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Specific binding of HLA-B44 to human macrophage MHC receptor 1 on monocytes

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ABSTRACT

Allograft (H-2D^{d}K^d)-induced macrophages (AIM) in C57BL/6 (H-2D^{b}K^b) mice exhibit major histocompatibility complex (MHC) haplotype-specific killing of allografts in a macrophage MHC receptor 1 (MMR1; for H-2D^d)- and MMR2 (for H-2K^d)-dependent manner. Recently, we showed HLA-B62 to be a ligand for the human homologue of mouse MMR2. In the present study, we isolated a cDNA encoding the human homologue of mouse MMR1 and found HLA-B44 to be the sole ligand specific for the human MMR1 by using beads that had been conjugated with 80 kinds of HLA proteins. Flow cytometric analyses revealed that HLA-B44-conjugated beads are specifically bound to HEK293T cells expressing human MMR1, that HLA-B44 tetramers are bound to the human *MMR1*-transfected HEK293T cells with a dissociation constant of 3.0×10^{-9} M, and that the interaction was completely inhibited by the addition of R15 monoclonal antibody specific for mouse MMR1. The MMR1 cDNA (1537-bp) encoded a 473-amino acid polypeptide and was expressed at least in part in the brain and peripheral blood mononuclear cells (PBMCs) or monocytes, but not in granulocytes or lymphocytes. PBMCs from 7 non-H-2D^d (non-self), but none from 5 H-2D^d (self), in-bred mice expressed mouse MMR1 specific for H-2D^d. In contrast, PBMCs from none of the 16 human volunteers expressed HLA-B44; whereas those from only 3 of these 16 volunteers expressed human MMR1.
These results reveal that human MMR1 on monocytes is a novel receptor specific for HLA-B44.

1. Introduction

In 1936, a mouse major histocompatibility complex (MHC) was discovered by Gorer (1936); and he named it H-2. Mintz and Silvers (1967, 1970) showed, using skin grafts from tetraparental (allophenic) donors, that the rejection process that destroyed the allogeneic cells left the syngeneic cells intact, suggesting specific cytotoxicity of recipient cells against allogeneic, but not syngeneic, cells. Thereafter, it was demonstrated on one hand that cytotoxic T lymphocytes (CTLs) exhibited the cytotoxic activity against target cells in a self MHC-restricted manner (Zinkernagel and Doherty, 1974); and it has been assumed on the other hand that rejection of the allografts expressing non-self MHC is mediated by CTLs (Engers, et al., 1982; Hall, 1991). In 1992–1996, several groups using β_{2m} (Zijlstra et al., 1992), CD8 (Dalloul et al., 1996) or CD4 (Krieger et al., 1996) knockout mice reported that neither CTL nor natural killer cells were essential for the rejection of skin or organ allografts and that non-cytotoxic Th1 cells were absolutely required for initiating allorejection. Similarly, in 1988, we found that when Meth A tumor cells of BALB/c origin, a CTL-resistant cell line (Noguchi et al., 1994), were transplanted i.p. into C57BL/6 mice, they were unexpectedly rejected (Yoshida et al., 1988). In 1991, among various kinds of cells infiltrating into the rejection site, allograft-induced macrophages (AIM) were found to be the major population of effector cells responsible for this rejection (Yoshida et al., 1991).

Of particular interest, AIM (H-2^b) exhibit H-2^d haplotype-specific cytotoxic activity against allografts (H-2^d; e.g., BALB/c skin components and Meth A tumor cells) in a cell-to-cell contact-dependent, but Fas-, perforin-, cytophilic antibody (Ab)-, and soluble factor (e.g., TNF-α, NO)-independent manner (Lee et al., 2004; Nomi et al., 2007a,b; Ushio et al., 1996; Ushio-Umeda and Yoshida, 1997; Yamaguchi et al., 2005; Yamamoto et al., 1998; Yoneda and Yoshida, 1998; Yoneda et al., 2008; Yoshida et al., 1997a,b, 1999, 2000), whereas they are inactive toward donor (H-2^d)-type concanavalin A blasts (Yamamoto et al., 1998). In contrast, allograft (H-2^d)-induced CTLs (H-2^b) are cytotoxic against the donor (H-2^d)-type lymphoblasts mainly in a perforin-

Abbreviations: Ab, antibody; AIM, allograft-induced macrophages; bp, base pair; CTL, cytotoxic T lymphocyte; FACS, fluorescence-activated cell sorter; HLA, human leukocyte antigen; ITAM, immunoreceptor tyrosine-based activation motif; K_d, dissociation constant; mAb, monoclonal Ab; MFI, mean fluorescence intensity; MHC, major histocompatibility complex; MMR, macrophage MHC receptor; PBMCs, peripheral blood mononuclear cells; PBS, phosphate-buffered saline; PE, phycoerythrin; RT-PCR, reverse transcription-polymerase chain reaction; TLR, Toll-like receptor.

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dependent manner, whereas they are inactive toward the allografts (Lee et al., 2004; Yamaguchi et al., 2005; Yoshida et al., 1997a,b). Recently, we isolated 2 cDNA clones encoding novel MHC receptors on AIM for allogeneic MHCs (H-2D^d and H-2K^d) by using anti-AIM monoclonal Abs (mAbs; R15 and R12) and H-2D^d and H-2K^d tetramers and called them macrophage MHC receptor 1 (MMR1) and MMR2 (Tashiro-Yamaji et al., 2006a,b). More recently, we isolated a cDNA encoding the human homologue of mouse MMR2, a ligand of which is human leukocyte antigen (HLA)-B62 (Shimizu et al., 2010).

In the present study, we isolated a cDNA encoding the human homologue of mouse MMR1 protein and found HLA-B44 to be a specific ligand of it.

2. Materials and methods

2.1. Animals

Two pairs of specific pathogen-free BALB.B/O1a mice were donated from RIKEN BioResource Center (Tsukuba, Japan). Specific pathogen-free C57BL/10, B10D2, DBA/2, B10A, B10S, B10Q, B10BR, B10M, BALB/c, C3H/He, and A/J mice (7 weeks of age) were purchased from Japan SLC (Hamamatsu, Japan). The animals were housed in our animal facility under specific pathogen-free conditions in an air-conditioned room at 23 ± 2 °C and $\approx$50% humidity. The experiments were carried out in accordance with the Guidelines on Animal Experiments of Osaka Medical College and the Japanese Government Notification on Feeding and Safekeeping of Animals (Notification No. 6 of the Prime Minister's Office). The experimental protocol was approved by the Review Committee for Animal Experiments of Osaka Medical College.

2.2. Reagents

T-select HLA-B4403 tetramers were purchased from Medical & Biological Laboratories Co. Ltd. (Ina, Japan). HLA-A0201, HLA-A1101, HLA-A2402, HLA-A2902, HLA-B0702, and HLA-B1501 (or HLA-B62) pentamers were purchased from ProImmune (Oxford, U.K.). The peptide sequence for the HLA-B4403 tetramer was constructed according to the HLA Peptide Binding Predictions (http://bimas.dcrt.nih.gov/molbio/hla_bind/). Premium RNA™ product (total RNA from human brain) was obtained from Takara Shuzo, Co., Ltd. (Otsu, Japan).

2.3. Homology search

A search for sequence homology was conducted by using BLAST (http://blast.genome.jp/), and the sequence alignment was performed by the use of CLUSTAL W Multiple Sequence Alignment Program (http://align.genome.jp/).

2.4. Reverse transcription-polymerase chain reaction (RT-PCR) analysis of full-length human MMR1

The total RNA of human PBMCs was reverse transcribed to synthesize the first-strand cDNA by using SuperScript II reverse transcriptase (Invitrogen, CH Groningen, Netherlands). An oligonucleotide primer set (forward: 5′-CTA GGT GAG CTC ATG CCA CTA ATG ATG TCT GAA GA-3′ and reverse: 5′-CGG GAT CCA CCA ACC TTA TTT TTT GGA TTG G-3′; KURABO, Osaka, Japan) was used to amplify full-length human MMR1 cDNA (1537-bp); and 35 cycles of PCR were then conducted in a GeneAmp PCR System apparatus (9700; PE Applied Biosystems). A human β-actin primer set (forward: 5′-TGA ACC CCA AGG CCA ACC GC-3′; reverse: 5′-TTG TGC TGG GTG CCA GGG CA-3′) (TOYOBO, Osaka, Japan) was used to amplify a 645-bp fragment by 30 cycles of PCR. The PCR products were electrophoresed on 1% agarose gels (Funakoshi, Tokyo) and analyzed after ethidium bromide staining. The full-length human MMR1 cDNA was sequenced by the use of an Applied Biosystems 3130 Genetic Analyzer (Life Technologies, Carlsbad, CA).

2.5. Real-time RT-PCR

The primer and fluorogenic probe sequences for LightCycler real-time PCR detection of human MMR1, human ATPase II type 9B, and β-actin were designed as described previously (Okada et al., 2005). The primers and the probes were purchased from Roche (GmbH, Mannheim, Germany). The MMR1 or ATPase II type 9B (10^2–10^6 copies/μl) standard was a PCR product; and the β-actin (10^2–10^6 copies/μl) standard was obtained from Funakoshi (Tokyo, Japan). PCR amplification was undertaken by using the LightCycler system (Roche). Each reaction was carried out in a total volume of 20 μl in glass capillaries containing 1 μl cDNA sample, LightCycler TaqMan Master, 200 nM primer (forward: 5′-GTC GTT CGG ACC AGC ACT AT-3′ and reverse: 5′-GAA AAG CAG GGG TGA GGT TC-3′ for human MMR1; forward: 5′-TCT GAC AAA TTG TTC TCA AAA AGG-3′ and reverse: 5′-TTC ATC CAA ATG CGC AGA C-3′ for human ATPase II type 9B; or forward: 5′-CCA ACC GCG AGA AGA TGA-3′ and reverse: 5′-CCA GAG GCG TAC AGG GAT AG-3′ for human β-actin), and 100 nM probe (Roche Universal Probe Library #6 for human MMR1, Roche Universal Probe Library #65 for human ATPase II type 9B or Roche Universal Probe Library #64 for human β-actin). The reaction for human MMR1 and β-actin was carried out under the following conditions: 1 cycle of 95 °C for 10 min, followed by 40 cycles of 95 °C for 10 s and 60 °C for 30 s, and then 1 cycle of 40 °C for 30 s. Analysis was carried out with the LightCycler 3.5 software (Roche).

2.6. DNA sequencing

A cDNA fragment was sequenced by the use of a DYEnamic ET terminator kit (Amersham, Piscataway, N.J.) and analyzed with an Applied Biosystem 3130 Genetic Analyzer (Life Technologies).

2.7. Preparation of peripheral blood leukocytes

Human peripheral blood was diluted with an equal volume of phosphate-buffered saline (PBS) containing 5 mM EDTA. Two time-diluted peripheral blood was centrifuged at $2260 \times g$ for 30 min at 20 °C to obtain the buffy coat (leukocyte-rich) fraction, and peripheral blood leukocytes were obtained by removing contaminating red blood cells from the buffy coat by treatment with buffered ammonium chloride solution.

2.8. Preparation of peripheral blood mononuclear cells (PBMCs)

Human (or mouse) peripheral blood was diluted with an equal volume of PBS containing 5 mM EDTA; and red blood cells and most of the granulocytes were removed by gradient centrifugation ($1420 \times g$ for 15 min at 20 °C) in PBS/Lympholyte®-H (or Lympholyte®-M; Cedarlane, Ontario, Canada). With the gate set in the forward scattering-side scattering mode, monocytes, lymphocytes, and granulocytes were sorted by using a fluorescence-activated cell sorter (FACS; FACSAria, Becton Dickinson, Mountain View, CA).

2.9. Transfection of HEK293T cells with human MMR1 cDNA

The full-length cDNA of human MMR1 was inserted into the pEGFPNI vector; and HEK293T cells were transfected with the linearized plasmid by using Lipofectamine™ 2000 (Invitrogen). Stable transfectants were selected for their resistance to G418 (1 mg/ml; Katoh et al., 1987).