proteins. Although the transmembrane regions of CD1.1 and CD1.2 were identical, there was no significant homology between murine and human CD1 in the transmembrane region.

The protein structure of the murine CD1.1 molecule was assessed by immunoprecipitation. A rat anti-mouse CD1.1 monoclonal antibody, 1H1, immunoprecipitated a molecule of about 48 kDa in association with β2-microglobulin from the cell surface of CD1.1-transfected cells (Fig. 5). These studies confirm that the murine CD1.1 gene encodes a β2-microglobulin associated transmembrane glycoprotein, analogous to the human CD1 molecules.

Despite the homology between the human and murine CD1, their tissue distribution is distinct. A previous report showed that low levels of CD1 message could be detected in thymus, spleen, and liver from BALB/cJ mice (24). This report confirms that thymic expression of CD1 message is low or undetectable in TL^- as well as TL^+ strains of mice. Consistant with this result, antibodies against CD1.1 demonstrate only very low or undetectable levels of CD1 protein expressed on the surface of murine thymocytes (data not shown). The only tissues found to express high levels of murine CD1.1, based on immunoperoxidase staining with a rat anti-CD1.1 mAb, are in the murine gastrointestinal tract. CD1.1 was identified

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CCCCAGCAAAACCCCAGAGCCTTTGTGTACCAGTCCGGGAGCAGAGTAAAGCGCAGAAGTCGGAGCCGAGGGCT--GTAGAACTCTGGCGCT-leader CD1.1

GGG.CAA...C.....................G.T.............T...........A..C......GT.............-leader CD1.2

A......GG..AGGG.CGTGAGC..A.----C.CGG.....A.....---.....--...A...GG.G.C..G..CAGTCCGCGA..TC.CCACGCCGGGCGAT-leader CD1d
```

Figure 3. Homology in the 5′ untranslated region between the murine CD1 cDNA and CD1d. The nucleotide sequence 5′ of the initiation ATG is shown for each cDNA. Nucleotides that differ from the CD1.1 sequence are shown and *dashes* are introduced to optimize the homology.

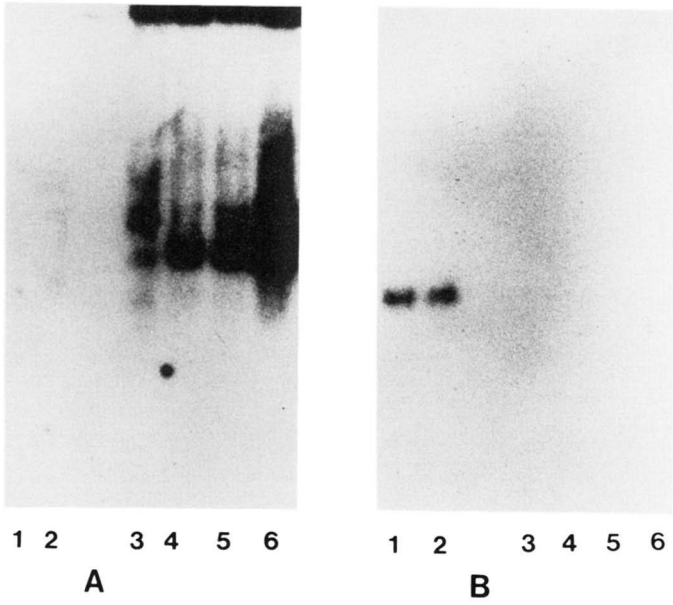

Figure 4. Northern blot analysis of thymocyte RNA from C57B16. Thymocytes from 2-wk-old C57B16/J mice (predominantly immature cortical thymocytes, *lane 1*) or from littermates treated with 2.5 mg of i.p. hydrocortisone 2 days before death (mature medullary thymocytes, *lane 2*) were isolated. RNA was extracted from these thymocytes and from several murine L cell clones stably transfected with the murine CD1.1 cDNA (*lanes 3* to *6*) (see *Materials and Methods*). Eight micrograms of whole cellular RNA from each sample were analyzed with a CD1.1 probe and exposed with an intensifying screen for 3 days at −80°C (*A*). The blot was then stripped, hybridized with a murine CD3ε probe and exposed overnight as above (*B*).

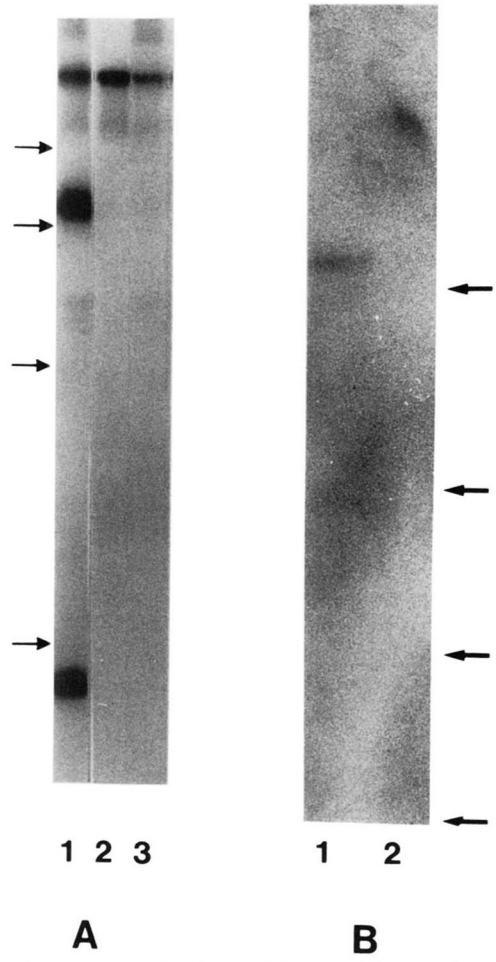

Figure 5. Immunoprecipitation and Western blotting of murine CD1.1 *A*, CD1.1 transfected or control cells were surface labeled and immunoprecipitated with a rat anti-mouse CD1.1 monoclonal antibody (1H1), as described in *Materials and Methods*. *Lane 1*, CD1.1 transfected BY155.16 cells immunoprecipitated with the 1H1 antibody; *lane 2*, control cells immunoprecipitated with 1H1; *lane 3*, transfected cells immunoprecipitated with normal rat serum. Samples were analyzed by SDS-PAGE on a 12.5% nonreducing gel. *B*, CD1.1 transfected BY155.16 cells were immunoprecipitated with an anti-murine β2-microglobulin antibody and the immunoprecipitate was Western blotted with the 1H1 antibody, as described in *Materials and Methods*. *Lane 1*, anti-β2-microglobulin immunoprecipitate; lane 2, control normal rat serum immunoprecipitate.

on epithelial cells in murine stomach, small intestine, and large intestine and in liver (see footnote 4). These studies demonstrate a distinct tissue distribution of murine versus human CD1, suggesting that they may serve different functions.

Although human CD1a, b, and c are expressed at high levels on the surface of immature thymocytes, the role these molecules play during thymocyte maturation is unknown. The expression of MHC class I molecules on immature thymocytes is low (31–33) and it has been suggested that there is some requirement for the expression of an MHC class I-like molecule (such as CD1 or murine TL) on immature thymocytes. The low or undetectable levels of murine CD1 on thymocytes from TL^- strains (Fig. 4) suggests that high level expression of an MHC class I-like molecule may not be critical during thymocyte maturation. Alternatively, other MHC class I-like molecules may be expressed by murine thymocytes or there may be subtle differences in human and murine thymocyte development.

In contrast to CD1a, b, and c, CD1d message is expressed at low or undetectable levels in human thymus (16). The human CD1d molecule appears to be more closely related by sequence to murine CD1 (25) than to CD1a, b, or c (16). Nucleotide sequences in the 5′ untranslated region of CD1d and murine CD1.1 and CD1.2 are also conserved (Fig. 4). It is possible that these conserved sequences in the 5′ untranslated region play some role in regulating CD1 expression. These findings suggest that the CD1 gene family has evolved into two classes of molecules (CD1a, b, and c vs CD1d and murine CD1) which can be distinguished by sequence (16, 24), tissue distribution and perhaps function. Experiments are in progress to identify tissues expressing significant levels of CD1d and to determine whether CD1d has a tissue distribution similar to murine CD1.

DNA blot hybridization indicates that there are only two CD1 genes present in the murine genome, CD1.1 and CD1.2 (data not shown). CD1 homologues that are CD1a, b, c-like, based on their sequence or expression on thymus and skin have been identified in rabbits and sheep (34–36). This suggests that the CD1a, b, c-like CD1 molecules were deleted from the murine genome and the CD1d-like gene was duplicated. The murine *CD1* locus is located on chromosome 3 in a region of the chromosome that may have been translocated from chromosome 1 during the divergence of humans and mice (37). It is possible that the CD1a, b, and c-like genes were deleted during such a translocation.

It is of interest to consider whether there are any immunological consequences of the apparent deletion of the CD1a, b, and c-like genes from the murine genome. One noteworthy distinction between the human and murine immune system is the distribution of T lymphocytes in the epidermis. Murine epidermal lymphocytes use pre-

dominantly a single $\gamma\delta$ TCR (38, 39). The human epidermal lymphocyte compartment is not well defined but is clearly dominated by T cells expressing the $\alpha\beta$ TCR (40–42); many of these epidermal $\alpha\beta$ T cells also appear to have the $CD4^-$, $CD8^-$ (double negative) phenotype (40). CD1a and c are expressed on Langerhans cells in the epidermis and double negative, $\alpha\beta$ T cell clones reactive with CD1a (22) and CD1c (S. Porcelli and M. Brenner, personal communication) have been identified. These findings suggest that CD1 expression may influence the development of the epidermal immune system.

The T cell distribution in gastrointestinal tract and perhaps other epithelial surfaces (41–47) is also distinct in humans versus mice. It is not clear whether the T lymphocyte distribution in these sites is influenced by differences in the expression of MHC or MHC-like Ag-presenting molecules or other factors. Further studies of CD1 in the human and murine systems will be needed to determine what role these molecules play in the immune system.

Acknowledgments. We thank Dr. Richard Blumberg for assistance with the immunoprecipitations and Dr. Joyce Fingeroth for critical review of the text.

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