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Two Human Monoclonal HLA-Reactive Antibodies Cross-React with Mamu-B*008, a Rhesus Macaque MHC Allotype Associated with Control of Simian Immunodeficiency Virus Replication

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MHC class I molecules play an important role in adaptive immune responses against intracellular pathogens. These molecules are highly polymorphic, and many allotypes have been characterized. In a transplantation setting, a mismatch between MHC allotypes may initiate an alloimmune response. Rhesus macaques (*Macaca mulatta*, *Mamu*) are valuable as a preclinical model species in transplantation research as well as to evaluate the safety and efficacy of vaccine candidates. In both lines of research, the availability of nonhuman primate MHC-reactive mAbs may enable in vitro monitoring and detection of presence of particular Mamu molecules. In this study, we screened a collection of thoroughly characterized HLA class I–specific human mAbs for cross-reactivity with rhesus macaque MHC class I allotypes. Two mAbs, OK4F9 and OK4F10, recognize an epitope that is defined by isoleucine (I) at amino acid position 142 that is present on the Indian rhesus macaque Mamu-B*008:01 allotype, which is an allotype known to be associated with elite control of SIV replication. The reactive pattern of a third mAb, MUS4H4, is more complex and includes an epitope shared on Mamu-A2*05:01 and -B*001:01–encoded Ags. This is the first description, to our knowledge, of human HLA-reactive mAbs that can recognize Mamu allotypes, and these can be useful tools for in vitro monitoring the presence of the relevant allelic products. Moreover, OK4F9 and OK4F10 can be powerful mAbs for application in SIV-related research. ***The Journal of Immunology*****, 2021, 206: 1957–1965.**

Humans and rhesus macaques (*Macaca mulatta*, *Mamu*) share a common ancestor that lived ∼25–33 million years ago (1, 2). The common ancestry of these two species is reflected by a highly similar immune system, and therefore, macaques are often used as preclinical models to study various aspects of human infectious and chronic inflammatory diseases such as AIDS, malaria, rheumatoid arthritis, and multiple sclerosis, as well as in transplantation research (3–5). The MHC maps in humans to chromosome 6 and is designated as HLA. It comprises a large genomic region occupied by genes that encode for several molecules, many of which play a central role in the adaptive immune response. In a transplantation setting, the disparity involving MHC molecules between donor and recipient may be responsible for triggering an alloimmune response. Matching for MHC allotypes has been shown to result in a significant elongation of graft survival (6–10).

The classical MHC class I molecules in humans are designated HLA-A, -B, and -C. In rhesus macaques, orthologs of HLA-A and -B have been identified and designated Mamu-A and -B, respectively (11, 12). Macaques, however, do not possess an ortholog of HLA-C (13). In humans, an MHC class I haplotype (defined as the combination of genes segregating on a single chromosome) contains one *HLA-A*, *-B*, and *-C* gene. In rhesus macaques, the situation appears to be more complex. In this species, a haplotype can comprise several *Mamu-A* and *-B* genes (12, 14–17), which can be differentiated in high (major) and low (minor) transcribed/expressed genes (12, 18, 19). As a result, the combination and number of *A* and *B* genes may differ per haplotype/region; to make a distinction, the term "haplotype configuration" was introduced (20). The *Mamu-A* genes are named *A1* to *A7*, in which the *A1* gene exhibits the highest level of polymorphism and is considered a major (21, 22). The *A2* to *A7* genes show less polymorphism and often a lower transcription level and are considered as minors. In general, one to two major and up to five minor transcript *A* genes can be present on a chromosome. The haplotype configurations of the highly related *Mamu-B* genes, which most likely arose through a series of expansions, are even more complex. At present, it is difficult to ascribe alleles to a specific *B* gene/locus because of the absence of physical genomic mapping data (12, 21). Therefore, most *Mamu-B* genes are not yet distinguished by locus numbering, such as is done for the *Mamu-A* genes. A few exceptions do exist,

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Abbreviations used in this article: BPRC, Biomedical Primate Research Centre; gMFI, geometric mean fluorescence intensity; HIV-1, HIV type-1; Mamu, *Macaca mulatta*; NHP, nonhuman primate; SAL, single Ag–expressing cell line.

namely for *Mamu-I* (previously designated *Mamu-B3*) (23) and, for example, the pseudogene *Mamu-B11* (22). One to six major and one to ten minor transcribed *Mamu-B* genes can be found on a haplotype (14, 15, 24). Overall, most macaque haplotypes encode one to three major *B* genes, which are generally accepted to act as the classical MHC class I molecules that execute the classical Ag presentation function (12, 25).

The preclinical application of the rhesus macaque as a model species in the field of transplantation and in SIV studies would greatly benefit from the availability of nonhuman primate MHC class I allotype reactive mAbs. Thus far, only one rhesus macaque MHC class I–specific mAb, detecting the Mamu-A1*001:01 allotype, is available to the scientific community (26). In the present communication, we have identified three human monoclonal HLA-reactive Abs that have the ability to cross-recognize particular rhesus macaque MHC class I allotypes, and comparative analysis of the allotype amino acid sequences has allowed us to pinpoint the relevant epitope.

Materials and Methods

Cell lines

A panel of 18 stable single Mamu-A and -B allotype–expressing K562 cell lines (single Ag–expressing cell lines [SALs]) was constructed as described previously (27). The cell lines were individually cultured in IMDM supplemented with 0.2 mg/ml G418 and 5% FCS. The cultures were routinely checked for MHC class I cell surface expression and mycoplasma infection as described previously (28).

The Biomedical Primate Research Centre (BPRC) repository also contains a large panel of lymphoblastoid B cell lines of rhesus macaque, of which the MHC class I and II typing is known. These B cell lines were individually cultured in RPMI 1640 supplemented with 10% FCS, GlutaMAX-I (final concentration of 2 mM in culture), and the antibiotics penicillin/streptomycin (final concentration of 100 U/ml in culture).

HLA class I–specific mAbs

The human HLA-reactive mAbs producing hybridomas (n = 43) were derived by EBV transformation and fusing B cells isolated from multiparous women. Subsequently, the supernatants of cloned hybridomas were screened for the presence of HLA-reactive Abs. The specificity of the HLA mAbs was determined by complement-dependent cytotoxicity assays against large panels of molecularly HLA-typed human PBMCs and was confirmed in binding assays using beads coated with single HLA class I Ags (29–31). From this panel of human HLA-reactive mAbs, seven were selected for testing their cross-reactive capacity to rhesus macaque MHC allotypes. This selection was based on the comparison of the deduced Mamu-A and -B allotype amino acid sequences to the empirically defined functional epitope of an HLA-reactive mAb using the program AASC-Mamu-new, in which the functional epitope (eplet) is defined as the amino acids of the MHC molecule that interact with the CDR H3 domain of the mAb (32). The program does not consider the amino acids that may interact with other parts of the mAb (structural epitope).

Flow cytometry analyses

The ability to detect the cell surface expression of Mamu-A and -B allotypes on K562 SALs in vitro was determined for the seven HLA mAbs by flow cytometry. In a 96-well plate, the washed cells of Mamu-A or -B SALs or K562-only cells (1×10^5 cells per well) were resuspended in FACS buffer (PBS + 0.5% BSA made filter sterile) and incubated with 25 μl of any of the mAbs DK7C11 (36 μg/ml), MUS4H4 (15 μg/ml), OK4F9 (2 μg/ml), OK4F10 (28 μg/ml), OK2H12 (12 μg/ml), OUW4F11 (128 μg/ml), or with VDK1D12 (122 μg/ml) on ice for 1 h. After two washes, cells were incubated for 30 min on ice with either $F(ab')_2$ goat anti-human IgG/PE (Jackson ImmunoResearch Laboratories) or with $F(ab')_2$ rabbit anti-human IgM/FITC (Dako), depending on the isotype of the mAb being tested. Cells were washed twice and fixed with 2% paraformaldehyde. Conjugate-only samples were included, and the total MHC class I expression of the Mamu-A and -B transfectants was assessed with the fluorochrome-labeled Ab HLA-ABC/RPE (clone W6/32; Dako). FACS analyses were performed on the LSRII (BD Biosciences), and data analyses were performed using FlowJo software Version 9.9.6 and 10.6 (Tree Star).

Similarly, rhesus macaque B cell lines (1×10^5 cells per well) were stained with 100 μl of MUS4H4 (15 μg/ml) or with 25 μl of OK4F9 (2 μg/ml) or OK4F10 (28 μg/ml) as described above. Conjugate-only samples were included, and the total MHC class I expression of the B cell lines was assessed with the fluorochrome-labeled Ab HLA-ABC/RPE (clone W6/32). The FACS and data analyses were performed as described above.

Program, calculations, and statistics

The reactivity of the MHC class I Ags with the mAbs was defined as follows:

gMFI(mAb) – gMFI(conjugate-only control).

The reactivity of the MHC class I Ags with the fluorochrome-labeled Ab HLA-ABC/RPE (clone W6/32) was defined as follows:

gMFI(W6/32) – gMFI(background).

The gMFI is denoted as geometric mean fluorescence intensity, and mAb, conjugate-only control, and background refer to the relevant incubations. For those Mamu-A and -B transfectants that reacted positively with one of the mAbs, the mean of expression in three independent determinations with SD was plotted using GraphPad Prism Version 8.0.1 (GraphPad Software). For all B cell lines tested, the mean of expression in three independent determinations with SD was plotted using GraphPad Prism Version 8.0.1.

The program AASC-Mamu-new is a locally developed software program based on the HLAMatchmaker principle (33), which includes the Mamu class I sequences, and it enabled analysis of *Mamu* class I alleles on single amino acid level. The deduced amino acid sequences of 17 *Mamu-A* and 43 *Mamu-B* alleles used comprise the repertoire of MHC haplotypes from a panel of 13 founder animals of the Indian rhesus macaque breeding colony housed at the BPRC. The Mamu-A and -B allotype frequencies in the BPRC Indian rhesus macaque colony formed the basis for the selection of the allotypes from which the 18 SALs also were constructed (14).

Results

Prediction for potential cross-reactivity of monoclonal HLA-reactive Abs with specific Mamu-A and -B allotypes

In the search for reagents to detect the cell surface expression of specific rhesus macaque MHC allotypes, we checked our collection of human monoclonal HLA-reactive Abs (n = 43), the human functional epitope of which is established, for their possible capacity to cross-react with rhesus macaque MHC class I molecules. For this purpose, deduced amino acid sequences of a panel of frequently observed MHC class I allotypes in the Indian rhesus macaque population, pedigreed at the BPRC facilities, were manually entered into the program AASC-Mamu-new. The panel comprised 17 Mamu-A and 43 Mamu-B allotypes (Table I). Subsequently, the program compared the macaque amino acid sequences with the empirically defined functional epitopes recognized by the HLA-reactive mAbs on HLA class I molecules (30, 31). Based on this comparison, several HLA mAbs were identified that were expected to cross-react with a number of different Mamu-A and -B allotypes. However, only a few of the HLA-reactive mAbs recognize functional epitopes that are also apparently predicted to be unique for a particular Mamu-A or -B allotype. Finally, we selected seven candidate mAbs for further in vitro analyses (Table II). Three of these mAbs are IgG (DK7C11, MUS4H4, and OUW4F11), and four are IgM (OK2H12, OK4F9, OK4F10, and VDK1D12) (www.epregistry.com.br) (30, 34–38).

Three out of the seven HLA-reactive mAbs show cross-reactivity with particular Mamu-A and -B allotypes

Eighteen Mamu-A and -B allotype SALs were used to test the cross-reactivity of the seven selected HLA-reactive mAbs and to determine whether these mAbs are applicable for defining particular Mamu-A or -B allotypes. An overview of the selected human mAbs with their key reactive amino acid residues recognized on HLA, and the distribution of those residues in the rhesus macaque MHC class I allotypes for which SALs were available, is provided (Fig. 1). The mAbs DK7C11 and MUS4H4, both IgG, show permissiveness for two different amino acids at one and two

Table I. Overview of the panel of 17 Mamu-A and 43 Mamu-B allotypes

	Allotype
Mamu-A	**A1*001:01,** A1*001:02, **A1*002:01,** A1*003:06, **A1*004:01,** A1*006:01, A1*006:02, **A1*008:01:01,** A1*026:01, **A2*05:01,** A2*05:03:01, A2*05:04:01, A2*24:01, A3*13:02, **A3*13:03,** A3*13:05, A4*14
Mamu-B	***001:01:01,** *006:01, *007:01, ***008:01, *012:01, *015:05 (*40:02),** *017:01, ***019:01,** *022:01, ***024:01,** *029:01, ***030:01, *030:02,** *036:01:01, *037:01, *038:01, *041:01, ***045:01,** *046:01:01, *046:04, *046:09, *046:11, *046:13, *046:15, *046:19, ***047:01, *048:01,** *049:01, *050:02, *051:05, *053:02, *057:01, *060:01:01, *060:02, *061:01, ***064:01,** *068:02, *069:03, *070:01, *072:01:01, *075:01, *082:02, *167:01

SALs are available for the allotypes indicated in boldface.

residues, respectively, namely at position 167 serine (S)/glycine (G) and positions 80 threonine (T)/isoleucine (I) and 81 alanine (A)/leucine (L). For mAb VDK1D12 (IgM), it is described at recognizing either 44 lysine (K), 150 valine (V), 158 valine, or a combination of these residues (37). Although for mAbs DK7C11 and VDK1D12, only some of the key residues were present on particular SALs; with these mAbs, it was possible to test whether at key positions amino acid substitutions other than those found in human HLAs are acceptable, and if so, these two mAbs might show specific cross-reactivity with some of the SALs.

Flow cytometry analyses were performed to identify the actual cross-reactivity of the selected HLA-reactive mAbs to particular Mamu-A and -B allotypes. Three out of seven HLA mAbs—namely, OK4F9 (IgM), OK4F10 (IgM), and MUS4H4 (IgG)—showed cross-reactivity with specific Mamu-A and/or -B allotypes (Fig. 2A). For the other four mAbs, two IgG (DK7C11 and OUW4F11) and two IgM (OK2H12 and VDK1D12), which were all tested against a selection of the 18 SALs, no cross-reactivity was observed (data not shown).

*OK4F9 and OK4F10 show cross-reactivity with Mamu-B*008:01*

The HLA-reactive mAbs OK4F9 and OK4F10, which were both tested against a panel of SALs covering eight different Mamu-A and -B allotypes, showed, as predicted using the program AASC-Mamu-new, a positive reaction with Mamu-B*008:01 (Fig. 2A). Based on the key residues defined in humans for OK4F9 and OK4F10, the positive reaction with the functional epitope on Mamu-B*008:01 correlates with the presence of isoleucine at position 142, most likely in combination with glycine at position 79 (Fig. 1).

Next, it was investigated whether OK4F9 and OK4F10 could detect the presence of Mamu-B*008:01 on cells that express a natural repertoire of MHC molecules at the cell surface. All known and annotated *Mamu-A* and *-B* alleles are archived in the IPD-MHC nonhuman primate (NHP) Database, which presently comprises 263 *Mamu-A1*, 81 *Mamu-A2*, 15 *Mamu-A3*, 67 *Mamu-A4*, 10 *Mamu-A5*, 11 *Mamu-A6*, 8 *Mamu-A7*, and 672 *Mamu-B* alleles (numbers are according IPD-MHC NHP Database release v. 3.5.0.0) (22, 39–41). In addition to *Mamu-B*008:01*, key residue 142I is encoded by the lineages/alleles *Mamu-B*003*, *-B*046*, *-B*054:03*, *-B*060*, *-B*063*, *-B*070:01/B*070:06*, *-B*079*, and *-B*188* (Supplemental Fig. 1) (IPD-MHC NHP Database release v. 3.5.0.0). Within our rhesus macaque breeding colony, MHC haplotypes comprising *Mamu-B*046*, *-B*060*, *-B*063*, or *-B*079* lineage alleles are present as well as allele *Mamu-B*070:01*. From the available set of B cell lines, 10 were selected that are derived from animals with MHC haplotypes that do or do not express Mamu-B*008:01 (Fig. 3A). In addition, the other MHC class I molecules expressed by these animals have been determined, and members of the *Mamu-B*046*, *-B*060*, *-B*063*, or *-B*079* lineage and allele *Mamu-B*070:01* are distributed among the different haplotypes in this carefully composed panel (Fig. 3A). The flow cytometry results demonstrated that OK4F9 and OK4F10 only reacted with the B cell lines of the animals that express Mamu-B*008:01 (Fig. 2B). No reaction was observed, with the B cell lines containing only members of the minor transcribed *Mamu-B*046*, *-B*060*, *-B*063*, or *-B*079* lineage or *Mamu-B*070:01* allele, which suggests that transcription or translation of these minors is at such a low level (if at all) that their antigenic products are not detectable on the cell surface. Therefore, the results indicate that for cells that express a natural repertoire of MHC molecules on their cell surface, OK4F9 and OK4F10 are excellent reagents to monitor the presence/absence of the expression of Mamu-B*008:01.

MUS4H4 shows a complex cross-reactivity in relation to Mamu allotypes

For MUS4H4, tested against all 18 Mamu-A and -B SALs, cross-reactivity was observed with Mamu-A2*05:01 and Mamu-B*001:01 (Fig. 2A). This cross-reactivity is complex, however, and can be correlated to amino acid residues in the 76–83 region of Mamu-A and -B allotypes (Fig. 1). Based on the recognition of HLA allotypes, the key residues for MUS4H4 are 79R, 80T/I, 81A/L, 82L, and 83R (35). Of these five residues, 79R, 82L,

Table II. The seven selected HLA-reactive mAbs with their isotype and HLA specificities

Human HLA mAb	Isotype	HLA Specificity
DK7C11	IgG1,κ	B*45:01, B*15:12, B*44:03, B*44:02
MUS4H4	IgG1,λ	A*23:01, A*24:02, B*59:01, B*27:05, B*49:01, A*23:02, B*51:02, B*57:02, A*25:01, B*52:01, B*38:01, B*13:01, B*58:01, A*32:01, B*57:01, B*13:02, B*51:01, A*24:03, B*57:03, B*27:03, B*15:16, B*58:02, B*27:01, B*53:01, B*15:13, B*44:03, B*37:01, B*44:02, B*47:01
OK2H12	IgM,κ	A*11:01, A*11:02, A*36:01, A*03:01, A*32:01, A*01:01, A*74:01
OK4F9	IgM,κ	A*66:01, A*66:02, A*11:01, A*33:03, A*31:01, A*11:02, A*26:01, A*30:01, A*34:02, A*43:01, A*80:01, A*03:01, A*33:01, A*74:01, A*30:02, A*29:01, A*29:02, A*34:01, A*36:01, A*01:01
OK4F10	IgM,κ	A*66:01, A*66:02, A*11:01, A*33:03, A*31:01, A*26:01, A*30:01, A*11:02, A*80:01, A*43:01, A*34:02, A*03:01, A*33:01, A*74:01, A*29:01, A*29:02, A*30:02
OUW4F11	IgG1,λ	B*39:01, B*14:05, B*15:02, B*08:01, B*15:10, B*15:03, C*08:01, B*14:02, B*40:02, B*15:12, B*18:01, B*14:06, B*35:05, B*40:01, C*03:04, B*39:05, B*15:01, B*78:01, B*55:01, B*46:01, C*14:02, C*07:01, C*01:02, B*27:08, C*12:02, B*35:08, B*15:18, C*03:02, C*08:02, B*35:01, B*42:01, B*14:01, C*07:02, B*15:08, B*45:01
VDK1D12	IgM,κ	A*36:01, A*01:01

		IgG								
		DK7C11		MUS4H4						OUW4F11
		163L	167S/G	76E	79R	80T/I	81A/L	82L	83R	80N
Allotype (SAL)	Mamu-A1*001:01	Q	W	V	-	T	L	-	-	T
	Mamu-A1*002:01	E	W	V	-	N	L	R	G	-
	Mamu-A1*004:01	E	W	V	-	N	L	R	G	-
	Mamu-A1*008:01	E	W	-	Q	N	L	R	G	-
	Mamu-A2*05:01	E	S	-	-	T	L	-	-	T
	Mamu-A3*13:03	E	W	V	-	N	L	R	G	-
	Mamu-B*001:01	R	W	-	-	I	A	-	S	I
	Mamu-B*008:01	T	W	A	G	T	L	R	G	T
	Mamu-B*012:01	R	W	V	G	N	L	R	-	-
	Mamu-B*015:05	R	W	V	-	T	A	-	-	T
	Mamu-B*019:01	E	W	G	-	T	L	-	-	T
	Mamu-B*024:01	T	W	V	G	N	L	R	G	-
	Mamu-B*030:01	T	S	V	-	T	V	P	G	T
	Mamu-B*030:02	T	S	V	-	T	V	P	G	T
	Mamu-B*045:01	E	W	G	-	T	L	R	G	T
	Mamu-B*047:01	T	W	V	E	T	L	R	G	T
	Mamu-B*048:01	R	W	V	G	I	L	R	G	I
	Mamu-B*064:01	E	W	A	G	T	L	-	-	T

		IgM							
		OK2H12			OK4F9 and OK4F10		VDK1D12 #		
		56G	62Q	63E	79G	142I	44K	150V	158V
Allotype (SAL)	Mamu-A1*001:01	-	R	-	R	N	R	A	A
	Mamu-A1*002:01	-	R	-	R	N	R	A	T
	Mamu-A1*004:01	-	R	-	R	N	R	A	T
	Mamu-A1*008:01	-	R	N	Q	N	R	A	T
	Mamu-A2*05:01	-	-	N	R	N	R	A	A
	Mamu-A3*13:03	-	R	-	R	N	R	A	A
	Mamu-B*001:01	-	R	-	R	N	R	A	A
	Mamu-B*008:01	-	E	-	-	-	R	A	A
	Mamu-B*012:01	-	E	Q	-	N	R	D	A
	Mamu-B*015:05	-	E	Q	R	N	R	A	A
	Mamu-B*019:01	-	R	N	R	F	R	A	A
	Mamu-B*024:01	-	R	-	-	N	R	A	A
	Mamu-B*030:01	-	E	Q	R	N	R	T	A
	Mamu-B*030:02	-	E	Q	R	N	R	T	A
	Mamu-B*045:01	-	E	-	R	N	R	A	A
	Mamu-B*047:01	-	R	-	E	N	R	D	A
	Mamu-B*048:01	-	E	-	-	N	R	-	A
	Mamu-B*064:01	-	E	Q	-	N	R	D	A

FIGURE 1. The key amino acid residues defining the functional epitope of the human HLA-reactive mAbs DK7C11, MUS4H4, OUW4F11, OK2H12, OK4F9, OK4F10, and VDK1D12 and the distribution of these residues in the 18 informative Mamu-A and -B allotypes (SALs). The mAbs are categorized based on their IgG or IgM isotype. For each key reactive amino acid, the respective position number in the HLA-polypeptide chain is indicated. For the 18 informative Mamu-A and -B allotypes identity to the key residue of the HLA mAb target epitope (presented at the top) is indicated by dashes, whereas amino acid replacements are given using the conventional one-letter code. The light gray box indicates the permission of serine at position 83 in the functional epitope for MUS4H4. #See text for an explanation for the epitope recognition of this mAb.

and 83R are present in four of the 18 SALs—namely Mamu-A1*001:01, -A2*05:01, -B*015:05 (previously designated as Mamu-B*040:02), and -B*019:01—with only the second of these four SALs being MUS4H4 reactive. This reactivity can be explained by the fact that Mamu-A2*05:01 shares, in addition, glutamic acid (E) at position 76 with the HLA-reactive allotypes (Fig. 1), whereas Mamu-A1*001:01, -B*015:05, and -B*019:01 either have a valine or a glycine at this position, which is apparently deleterious to the epitope. Mamu-B*001:01 also shares 76E as well as the key residues 79R and 82L but has a substitution of an arginine (R) to serine at position 83. The positive reaction of MUS4H4 with Mamu-B*001:01 illustrates that 83S is apparently permitted in the functional epitope and that the reactive amino acids within the functional epitope for MUS4H4 in rhesus macaques can therefore be described as 76E, 79R, 80T/I, 81A/L, 82L, and 83R/S (Fig. 1). The 83S substitution within this epitope is currently documented for

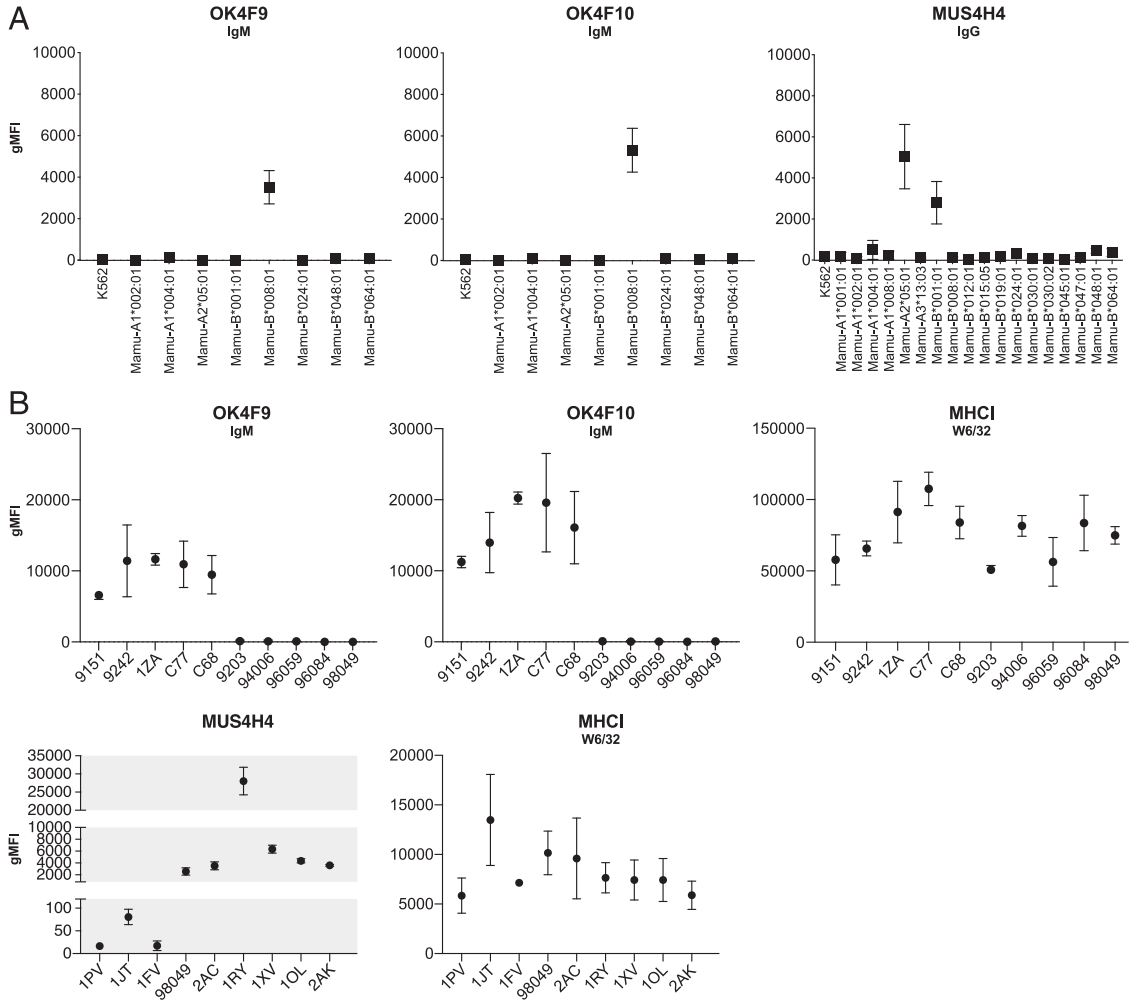

FIGURE 2. Flow cytometry results of the human HLA mAbs OK4F9, OK4F10, and MUS4H4. The isotype of the mAbs is indicated. (**A**) Plots show the gMFI of the three mAbs tested against their designated panel of K562 transfectants expressing either a single Mamu-A or Mamu-B allotype. K562 only was incorporated as MHC class I–negative control. For those Mamu allotypes that reacted positively with one of the mAbs the plots show the mean with the SD of three experiments. (**B**) Plots show the gMFI of the three mAbs tested against a selected panel of rhesus macaque B cell lines. For OK4F9 and OK4F10, the B cell lines of 9151, 9242, 1ZA, C77, and C68 express Mamu-B*008:01, whereas 9203, 94006, 96059, 96084, and 98049 are negative for this allotype. For MUS4H4, the B cell lines of 1PV, 1JT, and 1FV do not express MHC allotypes comprising the 76E, 79R, 80T/I, 81A/L, 82L, and 83R/S epitope, whereas the cell lines of 98049, 2AC, 1RY, 1XV, 1OL, and 2AK contain one or two allotypes comprising the 76E, 79R, 80T/I, 81A/L, 82L, and 83R/S epitope (Fig. 3). Plots showing the gMFI of the total MHC I expression (W6/32) are also provided. Plots show the mean with the SD of three experiments.

only one HLA allele: *HLA-A*24:241* (41). The reactivity of MUS4H4 with the allotype coded by this rare allele, however, has yet to be explored.

A database search showed that the rhesus macaque reactive functional epitope for MUS4H4 is present not only in Mamu-A2*05:01 and -B*001:01 but is also encoded by all other *Mamu-A2*05* ($n = 53$) and *-B*001* ($n = 7$) lineage alleles, as well as by all *Mamu-A1*011* ($n = 8$), *-A1*028* ($n = 8$), *-A1*052* ($n = 3$), *-A1*074* ($n = 6$), *-A1*108* ($n = 2$), and *-B*017* ($n = 6$); some *Mamu-A1*007* ($n = 3$ out of 8) *-A1*056* ($n = 2$ out of 6), *-A2*24* ($n = 3$ out of 4), *-A7* ($n = 3$ out of 8), and *-B*015* ($n = 3$ out of 7) lineage alleles; and by the alleles *Mamu-A1*057:01*, *-A1*066:01*, *-A1*073:01*, *-A1*091:01*, *-A1*092:02*, *-A1*118:01*, *-A1*119:01*, and *-A1*122:01* (IPD-MHC NHP Database release v. 3.5.0.0). Except for *Mamu-A2*05*, these alleles/lineages are defined as majors (15). The highly conserved gene encoding the Mamu-A2*05 allotype, however, is present on ~62% of the rhesus macaque haplotype configurations. Because homozygosity for a particular haplotype configuration is rarely observed, this may result in only a small number of animals that do not express an allotype of the *Mamu-A2*05* lineage, whether in combination with allotypes encoded by the other above-mentioned alleles/lineages (14, 15). Therefore, the application of MUS4H4 as a typing tool in rhesus macaques is not straightforward, and the large number of allotypes that may be recognized by this mAb might require the complete MHC typing of samples beforehand.

The flow cytometry analyses on a thoroughly selected panel of B cells expressing a natural repertoire of MHC molecules, however, showed that MUS4H4 may indeed discriminate between cells either lacking or expressing allotypes comprising the 76E, 79R, 80T/I, 81A/L, 82L, and 83R/S epitope. Three cell lines—1PV, 1JT, and 1FV—showed no cell surface staining with MUS4H4, which in these animals, corresponds to the lack of MHC allotypes expressing the MUS4H4 functional epitope (Figs. 2B, 3B). Six cell lines showed cell surface staining with MUS4H4. For five of the cell lines (98049, 2AC, 1XV, 1OL, and 2AK), the staining corresponds to the presence of either the Mamu-A2*05, -B*001:01, or -B*017:01 allotype that expresses the MUS4H4 epitope. In the case of 1RY, cell surface staining with MUS4H4 was of a higher magnitude and corresponds to the presence of Mamu-A2*05 combined with the Mamu-A1*011:01 allotype, which is denoted as major, and both express the MUS4H4 epitope (Figs. 2B, 3B).

A

Animal ID	*Mamu-A1**	*Mamu-A2**	*Mamu-A3**	*Mamu-A4**	*Mamu-B* (major)*			*Mamu-B* (minor)*							
9151	*004:01:01*	*05*		*14:03*	*012:01*	*038:01*	*030:01*	*049:01*	*053:02*	*057:01*	*070:01*				
	011:01	*05*		*14!*	***008:01***	*006:01*		*072!*	*079!*	*082!*					
9242 #	*006:02*	*05*		*14!*	***008:01***	*006:01*		*072!*	*079!*	*082!*					
	041:01				*065:01*			*050:02*							
1ZA	*011:01*	*05*		*14!*	***008:01***	*006:01*		*072!*	*079!*	*082!*					
	008:01:01	*05*	*13:03*		*045:01*	*037:01*	*036:01*	*035!*	*050!*	*060!*	*063!*	*167!*			
C77	*028:01*	*05!*		*14*	***008:01***	*006:01*		*072!*	*079!*	*082!*					
	007:03	*05:07*			*024:01*	*019:01*		*046!*	*051!*	*057!*	*072!*	*082!*	*109!*		
C68	*012:01*	*05:11*		*14!*	***008:01***	*006:01*		*072!*	*079!*	*082!*					
	007:03	*05:07*			*024:01*	*019:01*		*046!*	*051!*	*057!*	*072!*	*082!*	*109!*		
9203	*008:01*	*05*	*13*		*047:01*			*038:01*	*046*	*072!*	*082!*				
	004:01:01	*05!*		*14!*	*069:01*	*065:01*		*050*	*046!*	*070!*	*072!*	*079!*	*082!*	*098!*	*100!*
94006	*001:01/02*	*05:04*			*047:01*			*038:01*	*046!*	*072!*	*082!*				
	004:01:01	*05!*		*14*	*055:01*	*058:02*	*052:01*	*063:01*	*072!*						
96059	*008:01:01*	*05!*	*13:03*		*047:01*			*038:01*	*046*	*072*	*082*				
	016:02		*13:06*		*024:01*	*019:01*		*046!*	*051!*	*057!*	*072!*	*082!*	*109!*		
96084	*001:01/02*	*05:04*			*012:01*	*038:01*	*030:01*	*049:01*	*053:02*	*057:01*	*070:01*				
	001:01/02	*05:04*			*012:01*	*022:01*	*030:01*	*049:01*	*053:02*	*057:01*	*070:01*				
98049	*016:02*		*13:06*		*024:01*	*019:01*		*046!*	*051!*	*057!*	*072!*	*082!*	*109!*		
	001:02	*05:04*			*048:01*	*041:01*		*046!*	*064:01*	*072!*	*079!*	*109!*	*116!*	*134!*	

B

Animal ID	*Mamu-A1**	*Mamu-A2**	*Mamu-A3**	*Mamu-A4**	*Mamu-B* (major)*			*Mamu-B* (minor)*							
1PV	*002:01*		*13!*	*14!*	*024:01*	*019:01*		*046!*	*051!*	*057!*	*072!*	*082!*	*109!*		
	002:01		*13!*	*14!*	*055:01*	*058:02*	*063:01*								
1JT	*002:01*		*13!*	*14!*	*012:01*	*022:01*	*030:01*	*049:01*							
	023:01			*14!*	*024:01*	*019:01*		*046!*	*051!*	*057!*	*072!*	*082!*	*109!*		
1FV	*002:01*		*13!*	*14*	*012:01*	*022:01*	*030:01*	*049:01*	*053:02*	*057:01*	*070:01*				
	002:01		*13!*	*14!*	*ND*										
98049	*016:02*		*13:06*		*024:01*	*019:01*		*046!*	*051!*	*057!*	*072!*	*082!*	*109!*		
	001:02	***05:04***			*048:01*	*041:01*		*064:01*							
2AC	*001:01*	***05***			*055:01*	*058:02*	*063:01*								
	004:01:01			*14*	*012:01*	*022:01*	*030:01*	*049:01*	*053:02*						
1RY	***011:01***	***05***			*055:01*	*058:02*	*063:01*								
	002:01		*13!*	*14!*	*012:01*		*030:01*	*049:01*		*057:01*					
1XV	*002:01*		*13!*	*14!*	***001:01***	*007:02*	*030:02*	*045!*	*046!*	*057!*	*060!*	*072!*	*082!*		
	002:01		*13!*	*14!*	***001:01***	*007:02*	*030:02*	*045!*	*046!*	*057!*	*060!*	*072!*	*082!*		
1OL	*002:01*		*13!*	*14!*	***001:01***	*007:02*	*030:02*	*045!*	*046!*	*057!*	*060!*	*072!*	*082!*		
	016:01		*13:06*		*024:01*	*019:01*		*046!*	*051!*	*057!*	*072!*	*082!*	*109!*		
2AK	*002:01*		*13!*	*14!*	***017:01***	*029:01:02*	*060:02*	*061*	*068*						
	002:01		*13!*	*14!*	***017:01***	*029:01:02*	*060:02*	*061*	*068*						

FIGURE 3. MHC haplotype repertoire of *Mamu-A* and *-B* genes/alleles of the rhesus macaques from B cell lines that are selected. The *Mamu-A* and *-B* genes inherited together on one chromosome (haplotype) are presented on the same line, and for the *Mamu-B* genes, a division has been made based on their transcription status (major and minor). For each animal, two haplotypes are indicated, inherited from either the father or the mother. The *Mamu-A* or *-B* alleles indicated with an exclamation mark are retrieved from the haplotype table published by Karl and coworkers (15). (**A**) Rhesus macaques selected for testing mAbs OK4F9 and OK4F10. A dark gray box highlights the presence of *Mamu-B*008:01*, whereas a light gray box marks a minor transcribed *Mamu-B* gene that encodes isoleucine at position 142. For animals 94006 and 96084 *Mamu-A1*, typing was not unambiguous, indicated by *001:01/02*, which means that the animals are either positive for *Mamu-A1*001:01* or *-A1*001:02*. #The allele combination *Mamu-A1*041:01/-B*065:01/-B*050:02* is of Burmese descent and was inherited from the mother. (**B**) Rhesus macaques selected for testing mAb MUS4H4. A dark gray box highlights the alleles that encode the 76E, 79R, 80T/I, 81A/L, 82L, and 83R/S epitope.

Discussion

The close evolutionary relationship between humans and rhesus macaques was taken as an opportunity to investigate whether human HLA-reactive mAbs have the capability to cross-react with rhesus macaque MHC class I molecules. These tools are extremely useful for MHC typing and for monitoring the cell surface expression

of specific MHC molecules. With only one nonhuman primate MHC-reactive mAb currently available for rhesus macaques, which is specific for Mamu-A1*001, there is a need to expand this number. In this study, we describe three HLA-reactive mAbs—tested under saturated conditions—that are able to cross-react with certain rhesus macaque MHC class I allotypes. The mAbs OK4F9 and OK4F10 (both IgM) cross-react in Indian origin rhesus macaques uniquely with Mamu-B*008:01, and mAb MUS4H4 (IgG) was found to recognize an epitope present on Mamu-A2*05:01 and -B*001:01. This epitope, however, is not unique for these two allotypes, and therefore, MUS4H4 is also expected to cross-react with all other Mamu-A2*05 and -B*001 allotypes, as well as with all Mamu-A1*011, -A1*028, -A1*052, -A1*074, -A1*108, and -B*017 allotypes; some Mamu-A1*056, -A2*24, -A7, and -B*015 allotypes, and with the allotypes Mamu-A1*057:01, -A1*066:01, -A1*073:01, -A1*091:01, -A1*092:01, -A1*118:01, -A1*119:01; and -A1*122:01. Moreover, Mamu-A2*05 allotype-encoding genes are present on many haplotype configurations and can be found in combination with genes encoding the other above-mentioned allotypes, which complicates the applicability of MUS4H4 as typing tool and has to be chosen carefully in view of the question to be answered.

Potentially, mAbs such as OK4F9 and OK4F10 are powerful tools for MHC typing purposes, for monitoring the presence of Mamu-B*008:01 cell surface expression and, for instance, to study the inhibition of Ag presentation. Recently developed technologies that allow the production of rHLA-reactive mAbs may generate opportunities to engineer OK4F9 and OK4F10 or their complement determining region from an IgM into an IgG isotype, thus enabling in vivo utility (42). Other types of recombinant mAbs may also be produced, such as different IgG subclasses, mAbs of which the complement fixation capacity is removed or, for instance, bispecific mAbs.

In addition to Mamu-B*008:01, only a small number of carefully annotated *Mamu-B* lineage/alleles in the IPD-MHC NHP Database may encode residue 142I (Supplemental Fig. 1), which is considered the key reactive residue for OK4F9 and OK4F10 to recognize Mamu-B*008:01, most likely in concert with the presence of residues 79G. These lineages/alleles included the major *Mamu-B*003* and the minor transcribed *Mamu-B*046*, *-B*054:03*, *-B*060*, *-B*063*, *-B*070:01*, *-B*070:06*, *-B*079*, and *-B*188* (15). Within our panel of B cell lines lacking Mamu-B*008:01, no cross-reactivity of OK4F9 and OK4F10 was observed for the cells typed positive for *Mamu-B*070:01* or for members of the *Mamu-B*046*, *-B*060*, *-B*063*, or *-B*079* lineages (Figs. 2B, 3A). This suggests that OK4F9 and OK4F10 are not able to detect the presence of minors on the cell surface and/or that the signal was below the detection level. The lineages/alleles *Mamu-B*003*, *-B*054:03* (KF855184, https://www.ncbi.nlm.nih.gov), *-B*070:06* (LT623028, https://www.ncbi.nlm.nih.gov), and *-B*188* are encountered in rhesus macaques of Chinese origin (15). All rhesus macaques at the BPRC facilities are of Indian origin. Unfortunately, we have no access to material or transfectants (SALs) containing the lineages/alleles of Chinese origin. For that reason, we cannot rule out that OK4F9 and OK4F10 may cross-react with the major Mamu-B*003 allotype.

Rhesus macaques are often used as model species in biomedical research to evaluate HIV type-1 (HIV-1) vaccine candidates or components for HIV-1 vaccines (5, 43, 44). Over the past 37 y, research on HIV-1–infected humans has revealed that most of the individuals develop AIDS after 5–10 y of infection, but a particular group of individuals can control the virus naturally for over 20 y. This natural control of an HIV-1 infection, also referred to as elite control, appears to be strongly associated with the presence of particular MHC class I allotypes such as HLA-B*27:05 and -B*57:01 (45, 46). Elite control is also observed in the SIV rhesus macaque model for HIV-1 vaccine studies, and one of the prime candidates is Mamu-B*008:01 (47, 48). Therefore, the in vivo application of engineered OK4F9 and OK4F10 might facilitate the study of various aspects of elite control involving Mamu-B*008:01 in a preclinical rhesus macaque model system for AIDS.

Furthermore, equivalents of Mamu-B*008:01 are observed in the genetically well-characterized cynomolgus (*Macaca fascicularis*, *Mafa*) and pig-tailed (*Macaca nemestrina*, *Mane*) macaques (49–53), which are species that are also often applied as model in biomedical and transplantation research (54–57). A database search showed that in addition to the B*008 lineage, residue 142I is encoded also by other lineage/alleles in these two species, but it does not always associate with a G at position 79 (Supplemental Fig. 1). These particular lineages/alleles are more or less comparable to those observed in rhesus macaques, and most are distinguished as minors. The exceptions are *Mafa-B*003* and *-B*194*, which are known as majors (19, 49–51, 58–61). Therefore, this might suggest that mAbs OK4F9 and OK4F10 can cross-react with the equivalents of Mamu-B*008:01 in other macaque species as well.

For the remaining four mAbs (mAbs DK7C11, OUW4F11, OK2H12, and VDK1D12) no cross-reactivity was observed with their respective individual selected set of SALs. For two of the mAbs, DK7C11 and OK2H12, the reactive functional epitopes in HLA consist of a constellation of 2 or 3 aa, respectively (Fig. 1). The present set of SALs provided the opportunity to test mAbs on cells containing just part of a functional epitope. The results with the Mamu allotypes show that the entire epitope is required and that substitutions that do not occur in HLA are not permitted. In OK2H12, asparagine (N) at position 63 is apparently disrupting the epitope, with concomitant failure to react with Mamu-A2*05:01. Neighboring amino acids that have a deleterious effect on the epitope most certainly also play a role in the absence of cross-reactivity of mAb OUW4F11 to specific Mamu allotypes, and for mAb VDK1D12, the presence of only valine at position 150 does not seem sufficient to effect cross-reactivity with the Mamu-B*048:01 SAL. Therefore, with these additional Mamu data, we confirmed that the majority of the mAbs tested in this study are very stringent in their functional epitope requirements; only one, MUS4H4, turns out to be more permissive than was deduced from the HLA data alone.

The SALs in the current study were not specifically designed for the purpose described, and their number (18) is low when compared with the number of *Mhc* class I alleles present in the database (455 *Mamu-A* and 672 *Mamu-B*; IPD-MHC NHP Database release v. 3.5.0.0). To determine whether other human HLA-reactive mAbs might also show cross-reactivity with certain macaque MHC allotypes, it will be worthwhile to direct future endeavors toward the establishment of new SALs with alleles that harbor epitopes for which HLA mAbs are available. Nonetheless, our finding that three HLA-reactive mAbs show cross-reactivity with certain rhesus macaque MHC class I allotypes expands the mAb toolkit for biomedical studies for transplantation and SIV-related studies in rhesus macaques. Moreover, these mAbs might even be applicable to other macaque species.

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Disclosures

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