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ORIGINAL ARTICLE

Positional Effect of Amino Acid Replacement on Peptide Antigens for the Increased IFN-γ Production from CD4T Cells

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ABSTRACT

Background: Based on the fact that site-specific amino acid replacement on peptide antigens stimulated T cell clones to produce increased amount of IFN-γ, we investigated this structure-function relationship, using various peptide analogues.

Methods: We used three human Th0 clones (BC20.7,BC33.5 and BC42.1) that express distinct TCRα and TCRβ chains, but recognize the same TCR ligand ; *i.e.*, the same framework of peptide antigen BCGa p84-100 in the context of DRB1*1405. These T cells were stimulated with various peptide analogues, followed by determination of proliferative responses and IFN-γ production.

Results: Replacement of Leu at peptide position 2 (P2) by amino acids which are less hydrophobic than the wild type (Val, Ala) or those with similar structural or neutral charge (Thr, Ser), induced increased IFN-γ production from T cells. This phenomenon was associated with structural features of TCR, especially the length of CDR3 region of TCRα. Amino acid replacement at the other positions did not induce increased IFN-γ production.

Conclusions: Amino acid substitution at P2 frequently induces increased IFN-γ production in a clone-specific manner, which is associated with the structure of CDR3 in TCRVα chains.

KEY WORDS

analogue peptide, complementarity determining region 3, interferon gamma, peptide antigens, T-cell antigen receptor

INTRODUCTION

Recent studies showed that T cell activation is not an all-or-none type of event ; rather, qualitative changes in T cell responses can be induced by amino acid substitutions by either MHC molecules or antigenic peptides, *i.e.*, TCR ligands. Flexibility in recognition results in T-cell activation in the absence of a proliferative response, which is designated by the following terminology as demonstrated in previous studies by our group and others : partial agonism,[1] TCR antagonism,[2] anergy,[3] survival[4] and cytokine-specific up-regulation.[5,6]

Amino acid residues on antigenic peptides have been roughly divided into two groups ; one that is important for binding to TCR (T cell epitope), and the other that is important for binding to MHC (MHC anchor). However, the crystal structure of the human class II HLA-DR1 complexed with the influenza peptide reported by Stern *et al.*[7] demonstrated that all the amino acid residues of the influenza virus peptide physically made contact with both HLA and TCR, with the exception of only one residue at the amino terminus which is buried deeply in the groove of class II, hence, there is no possibility for interaction with TCR.

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In our previous studies, single amino acid substitutions on a group I allergen in the *Cryptomeria japonica* -derived peptide resulted in a significant increase in IFN-γ production, with no remarkable changes either in proliferative response or IL-4 production.[5] In this study, by using various analogue peptide species, we stimulated three human Th0 clones that express distinct TCRα and TCRβ chains, but recognize the same TCR ligand, and tried to determine the structure-function relationship that leads to increased IFN-γ production from T cells.

METHODS

SYNTHESIS OF PEPTIDES

The wild-type BCGa p 84-100 (EEYLILSARDVLAVVSK) and its analogue were synthesized using a solid-phase simultaneous multiple peptide synthesizer PSSM-8 (Shimadzu Corp., Kyoto, Japan), and were purified by C18 reverse-phase HPLC (Millipore).

T CELL CLONES

BCGa p84-100-specific T cell lines were established as described.[8] Three human CD4+ T cell clones (BC 20.7,BC33.5 and BC42.1) specific to BCGa p84-100+ DRA/DRB1*1405, yet bearing distinct TCRβ (BV13S3 ,BV6S1 and BV5S4, respectively ;)[8] established from PBMC of a BCG-primed healthy individual as described elsewhere,[4] were used throughout the study. T cells were fed 50 U/ml human rIL-2 and irradiated autologous PBMC prepulsed with the wild-type BCGa p84-100 on a weekly basis.

ASSESSMENT OF T-CELL RESPONSES

Antigen-induced proliferation of the T cell clones were assayed by culturing the T cells (3×10^4/well) in 96-well flat-bottomed culture plates in the presence of a peptide(s) and irradiated PBMC (1.5×10^5/well), using RPMI 1640 medium (Gibco, Grand Island, N.Y.) supplemented with 2 mM L-glutamine, 100 units/ml of penicillin, 100 μg/ml of streptomycin, and 10% heat-inactivated autologous plasma. For the proliferation assay, cells were cultured for 72 hr in the presence of 1 μCi/well of[^{3}H]thymidine during the final 16 hrs. Culture supernatants collected immediately before the addition of[^{3}H]thymidine were used to determine lymphokine concentrations, using hGM-CSF ELISA kits (R&D systems) and hIFN-γ ELISA kits (Otsuka,Tokyo,Japan).

DETERMINATION OF TCRA GENE USAGE BY T CELL CLONES

To determine TCRVA gene usage of the T cell clones that were cultured with irradiated autologous PBMC, RNA were extracted from the cell mixture, and converted to cDNA. TCRA variable region cDNA were amplified with anchored PCR as described previously for amplification of TCRA variable region cDNA.[9] A primary PCR was followed by two sequential nested PCR. TCRAC-specific primers used for primary PCR, nested PCR, and final PCR were CA4 (5'-CAG AAT CCT TAC TTT GTG AC), CA3 (5'-ATC GGT GAA TAG GCA GAC AG), and biotinylated CA5 (5'- CAC

Table 1 A panel of labeled TCRAV-specific oligonucleotide probes

AV gene	sequence	pool
AV4, 20	TGCTAAGACCACA/CCAGCC	A
AV11	TCTTCAGAGGGAGCTGTG	A
AV2	ATCCTTGAGAGTTTTACT	B
AV8a	CCATTCGAGCTGTATTTA	B
AV8b	GCATTCGAGCTTTATTTA	B
AV15	CATTTGCTGGATTTTCGT	B
AV17	GATCTTAGGAGCATCATT	B
AV21	TGGGGGCATCAGTGCTGA	B
AV3	GAGAAGAGGATCCTCAGG	C
AV5	ACTATTCTCCAGCATACT	C
AV10	CCGTGTCCATTCTTTGGA	C
AV13	GAGAGGAATACAAGTGGA	C
AV19	CAATTTTTGTTGGCTATT	C
AV24	AGCATCTGACGACCTTCT	C
AV25	TCCTTGAACATTTATTAA	C
AV26	CCTAGGGATATTGGGGTT	C
AV27	GAAAAAACTATACCATCT	C
AV29	CAGGCACCTTGTTGTGGC	C
AV32	ACTCATCACATCAATGTT	C
AV18	CTTTGGCAGCCCCATTAC	D
AV23	GAGACCCTCTTGGGCCTG	D
AV28	ACTAACTTTCGAAGCCTA	D
AV30	GGAGTGTGCATTCATAGT	D
AV7	GGAGGCACTA/GCAGGACAA	E
AV6	ACAGCTTCACTGTGGCTA	F
AV12	TGCCAGCCTGTTGAGGGC	F
AV14	GTGA/GTCTCCACCTGTCTT	F
AV1a	CTCCTGTTGCTCATACCA	G
AV1b	CTCCTGCTGCTCGTCCCA	G
AV1c	CTCCTGGAGCTTATCCCA	G
AV9	AAGCCCACCCTCATCTCA	G
AV16	GCCTCTGCACCCATCTCG	G
AV22	CTGATACTCTTACTGCTT	G
AV31	CCTCTCTGGACTTTCTAA	G

Oligomucleotide probes specific to TCRAV family genes. Degenerated probes were used to specify AV4 and AV20, AV7, and AV14 families. Three probes for AV1 family, and two probes for AV8 family were required to specify all members of each family. These probes were grouped into seven pools (pool A to G) depending on sequence similarity.

Table 2 TCRVA and TCRVB usage of BC clones

BC clone	TCRVA	TCRVB
20.7	(AV25S1)FCAGHNAG(AJ14S3)	(BV12S3)CASRQAGTAYE(BJ2S7)
33.5	(AV3S1)FCATERGQ(AJ13S2)	(BV6S1A1)CASSPTGTANT(BJ1S1)
42.1	(AV8S1A1)FCAASLDNY(AJ126)	(BV5S1A1)CASRRSTGE(BJ2S2)

TCRVA and VB usage are shown, with amino acid sequences in the N(D)N region.

TGG ATT TAG AGT CTC TC), respectively. A panel of labeled TCRAV-specific oligonucleotide probes (Table 1) were used to study TCRAV gene usage with PCR-ELISA.[10] First, seven pools of the AV-specific probes were hybridized with immobilized PCR products in microplates to find out positive wells. Then, the products were hybridized with individual AV probes in another set of plates to pin-point the AV genes predominantly used by the cDNA. To clone the entire variable region cDNA, cDNA were amplified with CA4 and reamplified with a nested primer, CA2 (5'-ACG CGT CGA CAC TGG ATT TAG AGT CTC TC). The products were subcloned into pBluscript II SK+ (Stratagene, La Jolla), and recombinant clones with the dominant VA gene were selected with dot blot DNA hybridization using corresponding VA-specific oligonucleotides. After sequence determination of these clones, dominant clones were selected as cDNA for the T cell clones.

RESULTS

TCRVA AND VB SEQUENCES

TCRVA and VB sequences of three T-cell clones BC 20.7, BC33.5 and BC42.1 are shown in Table 2. The N (D)N region sequences are shown as one-letter codes for amino acids, between V and J segments in parentheses. As described in our earlier studies, these T-cell clones recognize BCGa p84-100 (EEYLILSARD-VLAVVSK ; with first anchor underlined), in the context of HLA-DRB1*1405.[4] It is especially important to note that N(D)N region consists of 8 and 11 residues at TCRVA and VB of BC20.7 and BC33.5, respectively, whereas that of BC42.1 consists of 9 and 9 residues, repectively.

STIMULATORY ACTIVITIES OF BCGA P84-100-DERIVED ANALOG PEPTIDE L87V TO BC20.7

To evaluate the effects of single amino acid substitutions, proliferation and lymphokine production in response to analogue peptides were determined and findings were compared with those seen with the wild-type peptide. Most of the analogue peptides that stimulated BC clones showed a pattern of lymphokine production similar to that for the wild-type peptide (not shown). However, IFN-γ production of BC20.7 was increased in response to several analogue peptides at high concentration (16 μM), especially peptide L87V in which Leu is replaced by Val at the 87 th residue of the peptide BCGa p 84-100, whereas neither T cell proliferation nor production of other lymphokines, showed any remarkable change ; *i.e.*, only the production of IFN-γ was affected for recognition of the analog peptide L87V. As shown in Figure 1, to determine whether or not the change of IFN-γ production was due to differences in the HLA-peptide or TCR-TCR ligand avidity between L87V and the wild-type peptide, responses of BC20.7 to several different concentrations of L87V were compared with those of the wild-type peptide. In the range of concentrations from 0.016 μM up to 16 μM, IFN-γ production in response to L87V constantly exceeded that of the wild-type peptide. Moreover, the plateau level of L87 V-driven IFN-γ production was significantly higher. Mean IFN-γ production of BC20.7 for L87V increased significantly in comparison to the wild-type, whereas no statistical differences were noted in proliferative responses between R21K and the wild-type at a range of 0.16 μM to 16 μM. The IL-4 production of BC20.7 for each analogue peptide was proportional to the proliferative response to each peptide, at a range of 0.0016 to 16 μM (not shown). In contrast, production of GM-CSF gradually increased, in a dose-dependent manner throughout the range of 0.016 to 16 μM. These data indicate that the plateau responses and proliferation of IFN-γ are not due to saturation of the TCR ligand on the APC surface.

STIMULATORY ACTIVITIES OF BCGA P84-100-DERIVED ANALOGUES TO THREE BC CLONES

All three T-cell clones were stimulated with analogues at 16 μM, with replacements at P1 (=86Y) through P9 (=94V). Table 3 summarizes the results, regarding proliferative responses and IFN-γ production. P1 (=86Y) replaced by Ala (A) indicates a peptide species EEALILSARDVLAVVSK. Relative IFN-γ responses are shown, where IFN-γ production was divided by proliferation. P1 replaced by A gave values of 96/100/98, indicating that BC20.7,BC33.5 and BC 42.1 exhibited 96%, 100% and 98% responses respectively, as compared with the wild-type. Asterisks indicate peptide species that did not exert full agonistic activity ; *i.e.*, peptide stimulation even at 16 μM did not give a plateau response.

Most of analogues that exhibited full agonistic activity, stimulated IFN-γ production at levels roughly similar to the wild-type peptide, *i.e.*, at around 100%. However, it is important to note that L87T,L87S,L87A, and L87V significantly ($p<0.01$) induced increased levels of IFN-γ production of BC20.7 and BC33.5, but not of BC42.1. Such a clone-specific phenomenon was

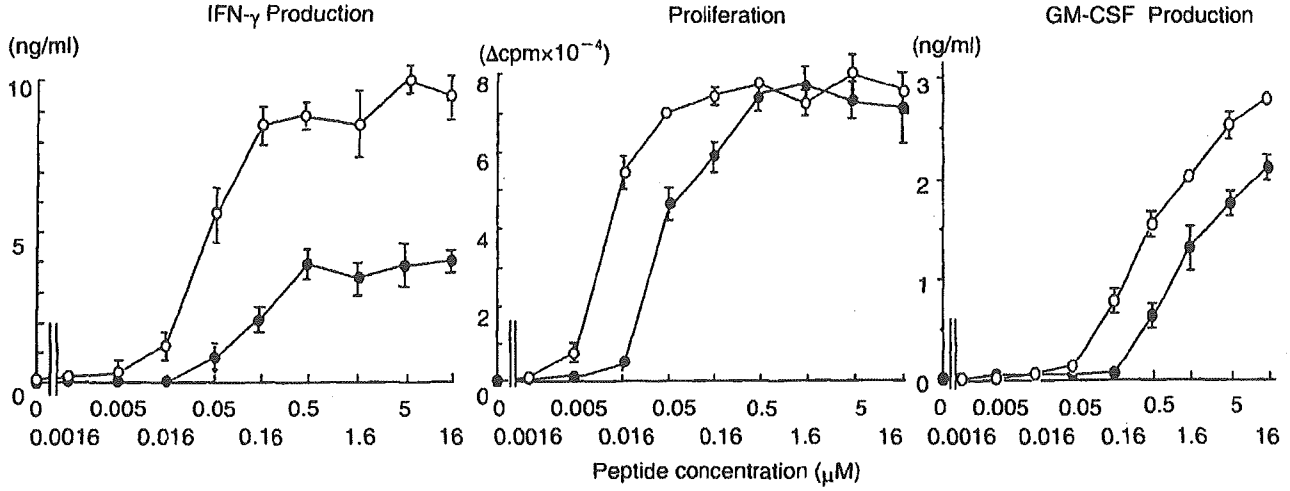

Fig. 1 IFN-γ production, GM-CSF production and proliferation of BC20.7 in recognition of either the wild-type peptide or L87V, at different concentrations. BC20.7 cells were cultured in triplicate with peptides and irradiated autologous PBMC, at the indicated concentrations. After 48-h incubation, supernatant fluids of triplicate cultures were collected. The remaining cells were pulsed with [^{3}H]-thymidine, harvested after 16h, and subjected to liquid scintillation counting. Closed circle, wild type peptide; open circle, L87V. Results are expressed as the geometric means ± standard error. IFN-γ production induced by L87V was significantly (p<0.01) higher than that induced by the wild-type peptide, at peptide concentrations ranging from 0.016 to 16 μM. On the other hand, plateau level of proliferation did not exhibit a significant difference, between 0.16 and 16 μM (p>0.05). GM-CSF production did not reach a plateau response even at 16 μM, without any statistical difference between L87V and the wild-type peptide, at 16 μM.

also observed when P5- and P8-substituted analogues were tested. Thus, S90E,S90G,S90M,D93Q,D93T and D93Y exhibited full agonism, in a clone-specific manner.

DISCUSSION

It is not very easy to identify TCR genes used by T cell clones, since they are usually cultured with irradiated autologous PBMC that includes polyclonal T cells. Random cloning of TCR cDNA derived from the cultured cells is minimally helpful in the identification, unless a large number of clones are examined. This problem was circumvented by the use of PCR-ELISA that was developed for TCRBV use,[9] and established in the present report for TCRVA usage. This technique allowed us to quantitate TCRV gene usage in the cDNA samples, and thus to identify the TCRV gene used by the T cell clones.

Three T-cell clones used in the present study recognize the same TCR ligand, as proven in our previous study. This is based on the fact that these clones recognize BCGa p84-100(^{84}EEYLILSARDVLAVVSK^{100}) in the context of DRB1*1405, and react to truncated peptides in a similar fashion.[11] Both BC20.7 and BC33.5 have 8 and 11 residues at N(D)N region of TCRVA and VB, respectively, whereas BC42.1 alone exhibits a different pattern, *i.e.*, 9 residues at N(D)N regions of TCRVA and VB. When peptide antigen is presented by class II MHC molecules, the N-terminal half of antigenic peptide is recognized mainly by CDR3 of TCRVA, whereas the C-terminal half is recoginized by CDR3 of TCRVB, which corresponds to N(D)N regions.[12] Interestingly, certain amino acid replacements on P2 induced increased IFN-γ production in BC20.7 and BC33.5 but not in BC42.1 cells, whereas those on P8 exhibited full agonism in BC 42.1 cells alone. It is thus likely that structural features of VACDR3 and VBCDR3 are responsible for specific responses induced by P2 and P8 analogues, respectively. Shuffling of N(D)N sequences between BC 42.1 and BC 20.7, or between BC 42.1 and BC 33.5 is underway to address this point.

Only L87T,L87S,L87A,and L87V induced IFN-γ enhancement. These arrangements are either smaller hydrophobic (A and V), or structurally similar neutral amino acids (T and S), indicating that close contact between P2 and TCRVA is taking place. Indeed, such a phenomenon is also seen in B-cell somatic hypermuation.[13] Thus, B-cell V region mutation in immunoglobulin heavy chain genes shows higher affinity than the germ-line sequence, usually associated with Gly, Ala, Val, Ser, Thr, or Cys, *i.e.*, small hydrophobic or small neutral residues. Apparently these mutations are not associated with static charges, but can affect either hydrogen bonding, van der Waar's force, or hydrophobic interactions.

In our previous studies using cedar pollen-derived peptides, T to V replacement on P2 also induced IFN-

Table 3 Increased IFN-γ production induced by peptide analogues

Replaced by	Relative IFN-γ response (BC20.7 / BC33.5 / BC42.1)								
	P1 =86Y	P2 =87L	P3 =88I	P4 =89L	P5 =90S	P6 =91A	P7 =92R	P8 =93D	P9 =94V
K	*/*/*	*/*/*	*/*/*	*/*/*	*/*/*	*/*/*	108/115/90	*/*/*	*/*/*
E	*/*/*	*/*/*	*/*/*	*/*/*	*/105/94	*/*/*	*/*/*	*/*/*	*/*/*
Q	*/*/*	88/79/90	*/*/*	92/79/81	*/*/*	*/*/*	*/*/*	*/*/87	*/*/*
N	*/*/*	105/97/95	*/*/*	88/92/97	*/*/*	*/*/*	*/*/*	*/*/*	*/*/*
T	*/*/*	177/210/86	110/92/81	*/*/*	77/97/108	*/*/*	*/*/*	*/*/95	85/96/91
S	*/*/*	155/187/90	95/95/99	*/*/*	100/100/100	81/87/97	*/*/*	*/*/*	93/75/99
G	*/*/*	110/98/79	90/100/92	*/*/*	*/*/115	77/69/93	*/*/*	*/*/*	94/99/100
A	96/100/98	189/202/94	105/94/83	107/93/83	88/104/110	100/100/100	*/*/*	*/*/*	80/81/92
V	91/91/85	271/259/92	91/84/86	105/96/86	99/100/101	90/76/85	*/*/*	*/*/*	100/100/100
L	93/88/102	100/100/100	100/90/101	100/100/100	*/*/*	*/*/*	*/*/*	*/*/*	*/*/*
Y	100/100/100	*/*/*	*/*/*	*/*/*	*/*/*	*/*/*	*/*/*	*/*/98	*/*/*
M	89/93/91	*/*/*	96/99/103	89/70/85	*/91/*	*/*/*	*/*/*	*/*/*	*/*/*
W	90/103/109	*/*/*	*/*/*	*/*/*	*/*/*	*/*/*	*/*/*	*/*/*	*/*/*

Positions 1 – 9 (P1 – P9) of BCGa p84-100 (EEYLILSARDVLAVVSK; with P1 underlined), was replaced by indicated amino acids. T cells were stimulated with peptide species at 16 μM. To obtain relative IFN-γ response values, plateau responses of IFN-γ (pg/ml) were first divided by plateau responses of proliferation (cpm). Then, the following calculation was performed; relative IFN-γ responses=100x[IFN-γ/proliferation to analogues]/[IFN-γ/proliferation to the wild-type BCGa p84-100]. The denominator was 0.0533. *Peptide that did not induce fully agonistic proliferation.

γ enhancement, whereas proliferation remained the same. Therefore, although not generalized, mutual replacement on G, A, V, L, S, or T at P2, tends to induce IFN-γ-specific enhancement. Such observations also have been reported in another study with different peptide species.[14] In this sense, analogue-induced clonal anergy is often observed, especially when residue replacement is made on P7 or P8.[11] Moreover, truncation of the C-terminal moiety of antigenic peptides, in general, exhibit TCR antagonism.[15] In other words, if a rule that applies to altered polyclonal novel responses induced by peptide analogues is established, it will lead us to novel therapeutic interventions using peptide analogues. Our observations on P2 replacement which is associated with increased IFN-γ production are imperative to furthering our understanding.

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ORIGINAL PAPER

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Identification of the MHC class I *B* locus in cynomolgus monkeys

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Abstract By determining the nucleotide sequences of more than 700 cDNA clones isolated from 16 cynomolgus monkeys, we identified 26 *Mafa-B* alleles. In addition, nine sequences with similarity to *Mamu-I* alleles were identified. Since multiple *Mafa-B* alleles were found in each individual, it was strongly suggested that the cynomolgus MHC class I *B* locus might be duplicated and that the *Mafa-I* locus was derived from the *B* locus by gene duplication, as in the case of the *Mamu-I* locus of rhesus monkeys.

Keywords Cynomolgus · MHC · *Mafa* · Allele

Introduction

It is well established that $CD8^+$ T-cell activation is triggered through recognition of the MHC class I molecule loaded with an antigenic peptide by an antigen-specific T-cell receptor. The MHC molecules of the mammals including primates are known to influence the outcome of many diseases such as infectious diseases, cancer, and metabolic disorders. HLA class I genes are divided into three different categories, classical (*HLA-A*, *-B*, and *-C*), non-classical (*HLA-E*, *-F*, and *-G*), and pseudogene (*HLA-H*, *-J*, *-K*, and *-L*), according to their degree of polymorphism and cell surface expression, and the presence of orthologues of the human *HLA-A*, *-B*, *-E*, *-F*, and *-G* genes were identified in several species of the Old World monkeys (Alvarez et al. 1997; Boyson et al. 1996a,b; Evans et al. 2000; Lafont et al. 2004; Otting and Bontrop 1993; Prilliman et al. 1996; Sidebottom et al. 2001; Uda et al. 2004). Cynomolgus monkeys as well as rhesus monkeys are preferentially used for biomedical research; however, cynomolgus MHC class I was not extensively studied compared with those in rhesus monkeys. We have previously reported the nucleotide sequences of cynomolgus MHC class I *A* locus and have shown that at least 14 *Mafa-A* alleles were present in cynomolgus monkeys (Uda et al. 2004). Although the MHC class I *B* locus is the most polymorphic MHC locus in primates, little information is available concerning the MHC class I *B* locus of cynomolgus monkeys. In this study, therefore, we have expanded our analysis on cynomolgus MHC class I genes and identified 26 *B* locus alleles by analyzing 16 monkeys. We have also found the presence of a novel locus that is very similar to MHC class I *I* locus recently identified in rhesus monkeys.

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Materials and methods

Animals

All cynomolgus monkeys were raised and reared in the Tsukuba Primate Center for Medical Science, the National Institute of Infectious Diseases (NIID). Both genders were involved, and the cynomolgus monkeys were between 5 and 24 years old. This study was conducted in accordance with the Guides for Animal Experiments Performed at the NIID.

RT-PCR and nucleotide sequencing

Preparation of mRNA from peripheral blood mononuclear cells (PBMC) and RT-PCR were performed as described before (Uda et al. 2004). Primers used in this study are listed in Table 1. 5′ MBS and 3′ MBS primers designed to amplify the gene products of the rhesus MHC class I *B*

Table 1 Primers used for the amplifications and sequencing of MHC class I cDNA from cynomolgus monkeys

Primers	Binding region (position)	Sequence[a]
Primers for amplification		
5′ Beta 3 XMO[b]	All loci exon 1 (–27-1)	5′-CGC TCG AGG ACT CAG AAT CTC CCC AGA CGC CGA G-3′
Mafa-B1a	*B* and I loci exon 8 (1089–1117)	5′-CCA CTT AAG ACA GTT TCA GGC TTT T-3′
5′ MBS[b]	*B* and I loci exon 1 (10–34)	5′-GCC TCG AGA ATT CAT GGC GCC CCG AAC CCT CCT CCT GC-3′
3′ MBS[b]	*B* and I loci exon 8 (1095–1116)	5′-GCA AGC TTC TAG ACC ACA CAA GAC AGT TGT CTC AG-3′
Primers for sequencing		
T7 primer	pCR4Blunt-TOPO vector (328–347)	5′-TAA TAC GAC TCA CTA TAG GG-3′
T3 primer	pCR4Blunt-TOPO vector (243–262)	5′-AAT TAA CCC TCA CTA AAG-3′
Ia698	All loci exon 4 (680–698)	5′-TAG AAG CCC AGG GCC CAG C-3′
Is437	All loci exon 3 (437–456)	5′-ATT ACA TCG CCC TGA ACG AG-3′

[a]*Xho*I, *Sal*I, and *Hin*dIII sites of 5′ beta 3 XHO, 5′ MBS, and 3′ MBS primers, respectively, are *underlined*
[b]From Boyson et al. (1996b)

locus by Boyson et al. (1996b) were also used to amplify the cynomolgus MHC class I *B* locus. PCR amplification was performed at least twice for each animal. PCR products were cloned into pCR4Blunt-TOPO plasmids (Invitrogen, Carlsbad, Calif., USA) and 48 clones were sequenced by 310 Capillary DNA Sequencer (Applied Biosystems, Foster City, Calif., USA) or 3100-Avant Capillary DNA Sequencer (Applied Biosystems). The *Mafa-B* nucleotide sequences were assembled with the Contig Manager of the DNASIS pro (Hitachi Software, Yokohama, Japan). The Clustal W algorithm provided in DNASIS PRO was used to align sequences.

Phylogenetic analysis

The full-length nucleotide sequences of *Mafa-B*, *Mafa-I*, *Mafa-A*, *Mamu-A*, *Mamu-B*, *Mamu-I*, *HLA-A*, and *HLA-B* were aligned using Clustal W provided online by the DNA Data Bank of Japan [(DDBJ) http://www.ddbj.nig.ac.jp]. A phylogenetic tree of these nucleotide sequences was constructed by the neighbor-joining method of the Molecular Evolution Genetics Analysis, version 2.1 (MEGA 2.1). Genetic distances were estimated using the method of Jules-Canter. At the sites in which alignment indicated a gap, nucleotides at this position in all the sequences were deleted. The reliability of the tree topology was tested by the bootstrap method. Thousand relationships and 64,238 random seeds were used for determining bootstrap values (Fig. 2a, b). Since the bootstrap values of less than 50% were unreliable, the values of less than 50% were not shown in Fig. 2a, b.

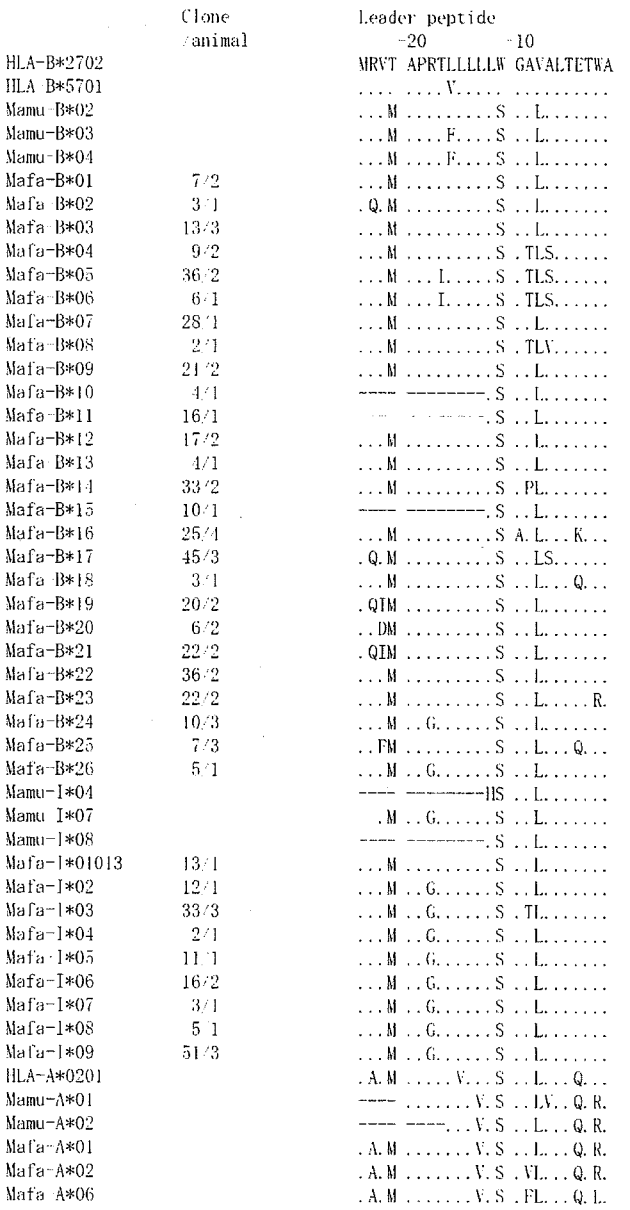


Fig. 1 Deduced amino acid sequences of *Mafa-B* and *Mafa-I* alleles. Amino acid sequences of *HLA-A*, *HLA-B*, *Mamu-A*, *Mamu-B*, *Mamu-I*, *Mafa-A*, and *Mafa-E* alleles were also included. Amino acids identical to those of *HLA-B*2702* are indicated by *dots*. The deletions of amino acids are indicated by *hyphens*. The total numbers of clones obtained and the numbers of animals having the allele were indicated *after the allele name*

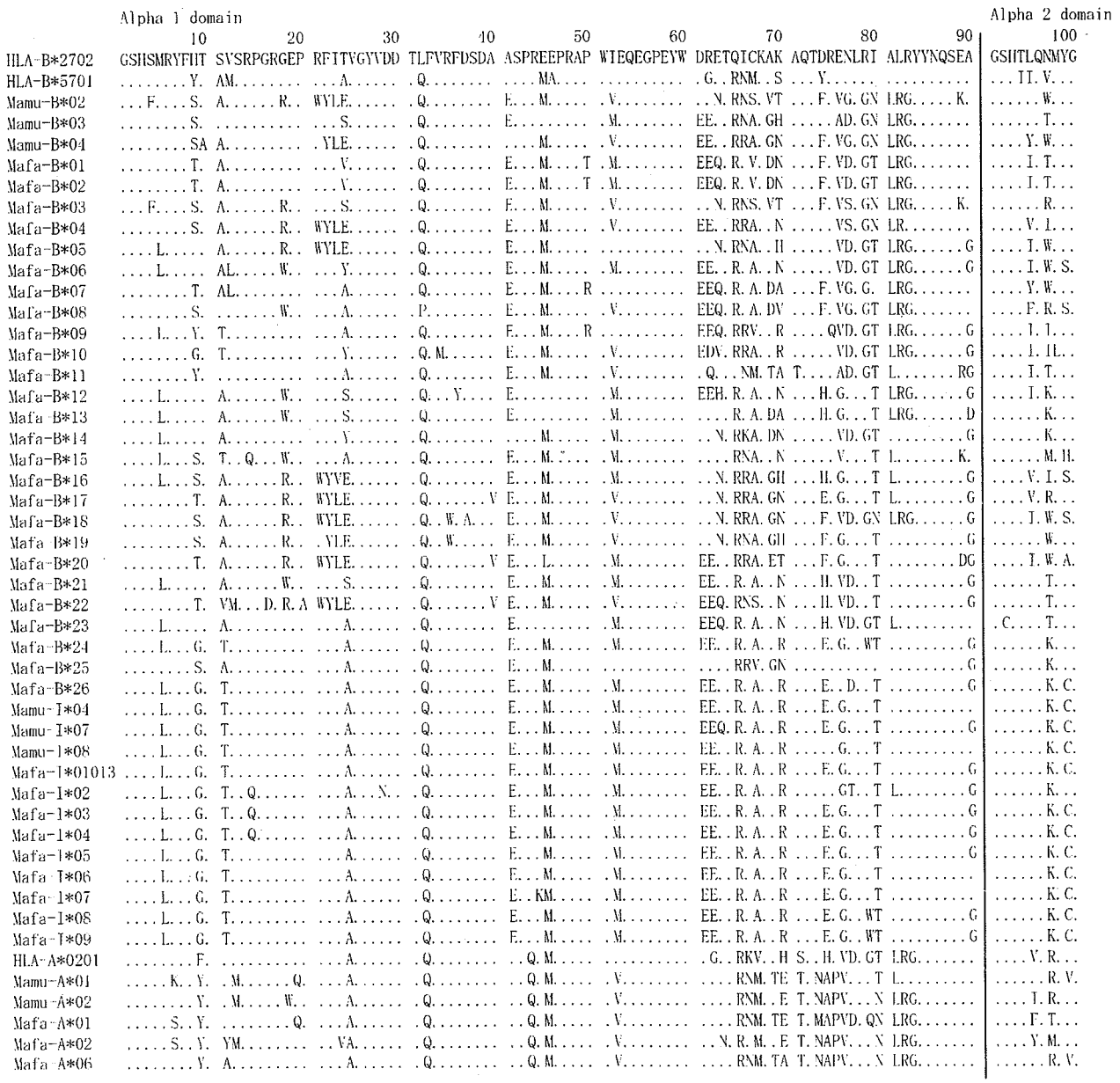


Fig. 1 (continued)

GenBank accession numbers

The *Mafa-B* and *Mafa-I* sequences described in this manuscript had been deposited in the DDBJ and were assigned accession numbers AB195431 to AB195465. We previously deposited *Mafa-A* alleles in the DDBJ, and these alleles were assigned accession numbers AB154760 to AB154773. The GenBank accession numbers for other sequences used in this study are as follows: *HLA-A*0201*, U07161; *HLA-B*2702*, L38504; *HLA-B*5701*, AJ458991; *Mafa-E*01*, U02976; *Mamu-A*01*, U50836; *Mamu-A*02*, U50837; *Mamu-A*03*, U41379; *Mamu-A*04*, U41380; *Mamu-B*02*, U41833; *Mamu-B*03*, U41825; *Mamu-B*04*, U41826; *Mamu-B*05*, U41827; *Mamu-B*06*, U41828; *Mamu-B*07*, U41829; *Mamu-B*08*, U41830; *Mamu-B*36*, AJ556886; *Mamu-I*01011*, AF161865; *Mamu-I*02012*, AF161869, *Mamu-I*04*, AF4161874; *Mamu-I*07*, AF161875; *Mamu-I*08*, AF161876; *Mamu-I*09*, AF161877; *Mamu-I*10*, AF161878; and *Mamu-I*11*, AF161879.

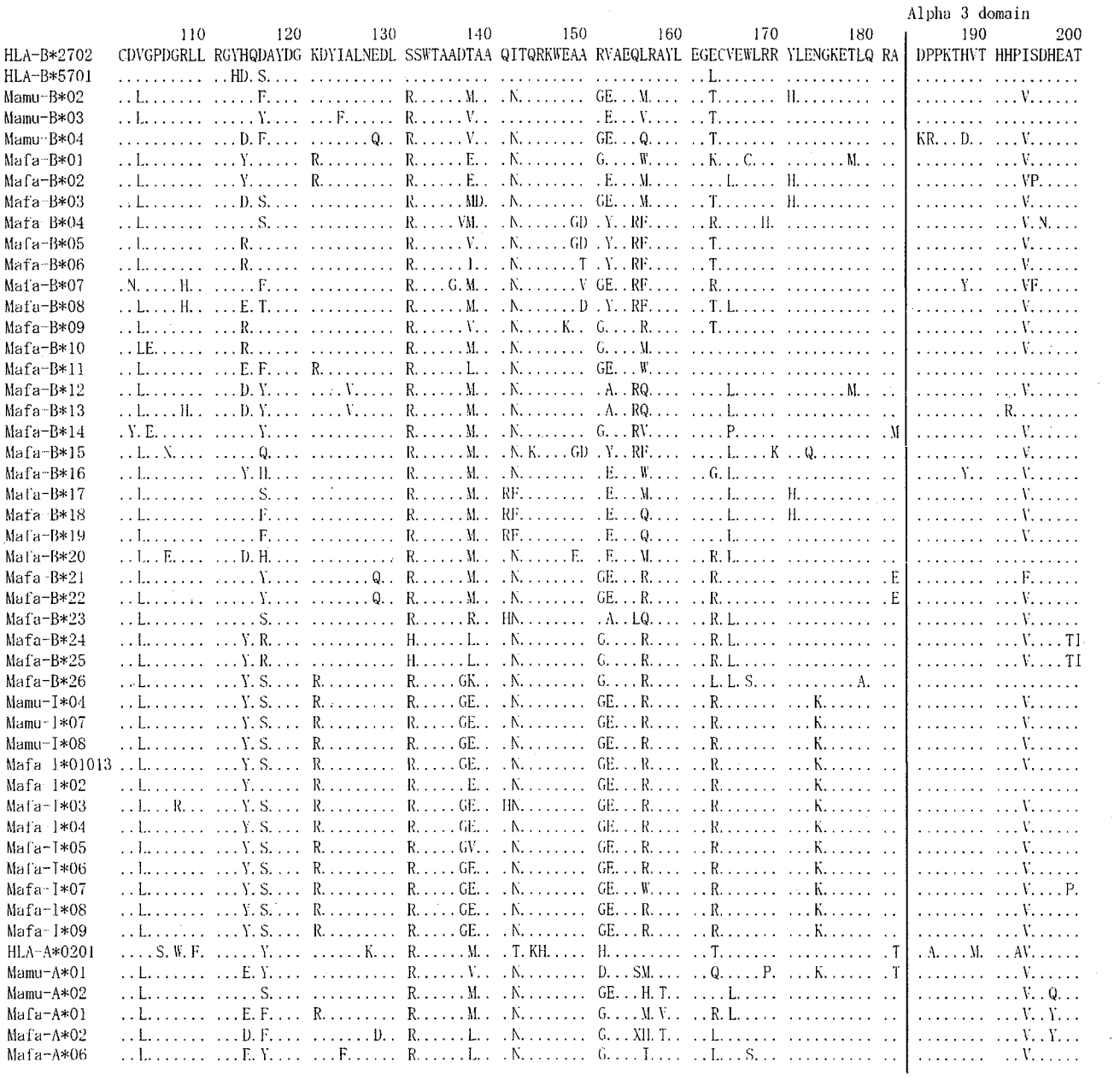


Fig. 1 (continued)

Results

Detection of 26 MHC class I *B* locus alleles and nine *I* locus alleles in cynomolgus monkeys

To amplify cynomolgus MHC class I *B* locus genes, PCR was carried out using primers that were successfully used for amplification of rhesus MHC class I *B* locus genes along with newly designed ones (Table 1; Boyson et al. 1996b). We obtained 48 clones from each animal. The nucleotide sequences that were found in just one clone were excluded from the subsequent analyses to avoid incorporation of artificial sequences generated by PCR error or during the cloning procedure into public databases. Ambiguous sequences were also excluded. When the nucleotide sequence was shared by more than two clones, regardless of whether they were derived from one animal or multiple animals, the sequences were regarded as a consensus sequences representing a particular alleles of each animal. Eventually, 43 candidate alleles were obtained, and 34 of 43 were found to have substantial homology with *Mamu-B* alleles. Amino acid sequences deduced from the nucleo-

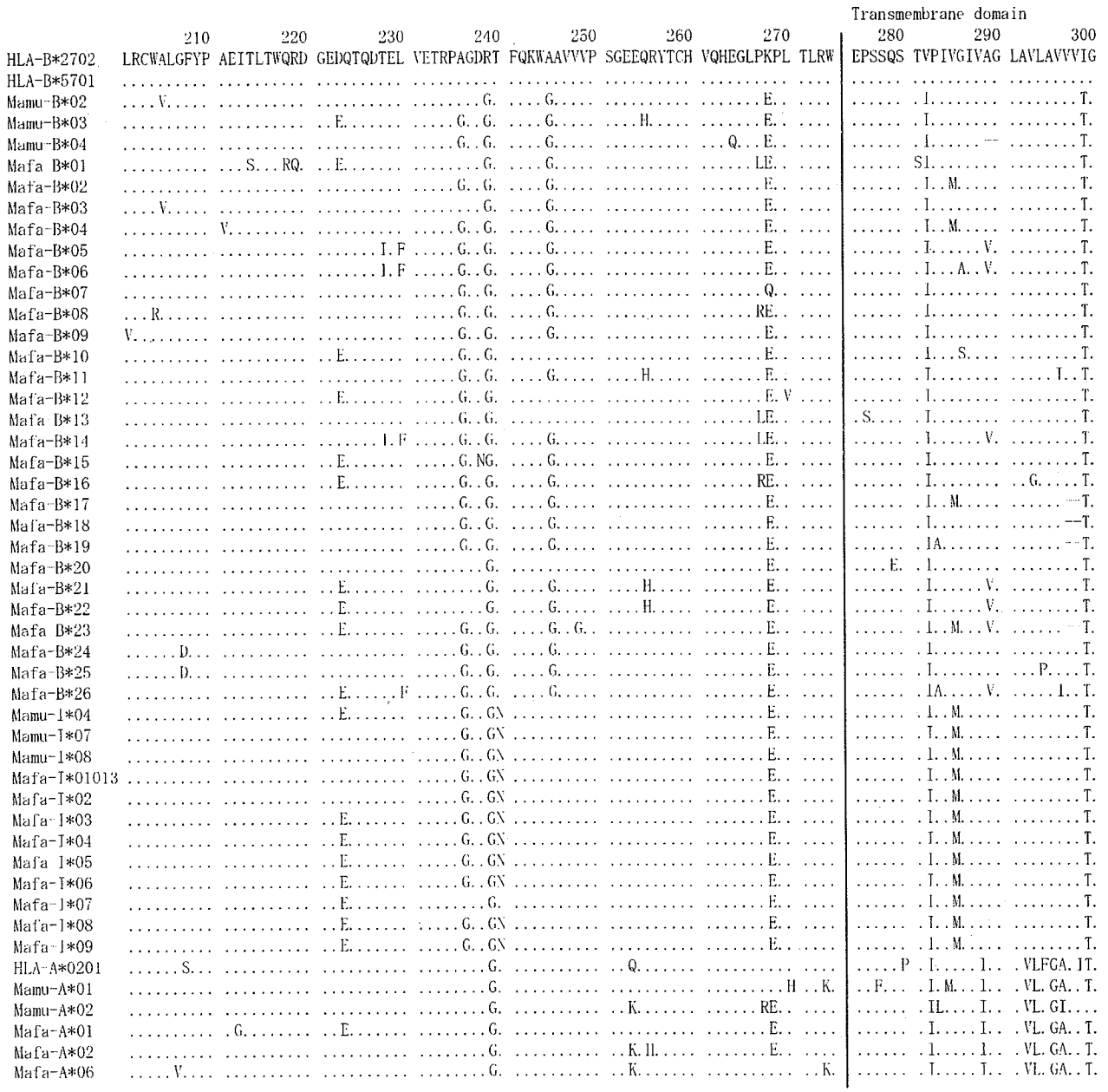


Fig. 1 (continued)

tide sequences of these 34 candidate *B* alleles were further subjected to phylogenetic analysis using the neighbor-joining method (Saitou and Nei 1987; data not shown). When the predicted amino acid sequence variation between two candidates was negligible (d<0.025), the amino acid sequence shared by a majority of the clones was regarded as representing a particular allele. The other sequence shared by a minority of clones was excluded from the subsequent analyses. As the result of the analysis, 26 *Mafa-B* alleles were identified. It was found that the remaining nine candidate alleles were closely related to those of *Mamu-I* locus reported by Urvater et al. (2000b). Since Urvater et al. also identified two *Mafa-I* alleles (*Mafa-I*01011* and *Mafa-I*01012*), we named tentatively alleles identified here *Mafa-I*01013* through *Mafa-I*09*. The *Mafa-I*01013* allele was identical in amino acid sequence with *Mafa-I*01011* and *Mafa-I*01012*, but there were several synonymous nucleotide changes scattered around the sequence. We therefore considered that this alleles was a variant of *Mafa-I*01*, although reported sequences of *Mafa-I*01011* and *Mafa-I*01012* were incomplete. The deduced amino acid sequences of *Mafa-B* and *Mafa-I* alleles were shown in Fig. 1

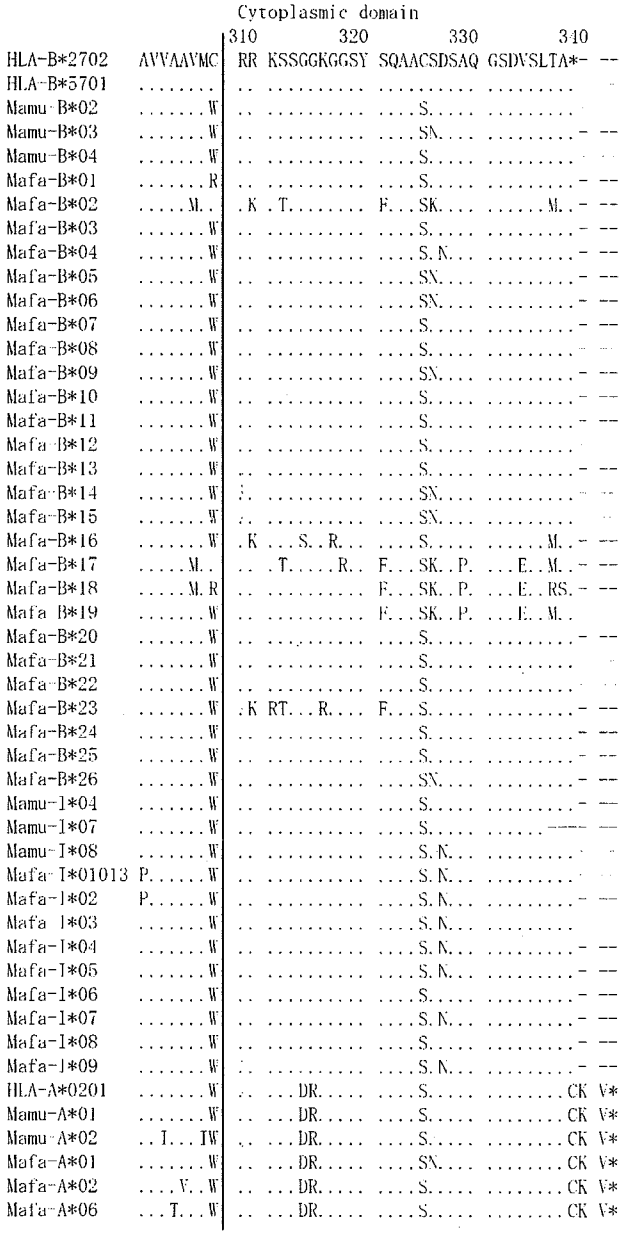


Fig. 1 (continued)

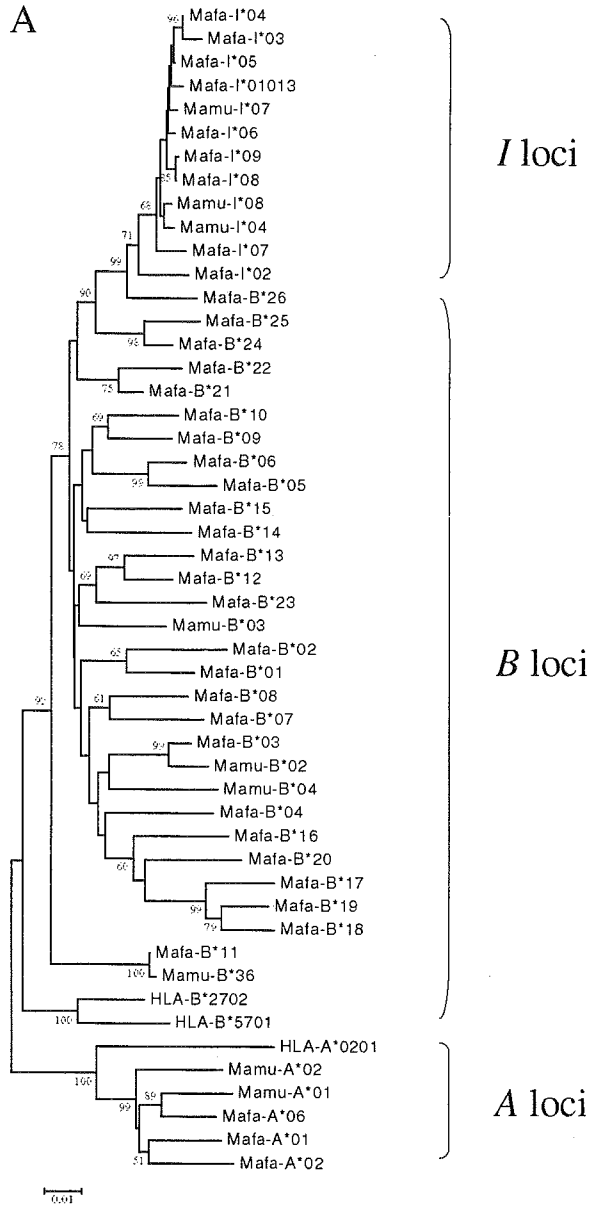
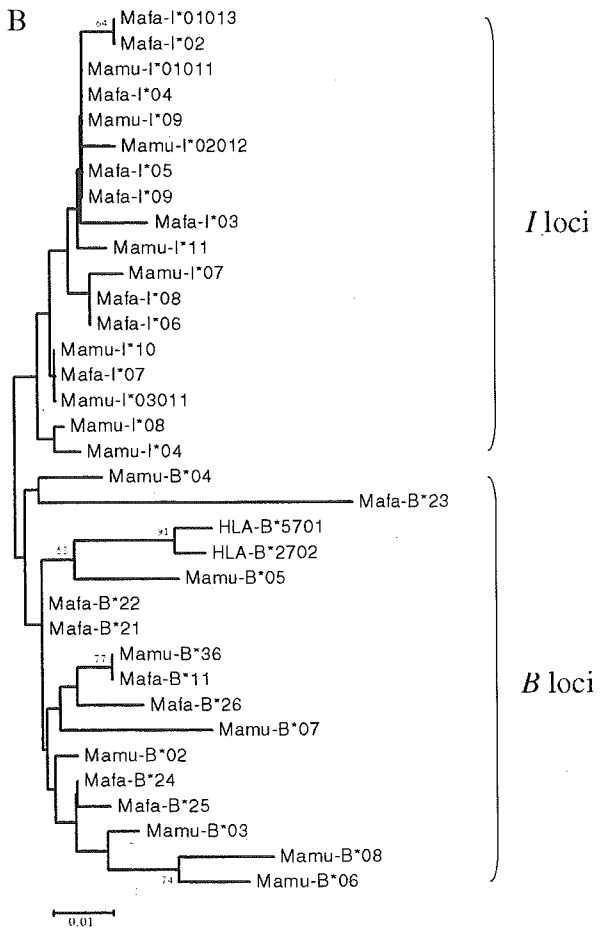


Fig. 2 Phylogenetic analysis of primate class I MHC molecules. The phylogenetic tree was constructed using **a** full-length and **b** exon five to eight nucleotide sequences by neighbor-joining method with MEGA2.1. The bootstrap values of more than 50% were shown

along with those of alleles reported for other primates. The total numbers of clones obtained and the numbers of animals having the allele were shown in the figure. The putative glycosylation site was located at residue 86, and the conserved cysteine residues occurred at positions 101 and 164 in $\alpha 2$ and at positions 203 and 259 in $\alpha 3$. To evaluate whether the nucleotide sequences of *Mafa-B* and *Mafa-I* alleles established in this study were gene products of class I *B* and *I* loci, respectively, *Mafa-B* and *Mafa-I* alleles were phylogenetically analyzed (Fig. 2a). The full-length nucleotide sequences of *Mafa-B*, *Mafa-I*, *Mafa-A*, *Mamu-A*, *Mau-B*, *Mamu-I*, *HLA-A*, and *HAL-B* were aligned by Clustal W. A phylogenetic tree was constructed by the neighbor-joining method of MEGA2.1 software. The reliability of the tree topology was tested by the bootstrap method, and the bootstrap values are shown in Fig. 2a. Since the bootstrap values of less than 50% were unreliable, the bootstrap values of greater than 50% are shown in Fig. 2a. Several *Mafa-B* alleles (*Mafa-B*21*, *22*, *24*, *25*, and *26*) appeared to cluster with *Mamu-I* or *Mafa-I* allele rather than *B* locus alleles. Since amino acid difference between alleles of *I* and *B* loci were more apparent at the carboxy half of the protein, we recon-



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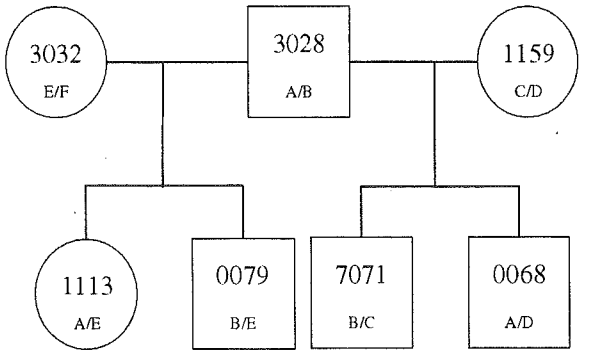

Fig. 3 The family pedigree demonstrating the inheritance of alleles of the MHC class I *B* and *I* loci of cynomolgus monkeys. Male and female are denoted by *squares* and *circles*, respectively. The animal number assigned to the animal is shown. The haplotypes of each animal are given by *capital letters*

structed the phylogenetic tree using the amino acid sequences of the exons 5 to 8. The result clearly showed that these nine alleles clustered with *Mamu-I* alleles (Fig. 2b). These results strongly suggested that these cDNA clones were derived from distinct alleles on MHC class I *B* and *I* loci of cynomolgus monkeys.

Inheritance of *Mafa-B* and *Mafa-I* in a family of cynomolgus monkeys

A family consisting of three parents (one sire and two dams) and four offspring was subjected to genetic analysis to study inheritance of *Mafa-B* and *Mafa-I* alleles. By nucleotide sequence analysis, ten *Mafa-B* alleles and four *Mafa-I* alleles were detected in this family as shown in Table 2. Since certain alleles appeared to be inherited in this family as a complex, we considered those gene complexes as haplotypes and assigned letters A through F to those combinations of alleles (Table 2). Haplotype A (*Mafa-B*03* and *Mafa-I*09*)

Table 2 Segregation of alleles with haplotypes

Haplotypes	Alleles	Animal no.						
		3032	3028	1159	1113	0079	7071	0068
A	*Mafa-B*03*		○		○			○
	*Mafa-I*09*		○		○			○
B	*Mafa-B*24*		○			○	○	
	*Mafa-B*25*		○			○	○	
C	*Mafa-B*17*			○			○	
	*Mafa-B*20*			○			○	
	*Mafa-I*06*			○			○	
D	*Mafa-B*01*			○			○	
	*Mafa-B*04*			○				○
E	*Mafa-B*16*	○			○	○		
	*Mafa-I*03*	○			○	○		
F	*Mafa-B*06*	○						
	*Mafa-B*23*	○						
	*Mafa-I*02*	○						

○ :Alleles were detected in each individual

Table 3 The presence of multiple *Mafa-B* alleles in cynomolgus monkeys

Animal no.	Alleles	Number of copies	Primers
2010	*Mafa-B*09*	5	5′MBS/3′MBS, 5′Beta 3 XHO/Mafa-Bla
	*Mafa-B*11*	16	5′MBS/3′MBS, 5′Beta 3 XHO/Mafa-Bla
	*Mafa-B*12*	7	5′MBS/3′MBS, 5′Beta 3 XHO/Mafa-Bla
	*Mafa-B*19*	2	5′MBS/3′MBS, 5′Beta 3 XHO/Mafa-Bla
5076	*Mafa-B*10*	4	5′MBS/3′MBS
	*Mafa-B*14*	17	5′MBS/3′MBS, 5′Beta 3 XHO/Mafa-Bla
	*Mafa-B*15*	10	5′MBS/3′MBS
	*Mafa-I*010103*	13	5′MBS/3′MBS, 5′Beta 3 XHO/Mafa-Bla

was detected in 3028, 1113, and 0068, whereas haplotype B (*Mafa-B*24* and *Mafa-B*25*) was carried by 3028, 0079, and 7071 (Fig. 3). Haplotype C (*Mafa-B*17*, *Mafa-B*20*, and *Mafa-I*06*) was found in 1159 and 7071, haplotype D (*Mafa-B*01* and *Mafa-B*04*) in 1159 and 0068, haplotype E (*Mafa-B*16*, *Mafa-I*03*) in 3032, 0079, and 1113, and haplotype F (*Mafa-B*06*, *Mafa-B*23*, and *Mafa-I*02*) in 3032 (Fig. 3). We could not detect *Mafa-I* alleles in monkeys bearing haplotypes B and D. It was evident that *Mafa-B* alleles were inherited in a Mendelian fashion. Moreover, cynomolgus monkeys in this family were shown to have two to four *Mafa-B* alleles. The presence of multiple *Mafa-B* alleles was confirmed by nucleotide sequences analysis of two additional cynomolgus monkeys unrelated to this family. Table 3 showed that 2010 had four *Mafa-B* alleles and 5076 had three *Mafa-B* alleles. These results indicated that MHC class I *B* locus of cynomolgus monkeys was duplicated as in the case of rhesus monkeys (Boyson et al. 1996b).

Discussion

Although cynomolgus monkeys are widely used as animal models in a variety of biomedical researches, there are no nucleotide sequence data on cynomolgus MHC class I *B* locus. In this study, we tried to identify the alleles of cynomolgus MHC class I *B* locus, using PBMC cDNA from 16 cynomolgus monkeys.

Nucleotide sequence analyses and following phylogenetic analysis identified 26 *Mafa-B* alleles (Figs. 1, 2a). We also found nine clones with the nucleotide sequences showing high homology with those of *Mamu-I* alleles. Phylogenetic analysis showed that these clones were derived from nine *Mafa-I* alleles. It was reported that novel MHC class I *I* locus in rhesus monkeys, *Mamu-I*, could be amplified with *B* locus-specific primers, and that the *I* locus was recently evolved from a classical MHC class I *B* locus by duplication (Urvater et al. 2000b).

The haplotypes of rhesus MHC class I composed of at least one *A* locus and at least two *B* loci (Boyson et al. 1996b). In cynomolgus monkeys, we previously reported that the *A* locus had been duplicated, because one to four *Mafa-A* alleles were found in an animal (Uda et al. 2004). The presence of up to six *Mamu-B* alleles in a rhesus monkey (Urvater et al. 2000a) indicates that rhesus monkeys have three class I *B* loci. In this study, we also showed that two to four *Mafa-B* alleles were present in each individual, strongly suggesting that cynomolgus monkeys have multiple MHC class I *B* loci. Regarding the *I* locus, it seemed possible that at least one locus was present in each animal, although some individual appeared not to have the locus. The apparent lack of the *I* locus in some individual was probably due to low efficiency of amplification of the *I* locus because of the presence of the multiple *B* loci.

Information on MHC class I molecule is particularly important in better understanding of pathogenesis of various infectious diseases including HIV infection. So far the nucleotide sequence data are available for the alleles of *Mafa-A* (Uda et al. 2004), *Mafa-E* (Alvarez et al. 1997; Boyson et al. 1995), *Mafa-G* (Arnaiz-Villena et al. 1997; Castro et al. 1996), *Mafa-I* (Urvater et al. 2000b), *Mafa-DRB* (Gaur et al. 1997; Kriener et al. 2000; Leuchte et al. 2004), *Mafa-DQA* (Kenter et al. 1992), and *Mafa-DQB* (Otting et al. 2002) in cynomolgus monkeys. The identification of *Mafa-B* alleles would, therefore, greatly help understand the pathogenesis of various pathogens that naturally or experimentally infect cynomolgus monkeys.

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Lecture

解　説

インバリアントNKT細胞によるTh1/2バランスの調節*

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Key Words：invariant NKT cells, α-GalCer, CD1d, Th1/2, dendritic cells

はじめに

インバリアントNKT(iNKT)細胞は，CD1d分子によって提示された糖脂質抗原を認識し，TCRα鎖に可変性のないインバリアントT細胞抗原受容体(iTCR：マウスではVα14Jα281，ヒトではVα24JαQ)を発現する．iNKT細胞はTCRを介した抗原刺激により活性化されると短時間で大量のIFN-γとIL-4を産生し，自然免疫，および獲得免疫応答を担当する種々の細胞の多様な生物活性を誘導するとともに，自己免疫疾患や感染症，腫瘍免疫，移植免疫などの免疫制御において重要な役割を示すことで注目視されている．最近，iTCRのリガンドであるCD1d/α-ガラクトシルセラミド(α-GalCer)複合体の立体構造が明らかにされるとともに，ヒトとマウスのiNKT細胞が共通して認識する自己および微生物由来の糖脂質抗原が同定され，iNKT細胞が有する多様な生物活性との因果関係が明らかにされつつある．本稿ではiNKT細胞によるヘルパーT(Th)応答制御における役割について最近の知見を踏まえて解説する．

CD1d/糖脂質抗原複合体の構造とiTCR認識の多様性

近年，ヒトとマウスのiNKT細胞がCD1d拘束性に，α-GalCerを認識して活性化を受けることが明らかとなり，iNKT細胞の有する生物活性が種々の観点から解析されてきた．さらに最近，ヒトおよびマウスにおけるCD1d/α-GalCer複合体の立体構造が解明され，iTCRの抗原認識における構造基盤が明らかとなった[1)2)]．これによるとα-GalCerのアシル鎖がCD1d分子のA′ポケットに反時計回りに回転しながら収容され，A′ポケットを安定化する．一方，スフィンゴシン鎖はF′ポケットに収容される．親水性頭部のガラクトースは，これら2つの疎水性のポケットの中央に位置しながら溶媒に露出する(図1A)．iTCRのTCRVα領域はCD1d分子のA′ポケットの上方でα2ヘリックス側に，TCRVβ領域はCD1d分子のF′ポケット上方でα1ヘリックス側に位置するようにCD1d分子と会合し，主にTCRα鎖のCDR3領域でα-アノマータイプのガラクトースを認識することが予想される．さらに，MHC class I分子がペプチドを結合する際にα1とα2のヘリックスの間隔が狭くなるのと同様に，α-GalCerの結合によりCD1d分子の両ヘリックスの間隔が狭くなるなどの特徴からiTCRの抗原認識は，基本的にαβTCRとMHC class I分子の相互作用に類似していることが予想される．しかし，CD1d分子に結合したα-GalCerのガラクトースの位置がヒトとマウスで異なっていることから(図1B)，ヒトとマウス

* Regulation of T helper type 1 and type 2 immune responses by invariant natural killer T cell.

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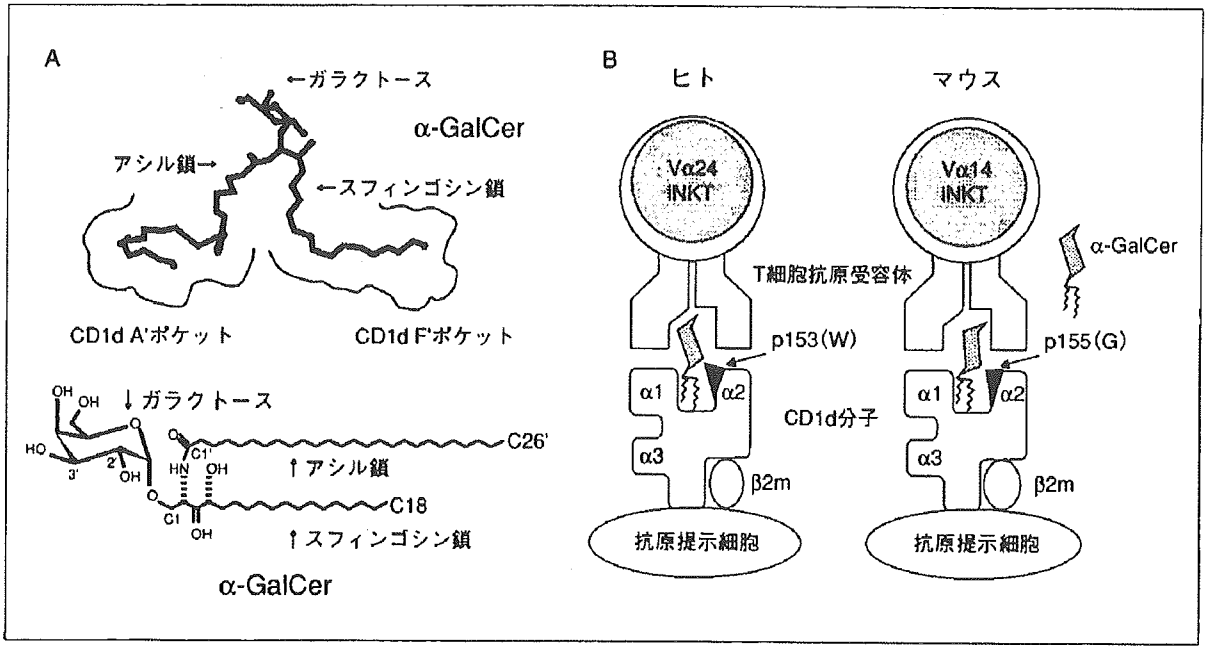

図1 iNKT細胞が認識する糖脂質抗原とCD1d分子

A：α-GalCerの構造とCD1d/α-GalCer複合体の模式図．α-GalCerのアシル鎖がCD1d分子のA'ポケットに，スフィンゴシン鎖がF'ポケットに収容される様子を示す．親水性のガラクトース部分が溶媒に露出し，iTCRに認識されると考えられる．

B：ヒトおよびマウスのiTCR認識における抗原認識の違い．α-GalCerのガラクトース部分に近接するヒトCD1d p153のアミノ酸残基はトリプトファン(W)であり，これに相当するマウスCD1d p155はグリシン(G)となっている．ヒトCD1d p153 Wは側鎖構造が大きいため，α-GalCerの頭部ガラクトースを押し出している．これによりガラクトースの位置がヒトとマウスで約3Åずれている．したがってヒトとマウスのiTCRの認識様式が若干異なることが予想される．

のiTCRが認識可能な抗原プロフィールが異なることが予想される[3]．また，α-GalCerとは大きな構造の違いを有する自己糖脂質抗原，isoglobotrihexosylceramide(iGb3)(図2)をヒトとマウスの両方のiTCRが認識することなどを考慮すると，iTCRの認識はこれまで考えられていた以上に可塑性を有しており，多様な糖脂質抗原を認識することが予想される．今後，iTCRが，CD1d/糖脂質抗原分子複合体とどのようにして会合するのか，また，多様なTCRβ鎖の影響，およびiTCR認識における可塑性の程度が，iTCR/糖脂質抗原/CD1dの三分子複合体の構造解析により明らかにされるであろう．

iNKT-TCRリガンドを用いたTh応答制御

MHC class Ⅰ，あるいはclassⅡ拘束性のαβTCR認識は多様であり，アミノ酸配列のまったく異なるペプチドをも認識することが明らかとなっている[4]．さらにこれらのペプチドの中には，T細胞のサイトカイン産生性を変化させるものが存在する[5]．前述のように，iTCRの認識も多様であることが予想されるが，すでにα-GalCerの変異アナログを用いることによってTh応答を制御しようとする試みが行われている(図2)．α-GalCerのα-C-グリコシドアナログを認識したマウスiNKT細胞は選択的にIFN-γ産生を誘導する[6]．一方，α-GalCerのスフィンゴシン鎖の炭素9個，アシル鎖の炭素2個を短くしたOCH，あるいはアシル鎖の炭素18個を短くしたPBS-25では，IFN-γに対するIL-4の産生比が上昇する．OCHによって誘導されるサイトカインバランスの変化は，スフィンゴシン鎖が短縮することによりCD1d分子との結合がα-GalCerより不安定となることに起因すると考えられている[7]．一方，PBS-25は水性溶媒に対する溶解性が高く，アシル鎖が著しく短いにもかかわらずCD1d分子との結合安定性に優れている．CD1d-PBS-25複合体の構造解析では，PBS-25の短いアシル鎖を補償するとともにCD1d

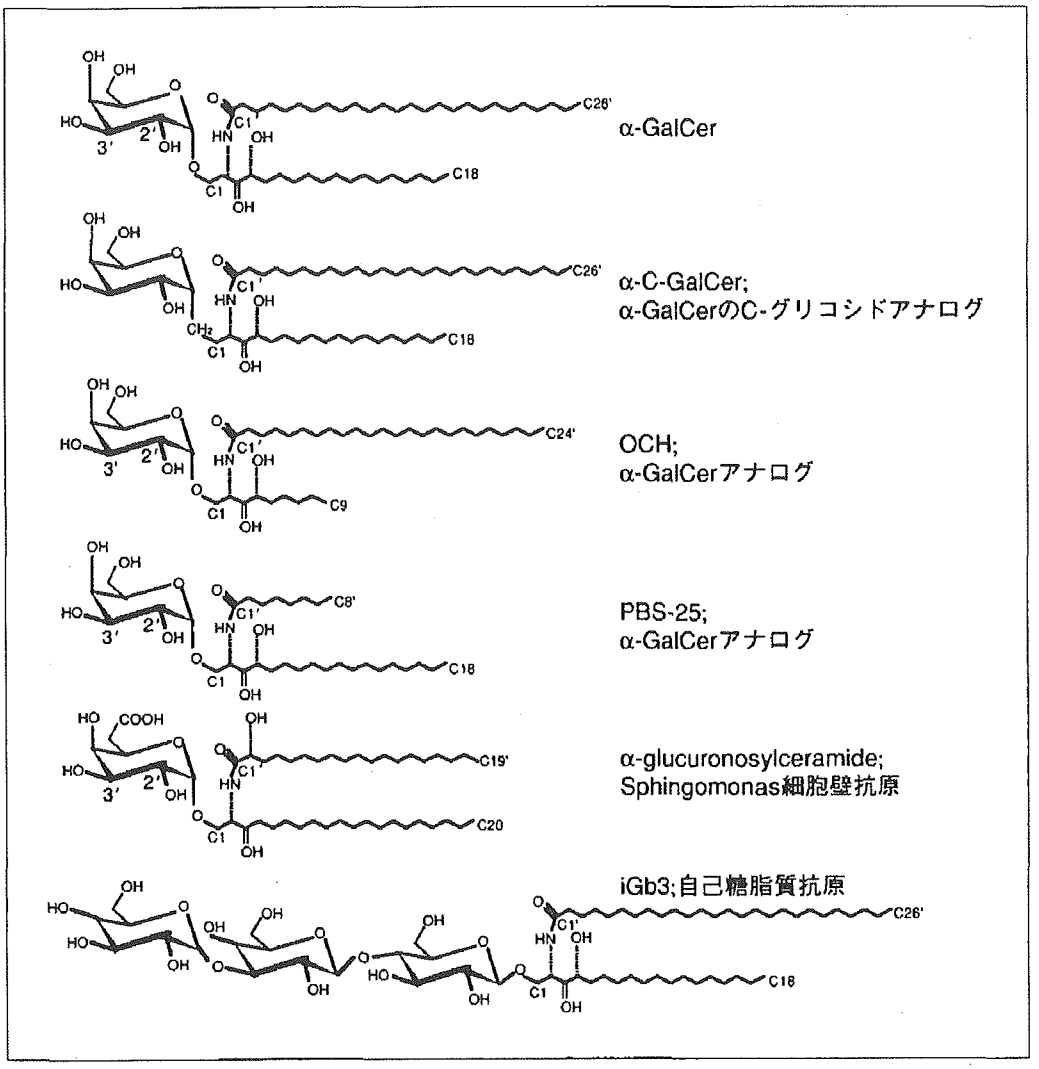

図2　iNKT細胞が認識する糖脂質抗原の構造
自己糖脂質抗原であるiGb3のガラクトースだけが，βフォームをとっているがこれもiNKTによって認識される．

分子の安定化を促進する内因性のスペーサーリピッドがA′ポケットに存在することが明らかとなっている[2]．α-GalCerがCD1d分子と会合するには，このスペーサーリピッドをlipid-transfer proteinの作用によりA′ポケットから取り出す必要がある[8]．CD1d分子は，MHC分子のように個人差がないため，これら糖脂質抗原のアナログはiNKT細胞のサイトカイン産生性を制御する格好のワクチンとなりうる．水性溶媒への溶解性，エンドソームにおけるlipid-transfer proteinの関与，受容体を介した抗原の取込み，CD1d分子との結合親和性，安定性などがiNKT応答を制御するワクチンデザインの重要な要素となる．

Th応答性における iNKTサブセットの役割

iNKT細胞には，Th2免疫応答を促進して自己免疫応答を抑制する機能を有するという報告がある一方，樹状細胞(DC)などのIL-12産生を促進することにより誘導されるTh1免疫応答を促進し，腫瘍細胞の拒絶，あるいは感染防御に貢献するという報告もある[9]．この矛盾する観察は，iNKT細胞には異なる機能を有するサブセットが存在し，これらのバランスの変化によって免疫応答性が制御されていることを示唆している．マウスiNKT細胞には，組織の違いによりそれぞれ異なるサイトカイン産生性や共刺激シグナル依存

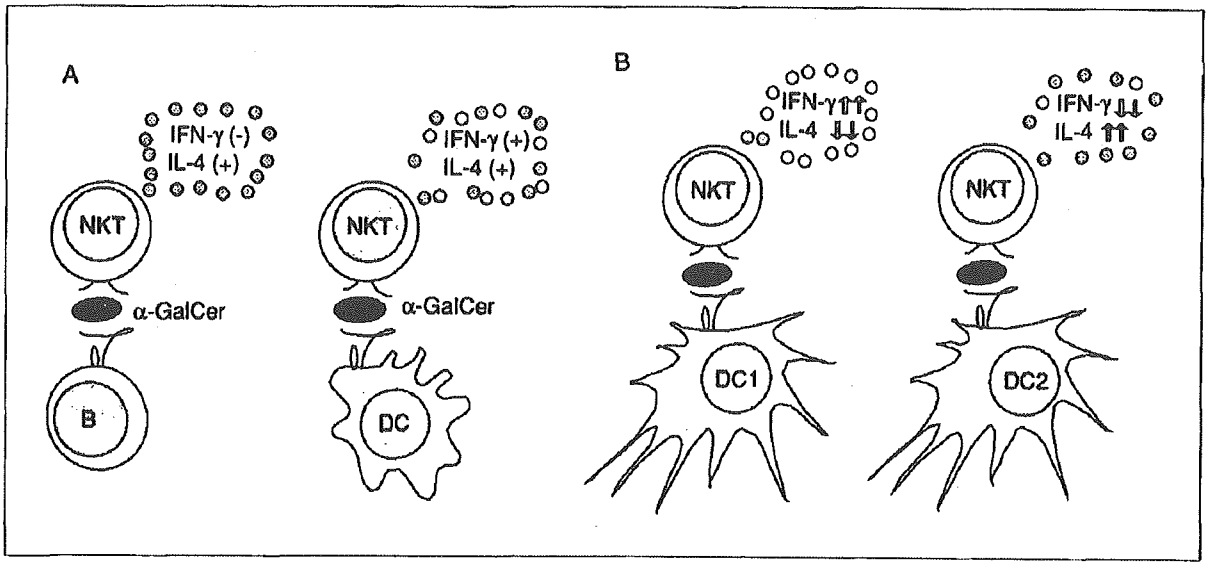

図3　抗原提示環境とiNKT細胞の応答性

A：抗原提示細胞とiNKT細胞応答．DCによって提示されたα-GalCerを認識したマウスiNKT細胞はIFN-γとIL-4の両方を産生する．これに対してB細胞によって提示されたα-GalCerを認識したiNKT細胞はIFN-γを産生せずに低レベルのIL-4を産生する．

B：DCサブセットとiNKT細胞応答．Th1誘導性のDC(DC1)によって提示されたα-GalCerを認識したヒトiNKT細胞はTh1サイトカインを優位に産生するが，Th2誘導性のDC(DC2)によって提示されたα-GalCerを認識したヒトiNKT細胞はTh2サイトカインを優位に産生する．このiNKTの応答性は可逆的である．

性を示すサブセットが存在するという報告がある[10]．

ヒトiNKT細胞は，$CD4^+$，$CD8^+$，および$CD4^-CD8^-$ (double negative；DN)の3つのサブセットから構成されており，サイトカイン産生性やケモカイン受容体の発現などがそれぞれ異なっている[11]．CD4 iNKTはTh2サイトカイン(IL-4, IL-13)の高産生性を示し，IL-2受容体α鎖(CD25)を発現する．DN iNKTは，Th1サイトカイン(IFN-γ, TNFα)を主に産生する．2B4, CD94, NKG2A, CD161などのNK受容体，およびCCR5, CCR6, CXCR6などのケモカイン受容体は，DNサブセットに特異的に発現する[12][13]．したがってCD4/DNバランスがTh1/Th2サイトカインバランスに直接的に影響を与える可能性がある．近年，全身性強皮症，1型糖尿病，全身性エリテマトーデス，関節リウマチなどの自己免疫疾患の患者群において，DN iNKTサブセットの減少，あるいは機能不全が報告され，これらの疾患の病因，病態に深く関与していることが示唆されているが，これらの現象が上記の疾患にどのように関与しているかについては明らかとされていない[14]~[16]．

抗原提示細胞環境の違いによるiNKT細胞のTh応答性

α-GalCerを提示する抗原提示細胞(APCs)がDCかBリンパ球(B細胞)であるかでマウスiNKT細胞の産生するサイトカインバランスが変化するという報告がある．B細胞上のCD1d分子により提示されたα-GalCerを認識したiNKT細胞は低レベルではあるがIL-4だけを産生し(図3A)，DCの機能不全が認められるNODマウスのTh1優位な免疫応答性をTh2傾向にすることで自己免疫性膵臓病を抑制する[17]．

一方，アジュバントの作用により成熟分化したDCを用いてヒトiNKT細胞のサイトカイン産生性を変化させることができる(図3B)．Th1誘導性のDC(DC1)とIL-15を組み合わせることにより，Th1サイトカイン(IFN-γ, TNFα, GM-CSF)産生能が高く，α-GalCer依存性に細胞傷害活性を示す1型(Th1型)iNKT細胞を誘導することができる．さらに，DC2によって誘導された2型(Th2型)iNKT細胞をDC1＋IL-15を用いて刺激することにより，1型の性質を示すiNKT細胞に変化させることができる．つまり，iNKT細胞はTh1/Th2 po-

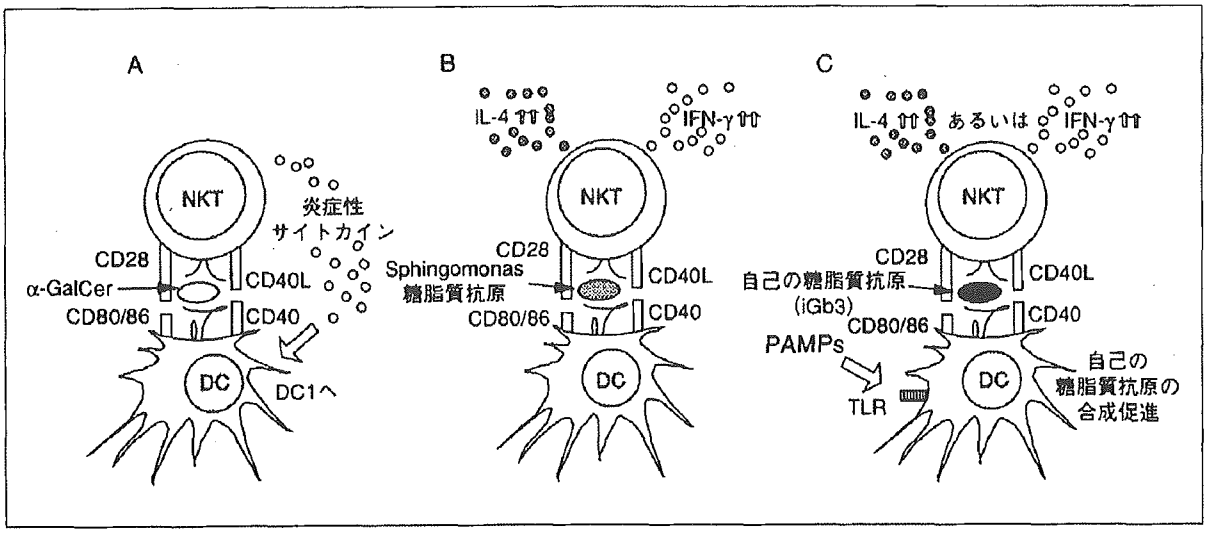

図4　iNKTのアジュバント活性と微生物感染感知機構

A：iNKT細胞のアジュバント活性．α-GalCerによって活性化されたiNKT細胞が産生する炎症性サイトカインとCD40Lの作用によりDCの成熟が誘導される．

B：微生物由来の糖脂質抗原を直接感知するシステム．*Sphingomonas*由来の糖脂質抗原をCD1d拘束性に直接認識してiNKT細胞が活性化される．

C：DCの活性化を介した微生物感染感知機構．微生物の特有の分子構造(PAMPs)をTLRを介して認識したDCは，自己糖脂質抗原の合成を促進し，これをCD1d分子により提示する．さらにこれを認識したiNKT細胞は活性化される．PAMPsの構造の違いにより，DCの異なる機能的修飾が誘導され，間接的にiNKT細胞の産生するThサイトカインバランスが変化するかもしれない．

larizationの可塑性を有しており，DC1/2-iNKT細胞の相互作用により免疫応答を変化させることができる[18]．

iNKT細胞が有するアジュバント活性と微生物感染感知機構

DCを炎症性サイトカインで刺激するだけで成熟が誘導されることは以前より知られていたが，この成熟は獲得免疫応答を誘導するには不十分であることが最近報告されている．しかし，iNKT細胞は，その活性化を通してDCの完全な成熟を誘導し，獲得免疫応答を促進するアジュバントとしての活性を有している．α-GalCerによって活性化されたiNKT細胞のCD40L(CD154)分子がDC上のCD40分子をligationすることでDCの成熟を誘導してTh1応答を促進する(図4A)[19]．最近，ヒトおよびマウスのiNKT細胞が，LPS陰性・グラム陰性の細菌である*Sphingomonas*の細胞壁に存在する糖脂質抗原をCD1d拘束性に認識することが明らかとなった(図4B)[20][21]．*Sphingomonas*にはLPSが存在しないため，DCはTLR4を介してこれを感知することができないが，iNKT細胞が*Sphingomonas*由来の糖脂質抗原を認識して産生するサイトカインとCD40Lの作用によってDCの成熟が誘導される．一方，LPS刺激により成熟分化したDCは自己の糖脂質抗原iGb3の合成を促進して，これをCD1d分子により提示し，さらにiNKT細胞がこれを認識した際にはIFN-γを優位に産生するという間接的なシステムによって感染を感知している(図4C)[22]．

おわりに

これまでα-GalCerや変異α-GalCerにより活性化されたiNKT細胞が直接産生するThサイトカインバランスと，これらが疾患に及ぼす影響について主に解析されてきた．iNKT細胞は免疫賦活，あるいは免疫抑制作用という相反する機能を示すことが報告されているが，抗原として広く解析に用いられてきたα-GalCerがiNKTに対して強力なTCRアゴニスト(スーパーアゴニスト)活性を示し，IFN-γとIL-4を同時に産生させてしまうため，その生理的機能を反映した解析を困難にしてきたように思われる．最近，異なるTLRシグナルの活性化を通して機能的修飾を受けたDCが，