TISSUE ANTIGENS
IMMUNE RESPONSE GENETICS

Tissue Antigens ISSN 0001-2815

TRIM5 allelic polymorphism in macaque species/populations of different geographic origins: its impact on SIV vaccine studies

N. G. de Groot[1], C. M. C. Heijmans[1], G. Koopman[2], E. J. Verschoor[2], W. M. Bogers[2] & R. E. Bontrop[1,3]

1 Department of Comparative Genetics & Refinement, Biomedical Primate Research Centre, Lange Kleiweg 161, 2288 GJ Rijswijk, the Netherlands
2 Department of Virology, Biomedical Primate Research Centre, Lange Kleiweg 161, 2288 GJ Rijswijk, the Netherlands
3 Theoretical Biology and Bioinformatics, Utrecht University, 3584 CH Utrecht, the Netherlands

Key words
cynomolgus macaque; rhesus macaque; simian immunodeficiency virus; tripartite motif 5α

Correspondence
Natasja G. de Groot
Department of Comparative Genetics and Refinement
Biomedical Primate Research Centre
Lange Kleiweg 161
2288 GJ Rijswijk
the Netherlands
Tel: +31 15 2842500
Fax: +31 15 2842600
e-mail: groot@bprc.nl

Received 11 July 2011; revised 8 August 2011; accepted 11 August 2011

doi: 10.1111/j.1399-0039.2011.01768.x

Abstract

Tripartite motif 5α (TRIM5α) is a potent antiretroviral immune factor present in the cytoplasm of cells of most tissue types. The rhesus macaque *TRIM5* gene has been shown to display polymorphism, with different variants being divided into three groups ($TRIM5^{TFP}$, $TRIM5^{Q}$, and $TRIM5^{CypA}$), which may have divergent retroviral effects on infection. Along with rhesus macaques, cynomolgus macaques are also used in simian immunodeficiency virus (SIV) infection studies. As a consequence, *TRIM5* genotyping of these animals will contribute to interpreting the outcome of such studies. The present communication covers Burmese, Chinese, and a large cohort of Indian-origin rhesus macaques, and describes the first large cohort study on *TRIM5* polymorphism in outbred cynomolgus macaques. We demonstrate the presence of the $TRIM5^{TFP}$ group in cynomolgus macaques. In addition, we have re-evaluated historical samples of rhesus macaques challenged with SIV_{mac251}, a virus that has been reported to be partially suppressed by particular rhesus macaque TRIM5 variants.

Introduction

Acquired immunodeficiency syndrome, caused by the human immunodeficiency virus type 1 (HIV-1), poses a major threat to humankind. Therefore, a safe and effective prophylactic vaccine is needed urgently to curb this epidemic. The rhesus macaque simian immunodeficiency virus (SIV_{mac}) infection model has been of great value to study the immunopathogenesis of these types of viruses as well as to evaluate new HIV-1 vaccine candidates or vaccine components (1, 2).

Different host immune factors, such as the major histocompatibility complex (MHC) class I molecules and killer-cell immunoglobulin-like (KIR) receptors, were shown to play a role in the immune response against an HIV-1 or an SIV infection (3–6). In rhesus macaques, the intrinsic innate immune factor named tripartite motif (TRIM) protein, TRIM5α, has potent antiretroviral activity (7). In a follow-up study, TRIM5α from different primate species was shown to have antiretroviral activity similar to several different retroviruses (8). TRIM5α is the product of the longest alternative spliced transcript of the *TRIM5* gene, and is expressed in the cytoplasm of many cell types (9). It is thought to interact with the incoming retroviral capsid, resulting in the premature disassembly of the capsid prior to reverse transcription, and as such it hampers the integration of the viral DNA into the host genome (10, 11). However, the exact mechanism of interaction still needs to be revealed (11, 12). A recent study claims that TRIM5 has additional activities in that it promotes innate immune signalling and can act as a pattern recognition receptor (13).

The human genome contains about 70 genes of the *TRIM* family, of which only a few are well characterised. Generally, *TRIM* genes encode three different protein domains: namely, the amino-terminal RING domain, one or two B-box domains, and a long coiled-coil (14, 15). Certain *TRIM* genes, including *TRIM5*, encode a fourth domain, B30.2, which is also referred to as SPRY, PRYSPRY, or B30.2/SPRY (14–16). Every domain has its own distinct function in the antiretroviral activity of the TRIM molecule (17). In that respect, the B30.2/SPRY domain is described to bind to the viral capsid, and mediates specific retroviral restriction. Different studies have illustrated that sequence

variation within this domain influences the capsid-binding specificity (18–20).

The macaque *TRIM5* gene displays considerable polymorphism, with much of the variation localised to particular parts coding for the C-terminal B30.2/SPRY domain (21–23). On the basis of genomic sequence similarity, the alleles can be grouped into three classes, designated $TRIM5^{TFP}$, $TRIM5^{Q}$, and $TRIM5^{CypA}$. The first two classes are discriminated due to a six-nucleotide insertion/deletion resulting in a $TFP^{339-341}/Q^{339}$ alteration. The third class, $TRIM5^{CypA}$, is recognised by a single-nucleotide polymorphism (SNP) at the terminal nucleotide of intron 6, which changes the acceptor site upstream of exon 7. As a result an alternative spliced form of *TRIM5* in which the B30.2 domain is replaced by a cyclophilin-A (CypA) domain is formed (24–26). Within a population, the three classes can give rise to six different genotypes: $TRIM5^{TFP/TFP}$, $TRIM5^{TFP/Q}$, $TRIM5^{TFP/CypA}$, $TRIM5^{Q/Q}$, $TRIM5^{Q/CypA}$, and $TRIM5^{CypA/CypA}$. These genotypes have been shown to display divergent antiretroviral restriction characteristics (21–23).

In this study, the aim was to characterise the *TRIM5* polymorphism in rhesus macaque populations of Burmese, Chinese, and Indian origin. In addition, the cynomolgus macaque, a species that shares with rhesus macaques a common ancestor that lived approximately 1.3 million years ago (27), has been of interest in the field of SIV infection studies (28–30). The Biomedical Primate Research Centre (BPRC) houses a large pedigreed cynomolgus macaque population, and the intention was to characterise the *TRIM5* polymorphism in this group as well. Moreover, historical samples from SIV-infected rhesus macaques have been analysed and challenge data were re-evaluated.

Materials and methods

Source of DNA

The macaque breeding colonies housed at the BPRC comprise approximately 650 Indian-origin rhesus macaques and 160 cynomolgus macaques. For this study 186 Indian-origin rhesus and 85 cynomolgus macaques were selected, covering most of the variation expected to be present in these populations. These animals have been pedigreed based on the segregation of MHC and microsatellite markers (31–34). Rhesus macaques of Burmese (18 unrelated animals) and Chinese (30 unrelated animals) origin were included as well. MtDNA analysis was used to define the origin of the rhesus macaques (35, 36). The outbred cynomolgus macaque colony is characterised by a long history of isolation. Recent mtDNA-typing data have shown that the animals originated either on the mainland of Indochina or on the Indonesian islands (31). Two animals in the cohort were introduced from the cynomolgus macaque population from the island of Mauritius.

TRIM5 genotyping

Genotyping of *TRIM5* in rhesus and cynomolgus monkeys was performed using genomic DNA (gDNA). For rhesus macaques, a 525-bp polymerase chain reaction (PCR) fragment, including a portion of the B30.2 domain of TRIM5α and the G/T SNP in intron 6 to discriminate for the presence of the $TRIM^{CypA}$ allele, was generated (50 µl reaction) using 100 ng gDNA, 0.5 µM of each species-specific primer (Table 1), 1.5 mM $MgCl_2$, 0.2 mM each dNTP, 5 µl 10× PCR buffer, and 1 unit Platinum *Taq* DNA polymerase (Invitrogen, Carlsbad, CA, USA). The PCR protocol used was as previously described (21). The DNA concentrations of purified PCR products were determined using a Nanodrop Spectrophotometer (Nanodrop Technologies, Wilmington, NC), and directly sequenced on a 3130XL ABI automatic sequencer (Applied Biosystems, Foster City, CA). Sequences were analysed using the MacVector™ program version 12.0.2 (MacVector Inc., Cambridge, UK). For cynomolgus monkeys a 779-bp PCR fragment, comprising exon 7, intron 7, and a part of exon 8 of the *TRIM5* gene, was amplified as described above. The PCR protocol comprised an initial denaturation step of 1 min at 94°C, followed by 30 cycles of 15 s at 94°C, 30 s at 55°C, 1 min at 72°C, and a final extension step of 10 min at 72°C. The PCR products were purified and sequenced directly as described above.

Background information on SIV studies

For three different SIV studies, the *TRIM5* genotypes were analysed retrospectively. Study 1 involved 10 rhesus macaques of Chinese origin challenged intravenously with SIV_{mac251} (obtained from Dr N. Letvin, BIDMC, Harvard, USA); study 2 involved six Indian-origin rhesus macaques challenged intrarectally with a SIV_{mac251} stock obtained from Dr C. Miller (California National Primate Research Center, Davis, USA), and that had been passaged once through an Indian-origin rhesus macaque from the BPRC colony; and study 3 comprising 24 Indian-origin rhesus macaques challenged intravenously with $SIV_{mac32H/1XC}$. $SIV_{mac32H/1XC}$ is an isolate from macaque 1XC infected with SIV_{mac251} that had been passed through macaque 32H. All animal studies have been approved by the ethical committee as demanded by Dutch law. Data on plasma viral loads were

Table 1 Species-specific primers used for the amplification of parts of the TRIM5 gene

Primer name	Primer sequence	Species
SPRY-006	5′-CAGTGCTGACTCCTTTGCTTG-3′	*Macaca*
T5α1087R	5′-GCTTCCCTGATGTGATAC-3′	*mulatta*
Mafa-Trim5αF-ex7	5′-AGGAATGCTAGACATGTTTAG-3′	*Macaca*
Mafa-Trim5αR-ex8	5′-GYTTCCCTGATGTGATAC-3′[a]	*fascicularis*

[a]Y represents a cytosine (C) or a thymine (T).

available for all the three studies. Graphs were drawn using column statistics and plotting the median with GraphPad Prism Version 5.0d for Mac OS X (GraphPad Software, San Diego, CA, www.graphpad.com). Statistical analyses were performed using a nonparametric *t*-test (Mann–Whitney test).

Results

TRIM5 genotyping in rhesus macaques of different origin

Polymorphism in the coiled-coil and B30.2/SPRY domains of the rhesus macaque *TRIM5* gene is reported, and thus far 12 alleles have been described (21, 22). In this study, we characterised the polymorphisms in the B30.2/SPRY domain (including the SNP in intron 6), because this method has been shown to discriminate the three different TRIM5 classes (21). In the 186 Indian-origin rhesus macaques that had been analysed, five previously identified alleles were observed: *Mamu*-1 and -3 (EF113914 and EF113916) that belong to the $TRIM5^{TFP}$ class, *Mamu*-4 and -5 (EF113917 and EF113918) clustering into the $TRIM5^{Q}$ class, and the allele that constitute the $TRIM5^{CypA}$ class (EU359036). In the cohort analysed, all six genotypes ($TRIM5^{TFP/TFP}$, $TRIM5^{TFP/CypA}$, $TRIM5^{TFP/Q}$, $TRIM5^{Q/CypA}$, $TRIM5^{Q/Q}$, and $TRIM5^{CypA/CypA}$) that can be present theoretically were observed (Figure 1). This is the second large *TRIM5*-typing study on a captive population of Indian-origin rhesus macaques. Genotype frequencies from both studies were compared, and it is evident that both populations show similarities but are not identical (Figure 2). This illustrates that founder effects and breeding policies can shift the numbers, and that the contemporary results may not reflect the *TRIM5* genotype frequencies of rhesus macaque populations living in the wild.

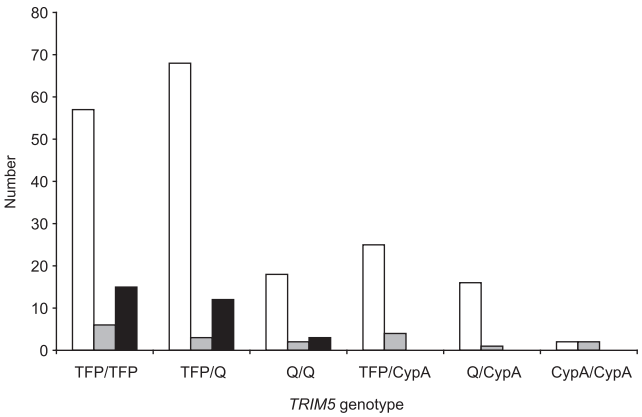

Figure 1 The number of *TRIM5* genotypes present in the rhesus macaque populations of different geographic origin. White bars represent the data in animals of Indian origin ($N = 186$), grey bars in animals of Burmese origin ($N = 18$), and black bars in animals of Chinese origin ($N = 30$). *N* is the number of animals analysed.

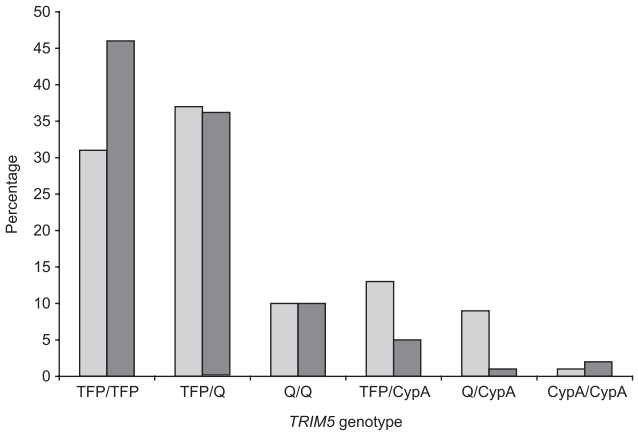

Figure 2 *TRIM5* genotype frequencies from two captive Indian-origin rhesus macaque populations. Light-grey bars represent the data from this study in animals housed at the Biomedical Primate Research Centre. Dark grey bars are data obtained from Kirmaier et al. (21), and represent data obtained in animals housed at the New England Primate Research Center.

As well as rhesus macaques of Indian origin, there are also those that have a Burmese or Chinese origin, and animals from both of these latter two groups were genotyped for *TRIM5* polymorphism (35, 36). The Burmese-origin animals share the same five alleles as described for the Indian cohort. As a result, all six possible genotypes are observed in the Burmese population (Figure 1). It is worth noting that compared with the Indian cohort, the small number of Burmese-origin animals analysed showed a high percentage of $TRIM5^{CypA}$-positive genotypes. In Chinese-origin rhesus macaques, the presence of the $TRIM5^{CypA}$ variant has never been recorded (24, 25), and this also applies to the animals analysed recently. In the studied Chinese cohort, six different alleles have been observed: the previously described *Mamu*-1, -3, -4, and -5, and two formerly undescribed alleles that group into the $TRIM5^{TFP}$ class and are most closely related to *Mamu*-3. The first newly detected allele contains a synonymous mutation: triplet 239 is changed from ACG to ACA. The second allele encodes an amino acid substitution (D318G). Triplet 318 is changed from GAT to GGT, resulting in the replacement of an aspartic acid (D) by a glycine (G). Both new alleles were submitted to EMBL and have been assigned the accession numbers FR873279 and FR873280, respectively. Most frequently observed in the Chinese cohort studied are the previously described *Mamu*-3 and -4 alleles.

TRIM5 genotyping in cynomolgus macaques

A few studies on cynomolgus *TRIM5* were performed, and focused either on defining its antiretroviral potency or on a description of the presence of a $TRIM5^{CypA}$ variant in

	CypA	EXON 7			EXON 8							TRIM5
	SNP	296	299	300	311	313	332	333	334	339	340	341	Group
Mafa-Trim-HM468438	G	A	Y	W	S	A	Q	S	P			Q	*TRIM5*^{Q}
Mafa-Trim-HM468439	G	A	Y	W	S	A	Q	S	P			Q	*TRIM5*^{Q}
Mafa-Trim-HM468440	G	A	Y	W	S	A	Q	S	P			Q	*TRIM5*^{Q}
Mafa-Trim-HM468441	T	V	C	W	S	V	R	T	Q			Q	*TRIM5*^{Cyp}
Mafa-Trim-HM468442	T	V	C	**R**	S	V	R	T	Q			Q	*TRIM5*^{Cyp}
Mafa-Trim-HM468443	T	V	C	W	S	V	R	T	Q			Q	*TRIM5*^{Cyp}
Mafa-Trim-FR873281	G	A	Y	W	**L**	A	Q	S	P			Q	*TRIM5*^{Q}
Mafa-Trim-FR873282	G	A	Y	W	S	A	Q	**A**	P	**T**	**F**	**P**	*TRIM5*^{TFP}

Figure 3 Deduced polymorphic amino acid positions in the B30.2 (SPRY) domain of the *TRIM5* alleles present in the cynomolgus macaque (*Mafa*). Black boxes represent the polymorphic amino acid residues at a particular position for that specific allele. The upper six alleles have been previously reported (24): the polymorphisms for the alleles HM468438 up to HM468440 as well as for HM468441 and HM468443 are located in intron 6 (data not shown). The cyclophilin-A single-nucleotide polymorphism is located in intron 6. *Mafa-Trim*-FR873281 and *Mafa-Trim*-FR873282 are the newly described alleles.

this species (24, 37, 38). Nonetheless, a population analysis of the *TRIM5* polymorphism in cynomolgus macaques is currently lacking. In that respect, a large cohort of 85 cynomolgus macaques was analysed for their *TRIM5* polymorphism, which covers most genetic variation expected to be present in this particular population. This resulted in the detection of eight alleles, of which five are newly described (FR873281-FR873285). Only two of these alleles contain non-synonymous mutations (Figure 3, FR873281 and FR873282). One of the newly described alleles groups into the TRIM5^{TFP} cluster (FR872282), a class that was not previously described to be present in cynomolgus macaques. The other alleles cluster either in the TRIM5^{Q} or TRIM5^{CypA} class. Four *TRIM5* genotypes are present in the studied cohort (Figure 4). The *TRIM5*$^{Q/Q}$ and *TRIM5*$^{Q/CypA}$ genotypes are most frequently observed. As described for the rhesus macaques, the observed number of genotypes may be a result of a sampling effect, and may not be representative for the number of genotypes present in other cynomolgus populations or wild-living cynomolgus macaques.

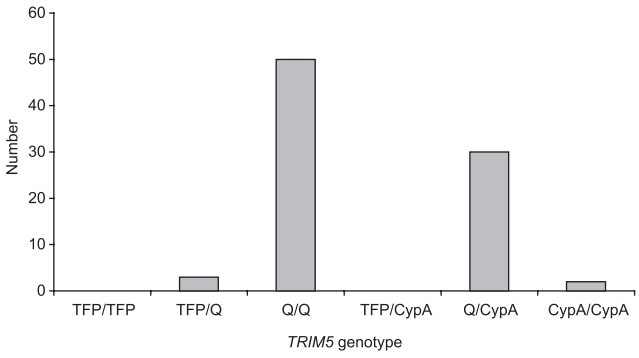

Figure 4 The number of *TRIM5* genotypes present in the cynomolgus macaque population.

Functional aspects of *TRIM5* polymorphism

Several groups have demonstrated that different *TRIM5* alleles have divergent antiretroviral specificities (21–23). In the past, various SIV studies were performed at the BPRC, and the animals were challenged either with different SIV$_{mac251}$ stocks or with SIV$_{mac32H/1XC}$. Recently, Lim et al. showed a correlation between *TRIM5* genotypes and viral replication in rhesus monkeys challenged with SIV$_{mac251}$. Animals that have at least one permissive allele (*TRIM5*^{Q}) present in their genotype appear to have a higher peak viral load than those animals lacking a permissive allele in their genotype (22).

From three studies, material was available to conduct retrospective analyses. The first study was performed in 10 animals of Chinese origin challenged with SIV$_{mac251}$. *TRIM5* analysis revealed that five animals had the *TRIM5*$^{TFP/TFP}$ genotype, two animals contained the *TRIM5*$^{TFP/Q}$ genotype, and two had the *TRIM5*$^{Q/Q}$ genotype. Plasma viral loads measured at 2 weeks post-infection were plotted against two *TRIM5* genotype groups (Figure 5, study 1): one that contains no permissive allele (TFP/TFP) *vs* one that contains at least one permissive allele (TFP/Q and Q/Q). The figure shows that no significant difference between the two groups could be observed in the peak viral load.

The second study comprised six Indian-origin animals challenged with SIV$_{mac251}$. *TRIM5* genotyping resulted in the observation that one animal was *TRIM5*$^{TFP/CypA}$, four were *TRIM5*$^{TFP/Q}$, and one was *TRIM5*$^{Q/CypA}$. Animals were grouped based on the presence of at least one permissive allele (*TRIM5*^{Q}) *vs* the absence of the permissive allele in their genotype. The peak viral loads (2 weeks post-infection) were plotted against these two groups (Figure 5, study 2 top). Although the number of animals per group was rather small and allowed no statistical analysis, a trend towards a higher peak viral load in animals that contain at least one permissive allele seemed to be present. The animals were also followed

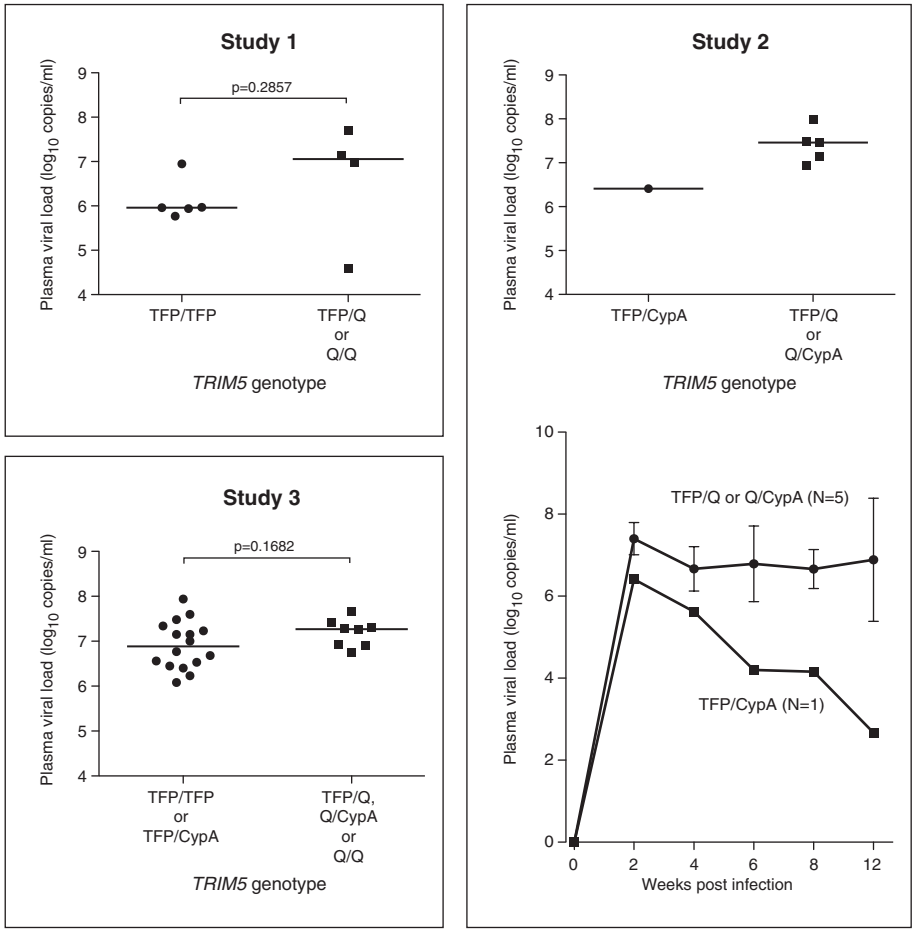

Figure 5 Plasma viral loads from three different SIV studies plotted against the *TRIM5* genotypes. Study 2, bottom graph, shows the plasma viral load in the different animals up to 12 weeks post-infection. For the TFP/Q or Q/CypA group, the mean with the standard deviation is plotted. *N* is the number of animals analysed.

in time, and plasma viral loads were measured every 2 weeks. Analysis of these data revealed that – up to 12 weeks post-infection – animals that contain at least one permissive allele (TFP/Q or Q/CypA) appear to continue to have a higher plasma viral load as compared to the animal that lack the permissive allele (Figure 5, study 2 bottom).

Because of the lack of statistical power and a seemingly contrasting result between the two studies, a third study, in which 24 Indian-origin animals had been challenged with the $SIV_{mac32H/1XC}$ stock was evaluated. *TRIM5* analyses within this cohort revealed 12 animals positive for $TRIM5^{TFP/TFP}$, four animals that contain the $TRIM5^{TFP/CypA}$ genotype, four animals with $TRIM5^{TFP/Q}$, one animal with $TRIM5^{Q/CypA}$, and three animals with the $TRIM5^{Q/Q}$ genotype. For the analysis, animals were divided again into a group that contains at least one $TRIM5^{Q}$ allele ($TRIM5^{TFP/Q}$, $TRIM5^{Q/CypA}$, and $TRIM5^{Q/Q}$) *vs* a group of animals that does not contain such an allele in their genotype ($TRIM5^{TFP/TFP}$ and $TRIM5^{TFP/CypA}$). Plotting the peak viral loads (2 weeks post-infection) did not show a significant difference between these groups (Figure 5, study 3). In addition, a separate evaluation of the reportedly most permissive $TRIM5^{Q}$ homozygous animals (only 3 animals) against the group of 16 least permissive $TRIM5^{TFP/TFP}$ and $TRIM5^{TFP/CypA}$ animals did not result in a significant difference in virus load (data not shown).

Discussion

The important role of the intrinsic immune factor TRIM5α in antiretroviral defence is well established (39). That rhesus macaques, often used in SIV studies, display polymorphism in their *TRIM5* gene, and that particular *TRIM5* genotypes correlate with control of replication of specific SIV viruses has been documented as well (21, 22). These observations clarify that *TRIM5* genotyping is essential and will contribute to the interpretation of the outcome of SIV vaccine studies in macaques.

The present communication provides a comprehensive analysis of the *TRIM5* gene polymorphism in four different macaque populations, and it documents the first large cohort study on *TRIM5* polymorphism in the cynomolgus macaque. We show that next to the $TRIM5^{Q}$ and $TRIM5^{CypA}$ group, the $TRIM5^{TFP}$ class is also present in this species. The fact that this allele appears to be rare may explain why it was not detected earlier, because in the past only a few animals

were analysed (24). It needs to be further investigated as to whether the high frequency of *TRIM5*^{Q} and *TRIM5*^{CypA} is due to an evolutionary advantage or to a bias based on the population analysed. For instance, all animals of the species *Macaca nemestrina* analysed so far appear to be homozygous for *TRIM5*^{CypA}, which may suggest that this allele could have been fixed in this species (25).

For rhesus macaques, we analysed animals of Burmese, Chinese, and Indian origin. Burmese and Indian-origin animals possess *TRIM5* alleles grouping into the three different classes. On the other hand, the Chinese-origin animals analysed did not possess the *TRIM5*^{CypA} variant. In general, it is known that Chinese-origin macaques are less susceptible to SIV infections than their Indian counterpart (40). Whether TRIM5α plays a role in this cannot be answered easily with the presently available data, as other genetic factors may contribute as well. In the small number of animals analysed, we did observe a high frequency of individuals that are homozygous or heterozygous for the least permissive *TRIM5*^{TFP} allele.

In contrast to earlier published data (22), in two of our SIV studies that comprised enough animals to allow statistical analyses (study 1 and 3), we do not observe a significant difference between the peak viral load in animals that contain at least one permissive *TRIM5*^{Q} allele *vs* animals that contain no permissive allele. For study 1, performed in animals of Chinese origin, other variations in the host genetics could have played a role in the observed differences between our study and the earlier published data. In study 3, the animals were challenged with $SIV_{mac32H/1XC}$. This isolate was derived from a SIV_{mac251} strain that has been passed through macaque 32H. This passage may have resulted in selection of viral variants with other mutations affecting viral fitness. This data exemplify that one needs to be careful in drawing general conclusions as to the effect of *TRIM5* polymorphism on the evolution of SIV in macaques, because observed effects may be cohort- and/or SIV strain-specific.

In conclusion, this study provides robust data on *TRIM5* genotyping in three geographically different rhesus macaque populations and in a large cohort of cynomolgus macaques. Moreover, the *TRIM5* genotyping in our SIV-infected macaque cohorts revealed no significant effect of *TRIM5* polymorphism on the replication of SIV_{mac251} and $SIV_{mac32H/1XC}$ in Chinese and Indian-origin rhesus macaques, respectively.

Acknowledgments

The authors wish to thank D. Devine for editing the manuscript, H. van Westbroek for preparing the figures, and S. Nieuwburg, N. Beenhakker, and I. Nieuwenhuis for technical assistance.

Conflict of interest

The authors confirm that there are no conflicts of interest.

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