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A Highly Conserved Major Histocompatibility Complex Class I-Related Gene in Mammals

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We report here a cDNA sequence of a murine homolog of the human major histocompatibility complex (MHC) class I-related gene, MR1. The analyses revealed unprecedentedly high conservation of MR1 in the α1 and α2 domains (corresponding to the peptide-binding domains in the classical MHC class I molecules) between human and mouse (predicted amino acid identity: 90 and 89% for the α1 and α2 domain, respectively), compared to MHC class I and other class I molecules. On the other hand, conservation in the α3 domain (73%) is comparable to those of others, suggesting domain-specific conservation of MR1. The localization of the mouse MR1 gene was determined to be chromosome 1H1, which corresponds to the human chromosomal region where the human MR1 gene is located (chromosome 1q25). High conservation of MR1 among mammals suggests that MR1 may be involved in critical conserved biological function(s).

Polymorphic classical MHC class I molecules (HLA-A, B and C in human) play crucial roles in the immune system by presenting antigenic peptides to $CD8^+$ T cells (1) and by serving as inhibitory molecules for natural killer cells (2). In addition to these and closely related oligomorphic molecules (HLA-E, F and G), there are other groups of divergent MHC class I-related molecules in human: CD1 family (3), Znα2gp (4), FcRn (5), MIC (or PERB11) family (6, 7), MR1 (8) and HLA-H (9). Some of these molecules have been shown to possess unique functions including presentation of lipophilic antigens such as mycolic acid and lipoarabinomannan (CD1b; 10, 11), and Fc receptor activity (FcRn; 5). Except for MIC (PERB11) family, homologs for human MHC class I-related groups have been reported to exist in mouse (murine homologs for CD1d (12), Znα2gp (13), FcRn (14), MR1 (8), and HLA-H (MR2) (15)), suggesting that each of these class I family may possess an important, conserved function in mammals.

Previously we reported the discovery of the human MR1 gene and mentioned the existence of a homolog in the mouse genome (8). Among MHC class I-related molecules, MR1 is unique in that, in the α1 and α2 domains (peptide-binding domains in the classical MHC class I molecules), it is closest to the class I group to which the vertebrate classical MHC class I molecules belong, although the human MR1 gene exists outside the MHC (8). In the present study, we isolated and analyzed a cDNA clone of mouse MR1 and determined its chromosomal localization in the mouse genome. The results revealed exceptional features of MR1 among MHC class I (-related) molecules with respect to conservation in mammals.

MATERIALS AND METHODS

General methods and probes. DNA and RNA were isolated as described (16). The sources of DNA are human placenta and livers of BALB/c mouse, Wistar rat, hamster, guinea pig, and bovine. RNA were isolated from organs including thymus of BALB/c and C3H/He mice (9 weeks old). mRNA were purified with mRNA purification kit (Pharmacia Biotech). The various probes for mouse MR1 were prepared as follows. The DNA fragment for the α1 domain probe could be amplified by polymerase chain reaction (PCR) (17, 18) from genomic DNA of BALB/c mouse liver with the two primers synthesized based on the beginning or the end of the α1 domain of the human MR1 sequence (8): No.105, 5′-GCAGAATTCGGACGCACTCTCTGAGATA (forward) and No.106, 5′-GACAAGCTTTGAGTGATTGTAGTGCCTCTG (reverse) with a restriction enzyme site (underlined). PCR products were then cloned into Bluescript vectors (STRATAGENE). The mouse α3 domain probe (mp18) that corresponds to the middle region of the α3 domain was prepared by PCR as described previously (8). The DNA sequences of the probes for the α1 domain and for the α3 domain sandwiched by the primers turned out to be the same as that of the mouse MR1 cDNA clone No.4 (described below). The probe for the α1 and α2 domains was prepared by PCR from the cDNA clone No.4 with the primers synthesized based on the beginning of the α1 domain of the mouse or the end of the α2 domain of the human and mouse MR1 sequences: No.124, 5′-GCAGAATTCGGACCCACTCGCTGAGATA (forward) and No.128, 5′-GCAGAATTCTGTTCTTT(C/G)TAGGGT(A/G)TC (reverse). Se-

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quencing was performed with an automated sequencer (4000L, LI-COR) and SequiTherm long-Read Cycle Sequencing Kits (EPICENTRE TECHNOLOGIES).

Library screening. A BALB/c mouse lung cDNA library in λgt11 (oligo(dT)- and random-primed; Clontech) was screened with the mouse MR1 α1 domain probe and the α3 domain probe, mp18 (16). The nucleotide sequence of a positive cDNA clone (No.4, ~1.5 kb in length) containing all coding regions and partial 5′- and 3′-untranslated regions was determined (GenBank accession No. U94989).

Reverse transcriptase (RT)-PCR. cDNA for PCR was synthesized using Super Script Preamplification System (GIBCO BRL). To amplify the entire coding region, PCR was performed on cDNA from 25 ng mRNA with the following primers: No.306, 5′-GCAGAATTCGCA-AAGGACTTCAGCACG (forward) and No.309, 5′-GCAGAATTC-TGGCCAAGACATGCGGAT. PCR reaction mixture (50 μl) contained 2.5 units of LA Taq polymerase (Takara), LA Taq buffer, 200 μM dNTP, 125 nM each primer and cDNA template. PCR conditions were: 94 °C 2 min, then 30 ~ 45 cycles of 94 °C 1 min, 55 °C 30 sec, 72 °C 2 min and finally 72 °C 8 min.

Southern and Northern blot analyses. Southern and Northern hybridization were performed essentially as described (16). For Southern blot analysis, 5 (Fig.2A) or 10 (Fig.2B) μg of genomic DNA digested with a restriction enzyme was electrophoresed through 0.8 % agarose gel and blotted onto a nylon membrane. After hybridization, the membrane was washed with 0.5 × (Fig.2A) or 0.1 × (Fig.2B) SSC/0.05 % SDS at 42°C (1× SSC = 0.15 M NaCl/0.015 M sodium citrate, pH 7) and autoradiographed for two days. For Northern blot analysis, a BALB/c mouse MTN blot membrane with polyadenylated RNA (2 μg) from various tissues was obtained from Clontech. For the analysis of expression in thymus, 5 μg of mRNA from BALB/c mouse was used. The membranes were washed with 0.1 × SSC/0.05 % SDS at 42°C and autoradiographed for four days.

FISH (fluorescence in situ hybridization) analysis. The probe for the mouse MR1 gene was cDNA clone No.4 described above. FISH analysis was performed by the method described previously (19). The biotinylated mouse cDNA probe (~1.5 kb) was hybridized to R-banded chromosomes from cultured splenocytes of male mice (BALB/c) prepared as described (20). After overnight hybridization, the slides were washed in 50 % formamide/2 × SSC at 42 °C for 10 min, followed by a wash in 1 × SSC at room temperature for 15 min. The slides were then blocked with 4 % bovine serum albumin/4 × SSC at 37 °C for 30 min. Signal amplification was achieved using rabbit anti-biotin (ENZO), fluorescein-labeled goat anti-rabbit IgG (ENZO) and Cy2-labeled donkey anti-goat IgG (Amersham). Chromosomes were counterstained with propidium iodide. Only twin-spot hybridization signals were scored.

RESULTS AND DISCUSSION

We isolated a murine homolog of human MR1 from a BALB/c mouse lung cDNA library and Fig.1 shows the comparison of the predicted amino acid sequences of MR1 between human and mouse. Percentages of amino acid identity between the two species are as follows: 40.9 %, 89.7 %, 89.1 %, 72.8 %, and 40.7 % for the leader peptide, α1, α2, α3 domain and transmembrane/cytoplasmic region, respectively. The cDNA sequence of MR1 from C3H/He mouse thymus was also determined by RT-PCR and was the same as that of BALB/c mouse with no polymorphism (data not shown). The sequences of the α1 and α2 domains of human MR1 determined by PCR also did not show polymorphic nature of these domains (unpublished results).

The results of Southern blot analysis with the α3 domain probe showed simple patterns of positive bands in the mouse genome with various restriction enzymes (Fig.2A). Fig.2B indicated that the probe for the α1 and α2 domains of mouse MR1 detected positive bands with various mammals including rat, hamster, guinea pig and bovine. These results suggest that MR1 is conserved in various mammalian species, although no apparent positive bands were observed with chicken genomic DNA (data not shown). Fig.2C shows the results of Northern blot analyses with murine tissues. Mouse MR1 is expressed in several tissues with two major transcripts although at low level, and expression in lung was relatively high. Relatively high expression was also observed in thymus compared with in liver, kidney and spleen (Fig.2C and data not shown). While expression in these tissues also could be detected in human, some disparity could be observed between human and mouse (e.g., expression in spleen and skeletal muscle; 8).

Comparison of MR1 between human and mouse revealed that similarity is especially high in the α1 and α2 domains (Fig.1). To gain some insight into this conservation, we compared sequences of various MHC class I (-related) molecules (described in Introduction) between human and mouse (Table 1). Among the known MHC class I (-related) molecules, MR1 shows unprecedentedly high conservation in the α1 and α2 domains. On the other hand, conservation of MR1 in the other domains is not high compared with those of the other molecules. Most of the MHC class I (-related) molecules including MR1 showed around 74 % of conservation in the α3 domain, with an exception of Znα2gp (46.2 %) that is a soluble protein and devoid of transmembrane and cytoplasmic regions. Thus, high conservation of MR1 in the α1 and α2 domains is domain-specific and not due to general, highly conserved nature of this molecule.

Reasons for conservation may vary with each molecule and with positions within a molecule. In the classical MHC class I molecules, the α1 and α2 domains constitute functionally important peptide-binding domains. In these domains, among MHC class I-related molecules, MR1 is closest to the class I group to which the classical MHC class I molecules belong (8). One possibility for the high conservation of MR1 in the α1 and α2 domains between human and mouse is that MR1 interacts with some conserved molecule(s) through these domains. Fig.3 shows a hypothetical structure of these domains of MR1 based on HLA-A2 molecule (21) and shows the amino acid positions where two MR1 molecules from human and mouse exhibit amino acid disparity. These positions are not evenly distributed, but tend to cluster to the top right and bottom right in Fig.3. High conservation of MR1 between human and mouse seems to be due to high conservation in the left half of the molecule, although

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Leader  human: MGELMAFLLPLIIVLMVKHSDS
        mouse: ****-ML----LA-FL--R-HT

α1      human: RTHSLRYFRLGVSDPIHGVPEFISVGYVDSHPITTYDSVTRQKEPRAPWMAENLAPDHWERYTQLLRGWQQMFKVELKRLQRHYNHS  87
        mouse: ----------A----GPV---------------------------K------------------------T--A--RH--------  87

α2      human: GSHTYQRMIGCELLEDGSTTGFLQYAYDGQDFLIFNKDTLSWLAVDNVAHTIKQAWEANQHELLYQKNWLEEECIAWLKRFLEYGKDTLQRT  179
        mouse: -L------------------------------I-----------M-Y---IT-------L---Q----------------------R---E--  179

α3      human: EPPLVRVNRKETFPGVTALFCKAHGFYPPEIYMTWMKNGEEIVQEIDYGDILPSGDGTYQAWASIELDPQSSNLYSCHVEHCGVHMVLQVPQ  271
        mouse: -H-V--TT-------I-TF--R---------S----------A--V---GV---------T-L-VN-----NDV---------RQ---EA-R  271

TM&CY   human: ESETIPLVMKAVSGSIVLVIVLAGVGVLVWRRRPREQNGAIYLPTPDR          319
        mouse: --GD-LR-STISGTT-*-I-A-------I---*SQ-LKEVM-Q--QVNEGSSPS    323
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FIG. 1. Comparison of the predicted amino acid sequences of MR1 from human and mouse. Dashes indicate the same amino acids as those in the human sequence. Asterisks indicate gaps in the sequences. The amino acid numbers for the mature proteins are indicated. L and TM & CY stand for the leader peptide and the transmembrane/cytoplasmic domains, respectively.

the total number of amino acid positions with synonymous and nonsynonymous substitutions in each half showed a similar number of ~30. The conserved region in the left half of MR1 (Fig.3) includes the region where variable loops of a T cell receptor α chain can contact with a peptide-bound classical MHC class I molecule (22, 23). Whether MR1 is expressed on the cell surface and can be actually recognized by some T cell receptor with conserved variable loops remains to be clarified. In this respect, it is interesting that $NK1^+$ T cells, by which CD1d can be recognized (24), possess an invariant T cell receptor α chain (Vα14-J281) (25) and similar human T cell subsets possess a homologous invariant α chain (Vα24-JQ) (26, 27). In this case, however, the

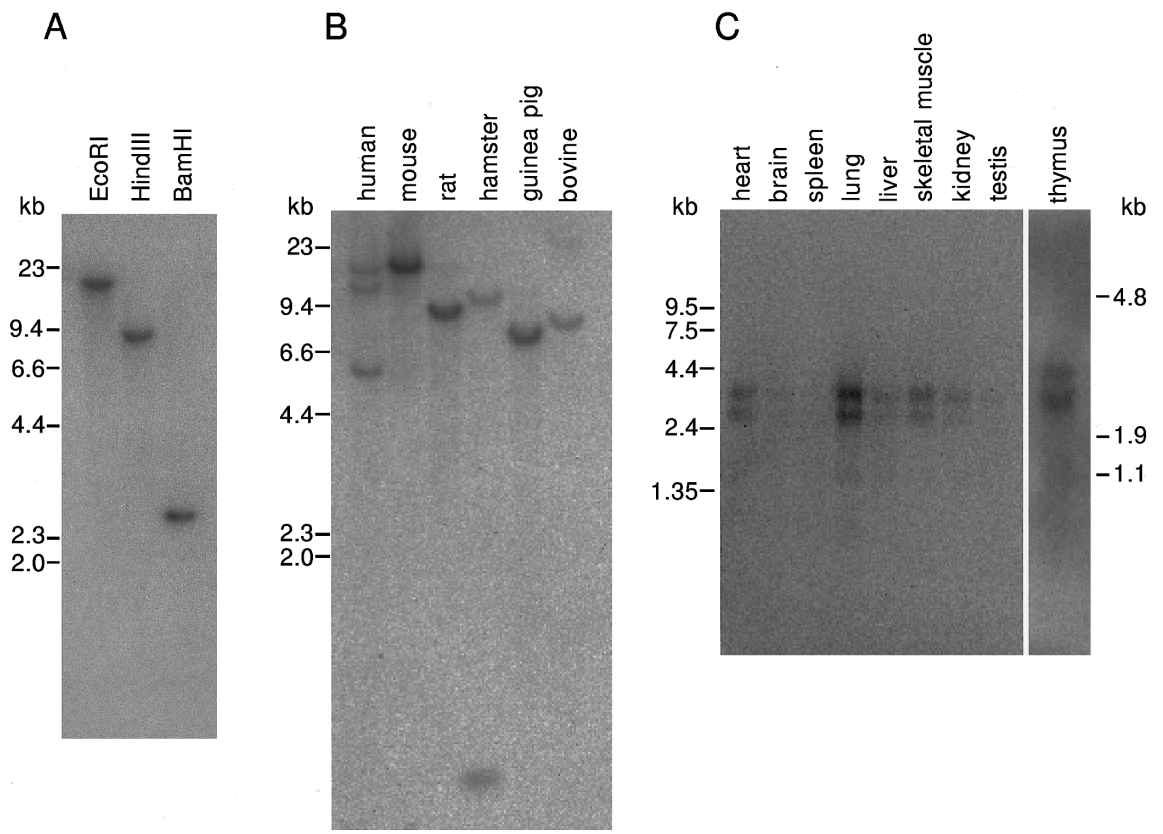

FIG. 2. Southern and Northern analyses of MR1. (A) Southern blot analysis of BALB/c mouse genomic DNA with the α3 domain probe of mouse MR1. Restriction enzymes used are indicated above lanes. Size markers are shown on the left. (B) Southern blot analysis with various mammals indicated. DNA were digested with EcoRI. The probe for the α1 and α2 domains of mouse MR1 was used. (C) Northern blot analysis of mouse MR1 expression in various tissues indicated. The probe for the α1 and α2 domains of mouse MR1 was used.

TABLE 1

Comparison of Conservation of Various MHC Class I (-Related Molecules) between Human and Mouse

Human / mouse	L	α1	α2	α3	TM&CY
MR1(h) / MR1(m)	40.9	89.7	89.1	72.8	40.7
	(59.1)	(85.8)	(87.0)	(81.5)	(59.0)
HLA-A2 / H2-k^d	62.5	68.9	76.1	76.1	37.7
	(62.5)	(79.6)	(81.9)	(83.0)	(60.2)
HLA-H / MR2	34.5	78.4	70.0	69.6	51.0
	(47.1)	(79.2)	(73.7)	(74.6)	(71.2)
FcRn(h) / FcRn(m)	50.0	70.9	65.2	73.3	57.3
	(58.3)	(70.5)	(71.7)	(72.2)	(64.0)
CD1d(h) / CD1d1(m)	60.0	59.3	62.4	76.3	25.0
	(70.0)	(74.4)	(77.1)	(82.4)	(53.7)
Znα2gp(h) / Znα2gp(m)	77.3	58.6	73.9	46.2	
	(75.6)	(73.6)	(77.9)	(69.2)	

On the left, human (h) and mouse (m) molecules used for the comparison are indicated. Percentages of amino acid identity between human and mouse molecules are shown. Percentages of nucleotide identity are also shown in parentheses. L and TM & CY stand for the leader peptide and the transmembrane/cytoplasmic domains, respectively. In the comparison of HLA-H/MR2, the values for the α2 domain would be 76.2 and 80.1 for amino acid and nucleotide identity, respectively, if the mouse sequence corresponding to the eight amino acid residues in the beginning of the α2 domain is not included in the comparison. The sequences used for the comparison are as follows: human MR1 (GenBank Accession No.U22963), mouse MR1 (the present study, U94989), HLA-A2 (M84379), H2-K^d (J00402), HLA-H (U60319), MR2 (a murine homolog of HLA-H) (U66849), human FcRn (U12255), mouse FcRn (L17022), human CD1d (J04142), mouse CD1d1 (M63695), human Znα2gp (M76707), and mouse Znα2gp (D21059).

general characteristic of the positions of amino acid residues that are variable between human and mouse CD1d is distinct from that of MR1 (data not shown), although mouse CD1d1 adopts a structure generally similar to MHC class I molecules with some modifications (28). Requirements for amino acid conservation appear to be quite different between MR1 and CD1.

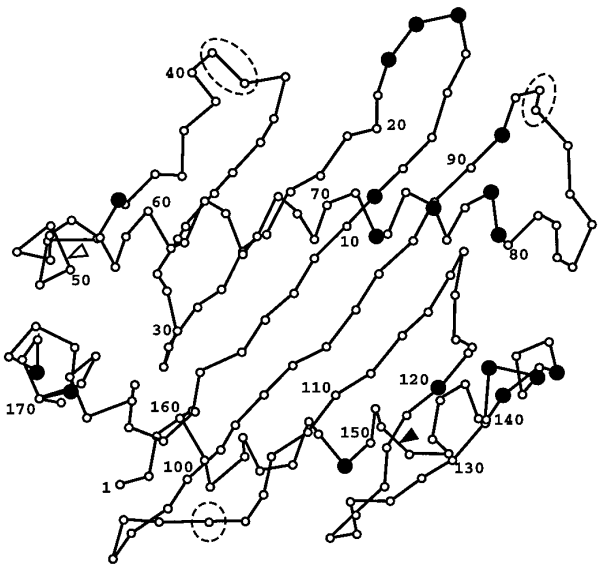

FIG. 3. A hypothetical structure of MR1 α1 and α2 domains based on the HLA-A2 structure (21). Putative amino acid positions deleted in MR1 molecule compared to HLA-A2 are indicated by dotted lines. Arrowheads indicate possible positions where an additional amino acid residue is present in MR1 compared to HLA-A2. The amino acid positions where human and mouse MR1 molecules exhibit amino acid disparity are indicated by black dots and a black arrowhead. Numbers are based on the sequence of MR1.

Chromosomal localization of mouse MR1 gene was determined by FISH analysis as shown in Fig.4. A total of 50 metaphase cells were examined. Of these, 5 cells exhibited twin-spot signals on both homologues of chromosome 1 at band H1. 17 other cells had twin spot signals on one chromosome 1H1 and a single spot on its homologue. As no specific signals could be detected on any other chromosomes, we assigned the MR1 gene to mouse chromosome 1H1. This region of the mouse chromosome is within a linkage group conserved between human chromosome 1 and mouse chromosome 1 (29), and corresponds to the region where human MR1 is located (chromosome 1q25.3). Another MHC class I-related family, CD1, is located at chromosome 1q22-23 in human and chromosome 3 in mouse (30, 31). The region in human chromosome 1 where MR1 and CD1 genes are present is supposed to have been split into two chromosomes 1 and 3 in mouse, and the human organization is thought to represent the ancestral one (29). Thus MR1 and CD1 genes seem to have been present on the same chromosome in the ancestral mammalian species.

Recently, human chromosomal region 1q21-25, where MR1 and CD1 genes exist, was reported to be paralogous to the MHC region (6p21.3) (32), as several paralogous genes can be found in the two regions. In addition, chromosomal regions 9q33-34 and 19p13.1-13.3 also appear to be paralogous to the MHC region (32, 33). It is speculated that these regions have been produced by duplications of the ancestral chromosomal region (32, 33). The two highly divergent class I fami-

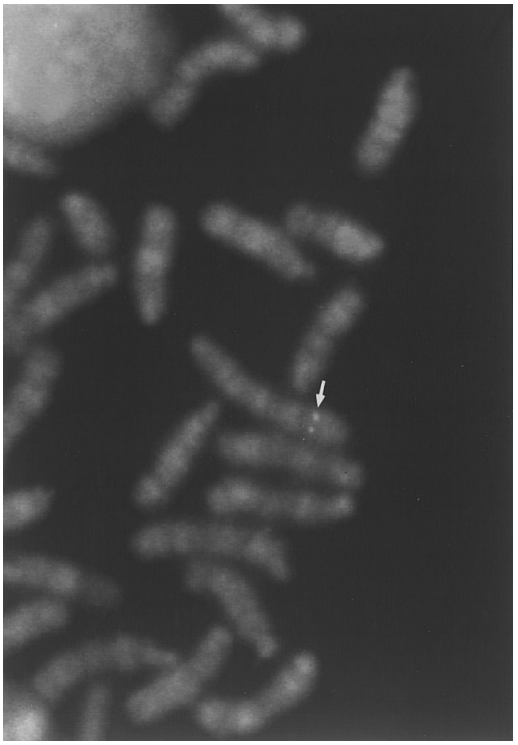

FIG. 4. Chromosomal assignment of the mouse MR1 gene. Partial R-banded metaphase chromosomes stained with propidium iodide, showing twin-spot signals on mouse chromosome 1 at band H1 (arrow).

lies, CD1 and MHC class I, have been thought to have diverged around the time when MHC class I genes separated from MHC class II genes (3) (early in vertebrate evolution at latest) and have been speculated to have diverged as a result of a chromosomal duplication (34) based on the observation described above (32), although a conclusion concerning MR1 has been suspended because a phylogenetic analysis was not decisive. In the α1 and α2 domains, the similarities of MR1 to the classical MHC class I molecules from various vertebrates including the cartilaginous fish, the most primitive vertebrates with jaws, are comparable (8; unpublished results). Further, the present study showed that these domains of MR1 possess highly conserved nature. These characteristics of MR1 molecule may be compatible with its possible ancient origin. Previously, we speculated that the fact that two MHC class I-related groups (MR1 and CD1) are present on the same human chromosome 1 is a consequence of a rational evolutionary history (8). Based on the characteristics of MR1, we speculate that MR1 together with CD1 may have been separated from the MHC class I group by a chromosomal duplication in ancient time.

The highly conserved nature of MR1 among mammals revealed in the present study suggests that it may interact with conserved molecule(s) and may possess a critical biological function. Studies at protein level and with gene-targeting should be needed and are now being investigated.

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