Immunogenetics (2001) 53:643–648
DOI 10.1007/s00251-001-0380-1

ORIGINAL PAPER

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Characterization of the MHC class I-related *MR1* locus in nonhuman primates

Received: 9 July 2001 / Accepted: 18 September 2001 / Published online: 6 November 2001

Abstract We characterized the MHC-related 1 (*MR1*) locus in two nonhuman primates species, *Pongo pygmaeus* and *Pan troglodytes*. *MR1* cDNA sequences encoding several isoforms generated through alternative splicing were observed in both species. Amino acid alignment between the five species in which *MR1* has been characterized to date – human, chimpanzee, orangutan, mouse, and rat – reveals a very high degree of conservation specially in the α1 and α2 domains of the molecule. The main differences concentrate in the transmembrane and cytoplasmic domains. In the three primates species there is a lysine residue inside the putative transmembrane domain which is not present in rodents. Furthermore, the MR1 cytoplasmic region is longer in rodents, with a conserved serine-containing motif that could be involved in endocytosis; remarkably, this motif is absent in the three primate species. We also describe the presence in the chimpanzee of a sequence homologous to the *MR1P1* pseudogene previously found in humans.

Keywords MR1 · HLALS · MR1P1 · MHC · Chimpanzee · Orangutan

Introduction

Human classical MHC class I genes (*HLA-A*, *-B*, *-C*) map to MHC loci on Chromosome (Chr) 6. Among human nonclassical genes, *HLA-E*, *-F*, *-G* (O'Callaghan and Bell 1998), *HFE* (Feder et al. 1996), and *MIC* (Bahram 2001) map to MHC loci, whereas *AZGP1* (Pendás et al. 1994), *PROCR* (Fukudome and Esmon 1994), *ULBP* (Cosman et al. 2001), *FCGRT* (Ghetie and Ward 2000), *CD1* (Porcelli and Modlin 1999), and *HLALS* (*MR1*) (Hashimoto et al. 1995) are sited outside the MHC complex.

MR1 was originally identified as a cDNA sequence by Hashimoto and co-workers (1995). The gene for human and mouse MR1 (Riegert et al. 1998; Yamaguchi et al. 1998) as well as a rat cDNA encoding MR1 (Walter and Günther 1998) have been described.

MR1 is not polymorphic (Parra-Cuadrado et al. 2000) and is ubiquitously expressed, as revealed in various Northern blot and RT-PCR experiments. Several mRNA isoforms generated through alternative splicing have been found in human, mouse, and rat (Hashimoto et al. 1995; Riegert et al. 1998; Walter and Günther 1998; Yamaguchi et al. 1997).

MR1 function is unknown, and of the nonclassical MHC class I proteins it shows the highest level of amino acid identity to classical molecules, specially in the α1 and α2 domains. The sequence conservation between human, mouse and rat is also very high. In humans, a *MR1*-related pseudogene (*MR1P1*) has been found containing exon 2, intron2, and exon 3 sequences (Parra-Cuadrado et al. 2000).

Here we present the isolation and characterization of *MR1* homologues in chimpanzee and orangutan.

Materials and methods

Cell lines

The following Epstein-Barr virus-transformed B-cell lines purchased from the ATCC (Manassas, Va.) were used: CARL (ATCC CRL 1857) from chimpanzee (*Pan troglodytes)* and PUTI (ATCC CRL 1850) from orangutan (*Pongo pygmaeus)*.

cDNA amplification

MR1 cDNA was obtained after RT-PCR amplification using oligonucleotides homologous to human *MR1* as previously described (Parra-Cuadrado et al. 2000).

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Genomic DNA amplification

MR1 genomic DNA encompassing exons 2 and 3 and the intervening intron was amplified using primers homologous to the human *MR1* gene as previously described (Parra-Cuadrado et al. 2000).

Cloning and sequencing

PCR-amplified products were cloned and automatically sequenced. Sequence analysis was performed using GCG (Genetics Computer Group) software. Multiple sequence alignment was obtained with ClustalW software at the EBI (European Bioinformatics Institute) server.

Results

PCR amplification of the chimpanzee and orangutan *MR1* cDNA

To characterize *MR1* cDNA sequences, RT-PCR experiments were carried out with primers previously used in our laboratory to amplify full-length human *MR1* cDNA (Parra-Cuadrado et al. 2000). Amplification was successful and we obtained several bands in samples from both species, as shown in Fig. 1.

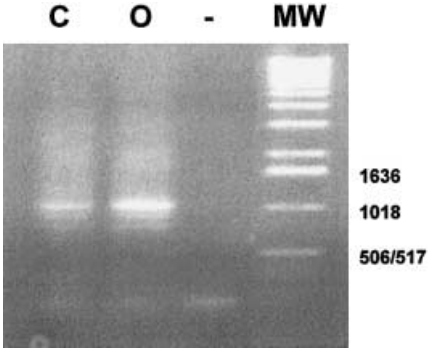

Fig. 1 RT-PCR analysis of *MR1* expression in orangutan and chimpanzee EBV B-cell lines (*C* chimpanzee sample, *O* orangutan sample, – negative control, *Mw* molecular-weight marker)

Orangutan *MR1* cDNA

In a total 12 clones we obtained three types of sequence in the orangutan samples (see Fig. 2). A 1066-nucleotide sequence containing information for the leader peptide, α1, α2, α3 transmembrane and cytoplasmic domains was found (*MR1B1*). This sequence was 97.8% homologous to the human *MR1* cDNA clone reported by Hashimoto and co-workers (1995). A second kind of clone, 790 nucleotides in length, named *MR1B2* was found that lacks exon4 encoding the α3 domain and is very likely generated through alternative splicing. The third *MR1* transcript (*MR1B3*) identified was 710 nucleotides long and identical to *MR1B2* except for a deletion of 79 nucleotides in the 3′ end of the α1 domain. A cryptic splice donor site located 77 bp from the 3′ end of the α1 domain generates this gap.

Chimpanzee *MR1* cDNA

In chimpanzee, we identified a 1066-nucleotide cDNA clone (*MR1B1*), 99.7% homologous to human *MR1* and 97.9% homologous to orangutan *MR1*. In addition, a chimpanzee *MR1* transcript lacking the α3 domain (*MR1B2*) was found (see Fig. 2).

Six out of 15 *MR1* chimpanzee clones show a point mutation in codon 197 (ACC→ATC) resulting in a substitution of threonine (accession numbers AJ275982, AJ275983) by isoleucine (accession number AJ275984, AJ275985). This indicates that CARL is a heterozygous cell line for the *MR1* gene. Orangutan and human MR1 sequences have T197, thus I197 is a chimpanzee-

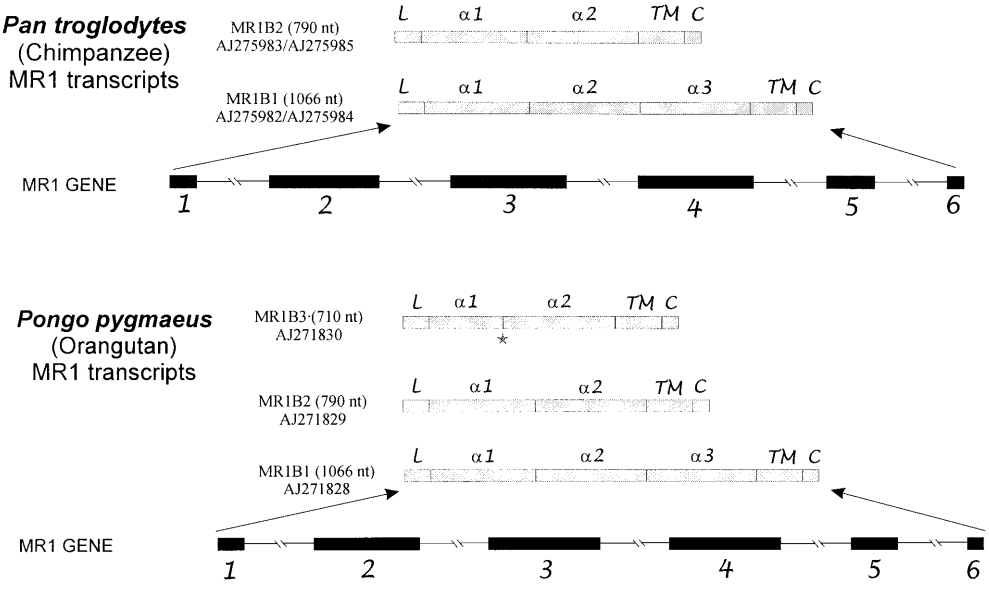

Fig. 2 Diagram representing the different cDNA isoforms found in chimpanzee and orangutan. EMBL/GenBank accession numbers are indicated for each isoform. The *asterisk* in the orangutan *MR1B3* isoform indicates a cryptic splice donor site

Fig. 3 Multiple alignment of the predicted MR1 amino acid sequences from primates (human, chimpanzee, and orangutan) and rodents (mouse and rat). *Asterisk* marks position 303: lysine in the three primate species. Identities with the MR1 consensus sequence are indicated by *dashes*, deletions/insertions are shown by *dots*. GenBank accession numbers are: human MR1, U22963; chimpanzee MR1, AJ275984; orangutan MR1, AJ271828; mouse MR1, U944989; rat MR1, Y13972

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                ▼Leader                  ▼α1 domain
consensus   MGELMAFLLP LIIVLMVKHS DSRTHSLRYF RLGVSDPI-G VPEFISVGYV
HUMAN       ---------- ---------- ---------- --------H- ---------- 50
CHIMPANZEE  ---------- ---------- ---------- --------H- ----------
ORANGUTAN   ---------- ---------- N--------- --------R- ----------
MOUSE           -ML--- -LA-FL--R- HT-------- --A----GPV ----------
RAT                                        - --A----GP- ----------

consensus   DSHPITTYDS VTRQKEPRAP WMAENLAPDH WERYTQLLRG WQQMFKVELK
HUMAN       ---------- ---------- ---------- ---------- ---------- 100
CHIMPANZEE  ---------- ---------- ---------- ---------- ----------
ORANGUTAN   ---------- --Q------- ---------- ---------- ----------
MOUSE       ---------- -------K-- ---------- ---------- ---T--A--R
RAT         ---------- ---------- ---------- ---------- --RT-QT--R

                        ▼α2 domain
consensus   RLQRHYNHSG SHTYQRMIGC ELLEDGSTTG FLQYAYDGQD FLIFNKDTLS
HUMAN       ---------- ---------- ---------- ---------- ---------- 150
CHIMPANZEE  ---------- ---------- ---------- ---------- ----------
ORANGUTAN   ---------- ---------- ---------- ---------- ----------
MOUSE       H--------- L--------- ---------- ---------- -I--------
RAT         H--------- L--------- ---------- ---------- -I--D-----

consensus   WLAVDNVAHT IKQAWEANQH ELQYQKNWLE EECIAWLKRF LEYGKDTLQR
HUMAN       ---------- ---------- --L------- ---------- ---------- 200
CHIMPANZEE  ---------- ---------- --L------- ---------- ----------
ORANGUTAN   ---------- --R------- ---------- ---------- ----------
MOUSE       ---M-Y---I T-------L- ---------- ---------- ----R---E-
RAT         ---M-----I T-R-----L- ---------- ---------- ----S-A-E-

                ▼α3 domain
consensus   TEPPLVRVNR KETFPGVTTL FCKAHGFYPP EIYMTWMKNG EEIVQE-DYG
HUMAN       ---------- --------A- ---------- ---------- ------I--- 250
CHIMPANZEE  ---------- --------A- ---------- ---------- ------I---
ORANGUTAN   ---------- ---------- ---------- ---------- ------M---
MOUSE       --H-V--TT- ---------F --R------- --S------- ---A--V---
RAT         --H-V--TT- ---------- --R------- --S-I-K--- ------V---

                                                        ▼Trans-
consensus   DILPSGDGTY QTWASVELDP QSSNLYSCHV EHCGVHMVLQ VPQESETIPL
HUMAN       ---------- -A-------- ---------- ---------- ---------- 300
CHIMPANZEE  ---------- ---------- ---------- ---------- ----------
ORANGUTAN   ---------- -----F---- ---------- ---------- ------A---
MOUSE       G--------- ---L--N--- --NDV----- ----RQ---E A-R--GD-LR
RAT         G--------- -M-V--D--- -TKDI----- ----LQ---E A----GNTL-

            membrane domain              ▼Cytoplasmic tail
consensus   VMKAVSGSIV LVIVLAGVGV LVWRRRPREQ NGAIYLPTPD -EGSSPS
HUMAN       ---------- ---------- ---------- ---------- R       347
CHIMPANZEE  ---------- ---------- ---------- ---------- R
ORANGUTAN   ---------- F----T---- ---------- ----------
MOUSE       -.ST---TT- ---A------ ---.--SQ-L KEVM-Q--QV N------
RAT         -ANT---T-- ---------A ------S--P KEVM-Q--QV N------
              *
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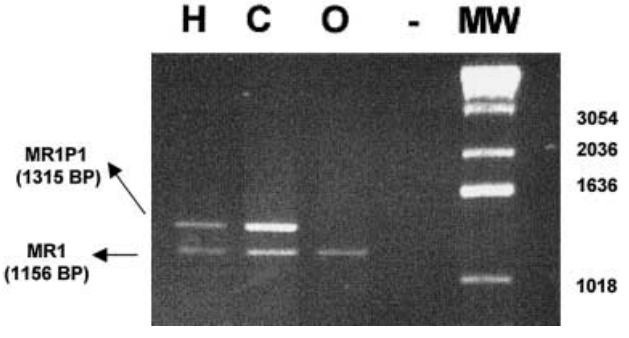
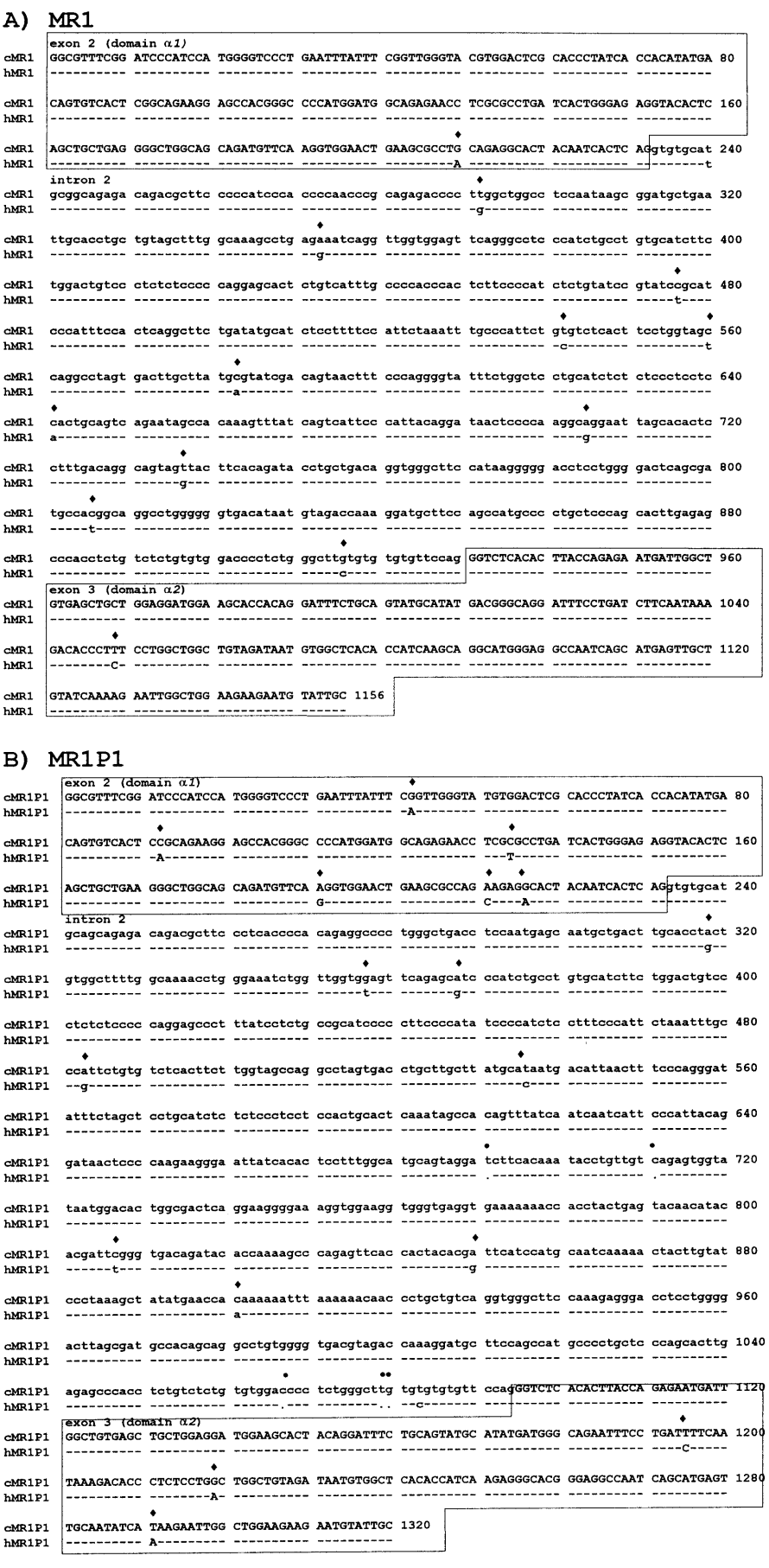


Fig. 4 PCR amplification of *MR1* and *MR1P1* fragments encompassing exon2, intron 2, and exon 3 from human (*H*), chimpanzee (*C*), and orangutan (*O*) genomic DNA. *MR1P1* sequences were not amplified in orangutan

specific substitution that alters the hydrophobicity in the α2 domain located outside the putative peptide-binding region.

A comparison of the MR1 predicted amino acid sequences from human, chimpanzee, orangutan, mouse, and rat reveals some interesting features as shown in Fig. 3. The highest similarities between species are found in the α1 and α2 domains, α3 and, in particular, the transmembrane and cytoplasmic domains being more divergent. In primates, there is a positively charged lysine residue (number 303 in Fig. 3) inside the putative transmembrane domain which corresponds to serine in mouse and asparagine in rat. The cytoplasmic domain of MR1 in primates is shorter than in rodents which display three serine residues absent in primates.

Fig. 5 Comparison between *MR1* sequences from chimpanzee (*cMR1*) and human (*hMR1*) genomic DNA encompassing exon 2, intron 2, and exon 3 (**a**) and comparison between *MR1P1* sequences from chimpanzee (*cMRP1*) and human (*hMRP1*) genomic DNA encompassing exon 2, intron 2, and exon 3 (**b**). Identities between sequences are indicated by *dashes*, deletions/insertions are shown by *dots*, single point substitutions are indicated by *diamonds*. *MR1* and *MR1P1* exons are boxed. Database accession numbers are: *cMR1*, AJ315655; *hMR1*, AF073484; *cMR1P1*, AJ315654; *hMR1P1*, AJ132011



MR1 genomic DNA amplification

We wanted to investigate whether the *MR1P1* pseudogene found in humans is also present in orangutan and chimpanzee genomes. To do this, PCR reactions using genomic DNA were carried out with primers used previously in our laboratory to amplify DNA fragments of the human *MR1* gene as well as *MR1P1* pseudogene, containing exon 2, intron 2, and exon 3.

In orangutan, a single band of 1156 nucleotides in length was observed (Fig. 4), DNA sequencing revealed that this PCR product corresponds to the MR1 gene fragment (Accession number AJ315656). MR1P1-related sequences were not found.

In chimpanzee two PCR products of 1320 and 1156 nucleotides in length respectively were observed (Fig. 4). The short product corresponded to the expected fragment of the *MR1* gene and was 98.87% homologous to the human *MR1* segment. The long product was 98.6% homologous to human *MR1P1*. This result indicates that there are two *MR1* sequences in chimpanzee as in humans. Comparison between chimpanzee and human *MR1* fragments (Fig. 5a) reveals a very high degree of conservation with just one point mutation in exon 2 and one in exon 3, both of them synonymous. In contrast, 11 substitutions can be seen in the intron sequence. This situation is very different from that observed in the *MR1P1*-like fragment (Fig. 5b). In the intron, there are nine point substitutions as well as five deletions that differentiate human and chimpanzee sequences. In exon 2, there are six point substitutions, only two of which are synonymous. In exon 3, there are three point substitutions (one synonymous). Interestingly, the stop codon (position 151) present in human *MR1* is tryptophan in chimpanzee.

Discussion

We have found that *MR1* orthologues exist in chimpanzee and orangutan. *MR1* cDNA isoforms generated by alternative splicing were observed in both species. Similar isoforms have also been found previously in human, rat, and mouse. Thus, the pattern of multiple cDNA isoforms generated through alternative splicing mechanisms is extended to the species analyzed here and reinforces interest in the possible functional implications of these isoforms.

Our results show that MR1 is a highly conserved molecule between primate and rodent species, especially in the α1 and α2 domains, the α3 domain and, in particular, the transmembrane and cytoplasmic regions showing higher variability. This indicates strong evolutionary pressure to maintain the α1 and α2 domains, suggesting that this molecule binds a very conserved ligand.

This pressure can be appreciated by comparing the chimpanzee and human genomic sequences we have obtained from the *MR1* gene and *MR1P1*-like sequences. In the *MR1* sequences, the differences between species accumulate inside intron 2, with the exons being almost identical in both species. In contrast, the differences between both species appear in both intronic and exonic sequences in *MR1P1*.

Interestingly, the stop codon found at position 151 in human *MR1P1* encodes a tryptophan in chimpanzee, suggesting the locus could be a functional gene. However, in our experiments with cDNA, we found no transcripts corresponding to chimpanzee *MR1P1*.

We did not find *MR1P1*-like sequences in orangutan. This could indicate that this locus does not exist in orangutan or that orangutan *MR1P1* cannot be amplified with the primers we used.

Multiple alignment of MR1 amino acid sequences from human, chimpanzee, orangutan, rat, and mouse demonstrates some interesting features with possible evolutionary and functional implications.

As already mentioned, there is a remarkable conservation of the α1 and α2 domains, with the α3 domain being more divergent. The most intriguing differences are located in the transmembrane and cytoplasmic domains. The three primate MR1 sequences exhibit a positively charged amino acid (lysine) inside the putative transmembrane region which is converted to serine in mouse and asparagine in rat. In contrast, both rodent species show longer cytoplasmic tails, with a conserved serine-containing motif (NEGSS) similar to that found in HLA class I (SD/EXSL) and pig CD1 (CDPSS) molecules (Chum et al. 1999). In HLA class I molecules, this motif is necessary for constitutive endocytosis (Vega and Strominger 1989) and the serine residue can be phosphorylated (Guild and Strominger 1984). Although the function of MR1 is currently unknown, these features invite some speculations. MR1 possibly binds to a ligand that has been highly conserved through evolution, such as a peptide or a membrane protein, through the α1 and α2 domains. MR1 is also conceivably involved in endocytosis and/or signal transduction. If so, the signal could be transduced using different mechanisms in rodents and primates. Rodents may phosphorylate the conserved serine in the cytoplasmic tail. In primates, this signal would be transduced using a coupled protein associated with MR1 through saline bridges between the lysine in MR1 and a negatively charged amino acid present inside the transmembrane region of the second protein, in a manner resembling immune receptors such as the T-cell receptor, some FcRs, or some natural killer receptors.

Acknowledgements This work was supported by grants from the Spanish Ministerio de Ciencia y Tecnología (BMC 2001-1382) and from Comunidad Autónoma de Madrid to E.M.-N. J.F.P.-C. is recipient of a predoctoral fellowship from Ministerio de Eduación y Ciencia. M.G.d.M. is recipient of a postdoctoral fellowship from Comunidad Autónoma de Madrid.

References

Bahram S (2001) MIC genes: from genetics to biology. Adv Immunol 76:1–57

Chum T, Wang K, Zuckermann FA, Gaskins HR (1999) Molecular cloning and characterization of a novel CD1 gene from the pig. J Immunol 162:6562–6571

Cosman D, Müllberg J, Sutherland CL, Chin W, Armitage R, Fanslow W, Kubin M, Chalupny NJ (2001) ULBPs, novel MHC class I-related molecules, bind to CMV glycoprotein UL16 and stimulate NK cytoxicity through the NKG2D receptor. Immunity 14:123–133

Feder JN, Gnirke A, Thomas W, Tsuchihashi Z, Ruddy DA, Basava A, Dormishian F, Domingo R, Ellis MC, Fullan A, Hinton LM, Jones NL, Kimmel BE, Kronmal GS, Lauer P, Lee VK, Loeb DB, Mapa FA, McClelland E, Meyer NC, et al (1996) A novel MHC class I-like gene is mutated in patients with hereditary hemochromatosis. Nat Genet 13:399–408

Fukudome K, Esmon CT (1994) Identification, cloning and regulation of a novel endothelial cell protein C/activated protein C receptor. J Biol Chem 269:26486–26491

Ghetie V, Ward WE (2000) Multiple roles of the major histocompatibility complex class I-related receptor FcRn. Annu Rev Immunol 18:739–766

Guild BC, Strominger JL (1984) Human and murine class I MHC antigens share conserved serine 335, the site of HLA phosphorylation in vivo. J Biol Chem 259:9235–9240

Hashimoto K, Hirai M, Kurosawa Y (1995) A gene outside the human MHC related to classical HLA class I genes. Science 269:693–695

O'Callaghan CA, Bell JI (1998) Structure and function of the human MHC class Ib molecules HLA-E, HLA-F and HLA-G. Immunol Rev 163:129–138

Parra-Cuadrado JF, Navarro P, Mirones I, Setién F, Oteo M, Martínez-Naves E (2000) A study on the polymorphism of human MHC class I-related MR1 gene and identification of an MR1-like pseudogene. Tissue Antigens 56:170–172

Pendás AM, Matilla JA, Uría JA, Freije JP, Fueyo A, Estivill X, López-Otin C (1994) Mapping of the human Zn-α2-glycoprotein gene (AZGP1) to Chr 7q22 by in situ hybridization. Cytogenet Cell Genet 66:263–266

Porcelli SA, Modlin RL (1999) The CD1 system: antigen-presenting molecules for T cell recognition of lipids and glycolipids. Annu Rev Immunol 17:297–329

Riegert P, Wanner V, Bahram S (1998) Genomics, isoforms, expression, and phylogeny of the MHC class I-related MR1 gene. J Immunol 161:4066–4077

Vega MA, Strominger JL (1989) Constitutive endocytosis of HLA class I antigens requires a specific portion of the intracytoplasmic tail that shares structural features with other endocytosed molecules. Proc Natl Acad USA 86:2688–2692

Walter L, Günther E (1998) Isolation and molecular characterization of the rat MR1 homologue, a non-MHC-linked class I-related gene. Immunogenetics 47:477–482

Yamaguchi H, Hirai M, Kurosawa Y, Hashimoto K (1997) A highly conserved major histocompatibility complex class I-related gene in mammals. Biochem Biophys Res Commun 238: 697–702

Yamaguchi H, Kurosawa Y, Hashimoto K (1998) Expanded genomic organization of conserved mammalian MHC class I-related genes, human MR1 and its murine ortholog. Biochem Biophys Res Commun 250:558–564