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In Vivo Examination of Mouse APOBEC3- and Human APOBEC3A- and APOBEC3G-Mediated Restriction of Parvovirus and Herpesvirus Infection in Mouse Models

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ABSTRACT

APOBEC3 knockout and human APOBEC3A and -3G transgenic mice were tested for their ability to be infected by the herpesviruses herpes simplex virus 1 and murine herpesvirus 68 and the parvovirus minute virus of mice (MVM). Knockout, APOBEC3A and APOBEC3G transgenic, and wild-type mice were equally infected by the herpesviruses, while APOBEC3A but not mouse APOBEC3 conferred resistance to MVM. No viruses showed evidence of cytidine deamination by mouse or human APOBEC3s. These data suggest that *in vitro* studies implicating APOBEC3 proteins in virus resistance may not reflect their role *in vivo*.

IMPORTANCE

It is well established that APOBEC3 proteins in different species are a critical component of the host antiretroviral defense. Whether these proteins also function to inhibit other viruses is not clear. There have been a number of *in vitro* studies suggesting that different APOBEC3 proteins restrict herpesviruses and parvoviruses, among others, but whether they also work *in vivo* has not been demonstrated. Our studies looking at the role of mouse and human APOBEC3 proteins in transgenic and knockout mouse models of viral infection suggest that these restriction factors are not broadly antiviral and demonstrate the importance of testing their activity *in vivo*.

Given the frequent encounters of vertebrates with viruses, it is not surprising that there has been selection for host defense systems. In 2002, human apolipoprotein B editing complex 3G (APOBEC3G) was discovered and shown to confer intrinsic immunity to HIV-1 (1). The APOBEC3G gene belongs to a family of genes encoding DNA- and RNA-editing enzymes characterized by the presence of at least one cytidine deaminase (CDA) domain (2). The number of APOBEC3 genes varies from species to species, from 1 gene in mice to 7 genes (the APOBEC3A, -3B, -3C, -3DE, -3F, -3G, and -3H genes) in primates (2). Moreover, there are multiple allelic differences in APOBEC3 gene coding regions; notably, there are at least 2 alleles in mice that differ in both their protein-coding regions and levels of expression (3, 4). These allelic variants confer weak or strong resistance to murine retrovirus infection *in vivo* (3, 5–7).

Several human APOBEC3 proteins inhibit HIV-1 lacking the *vif* gene, which encodes a protein expressed at high levels late in infection (8–12). In *vif*-deficient-HIV producer cells, APOBEC3 proteins are packaged into progeny virions via interaction with the nucleocapsid (NC) protein and viral RNA. Vif prevents packaging by binding APOBEC3 proteins, targeting them for ubiquitination and degradation in the proteasome (13–17). APOBEC3 proteins inhibit infection by deaminating deoxycytidine residues on minus-strand DNA, inducing hypermutation in newly reverse-transcribed HIV-1 DNA; this leads both to degradation of viral DNA prior to integration and to G-to-A coding-strand mutations in viral genes in the integrated provirus (18). APOBEC3 proteins also inhibit replication by a number of CDA-independent mechanisms (19). APOBEC3 proteins restrict animal retroviruses (20–23) and other human retroviruses, such as human T cell leukemia virus 1 (HTLV-1), but it has also been suggested that they prevent zoonotic transmission (18, 24, 25). Indeed, several human APOBEC3 proteins have been shown to be more effective at inhibiting mouse retroviruses than the murine APOBEC3 protein, both *in vitro* and *in vivo* (26–28).

The selective pressure placed on APOBEC3 genes, which led to the expansion of the human APOBEC3 locus through gene duplication as well as to polymorphisms in the genes in many species, may also be the result of infections by other viruses in addition to retroviruses. There have been numerous reports of APOBEC3 proteins restricting members of several virus families by both deaminase-dependent and -independent mechanisms (29–31). For example, APOBEC3A but not APOBEC3G was shown to inhibit infection by the parvoviruses adeno-associated virus and

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minute virus of mice (MVM) in cultured cells overexpressing these proteins, in this case by deaminase-independent means (32, 33). Evidence of APOBEC3A-, 3C-, and 3G-mediated editing of herpes simplex virus 1 (HSV-1) was detected when these factors were overexpressed in cultured cells, and HSV-1 and Epstein-Barr virus (EBV) showed signs of cytidine deamination in buccal swabs or immortalized cell lines, respectively (30). Moreover, a recent study showed that several human APOBEC3s, including APOBEC3A, inhibited replication of murine gammaherpesvirus 68 (MHV68) when the viral genome was transfected into cultured cells but not when it was introduced by infection (34). However, APOBEC3 knockout (A3KO) and wild-type (WT) mice were equally resistant to infection by this virus (34).

Here, we took advantage of A3KO and human APOBEC3A- and 3G-expressing transgenic mouse lines developed by our lab several years ago to study *in vivo* infection by HSV-1, MHV68, and MVM and to determine if the mouse or human proteins could contribute to inhibition of zoonosis (27). These transgenic mouse strains, each of which was backcrossed onto a mouse A3KO background, express myc-tagged human APOBEC3A and -3G under the control of the β-actin promoter, and thus the transgenes are expressed in multiple tissues, including the brain, spleen, and liver, as well as sentinel cells (macrophages, dendritic cells, and lymphocytes) and fibroblasts. Importantly, the level of expression of the transgenes was similar to that seen in normal human tissue (27). The human APOBEC3 proteins were fully functional *in vivo*; both APOBEC3A and APOBEC3G restricted infection by the murine retroviruses mouse mammary tumor virus (MMTV) and murine leukemia virus (MLV). Interestingly, the restriction occurred by different mechanisms: APOBEC3G was packaged into virions and caused extensive deamination of the retrovirus genomes, while APOBEC3A was not packaged, did not deaminate, and restricted infection when expressed in cells that were targets of infection. While the human APOBEC3 transgenic mice showed much lower infection by the murine retroviruses, here we show that this is not the case for mouse or human herpesviruses and that only the parvovirus MVM was modestly inhibited by APOBEC3A *in vivo*.

MATERIALS AND METHODS

Mice. A3KO, $A3A^{high}$, $A3G^{low}$, and $A3G^{high}$ transgenic mice on an A3KO (C57BL/6N) background were previously described (20, 27). All transgenic lines were maintained by breeding with mouse A3KO mice, so the transgenes were carried in heterozygotes on the A3KO background. This allowed us to generate nontransgenic, matched controls for infection studies. Tmem173 mice (C57BL/6J background) were obtained from the Jackson Laboratory, and BALB/cN and C57BL/6N mice were obtained from Charles River; all 3 strains were bred at the University of Pennsylvania. The mice were housed according to the policies of the Institutional Animal Care and Use Committee of the University of Pennsylvania. The experiments performed with mice in this study were approved by this committee (IACUC protocol 801594). All virus infections were carried out under animal biosafety level 2 conditions.

HSV-1 infections and analysis. Mice were intraocularly (i.o.) or intraperitoneally (i.p.) inoculated with 1×10^6 PFU of HSV-1 (McKrae strain), and then sacrificed at 5.7 days postinoculation (dpi) to harvest the tissue samples. Moribund mice (as defined by a hunched posture, dehydration, and lethargy) were sacrificed before 5.7 days, as noted below. For i.o. inoculations, mice were anesthetized with ketamine-xylazine, and then 2 to 10 μl of virus was pipetted onto the eyes. For mice inoculated by the i.o. route, DNA was isolated from the trigeminal ganglia, eyeballs, and spleen, and real-time quantitative PCR (qPCR) was performed using primers specific for the HSV-1 thymidine kinase (TK) gene (5′-GAG TTTCACGCCACCAAGAT-3′/5′-CTATGATGACACAAACCCCG-3′). HSV-1 values were normalized to mouse GAPDH (glyceraldehyde-3-phosphate dehydrogenase) (27). For mice inoculated by the i.p. route, daily body weight and survival were ascertained, and brain tissue samples obtained at euthanasia were homogenized in 1 ml culture medium. The supernatants of homogenates were 10-fold serially diluted, and titers were determined on Vero cells. At 2 h postinfection, the inoculum was replaced with culture medium containing 2% fetal calf serum (FCS) and 5% methylcellulose. The cells were fixed with methanol and stained with crystal violet at 3 dpi. Plaques were counted, and the viral titers were calculated as PFU/ml.

MHV68 infections and analysis. MHV68 (10^3 PFU; WUMS strain) was intranasally inoculated into mice. The mice were sacrificed at 7 and 16 dpi for acute- and latent-infection analysis, respectively (34). Plaque assays were conducted to measure the virus titers during acute infection in the lungs. The lungs were homogenized in 1 ml culture medium. The supernatants of homogenates were 10-fold serially diluted. The titer of each dilution was determined on NIH3T12 cells. At 2 h postinfection, the inoculum was replaced with culture medium containing 5% FCS and 2.5% methylcellulose. The cells were fixed with methanol and stained with crystal violet at 7 dpi. Plaques were counted, and the viral titers were calculated as PFU/ml. MHV68 latency was evaluated by qPCR of spleen and lung DNA, using the specific primer set for the MHV68 ORF12 gene (5′-GTCTACAACAGGATCTGCATTT-3′/5′-AAAACTCTACCGTGAC TGTGAA-3′), as described by Minkah et al. (34). The values were normalized to mouse GAPDH.

MVMi infections and PCR. Newborn C57BL/6N, BALB/cN, A3A, and A3KO mice were infected oranasally with equal amounts of the lymphotropic-specific variant of MVM, MVMi (35). Mice were sacrificed at 5, 9, or 13 days postinfection. Spleens, lungs, kidneys, and brains were isolated from the infected mice, and DNA was isolated from the organs using a DNeasy kit (Qiagen). qPCR was performed to examine the MVMi levels in the infected organs. MVMi primers used for qPCR detection were 5′-AAGGTACGATGGCGCCTC-3/5′-GTGCTCTTTGGCAGC-3′. MVMi values were normalized to GAPDH.

3DPCR and sequencing. PCRs to amplify edited viral DNA were performed using a gradient cycler (Veriti 96-well thermal cycle; Applied Biosystems) on DNA isolated from the indicated organs. To examine deamination in HSV-1, differential DNA denaturation PCR (3DPCR) on organ DNA was conducted using GoTaq Green master mix (Promega) with a specific primer set for the HSV-1 ICP22 gene, 5′-CGACGCGGGCCCGA GCRTATRCTYYAT-3′/5′-GGAAATGGCGGACACCTTCCTGGAYAY YAT-3′, as described by Suspène and colleagues (30). The denaturation temperature of the PCR cycle was programmed to range from 89 to 95°C, and reactions were carried out for 40 cycles. For MHV68, 3DPCR was performed using Q5 Hot Start high-fidelity DNA polymerase (New England BioLabs) with a specific PCR primer set for a GC-rich region of the MHV68 genome (bp 88884 to 89613) (5′-ACGACCCTGACAACATCAA C-3′/5′-TCTTGTTCCCAGGTGGCCTTAA-3′) (34). The denaturation temperature of the PCR cycle ranged from 83 to 98°C. For MVMi, a 946-bp region of the genome was amplified with the primers 5′-CTG TCCACTCAGCTGCAAGA-3′/5′-ACTCACCCAGTTAACCCCCA-3′. A temperature gradient of 85 to 89°C was applied. To increase the likelihood of detecting deamination events, the PCR products amplified at the lowest denaturation temperature for all the viruses were purified from agarose gels using a Qiagen QIAquick gel extraction kit and were cloned into pCR-Blunt II-TOPO vector as directed by the manufacturer (Invitrogen, Inc.). DNA was isolated from the resultant colonies using the TempliPhi kit (GE Healthcare Life Sciences, Inc.). Clones were then sequenced using a BigDye Terminator v3.1 cycle sequencing kit from Applied Biosystems. The numbers of target sites for the different APOBEC3s in the regions of HSV-1, MHV68, and MVMi sequenced are shown in Table 1.

TABLE 1 Numbers of APOBEC3 sequence hot spots found in the regions of HSV-1, MHV68, and MVMi sequenced

	No. of hot spots		
Virus	Mouse APOBEC3 (TCC/GGA)	APOBEC3A (TC/GA)	APOBEC3G (CCC/GGG)
HSV-1	39	106	83
MHV68	27	112	11
MVMi	19	100	NA[a]

[a] NA, not applicable.

Statistical analysis. Statistical analysis was performed using GraphPad Prism software. For Fig. 1, 3, and 4, the horizontal bar denotes the geometric mean of the linear values.

RESULTS

HSV-1 infection is not affected by endogenous mouse or human APOBEC3 proteins. Both the A3KO and human A3 transgenic mice were generated on a C57BL/6 background. Since this strain is not highly susceptible to HSV-1, we used the pathogenic McKrae strain, which is reported to more efficiently infect this mouse strain as well as to cause pathogenesis after i.o. inoculation (36). The transgenic strains used in all experiments were the A3G^{high}, A3G^{low}, and A3A^{high} strains described in our original publication and were expressed in a broad range of tissues (27). The A3G^{high} and A3A^{high} transgenic mice have equivalent levels of expression in mouse and human PBMCs, while the A3G^{low} transgenic mice express 10-fold lower levels in these cells (27). We first used i.o. inoculation (10^6 PFU/animal) and measured infection in the eyes and trigeminal nerve and spread to the spleen. We found no difference in infection in any of the mice (Fig. 1A and B). To confirm that the virus was indeed infectious in mice, we repeated the inoculations with both 10^6 and 10^7 PFU, this time including as a positive control STING mutant (Tmem173) mice on a C57BL/6 background, which are highly susceptible to HSV infection (37). The Tmem173 mice were more highly infected at both doses, while the other mice showed similar low levels of infection, both at the initial sites of infection (eyes) (Fig. 1C) and after spread to the spleen (Fig. 1D). Similar results for both sets of inoculations were seen in the trigeminal nerve (not shown). Interestingly, although the McKrae strain is reported to be pathogenic in C57BL/6 mice, we found that while some mice exhibited mild swelling around the forebrain after i.o. inoculation, most showed no symptoms, and with the exception of Tmem173 mice, all recovered from infection by 1 week postinfection (not shown).

To determine if the route of infection influenced the ability of the APOBEC3 proteins to affect infection, we also inoculated the A3KO, C57BL/6, A3A^{high}, A3G^{high}, and Tmem173 mice by i.p. injection. The Tmem173 mice showed a dramatic weight loss at 4 days after i.p. infection, while all the other strains maintained their weight through day 6 (Fig. 2A). Similarly, while all the Tmem173 mutant mice rapidly succumbed to HSV-1 infection, at most 50%

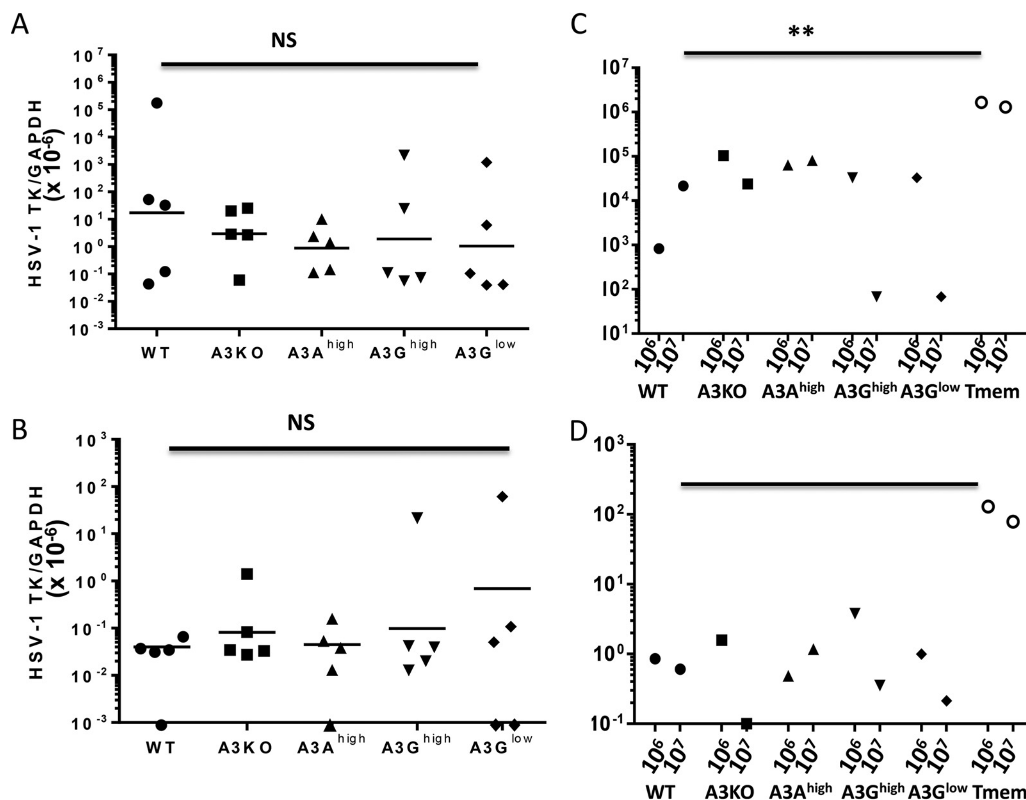

FIG 1 APOBEC3 does not protect mice from intraocular HSV-1 infection. (A and B) HSV-1 strain McKrae (10^6 PFU) was intraocularly applied to mice of the indicated genotypes, and at 5.7 dpi, their eyes (A) and spleens (B) were harvested and DNA was isolated for qPCR analysis ($n = 5$ for all genotypes). (C and D) An additional mouse of each genotype as well as Tmem173 was inoculated via the same route with 10^6 or 10^7 PFU of HSV-1, and infection in eyes (C) and spleen (D) was analyzed. Although not included in the calculation of the geometric mean, mice with values of 0 are shown on the plot. NS, not significantly different by one-way analysis of variance (ANOVA); **, $P \leq 0.002$; ****, $P \leq 0.0001$ (by two-tailed unpaired t test).

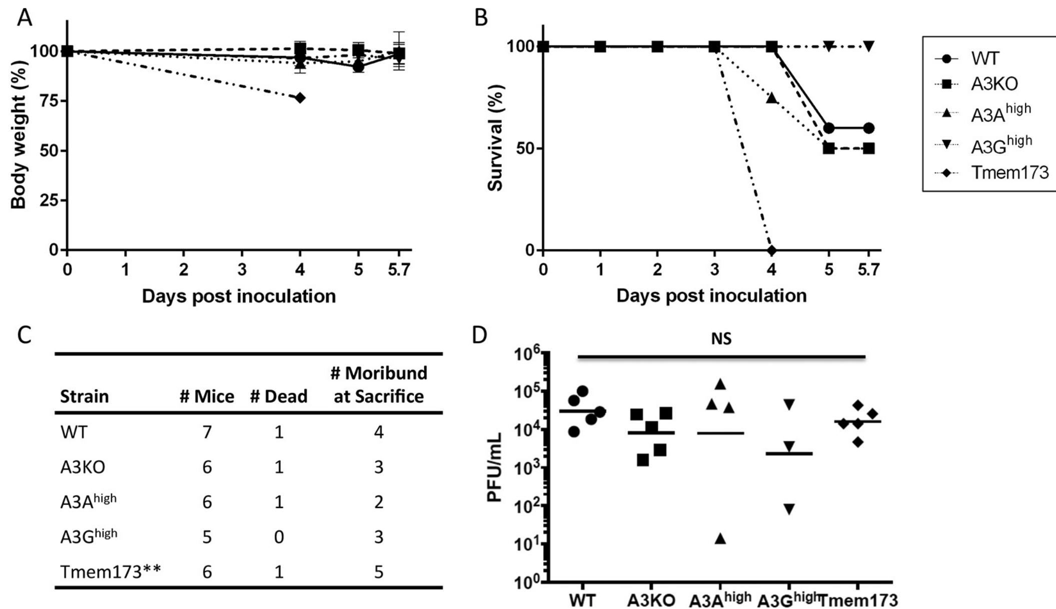

FIG 2 APOBEC3 does not protect mice from systemic HSV-1 infection. (A and B) HSV-1 McKrae strain (10^6 PFU) was intraperitoneally injected into mice of each genotype, as indicated. The mice died or were sacrificed by 5.7 dpi. Weight loss (A) and survival ratio (B) of mice were examined ($n = 5$ for C57BL/6 [WT]), 4 for A3KO and A3A^{high}, 3 for A3G^{high}, and 5 for Tmem173). (C) Additional mice were infected with 10^6 PFU of HSV-1, and the overall incidence of mortality and morbidity in the different strains was analyzed. **, all of the Tmem mutant mice were dead or moribund by 4 dpi. (D) Mice from the experiments for panels A to C were sacrificed, and brain tissue was collected at 4 to 5.7 dpi. The left hemisphere was homogenized in 1 ml culture medium, and titers in the supernatants of homogenates were determined on Vero cells. Because the Tmem173 mice were moribund by 3 dpi, they were sacrificed by day 4 ($n = 6$ for C57BL/6 [WT] and 5 for all the other strains). One WT and A3A^{high} mouse each and 2 A3G^{high} mice had no virus titers. NS, not significantly different by either two-tailed unpaired *t* test or one-way ANOVA, including the uninfected mice in the analysis.

of the C57BL/6, A3KO, or A3A^{high} mice and none of the A3G^{high} mice succumbed at later times after infection, and there was no statistical difference in the survival curves (Fig. 2B). Moreover, approximately equal numbers of the transgenic, C57BL/6, and A3KO mice were moribund at sacrifice (Fig. 2C). In contrast to the case for mice that received i.o. inoculations, HSV-1 infection levels, as measured by viral levels in the brain, were not significantly different between the mice at day 4 (Tmem173) or 5.7 (all other strains) after i.p. inoculation (Fig. 2D), suggesting that the HSV-1–mediated mortality in Tmem173 mice was due to infection of tissues other than brain. This is in contrast to work by others demonstrating that STING KO mice on a mixed 129SvEv × C57BL/6J background sustain higher levels of brain infection after intravenous infection by HSV-1 (37).

MHV68 infection is not affected by endogenous mouse or human APOBEC3 proteins. We next tested the mice for their susceptibility to the gammaherpesvirus MHV68; as discussed above, the human gammaherpesvirus EBV shows signs of APOBEC3-mediated cytidine deamination, and a recent report suggested that APOBEC3A but not mouse APOBEC3 was able to restrict MHV68 after DNA transfection of viral genomes but not virion infection in tissue culture cells (34). Wild-type, A3A^{high}, A3G^{high}, A3KO, and Tmem173 mice received intranasal inoculation of the virus, and at 7 dpi, virus titers in lung were measured (Fig. 3A). No differences in the infection levels were seen between any of the mice. To determine whether there was a difference in establishment of latent infection between the strains, a cohort of infected mice of each genotype was sacrificed at 16 dpi. There was no difference in infection in the lungs (Fig. 3B) or spleens (Fig. 3C) in any of the mice, including Tmem173 mutant mice. The latter finding is in accord with a recent study showing that MHV68 only weakly stimulates innate immune responses via the STING pathway, although in this study the STING mutant mice were infected at about 2-fold-higher levels at 2 days dpi (38).

Lack of cytidine deamination of herpesvirus genomes in WT or human APOBEC3 transgenic mice. Several human APOBEC3 proteins have also been implicated in deamination of herpesvirus genomes, including APOBEC3A and -3G, without affecting the level of viral DNA (30). To determine if the viral genomes of HSV-1 and MHV68 showed evidence of editing by these enzymes *in vivo*, we used differential DNA denaturation PCR (3DPCR) to amplify DNA isolated from the brains of the HSV-1-infected mice and the lungs of the MHV68-infected mice of different genotypes. The fragments amplified and sequenced had a significant number of target sequences for mouse APOBEC3, APOBEC3A, and APOBEC3G (Table 1). No difference was seen in the minimum denaturation temperature needed for amplification of the viral DNA by 3DPCR (not shown). When the PCR products amplified at the lowest temperatures were sequenced, no difference in deamination of the viral genomes was detected (Table 2).

MVMi is restricted by human APOBEC3A but not mouse APOBEC3. While previous studies indicated that restriction of herpesviruses and papillomaviruses occurred via cytidine deamination, human APOBEC3A restriction of parvoviruses is not dependent on cytidine deamination, at least in cultured cells (32, 33). We also tested whether mouse APOBEC3 or human APOBEC3A proteins restricted MVMi infection *in vivo*. MVMi is a lymphotropic variant that was reported to be lethal in BALB/c but not C57BL/6 mice (35), while the prototypic MVMp which was used to study APOBEC3 restriction in cultured cells primarily

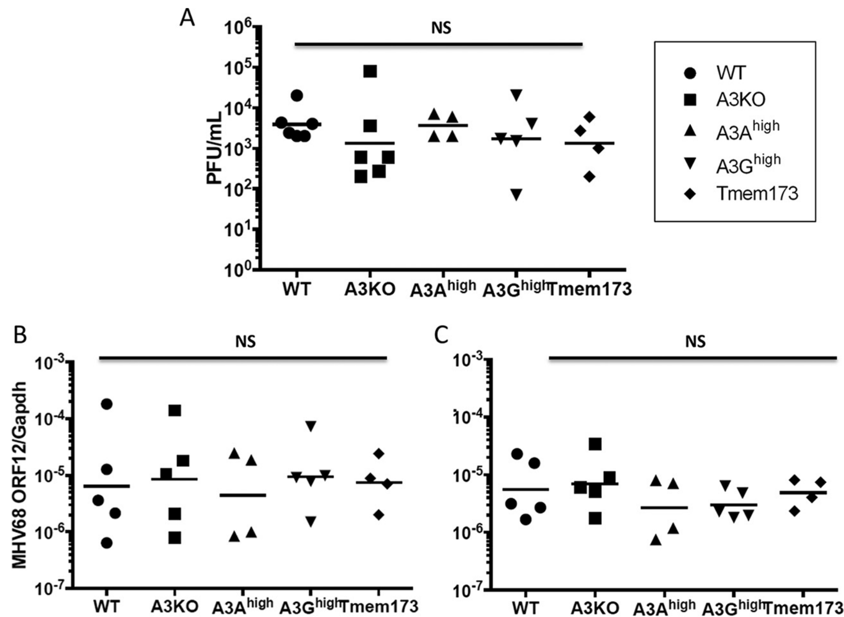

FIG 3 APOBEC3 does not protect mice from acute or latent MHV68 infection. (A) MHV68 (10^3 PFU) was intranasally introduced into each genotype of mice as indicated. The mice were sacrificed, and lung tissue was collected 7 dpi. Whole lung tissues were homogenized in 1 ml culture medium, and the supernatants of homogenates were 10-fold serially diluted. The titer of each dilution was determined on NIH3T12 cells, and they were stained and fixed at 5 dpi. Plaques were counted, and the viral titers are indicated as mean PFU/ml ($n = 6$ for C57BL/6 and A3KO, 5 for A3A^{high} and A3G^{high}, and 4 for Tmem173). (B and C) MHV68 (10^3 PFU) was intranasally injected into mice of each genotype as indicated. The mice were sacrificed, and the lung (B) and spleen (C) tissues were collected 16 days after injection. DNA was isolated and subjected to PCR with primers specific to ORF12. Values are indicated as MHV68 copy numbers normalized to mouse GAPDH ($n = 5$, 5, 4, 5, and 4 for WT, A3KO, A3A^{high}, A3G^{high}, and Tmem, respectively). NS, not significantly different by either two-way unpaired *t* test or one-way ANOVA.

infects fibroblasts and is not pathogenic *in vivo* (39). Interestingly, in past studies (35), susceptibility to MVMi in different mouse strains cosegregated with the weak APOBEC3 allele found in BALB/c mice, which is expressed at ~10-fold-lower levels than that found in C57BL/6 mice; the weak BALB/c allele confers increased susceptibility to MLV and MMTV infection (3, 5–7, 40). We speculated that allelic differences in the mouse APOBEC3 gene might also play a role in MVM pathogenesis. First, we did a

TABLE 2 Mutation analysis of HSV-1-, MHV68-, and MVMi-infected mice[a]

Virus and mouse strain	No. of: Mice	Total sequences	Total nucleotides	Mutations: Total	G to A plus C to T	Other	Ratio of mutations to total nucleotides: G to A plus C to T	Other
HSV-1								
C57BL6	3	16	13,621	72	28	44	0.0021	0.0032
A3KO	3	16	13,632	52	30	22	0.0022	0.0016
A3A^{high}	3	17	14,483	62	32	30	0.0022	0.0021
A3G^{high}	3	15	12,775	64	32	32	0.0025	0.0025
Tmem173	3	17	14,484	72	39	33	0.0027	0.0023
MHV68								
C57BL6	3	18	13,131	0	0	0		
A3KO	3	16	11,680	0	0	0		
A3A^{high}	3	15	10,945	4	1	3	9.14E−05	0.0003
A3G^{high}	3	12	8,761	1	0	1		0.0001
Tmem173	3	15	10,946	0	0	0		
MVMi								
C57BL/6	3	33	31,152	56	16	40	0.0005	0.0013
A3KO	3	31	29,264	59	12	47	0.0004	0.0016
A3A^{high}	3	29	27,376	74	15	59	0.0005	0.0022

[a] DNA isolated from the brains (HSV-1), lungs (MHV68), or spleens (MVMi) of the indicated mice was subjected to differential DNA denaturing PCR. The numbers of mouse APOBEC3, APOBEC3G, and APOBEC3A target sites in each of the DNA segments analyzed are presented in Table 1.

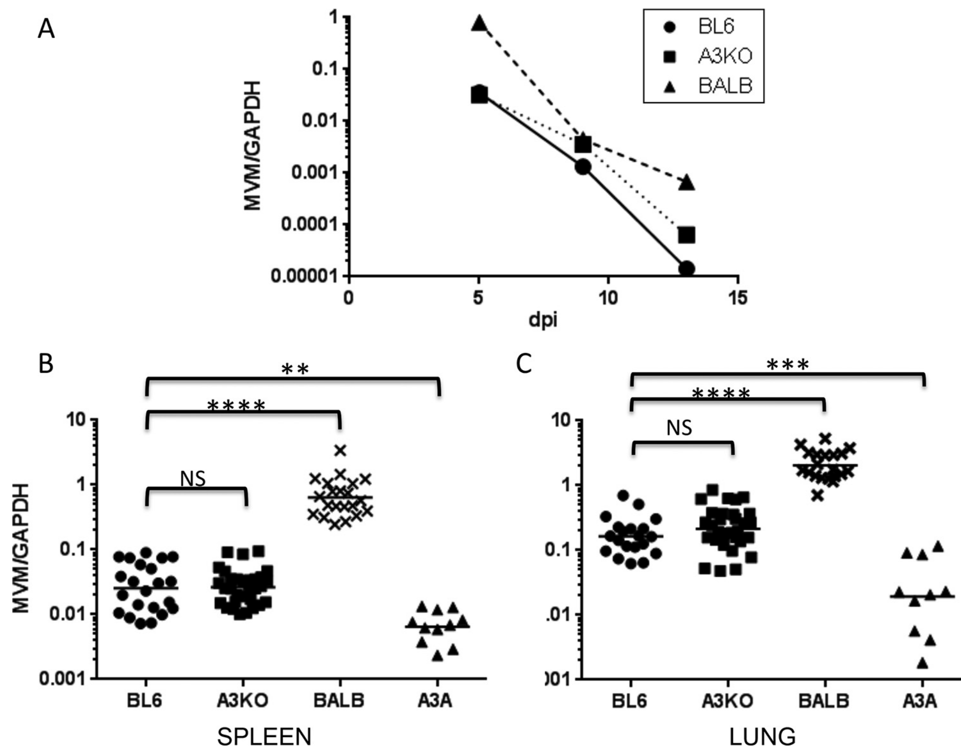

FIG 4 APOBEC3A restricts MVMi infection *in vivo*. (A) MVMi genome equivalents were calculated by alkaline gel electrophoresis followed by quantitative Southern blotting. Newborn C57BL/6, BALB/c, and A3KO mice were infected oranasally with 2×10^7 genome equivalents of virus in 5 μl. Mice were sacrificed at 5, 9, or 13 dpi, and DNA was isolated from their spleens and subjected to quantitative PCR (qPCR) to determine relative levels of viral DNA, using the primers 5′-AAGGTACGATGGCGCCTC-3/5′-GTGCTCTTTGGCAGC-3′. MVMi values were normalized to GAPDH, as previously described (45). (B and C) Newborn C57BL/6, BALB/c, A3KO, and A3A mice were infected oranasally with 2×10^7 genome equivalents of virus and sacrificed at 5 dpi. DNA isolated from the spleen (B) and lungs (C) was analyzed by qPCR. Each point represents an individual mouse. ****, $P < 0.0001$; **, $P < 0.0025$; ***, $P < 0.0035$ (by 2-tailed unpaired t test).

time course of infection of newborn C57BL/6, BALB/c, and A3KO mice after MVMi intranasal inoculation. To measure virus infection levels, we performed qPCR with DNA from different organs. There was no difference in the infection levels in A3KO versus C57BL/6 mouse spleen at all time points, indicating that mouse APOBEC3 does not restrict MVM infection (Fig. 4A); similar results were seen in lung and kidney (not shown). BALB/c mice were more highly infected than all the other mice at all dpi (Fig. 4A). This indicates that genes other than the APOBEC3 gene are responsible for the genetic susceptibility to infection in this strain. Next, we tested whether the presence of the human APOBEC3A transgene would diminish infection. Interestingly, we did find that MVMi infection was reduced by 5-fold on average in the spleens or lungs of APOBEC3A^{high} mice at 5 dpi (Fig. 4B and C); similar results were seen at 9 dpi (not shown). Surprisingly, none of the mice, including BALB/c mice, exhibited signs of pathogenesis at any time after infection up to 14 dpi, at which time all the mice had decreased infection by 2 logs or more compared to the initial infection levels (Fig. 4A); the original report for MVMi showed hemorrhaging in multiple organs of BALB/c mice by 9 dpi (35). In contrast to the previous study, all of the BALB/c mice survived infection in our study.

We looked for evidence of deamination of MVMi genomes in the APOBEC3A transgenic mice, also using 3DPCR and sequencing of the products generated by low annealing temperatures. No difference in deamination was detected (Table 2). This is in accord with studies done after infection of APOBEC3A-expressing tissue cultures with both adeno-associated virus and MVMp, demonstrating that virus restriction was independent of deaminase activity (33).

DISCUSSION

In addition to their well-established role in restricting retrovirus infection, there have been a number of reports that APOBEC3 proteins restrict other viruses, particularly DNA viruses that replicate in the nucleus. These include herpesviruses, where APOBEC3C overexpression reduced HSV-1 titers in tissue culture cells, and APOBEC3A, -3C, and 3G edited HSV-1 DNA when overexpressed, and HSV-1 and EBV in buccal swabs or immortalized cells lines, respectively, showed signs of cytidine deamination characteristic of APOBEC3 editing (30). There is also evidence that HPV is edited by APOBEC3A, -3B, and -3H, all of which are expressed in keratinocytes (41, 42). Several groups have shown that APOBEC3A, -3C, and -3H have access to the nucleus during telophase, while APOBEC3B is nuclear after mitosis (43); thus, some but not all APOBEC3 proteins can potentially affect viruses that replicate in the nucleus. Thus, it is not surprising that we found that APOBEC3G, which is found in the cytoplasm, had no significant effect on herpesvirus replication *in vivo*. We also found with a small cohort of mice that APOBEC3G had no impact on MVMi infection levels, although results with the low number of mice examined did not reach statistical significance (not shown).

However, based on the *in vitro* studies and its nuclear localization, we expected that APOBEC3A might restrict infection by both herpesviruses and parvoviruses. Contrary to this expectation, only the mouse parvovirus MVMi was inhibited *in vivo*. While this could possibly be due to lack of transgene expression in the appropriate cell types, we think this is unlikely because both the APOBEC3A and -3G transgenes were expressed in all tissues and cell types that we have examined, including brain, lymphoid tissue, macrophages, dendritic cells, lymphocytes, and fibroblasts (27) (not shown). However, it may be that herpesviruses but not parvoviruses encode viral proteins that counteract the action of APOBEC3A in particular, since it is found in the nucleus where these viruses replicate. Since the APOBEC3 transgenes are under the transcriptional control of the β-actin regulatory region and the β-globin 3′ untranslated region and polyadenylation site, the putative viral anti-APOBEC3 proteins would have to act at the level of protein degradation, relocalization, or direct inhibition of APOBEC3 enzymatic function. This could be tested in future experiments.

APOBEC3A but not APOBEC3G restricts infection by the human parvovirus adeno-associated virus, as well as by MVM (32, 33). Interestingly, we show here that APOBEC3A but not mouse APOBEC3 restricted MVM *in vivo*. One possibility for the lack of mouse APOBEC's antiviral activity is that the transgene is expressed in an MVM cellular target but the endogenous APOBEC3 is not; we think this unlikely because at least in lymphoid cells, we detected lower APOBEC3A transgene expression than that of the mouse APOBEC3 and yet splenic levels of MVMi infection were lower in the APOBEC3A transgenic mice (Fig. 4B). Another possibility is that the subcellular localization of the APOBEC3A (nuclear and cytoplasmic) allows it access to replicating MVM, unlike mouse APOBEC3, which is cytoplasmic (32, 44). We were surprised by the lack of MVMi lethality in any of the mouse strains tested, in contrast to previous reports (35). Moreover, the mice infected with McKrae strain HSV-1 also exhibited only modest signs of pathogenicity compared to those seen in earlier studies (36). This may be due to the purity of the virus preparations used here or perhaps because the original infections were performed with mice raised under less-stringent specific-pathogen-free criteria than are currently used.

Our previous work showed that retrovirus restriction by APOBEC3A and -3G transgenic mice provided good systems for the *in vivo* study of antiviral effects of these proteins. Indeed, we were able to recapitulate in our mice the ability of the HIV-1 Vif to counteract APOBEC3G (27). The results presented here suggest that only parvoviruses and not herpesviruses are likely to be targets for APOBEC3A *in vivo* and point to the importance of using systems that closely recapitulate infection of whole organisms to understand their biological functions. They also suggest that these and similar transgenic models might be useful for testing whether APOBEC3 proteins inhibit zoonotic viral infections.

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