AIDS RESEARCH AND HUMAN RETROVIRUSES
Volume 29, Number 7, 2013

DOI: 10.1089/aid.2012.0383

Susceptibility to Repeated, Low-Dose, Rectal $SHIV_{SF162P3}$ Challenge Is Independent of *TRIM5* Genotype in Rhesus Macaques

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Abstract

Infections following repeated, low-dose (RLD), mucal S(H)IV exposures of macaques are used to model sexual HIV exposures for biomedical prevention testing. Different susceptibilities among animals can complicate study designs. In rhesus macaques, *TRIM5* alleles *Q*, *CypA*, and *TFP* are resistance factors for infection with some S(H)IV strains, but not for SIV_{mac239} due to its capsid properties. SIV_{mac239}-derived $SHIV_{SF162P3}$ has been demonstrated to reproducibly infect mucosally in vaginal and rectal RLD models. To further test the suitability of $SHIV_{SF162P3}$ for RLD models, we studied the influence of the *TRIM5* genotype on susceptibility to rectal RLD infection and on plasma viremia by analyzing 43 male Indian rhesus macaques from control arms of completed studies. The median number of exposures required for infection was three (Q/Q, $n=4$) (*TRIM5* alleles, number of macaques, respectively), four (*Q/CypA*, $n=7$), three (*TFP/Q*, $n=15$), three (*TFP/TFP*, $n=15$), and two (*TFP/CypA*, $n=2$); $TRIM5^{CypA/CypA}$ was not represented in our study. Median peak viremia (log_{10} viral copies/ml) in infected animals was 7.4 (*Q/Q*, $n=4$), 7.2 (*Q/CypA*, $n=6$), 7.3 (*TFP/Q*, $n=13$), 7.1 (*TFP/TFP*, $n=15$), and 6.5 (*TFP/CypA*; $n=2$). Neither susceptibility nor peak viremia was significantly different (log rank test, Kruskal–Wallis test, respectively). Rhesus macaques' susceptibility to RLD $SHIV_{SF162P3}$ is independent of the *TRIM5 TFP*, *CypA*, and *Q* alleles, with the limitation that the power to detect any impact of *CypA/CypA* and *TFP/CypA* genotypes was nonexistent or low, due to absence or infrequency, respectively. The finding that *TRIM5* alleles do not restrict mucosal infection or ensuing replication rates suggests that $SHIV_{SF162P3}$ is indeed suitable for RLD experimentation.

The repeated-low dose (RLD) model of SIV or SHIV infection in nonhuman primates (NHPs), particularly macaques, has become a powerful tool to inform HIV biomedical intervention studies. There are many advantages to this approach as a model for HIV transmission, namely the similarity in inoculum and multiplicity in virus exposure before systemic infection to humans.[1] The use of higher physiological doses has been thought to dilute the effects of microbicide or vaccine-induced immunity, thereby underestimating the potential of candidate biomedical preventions. In the RLD model, it is possible to measure susceptibility as the number of challenges required to infect with the assumption that each animal is vulnerable to infection with each challenge.[2,3]

One concern as it relates to NHP models is the variability in susceptibility to infection. In humans, major histocompatibility complex alleles play a role in susceptibility to HIV infection[4] and they also impact the outcome of SIV infection in nonhuman primates.[5,6] Another aspect of variability is the control of retroviral infection by antiviral restriction factors that are a part of innate immunity. The tripartite motif protein, *TRIM5α*, belongs to the tripartite motif-containing (TRIM) superfamily of proteins and has been identified as an important post-entry host restriction factor[7] that inhibits retrovirus infection in a species-specific manner.[8–10] The exact mechanism of *TRIM5α* restriction is not clear but it is known that TRIM5α proteins aggregate on the incoming retroviral capsid and decrease the stability of the capsid.[9,11,12] In rhesus macaques, *TRIM5* gene variants can be grouped into three allelic classes, $TRIM5^{Q}$, $TRIM5^{CypA}$, and $TRIM5^{TFP}$, which yield six possible genotypes: $TRIM5^{TFP/TFP}$, $TRIM5^{TFP/Q}$, $TRIM5^{TFP/CypA}$,

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TRIM5$^{Q/Q}$, *TRIM5*$^{Q/CypA}$, and *TRIM5*$^{CypA/CypA}$. Cell culture assays and animal infections have been used to elucidate the role that allelic variation in the rhesus macaque *TRIM5* gene has in susceptibility to SIV infection and viral replication,[13–16] while other species such as cynomolgus macaques are also under investigation.[17] The goal of this study was to define the influence of the *TRIM5* genotype on susceptibility to $SHIV_{SF162P3}$ infection in a RLD model.

We analyzed 43 male Indian rhesus macaques from control arms of studies completed at the CDC.[18–21] They were purchased from diverse U.S. vendors, they were housed at the CDC according to the standards established in the Guide for the Care and Use of Laboratory Animals,[22] and their use was approved by the Animal Care and Use Committee (IACUC) of the CDC. All were rectally challenged with 10 $TCID_{50}$ of the same $SHIV_{SF162P3}$ stock [obtained in 2001, National Institutes of Health (NIH) AIDS Research and Reference Reagent Program (ARRRP)] for up to 14 or 16 exposures, once or twice a week. The virus stocks were not further propagated, which eliminated the potential variable of enriching a virus stock with capsid adaptations due to *TRIM5* selective pressure during propagation in peripheral blood mononuclear cells (PBMCs) from individual macaques, another potential confounder in NHP challenge models. Plasma viremia was assessed by polymerase chain reaction (PCR) with a detection limit of 50 copies/ml as described previously.[23] We determined the *TRIM5* genotype by isolation of genomic DNA from PBMCs using the QIAamp DNA Blood Mini kit (QIAGEN, Valencia, CA) and direct bulk sequencing of a PCR fragment as previously described.[13]

TRIM5 allelic frequencies in our cohort were as follows: 7% of the macaques were *TRIM5*$^{Q/Q}$ ($n=4$), 16% *TRIM5*$^{Q/CypA}$ ($n=7$), 35% *TRIM5*$^{TFP/Q}$ ($n=15$), 35% *TRIM5*$^{TFP/TFP}$ ($n=15$), and 5% *TRIM5*$^{TFP/CypA}$ ($n=2$); no macaque was *TRIM5*$^{CypA/CypA}$. These percentages were similar to distributions recently reported from another major U.S. facility for nonhuman primate research.[13,14] The median number of exposures required for infection was three (*TRIM5*$^{Q/Q}$), four (*TRIM5*$^{Q/CypA}$), three (*TRIM5*$^{TFP/Q}$), three (*TRIM5*$^{TFP/TFP}$), and two (*TRIM5*$^{TFP/CypA}$) (Fig. 1). For statistical analysis, the three macaques that remained uninfected were assessed at the maximum number of challenges received. Differences in the number of exposures required to infect were tested using global log rank statistics. There was no statistically significant difference in the number of exposures needed for infection between the genotype groups (4 degrees of freedom, $p=0.37$). This suggests that the *TRIM5* genotype has no marked effect on rhesus macaque susceptibility to $SHIV_{SF162P3}$ or the number of challenges it takes to infect using $SHIV_{SF162P3}$ in an RLD exposure model. Of note, the three uninfected animals in this study had *Q/CYPA* ($n=1$) and *TFP/Q* ($n=2$) alleles, and not *TFP/CypA* as reported for animals resistant to SIV_{smE660} infection.[14] Rather, the two macaques of the *TFP/CypA* phenotype were infected quickly, at exposures one and two, respectively. Moreover, animals requiring six or more exposures were found in each allele type except for *TFP/CYPA* ($n=2$).

The three uninfected macaques had Q/CYPA ($n=1$) and TFP/Q ($n=2$) alleles. The underlying resistance mechanisms remain unknown. MHC class I analysis for common Mamu alleles A*01, A*02, A*08, A*11, B*01, B*03, B*04, and B*17 revealed the presence of Mamu-A*08 in one resistant macaque, Mamu-B*01 in another, and none of these in the third macaque. Mamu-A*08 and Mamu-B*01 have not previously been associated with increased viral control.[5,6] It is possible that uncharacterized Mamu alleles contributed to resistance. Many other host factors can also contribute to resistance to infection; e.g., we have previously shown that high systemic RANTES levels can render macaques resistant to $SHIV_{SF162P3}$ infection in the RLD model,[18] although the RANTES status of the three resistant macaques in this study was not available.

Previous work has provided evidence that *TRIM5* alleles affect SIV_{smE660} RLD rectal acquisition[14] and penile acquisition.[15] The alleles did not impact SIV_{mac239}[14] or SIV_{mac251}[24]

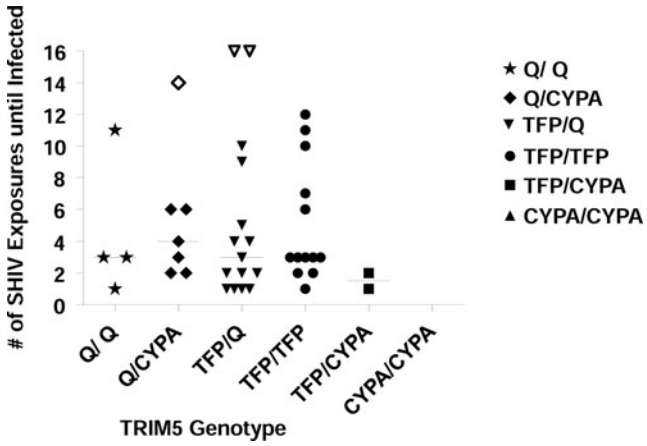

FIG. 1. *TRIM5* genotypes versus susceptibility (number of $SHIV_{SF162P3}$ challenges until Infection). The number of exposures until infection was compared in 43 rhesus macaques of indicated *Trim5* genotypes. There was no correlation between the genotype and the number of exposures with $SHIV_{SF162P3}$ that it took to infect ($p=0.37$, global log rank test). One week was subtracted for the eclipse phase when determining date of infection. The solid lines indicate the median number of SHIV exposures until infected. The open symbols represent the three uninfected animals in this study that had Q/CYPA (censored at 14 exposures) and TFP/Q (both were censored at 16 exposures) alleles.

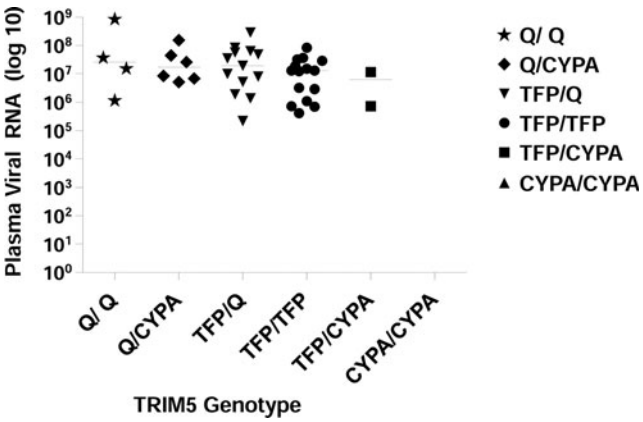

FIG. 2. *TRIM5* genotype versus peak plasma viremia with $SHIV_{SF162P3}$. There was no association between the genotype and the amount of plasma viral RNA after infection with $SHIV_{SF162P3}$ ($p=0.45$; Kruskal–Wallis test). The solid lines indicate median RNA copies/ml.

RLD rectal acquisition, although an effect on SIV_{mac251} replication after intravenous acquisition was found.[16,25] Contrary to SIV_{smE660} challenges, we found no evidence that Q alleles conferred increased susceptibility or that TFP and CypA alleles conferred resistance to $SHIV_{SF162P3}$ infection. In conclusion, $SHIV_{SF162P3}$ is an addition to the list of viable strains for rhesus macaque RLD infections not affected by *TRIM5* alleles. Finding a similarly suited virus strain may be the key to the development of RLD models in other species that have not been studied yet with regard to *TRIM5* or other genetic restriction.

For $SHIV_{SF162P3}$, peak viremia levels were also similar for macaques of different *TRIM5* genotypes (Fig. 2). The Kruskal–Wallis rank sum statistic was used to test for genotype group differences in peak viral load. Following peak viremia, there were no significant differences in viral load or in viral load decay between host *TRIM5* genotypes (4 degrees of freedom, $p = 0.45$; these analyses were done using all data within 10 weeks of or after the peak viral load). A mixed-effects regression model was used to assess group differences in rate of decay after peak viral load and identified no differences ($p > 0.05$). Last, to compare total viral burden by group, area under the curve (AUC) was determined and group comparisons were tested using Kruskal–Wallis tests, which again found no significant differences ($p = 0.20$).

A recent study by *Yeh et al.* reports that susceptibility to repeated penile SIV_{smE660} infection in rhesus macaques is highly dependent on the *TRIM5* genotype.[15] It is possible that penile or other transmission routes are more susceptible to the impact of *TRIM5*-mediated effects than during rectal transmission. *Kirmaier et al.* found that two restrictive alleles ($TRIM5^{TFP/TFP}$ and $TRIM5^{TFP/CypA}$) led to lower $SIV_{smE543\text{-}3}$ replication than in homozygous $TRIM5^{Q/Q}$ macaques.[13] Here, $SHIV_{SF162P3}$ replication peaked at medians of 7.1, 6.5, and 7.4 $\log_{10}$ viral copies/ml for *TFP/TFP*, *TFP/CypA*, and *Q/Q* alleles, respectively, but these differences were not statistically significant. It is possible that a subtle *TRIM5α* impact on $SHIV_{SF162P3}$ replication nevertheless exists, but could not be measured in our small sample macaque groups. Utilizing cell culture lines to measure infectivity and replication of SIV_{mac239} and $SHIV_{SF162P3}$ for each expressed common allele would shed further light on what role *TRIM5* may have in influencing susceptibility to infection.

In conclusion, our results demonstrate that rhesus macaques' susceptibility to RLD $SHIV_{SF162P3}$ is independent of *TRIM5 TFP*, *CypA*, or *Q* alleles, with the caveat that the power to detect any impact of rare $TRIM5^{CypA/CypA}$ and $TRIM5^{TFP/CypA}$ genotypes was nonexistent or low, due to absence or infrequency, respectively. That *TRIM5* alleles do not restrict mucosal infection or ensuing replication rates is perhaps not surprising because $SHIV_{SF162P3}$ is a derivative of SIV_{mac239}, which has previously been shown to infect cells and rhesus macaques irrespective of *TRIM5* genotype.[13,14] Our retrospective study served to provide an *in vivo* experimental demonstration that rhesus macaques can be reliably infected with rectal $SHIV_{SF162P3}$ exposure irrespective of *TRIM5* genotype. Although the result was expected due to similar studies with parent strain SIV_{mac239}[14] and due to *in vitro* infectivity experiments with SIV_{mac239},[13] our findings further document in more animals that *TRIM5* alleles do not confound macaque studies when $SHIV_{SF162P3}$ is used at the reported dose, and using the reported stock preparation with all potential virus adaptations that can happen during virus propagation. This further documents the usefulness of this unique RLD model for preclinical HIV prevention trials.

Acknowledgments

This work was supported by the Centers for Disease Control and Prevention (CDC) and partially supported by Interagency Agreement Y1-AI-0681-02 between the CDC and the National Institutes of Health (NIH). This work was also partially supported by grants AI083118 and AI095092 from the National Institutes of Health (NIH). We would like to thank the veterinarians and animal technicians who performed the studies from which samples were obtained retrospectively. We would also like to thank past members of our research team for residual specimens that were used for this study. All authors critically reviewed this manuscript. E.N.K. thanks Gil Kersh for his support.

The findings and conclusions in this report are those of the authors and do not necessarily represent the views of the Centers for Disease Control and Prevention.

Author's contributions: K.B. and E.K. coordinated samples, analyzed data, and wrote the manuscript. J.M. and W.J. genotyped the samples. D.H. performed statistical analyses. D.A., G.G., W.H., and D.E. gave samples with infection and viral load data. R.M.H., J.M.N., W.H., and W.J. provided input in the study design and result interpretation. E.K. designed and led the study. All authors read, commented on, and approved the final manuscript.

Author Disclosure Statement

No competing financial interests exist.

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