

in vivo での各免疫細胞の活性化および IFN- γ 産生を測定した。

(倫理面への配慮)

1) HIV の感染実験は P3 で行い、環境中への拡散が起こらないように配慮した。

2) 動物実験は、千葉工業大学動物実験規程を遵守し、極力動物の苦痛軽減に配慮して行う。

C. 研究結果

gag タンパク質をウイルス粒子表面および感染細胞内で発現する rBV-gag(AcCAG-gag/Ph-gp64-gag) は pAcCAGgag-Ph-gp64-gag とバキュロウイルスゲノムを sf9 細胞に形質導入し HIV-1gag 発現組換えバキュロウイルス(rBV-gag)を作製した。293T 細胞に感染させ、Gag タンパク質(p24)の発現をウエスタンブロットと Lumipulse (ELISA) にて確認した。

はじめに BMDC に野生型バキュロウイルス(wBV)または rBV-gag を感染させ、実際に BMDC が活性化し、活性化マーカーの発現及びサイトカインの産生が上昇することを FACS にて解析したところ、活性化マーカーである MHC クラス I、II、CD80 及び CD86 の発現が未刺激の BMDC と比較して顕著に上昇していることが確認された。また、ELISA にて培養上清中のサイトカイン IL-6、IL-12p70 及び IFN- α の産生も認められたが、IL-4、IL-10 の産生が見られなかったことから Th1 を強く誘導する可能性が示唆された。

つぎに、rBV-gag 感染 BMDC による免疫細胞の活性化確認を行った。rBV-gag 感染 BMDC と脾臓細胞を共培養し、NK 細胞、CD8⁺T 細胞、CD4⁺T 細胞および B 細胞の活性化マーカーを FACS にて解析した結果、未刺激脾臓細胞のものよりも活性化マーカーである CD69 の発現の上昇が確認できた。また、NK 細胞と CD8⁺T 細胞の IFN- γ 産生は未刺激の脾臓細胞よりも高い IFN- γ 産生が確認できたことから、組換えバキュロウイルス感染 BMDC によって活性化された NK 細胞は抗ウイルス活性を持つことが期待される。

一方 rBV-gag 感染 BMDC 投与 BALB/c マウスにおける Gag 特異的な CD8⁺T 細胞と NK 細胞の活性化の確認を rBV-gag 感染 BMDC を BALB/c マウスに投与し、脾臓細胞

を回収、Gag ペプチドにより再刺激し、活性化マーカーの確認を行った結果、未刺激の脾臓細胞より各細胞 (CD8⁺T、CD4⁺T 細胞、NK 細胞) の活性化が確認された。同様に、CD8⁺T 細胞と NK 細胞の IFN- γ 産生を確認した結果、未刺激脾臓細胞よりも IFN- γ 産生の上昇が確認できた。さらにターゲット細胞との共培養で HIV-1 Gag 特異的に働く CD8⁺T 細胞の活性化を確認した結果、未刺激脾臓細胞よりも HIV-1 Gag 特異的細胞障害性が上昇していることが認められた。

さらに、rBV 感染 BMDC と脾臓細胞を共培養した結果、NK 細胞、CD4⁺T 細胞、CD8⁺T 細胞及び B 細胞の活性化マーカーである CD69 の発現上昇及び NK 細胞と CD8⁺T 細胞での IFN- γ 産生が認められた。さらに rBV 感染 BMDC をマウスへ投与すると、NK 細胞、CD4⁺T 細胞、CD8⁺T 細胞及び B 細胞の活性化マーカーである CD69 の発現上昇が確認された。最終的に rBV 感染 BMDC を投与したマウスの脾臓細胞を用いて、HIV-1 Gag 特異的な細胞障害性を解析したところ、組換えバキュロウイルス感染樹状細胞投与したマウスでは Gag 特異的な細胞障害性が確認された。最終的に HIV-1 NL4-3-VSV-G 感染マウスモデルでの HIV-1 感染制御を確認できた。

以上の結果から rBV は自然免疫及び獲得免疫を誘導する樹状細胞ワクチンとして期待される。

D. 考察

rBV を BMDC に感染させることにより、MHC クラス I、II、CD80 及び CD86 の発現上昇、IL-6、IL-12p70 及び IFN- α の産生が確認された。また、バキュロウイルス感染 BMDC と脾臓細胞を共培養した結果、NK 細胞、CD4⁺T 細胞、CD8⁺T 細胞及び B 細胞の活性化マーカーである CD69 の発現上昇及び NK 細胞と CD8⁺T 細胞での IFN- γ 産生が認められた。また rBV 感染 BMDC をマウスへ投与すると、NK 細胞、CD4⁺T 細胞、CD8⁺T 細胞及び B 細胞の活性化マーカーである CD69 の発現上昇が確認された。さらに rBV 感染 BMDC を投与したマウスでは Gag 特異的な細胞障害性が確認されたことから、バキュロウイルス感染 BMDC から産生されたサイトカイン及び Gag タンパク質が NK 細胞、T 細胞及び B 細胞を活性化することを見出した。最終的

に HIV-1 NL4-3-VSV-G 感染マウスモデルでの HIV-1 感染制御をも確認できたことから、これらの知見が今後の樹状細胞ワクチン開発につながることを期待される。

E. 結論

rBV-gag はマウス樹状細胞を活性化させ多彩な自然免疫および適応免疫応答を発揮し、HIV-1 感染制御がマウスレベルでも確認され、新規 HIV-1 樹状細胞ワクチンの開発に対し有用な結果が得られた。

F. 研究発表

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G. 知的所有権の出願・取得状況（予定を含む） 特になし

III. 研究成果の刊行に関する一覧表

研究成果の刊行に関する一覧表

書籍

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IV. 研究成果の刊行物・別刷（抜粋）

ULBP4/RAET1E is highly polymorphic in the Old World monkey

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Received: 22 February 2011 / Accepted: 21 April 2011 / Published online: 7 May 2011
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Abstract Natural-killer group 2 member D (NKG2D) is an activating receptor that plays an important role in the immune response mediated by NK cells, $\gamma\delta^+$ T cells, and CD8⁺ T cells. In humans, MHC class I chain-related genes and UL-16 binding protein (ULBP)/retinoic acid early transcript 1 (REAT1) gene family encode ligands for NKG2D. The rhesus and crab-eating macaques, which belong to the Old World monkeys, are widely used as non-human primate models in medical researches on the immunological process. In the present study, we investigated the polymorphisms of *ULBP4/RAET1E*, a member of the *ULBP/RAET1* family, and found 25 and 14 alleles from the rhesus and crab-eating macaques, respectively, of which diversities were far more extended than in humans. A phylogenetic study suggested that the allelic diversification of *ULBP4/RAET1E* predated the divergence of rhesus and crab-eating macaques.

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Keywords Rhesus macaque · Crab-eating macaque · *ULBP4/RAET1E* · NKG2D · Polymorphism

Introduction

Non-human primates, such as rhesus and crab-eating macaques, are important animal models for the study of infectious diseases, autoimmune diseases, and organ transplantation. These macaques are members of the Old World monkeys, and it has been reported that the genetic diversity in the rhesus macaque is quite unique, that is, more than 60% of the rhesus macaque-specific expansions are found in the protein coding sequences (Gibbs et al. 2007). To evaluate the results of immunological experiments in the macaque models, it is essential to characterize the genetic diversity of immune-related molecules which may control the individual differences in the immune response against foreign antigens and/or pathogens. It has been reported that the gene copy number in the major histocompatibility complex (MHC) loci in the rhesus and crab-eating macaques is higher than that in humans (Kulski et al. 2004; Gibbs et al. 2007; Otting et al. 2007). In addition, the extent of genetic diversity differed, in part, depending on the geographic areas, and we have reported that the diversity of MHC class I genes in the rhesus macaque is considerably different depending on habitat (Naruse et al. 2010).

Because the innate immune system is involved in the response to environmental pathogens, it is necessary to consider the function of natural killer (NK) cells in the experimental animal models. Natural-killer group 2 member D (NKG2D), a C-type lectin molecule, is an activating receptor expressed on the cell surface of NK, $\gamma\delta^+$, and CD8⁺ T cells, which plays an important role in the immune response (Wu et al. 1999; Raulet 2003). In humans, MHC class I chain-related genes (MIC) and UL-16 binding protein (ULBP)/retinoic acid early transcript 1 (REAT1)

gene family are known to encode ligands for NKG2D (Bauer et al. 1999; Cosman et al. 2001; Chalupny et al. 2003; Bacon et al. 2004). These ligand molecules are usually stress-inducible, and their recognition by NKG2D can lead to the activation of NK cells, consequently killing virus-infected and tumor cells (Pende et al. 2002; Eagle et al. 2006; Pappworth et al. 2007; Ward et al. 2007).

The human *ULBP/RAET1* gene family is located on chromosome 6q24.2, which is composed of ten members including six functional genes, *ULBP1*, 2, 3, 4, 5, and 6, corresponding to *RAET1I*, *H*, *N*, *E*, *G*, and *L*, respectively (Radosavljevic et al. 2001; Chalupny et al. 2003; Eagle et al. 2009a, b). In addition, several sequence polymorphisms in each *ULBP* gene have been identified (Romphruk et al. 2009; Antoun et al. 2010). Although it is evident that the cell surface expression of the ligand molecules on target cells is differentially regulated (Eagle et al. 2006), genetic polymorphisms in the coding regions might have a functional impact. We have previously investigated the genetic polymorphisms of *ULBP/RAET1* genes and have found that the *ULBP4/RAET1E* gene is the most polymorphic, with the allelic distribution differing among ethnic groups (Romphruk et al. 2009).

On the other hand, rhesus macaque *ULBP4/RAET1E* (GenBank: NW_001116520) is mapped on the long arm of chromosome 4 (i.e., positions from 31,164,822 to 31,175,032 of chromosome 4 in the rhesus genome; data obtained from the UCSC Genome Browser at <http://genome.ucsc.edu/cgi-bin/hgGateway>; Gibbs et al. 2007). However, its genetic polymorphisms are poorly characterized, although the MIC gene polymorphisms are well studied in the rhesus macaque (Seo et al. 1999, 2001; Doxiadis et al. 2007; Averdarm et al. 2007). In the present study, we investigated the polymorphisms of *ULBP4/RAET1E* in rhesus and crab-eating macaques. This is the first report demonstrating the extreme diversity of the NKG2D ligand in the Old World monkey.

Materials and methods

Animals

A total of 38 rhesus macaques from seven lineages previously analyzed for the MHC polymorphisms (Naruse et al. 2010) and 24 crab-eating macaques from five lineages were the subjects. They were maintained in the breeding colonies in Japan. The founders of the rhesus macaque colonies were captured in Myanmar and Laos, whereas the founders of crab-eating macaque colonies were captured in Indonesia, Malaysia, and the Philippines. All care, including blood sampling of animals, were in accordance with the guidelines for the Care and Use of Laboratory Animals published by the National Institutes of Health (NIH

publication 85–23, revised 1985) and were subjected to prior approval by the local animal protection authority.

DNA extraction and sequencing analysis

Genomic DNAs from B lymphoblastoid cell lines of the rhesus macaque (Naruse et al. 2010) and from whole blood sample of the crab-eating macaque were extracted by using the QuickGene DNA kit (Fujifilm, Tokyo, Japan) according to the manufacturer's instructions. The genomic gene for *ULBP4/RAET1E* of rhesus and crab-eating macaques was amplified by polymerase chain reaction (PCR) with a primer pair designed for the region spanning from introns 1 to 3 of the rhesus gene (NC007861), *ULBP4F* (5'-TGGGCCTCTTCCCCTGTCC) and *ULBP4R* (5'-GTGGGAGGTGGGATGGG), using FastStart Taq DNA polymerase (Roche, Mannheim, Germany). The PCR condition was composed of the following steps: denaturation at 95°C for 4 min; 30 cycles of 95°C for 30 s, 63°C for 30 s, and 72°C for 45 s; and additional extension at 72°C for 7 min. The PCR products of about 1,200 bp in length were cloned into pSTBlue-1 AccepTer vector (Novagen, WI, USA) according to the manufacturer's instructions and were transformed to Nova Blue Single™ competent cells (Merck4Biosciences Japan, Tokyo, Japan). Ten to 20 independent transformant colonies were picked up for each sample and subjected to sequencing on both strands by using a BigDye Terminator cycling system and an ABI 3730 automated sequence analyzer (Applied Biosystems, CA, USA).

Data analyses

Nucleotide sequences of *ULBP4/RAET1E* from cloned DNAs were aligned using the Genetyx software package (version 8.0, Genetyx Corp., Japan). If at least three clones from independent PCR or from different individuals showed identical sequences, the sequences were submitted to the DNA Data Bank of Japan (DDBJ). Neighbor-joining trees were constructed with Kimura's 2-parameter method for a phylogenetic analysis of *ULBP4/RAET1E* sequences spanning from exons 2 to 3 including intron 2 by using the Genetyx software. Bootstrap values were based on 5,000 replications. The *ULBP4/RAET1E* sequences from humans (GenBank accession number AY252119), chimpanzees (AY032638), and rhesus (NC007861) were included in the analysis as references.

Structure model analysis

A three-dimensional (3-D) structure model of rhesus *ULBP4/RAET1E*, with amino acid positions from 1 to 178, was created by a molecular visualization software RasTop2.2 (<http://sourceforge.net/projects/rastop/>), and the

human RAET1B in complex with NKG2D (Radaev et al. 2001) from the Molecular Modeling Database (MMCB No. 18231) was used as the reference. Polymorphic sites were mapped on the 3-D structure model of macaque RAET1E by using the Cn3D 4.1 program (<http://www.ncbi.nlm.nih.gov/Structure/CN3D/cn3d.shtml>).

Table 1 Identified alleles of the ULBP4 gene in rhesus and cynomolgus

Species	Allele name	Accession no.	Reference animal	Identical sequence
Rhesus macaque	<i>Mamu-ULBP4*1.1</i>	AB568525	R228, R367	
	<i>Mamu-ULBP4*1.2</i>	AB568533	R492, R396, R465	
	<i>Mamu-ULBP4*2</i>	AB568526	R283, R384, R328, R337	
	<i>Mamu-ULBP4*3</i>	AB568527	R346, R361, R396, R379, R408	
	<i>Mmau-ULBP4*4</i>	AB568528	R320, R490, R321, R465, R367, R446, R328, R234, R237, R314	
	<i>Mamu-ULBP4*5</i>	AB568529	R430, R453, R325, R477, R439, R360, R379, R446, R355	
	<i>Mamu-ULBP4*6</i>	AB568530	R437, R350,	
	<i>Mamu-ULBP4*7.1</i>	AB568531	R325, R384, R491, R333, R337	
	<i>Mamu-ULBP4*7.2</i>	AB568544	R477	
	<i>Mamu-ULBP4*8</i>	AB568532	R408, R454, R241, R342, R316	
	<i>Mamu-ULBP4*9.1</i>	AB568534	R312, R314	
	<i>Mamu-ULBP4*9.2</i>	AB568535	R333	
	<i>Mamu-ULBP4*10</i>	AB568536	R316	
	<i>Mamu-ULBP4*11</i>	AB568537	R241	
	<i>Mamu-ULBP4*12</i>	AB568538	R342	
	<i>Mamu-ULBP4*13</i>	AB568539	R491	
	<i>Mamu-ULBP4*14</i>	AB568540	R495	<i>Mafa-ULBP4*1.1</i>
	<i>Mamu-ULBP4*15</i>	AB568541	R350	
	<i>Mamu-ULBP4*16</i>	AB568542	R492	
	<i>Mamu-ULBP4*17</i>	AB568543	R495	
	<i>Mamu-ULBP4*18</i>	AB568545	R454	
	<i>Mamu-ULBP4*19</i>	AB568546	R321	
	<i>Mamu-ULBP4*20</i>	AB568547	R355	
Crab-eating macaque	<i>Mamu-ULBP4*21</i>	AB571025	R437	
	<i>Mamu-ULBP4*22</i>	AB571026	R439	
	<i>Mafa-ULBP4*1.1</i>	AB578934	M01, P01, P02, C001, C003, C004, C005, C006	<i>Mamu-ULBP4*14</i>
	<i>Mafa-ULBP4*1.2</i>	AB578935	M02, C004	
	<i>Mafa-ULBP4*2</i>	AB578936	P04, M06, C010, C011, C013	
	<i>Mafa-ULBP4*3</i>	AB578938	M03, C007	
	<i>Mafa-ULBP4*4</i>	AB578939	M03, C006	
	<i>Mafa-ULBP4*5</i>	AB578940	P04, P05, M05, M06, C012, C013	
	<i>Mafa-ULBP4*6</i>	AB578941	M05, C010, C011	
	<i>Mafa-ULBP4*7.1</i>	AB578942	M01, C002	
	<i>Mafa-ULBP4*7.2</i>	AB578943	P03, C008	
	<i>Mafa-ULBP4*8</i>	AB578944	P03, M04, C008, C009	
	<i>Mafa-ULBP4*9</i>	AB578945	P01, C001, C002	
	<i>Mafa-ULBP4*10</i>	AB578946	M04, C009	
	<i>Mafa-ULBP4*11</i>	AB578947	P02, C007	
	<i>Mafa-ULBP4*12</i>	AB578948	M02, C005	

Fig. 1 Phylogenetic tree of *Mamu*- and *Mafa*-ULBP4/*RAET1E* alleles. A phylogenetic tree of *ULBP4/RAET1E* sequences spanning from exons 2 to 3, obtained in this study, was constructed by using the neighbor-joining method with bootstrap values of 5,000 replications. Values are indicated as percentages, and only those with more than 50% are shown. Sequences of human *ULBP4/RAET1E* (AY252119), chimpanzee *MICH3* (AY032638), and rhesus *ULBP4/RAET1E* (NC007861) were underlined and included in the analysis as reference sequences. Alleles represented with *broken underlines* had identical amino acid sequences predicted from the nucleotide sequences. The allele containing an in-frame termination codon was *boxed*

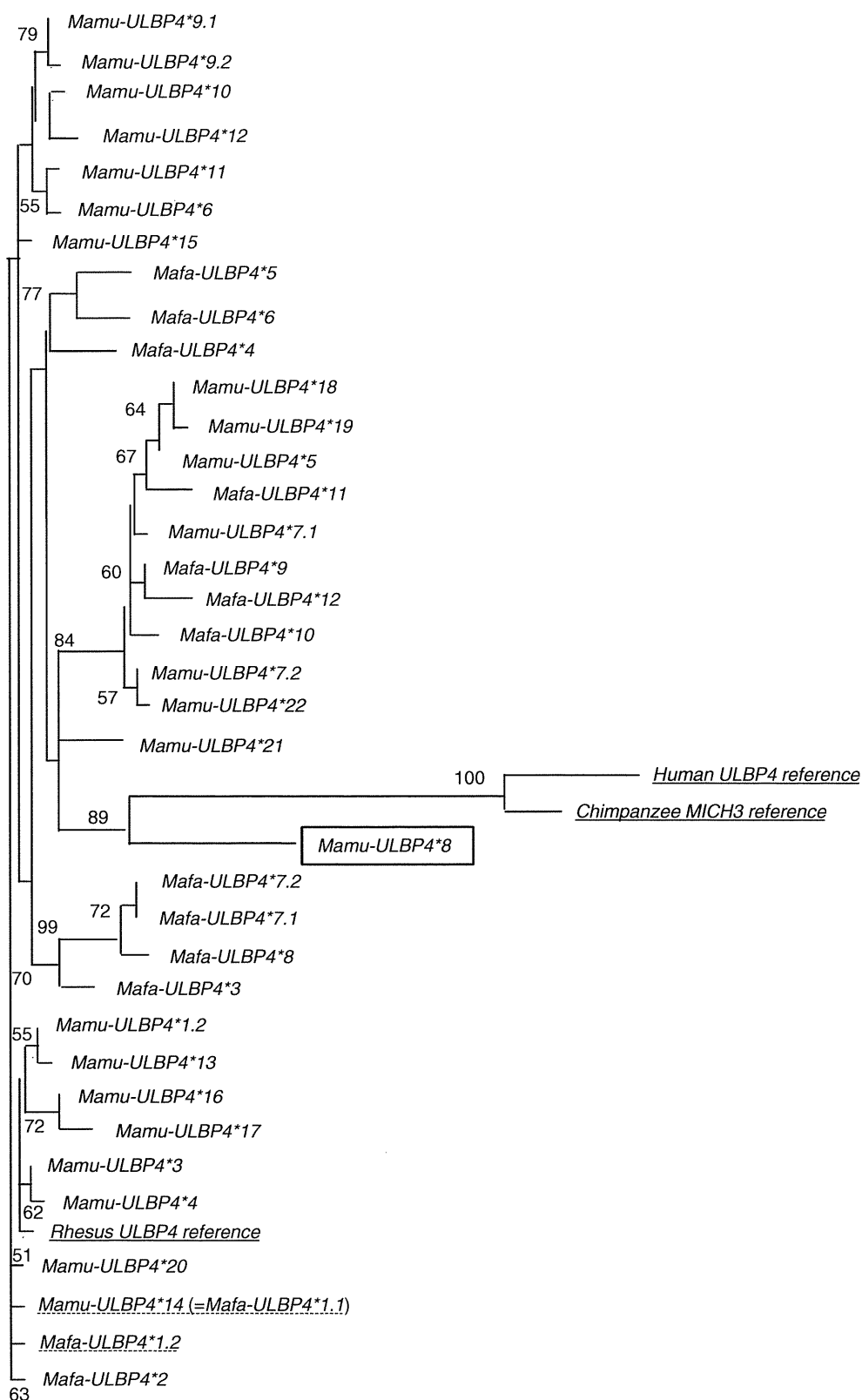


Table 2 Single nucleotide polymorphisms of ULBP4 gene among human and Old World monkeys

	Number of alleles	Exon 2		Intron 2	Exon 3	
		Polymorphism	Non-synonymous change (%)	Polymorphism	Polymorphism	Non-synonymous change (%)
Human	5	2	2 (100%)	3	3	3 (100%)
Rhesus macaque	25	9	5 (55.6%)	22	22	14 (63.6%)
Crab-eating macaque	14	17	9 (52.9%)	18	16	9 (56.3%)

eating macaques showed a higher degree of polymorphism in the analyzed region, namely, exon 2, intron 2, and exon 3, than in humans (Table 2). All polymorphisms found in exons of human *ULBP4/RAET1E* were non-synonymous, whereas a considerable part of the polymorphisms were synonymous in the Old World monkeys. On the other hand, the polymorphic sites in the rhesus macaque (positions 29, 46, 59, 64, 79, 88, 112, 121, 126, 135, 136, 144, 157, 158, 161, 168, 171, and 173) and the crab-eating macaque (positions 32, 39, 40, 59, 72, 73, 79, 91, 112, 136, 163, 164, 165, 171, 178, and 179) were shared at five positions (59, 79, 112, 136, and 171) by each other, whereas only one position (position 112) was shared with polymorphic sites in humans (positions 53, 99, 112, and 113) (Fig. 2). In addition, a termination at position 29 was found in a rhesus macaque allele *Mamu-ULBP4*8*; a single amino acid deletion caused by deletions of a total of three nucleotides was found in a crab-eating macaque allele *Mafa-ULBP4*6* [i.e., TGGCTCAGG sequences corresponding to codons 163–165 were changed to TGCTCA, which may be due to two different deletions at codons 163 (from TGG to TG) and 165 (from AGG to A)], whereas such polymorphisms were not observed in humans. These findings suggest that a selection pressure to generate and maintain the polymorphic sites might be considerably different between the lineages of humans and the Old World monkeys.

Discussion

It has been suggested that the ancestral gene for the ULBP/ REAT molecule of placental mammals was originally diverged and duplicated in each species after an emigration from the MHC region (Kondo et al. 2010). In humans, MHC genes (*HLA* genes) are clustered and mapped on the short arm of chromosome 6, 6p21.3, whereas the *ULBP/RAET1* genes are located on the long arm of chromosome 6, 6q25.1. As for the *MHC* genes in the macaque, it was previously reported that rhesus macaque MHC, e.g., *BAT1* gene, was localized to chromosome 6q24 by using fiber-fluorescence in situ hybridization (Huber et al. 2003) and cynomolgus (crab-eating) macaque MHC, e.g., *Mafa-A* and *Mafa-B* genes, was

cytogenetically mapped to chromosome 6p13 (Liu et al. 2007), although the rhesus macaque MHC is mapped on the short arm of chromosome 4 in the draft genome sequence database of rhesus macaques (Gibbs et al. 2007); e.g., *Mamu-A* and *BAT1* were mapped from positions 29, 517, 308 to 29, 520, 221 and from 31, 164, 822 to 31, 175, 032, respectively, on chromosome 4 (data were obtained from the UCSC Genome Browser at <http://genome.ucsc.edu/cgi-bin/hgGateway>). The discrepancy between the cytogenetic mapping and the assignment in draft genome sequence should be resolved in the future. On the other hand, it is interesting to note that each member of the *ULBP/RAET1* gene family, except for *ULBP6*, is completely or partially duplicated in the rhesus genome. As for the *ULBP4/RAET1E*, two related sequences, LOC695031 (NC007861) and LOC694265, have been identified as orthologs of human *ULBP4/RAET1E*. On the other hand, the configuration of *ULBP/RAET1* loci in the crab-eating macaque genome remained unknown. Because LOC694265 was a pseudogene lacking most part of the coding exons, we designed PCR primers by referring the NC007861 sequence. By using the designed primers, we could successfully amplify *ULBP4/RAET1E* alleles from both rhesus and crab-eating macaques.

In this study, we identified a total of 25 and 14 alleles from rhesus and crab-eating macaques, respectively. One of the rhesus macaque alleles had identical sequences to one of the crab-eating macaque alleles, and the phylogenetic analysis demonstrated that the *ULBP4/RAET1E* alleles were widely diverged. None of the alleles identified in this study were identical to the previously reported sequence NC007861, which was derived from an individual of Indian rhesus macaque. Given that we analyzed rhesus macaques of Burmese origin in this study, and allele distribution of MHC-related polymorphic genes are well known to be largely dependent on the habitat regions, the extent of diversity and variation in *ULBP4/RAET1E* may be further expanded.

It was demonstrated that the diversity of *ULBP4/RAET1E* in the Old World monkeys was much higher than that of human *ULBP4/RAET1E*. It is possible that the genes in the *ULBP/RAET1* locus, in particular, *ULBP4/RAET1E* and *ULBP/RAET1s*, might be highly polymorphic in the

Old World monkeys. We therefore investigated ten unrelated rhesus macaque subjects, in which we had detected 16 *ULBP4/RAET1E* alleles for polymorphisms in the adjacent *ULBP/RAET1* genes. We found one *ULBP1/RAET1I* allele, seven *ULBP2/RAET1H* alleles, and one *ULBP3/RAET1N* allele in these subjects. The observation suggested that *ULBP4/RAET1E* was highly polymorphic as compared to the adjacent *ULBP/RAET1* genes.

We revealed a high degree of polymorphism in the *ULBP4/RAET1E* of the rhesus and crab-eating macaques, although about half of the polymorphisms were synonymous changes (Table 2). Albeit the expression of the *ULBP4/RAET1E* molecule is known to be involved in the recognition of tumor cells by the NKG2D receptor (Cao et al. 2008; Kong et al. 2009), the functional significance of the polymorphisms in the extracellular domain of the *ULBP4/RAET1E* molecules remained unknown. To investigate a possible role of the polymorphisms, we have created a 3-D structure model of rhesus *ULBP4/RAET1E* molecule by using the structure data of human *ULBP3/RAET1N* in complex with NKG2D (Radaev et al. 2001) as the reference. As shown in Fig. 3, only one polymorphic site at 173 was on the surface of the α helix pointing to the NKG2D receptor, five sites at 59, 136, 144, 161, and 165 were positioned outside the α helix, and only two sites at 32 and 91 were mapped on the β sheet in the groove. The other polymorphic sites were on the β sheet outside of the groove or were not on the surface of the α helix. In addition, expression of *ULBP4/RAET1E* is predominantly found in the skin and tumor tissues and not induced by viral infection in normal cells (Chalupny et al. 2003; Eagle et al. 2006). These observations suggest that the polymorphisms are unlikely to be involved in the differential presentation

of characteristic small molecules bound by the *ULBP4/RAET1E* molecules, as found in the presentation of antigenic peptides by the MHC molecules. Nevertheless, highly prevalent polymorphisms leading to amino acid replacements suggest that a selection pressure had operated on the configuration of diversity in *ULBP4/RAET1E*.

Of particular interest in this study was the rhesus macaque allele *Mamu-ULBP4*8*, which was supposed to contain a stop codon in the exon 2 coding sequence that would truncate the most part of the molecule. This is the first report of a non-functional *ULBP/RAET1* allele in primates; however, a similar situation was reported for another NKG2D ligand gene, *MIC*. For example, a specific human *MIC* haplotype linked to HLA-B*048 consists of non-functional *MIC* genes, in which *MICA* was deleted and *MICB* contained a termination codon (Ota et al. 2000); the non-functional *MIC* haplotype is widely distributed in the East Asian populations (Komatsu-Wakui et al. 2001). It is interesting to note that there are two distinct and polymorphic genes for *MIC* in the rhesus macaque, *MICA* (previously designated as *MIC1* and *MIC3*) and *MICB* (previous *MIC2*); however, they are not considered to be orthologous to the human *MICA* and *MICB* genes, respectively (Seo et al. 1999, 2001; Doxiadis et al. 2007; Averdam et al. 2007). Because members of the *MIC* and *ULBP/RAET1* molecules are structurally related (Li et al. 2002), there is a functional redundancy in the recognition by NKG2D, and thus, the presence of a null allele had been allowed during the evolution of primates.

In the present study, we demonstrated the *ULBP4/RAET1E* allelic polymorphisms not only in the rhesus macaque but also in the crab-eating macaque. Although the localization of *ULBP4/RAET1E* in the crab-eating macaque

Fig. 3 Mapping of polymorphic sites on the structure model of the macaque *ULBP4/RAET1E* molecule. Polymorphic sites found in the Old World monkeys were mapped on the 3-D structure model of *ULBP4/RAET1E*. Residues on the upper and outer sides of the α helix structure were indicated by a circle and squares, respectively. Residues not found on the surface of the α helix were underlined, and those on the β sheet structure were represented by rhombi

