Human Immunology 73 (2012) 547–553

Contents lists available at SciVerse ScienceDirect

journal homepage: www.elsevier.com/locate/humimm

Comprehensive identification of high-frequency and co-occurring *Mafa-B*, *Mafa-DQB1*, and *Mafa-DRB* alleles in cynomolgus macaques of Vietnamese origin

Fei Ling [a], Min Zhuo [a], Chao Ni [a], Gui-qing Zhang [a], Tao Wang [a], Wai Li [b], Li-qiong Wei [a], Hong-li Du [a], Ju-fang Wang [a], Xiao-ning Wang [a,b,*]

[a] School of Bioscience and Bioengineering, South China University of Technology, Guangzhou 510006, P.R. China
[b] School of Life Science, General Hospital of PLA T, Beijing 100853, P.R. China

ARTICLE INFO

Article history:
Received 29 June 2011
Accepted 1 February 2012
Available online 9 February 2012

Keywords:
High-frequency
Co-occurring alleles
Major histocompatibility complex
Macaca fascicularis

ABSTRACT

High-frequency alleles and/or co-occurring human leukocyte antigen (HLA) alleles across loci appear to be more important than individual alleles, because they might be markers of disease risk that have clinical value as biomarkers for targeted screening or the development of new therapies. To better elucidate the major histocompatibility complex background and to facilitate the experimental use of cynomolgus macaques, *Mafa-B*, *Mafa-DQB1*, and *Mafa-DRB* alleles were characterized and their combinations were investigated from 30 macaques of Vietnamese origin by cloning and sequencing. A total of 48 *Mafa-B*, 22 *Mafa-DQB1*, and 42 *Mafa-DRB* alleles, were detected in this study, respectively. In addition, two *Mafa-DQB1* and eight *Mafa-DRB* alleles represented novel sequences that had not been documented in earlier studies. Our results also showed that the macaque from Vietnam might be valuable because >30% of the test animals possessed *Mafa-DRB*w304* (30%) and *-DQB1*0616* (30%). We report that the combination of major histocompatibility complex (MHC) class I and II alleles, including the combination of *DRB3*0403-DRB*w304*, *DRB1*1013-DRB*w304,* and *Mafa-B*007:01:01-DRB*w304*, which was in 17%, 13%, and 13% of the animals, respectively. Interesting, more than two *Mafa-DQB1* alleles detected in one animal in this study suggest that *Mafa-DQB1*, like *Mafa-DRB*, might be a duplication in the chromosome, which have ever been documented in cynomolgus monkeys but has not yet been observed in rhesus macaques or other primates. Our results for the high frequency of commonly co-occurring MHC alleles across loci in a cohort of the Vietnamese cynomolgus macaque emphasized the value of this species as a model for biomedical research.

1. Introduction

With the 1978 ban on exportation of rhesus macaques (*Macaca mulatta*) from India, the cynomolgus macaque (*Macaca fascicularis*) became an increasingly useful animal model for various diseases, including diabetes, severe acute respiratory syndrome (SARS), tuberculosis, simian immunodeficiency virus (SIV), renal transplantation, and pharmacodynamic evaluation [1–4]. Major histocompatibility complex (MHC) class I and class II molecules play key roles in immune regulatory processes by presenting peptides of intracellular or extracellular origin to $CD8^+$ or $CD4^+$ T cells, respectively. It has been suggested that certain co-occurring alleles might be markers of disease risk that have clinical value as biomarkers for targeted screening or the development of new therapies [5]. A number of research groups have suggested that *HLA-DRB1/DQB1* and/or HLA classμalleles and haplotypes are associated with many diseases, including type 1 diabetes [6–10], pemphigus [11], pure red cell aplasia [12], allergies [13], low hepatitis activity [14], multiple sclerosis [15], primary Sjögren's syndrome [16], Graves' disease in Koreans [17], familial generalized vitiligo and early disease onset [18], lichen sclerosus [19], and rheumatoid arthritis [20,21]. It has been reported that the combination *B*4402-DRB1*1101-DQB1*0301* was associated with an 11-fold increased risk of cervical cell cancer [5].

For a few diseases, in particular SIV infection, macaques have become the dominant preclinical model for human immunodeficiency virus (HIV) vaccine evaluation [22,23]. There are many reports that show polymorphism of MHC genes in the cynomolgus macaque affects the results obtained with drugs [24–26] and is associated with the control of viral diseases [27]. The cynomolgus macaque from Mauritius appears to be particularly valuable, because 88% of these animals have the MHC class I allele combination *Mafa-A*25-A*29* [28] and more than half of these have the combination *Mafa-B*43010-B*44010-B*460101* [29]. The increased sharing of the MHC I allele in the Mauritian cynomolgus macaque might dramatically reduce the overall number of animals needed to study cellular immune responses in nonhuman primates and simultane-

* Corresponding author.
E-mail address: xnwang88@163.com (X.-n. Wang).
F. Ling and M. Zhuo contributed equally to this work.

0198-8859/12/$36.00 - see front matter © 2012 American Society for Histocompatibility and Immunogenetics. Published by Elsevier Inc. All rights reserved.
doi:10.1016/j.humimm.2012.02.003

Table 1
Primers used to amplify *Mafa-B*, *Mafa-DRB*, and *-DQB1* alleles

Locus	Primer name	Primer sequence (5′ to 3′)	Temp (°C)	Product (bp)
Mafa-B	B-F	TGGCAGCTCTGACAGTGA	52	893
	B-R	CTGCCTGGATAGAAACCG		
Mafa -DRB	DRB1-F	TGGCAGCTCTGACAGTGA	52	450
	DRB1-R	CTGCCTGGATAGAAACCG		
Mafa -DQB1	DQB1-F	GAAGAAGGCTTTGCGGAT	55	420
	DQB1-R	GTCGCCGTTCCTAATAAG		

Temp, temperature.

ously reduce the confounding effects of genetic heterogeneity in HIV/AIDS research [28–31]. The high-frequency alleles might be high-priority targets for additional characterization of the immune function [32]. In addition, co-occurring MHC alleles across loci appear to be more important than individual alleles. So, to examine the combination of MHC class I and class II alleles in a cohort of the Vietnamese cynomolgus macaque, the transcribed Mafa class I and II genes were characterized and analyzed by sequencing the polymorphic exon 2 of *Mafa-DRB* and *Mafa-DQB1* genes and exons 2 and 3 of the *Mafa-B* gene.

2. Subjects and methods

2.1. Animals

Whole blood samples from 33 unrelated test animals (*M. fascicularis*), originally from Vietnam, were provided generously by South China Primates Research Central. Whole blood samples (3–5 ml) withdrawn from each monkey were collected into ethylenediaminetetraacetic acid (EDTA)-treated vacuum tubes. All of the monkeys were clinically normal with no known diseases.

2.2. RNA isolation, cDNA synthesis, and cloning of MHC class i and ii cDNAs

For all of the animals used in this study, RNA was isolated from peripheral blood (Blood RNA kit, Omega Bio-Tek, Guangzhou, China) and subjected to One-Step reverse transcription–polymerase chain reaction (RT-PCR), as recommended by the supplier (Takara). To identify and investigate the presence and expression of *Mafa-B*, *-DQB1* and *-DRB*, the first strand cDNA (1 μl) was amplified in a 25-μl reaction volume using coding region-specific forward and reverse primers to amplify *Mafa-DQB1* and *-DRB* alleles for exon 2 and *Mafa-B* for exons 2 and 3 (Table 1). In general, amplification was carried out for 3 minutes

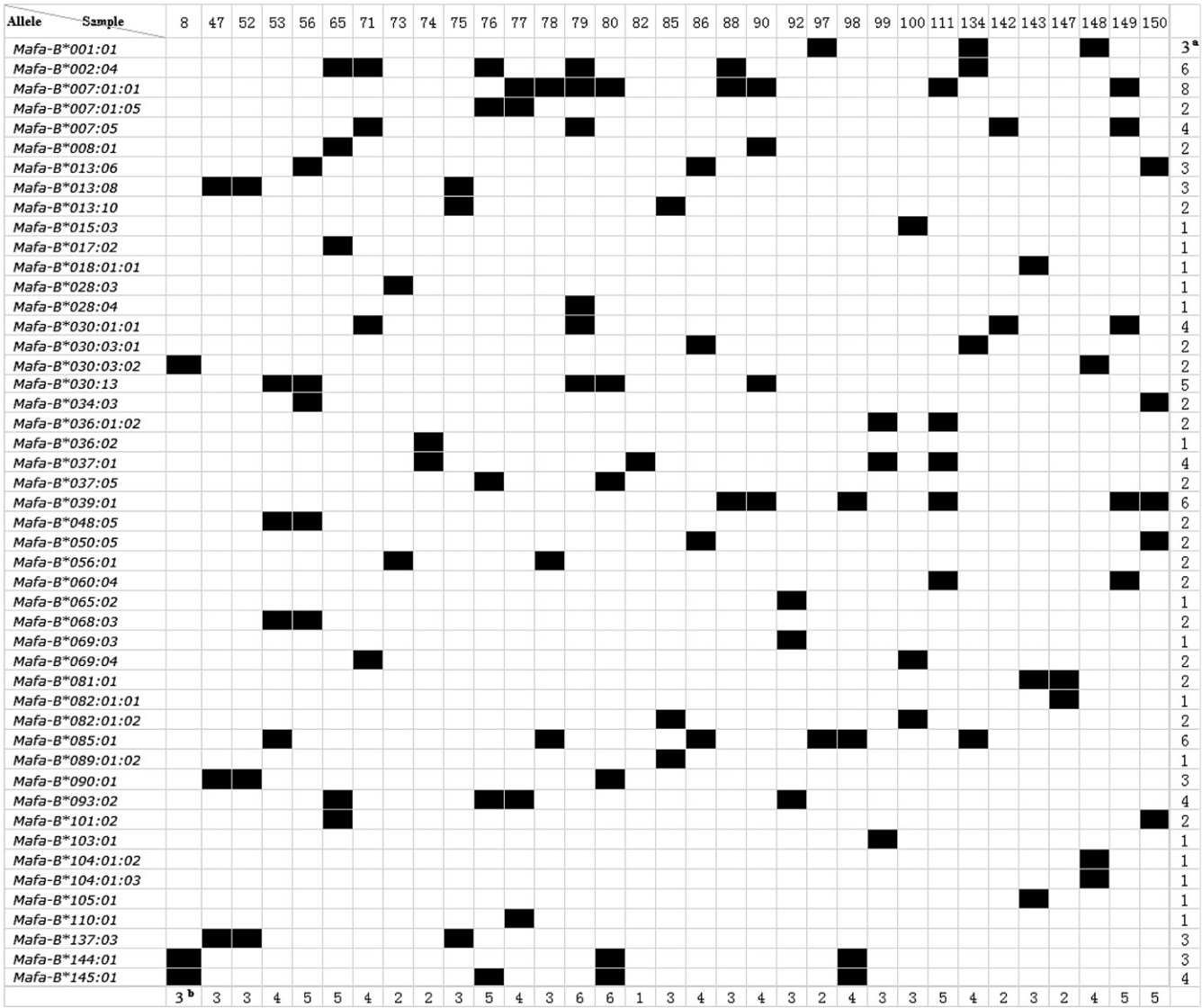

Fig. 1. Distribution of *Mafa-B* alleles detected in a cohort of Vietnamese cynomolgus macaques. [a]Number of animals sharing a certain allele. [b]Number of alleles in one animal.