AMERICAN SOCIETY FOR MICROBIOLOGY | Journal of Virology

Check for updates

Editor's Pick | Evolution | Full-Length Text

A single polymorphic residue in humans underlies species-specific restriction of HSV-1 by the antiviral protein MxB

Avraham Bayer,[1] **Stephanie J. Child,**[1] **Harmit S. Malik,**[2,3] **Adam P. Geballe**[1,4,5,6]

AUTHOR AFFILIATIONS See affiliation list on p. 15.

ABSTRACT Myxovirus resistance proteins A and B (MxA and MxB) are interferon-induced proteins that exert antiviral activity against a diverse range of RNA and DNA viruses. In primates, MxA has been shown to inhibit myxoviruses, bunyaviruses, and the hepatitis B virus, whereas MxB restricts retroviruses and herpesviruses. As a result of their conflicts with viruses, both genes have been undergoing diversifying selection during primate evolution. Here, we investigate how MxB evolution in primates has affected its restriction of herpesviruses. In contrast to human MxB, we find that most primate orthologs, including the closely related chimpanzee MxB, do not inhibit herpes simplex virus (HSV-1) replication. However, all primate MxB orthologs tested restricted human cytomegalovirus. Through the generation of human and chimpanzee MxB chimeras, we show that a single residue, M83, is the key determinant of restriction of HSV-1 replication. Humans are the only primate species known to encode a methionine at this position, whereas most other primate species encode a lysine. Residue 83 is also the most polymorphic residue in MxB in human populations, with M83 being the most common variant. However, ~2.5% of human MxB alleles encode a threonine at this position, which does not restrict HSV-1. Thus, a single amino acid variant in MxB, which has recently risen to high frequency in humans, has endowed human MxB with HSV-1 antiviral activity.

IMPORTANCE Herpesviruses present a major global disease burden. Understanding the host cell mechanisms that block viral infections, as well as how viruses can evolve to counteract these host defenses, is critically important for understanding viral disease pathogenesis. This study reveals that the major human variant of the antiviral protein myxovirus resistance protein B (MxB) inhibits the human pathogen herpes simplex virus (HSV-1), whereas a minor human variant and orthologous MxB genes from even closely related primates do not. Thus, in contrast to the many antagonistic virus-host interactions in which the virus is successful in thwarting the host's defense systems, here the human gene appears to be at least temporarily winning at this interface of the primate-herpesvirus evolutionary arms race. Our findings further show that a polymorphism at amino acid 83 in a small fraction of the human population is sufficient to abrogate MxB's ability to inhibit HSV-1, which could have important implications for human susceptibility to HSV-1 pathogenesis.

KEYWORDS MxB, evolution, species specificity, herpes simplex virus, primates, interferons

Upon exposure to interferon, cells generate a robust antiviral response composed of hundreds of different interferon-stimulated genes (ISGs) capable of restricting different stages of the viral life cycle (1). Some ISGs differentially target specific viruses, while others exhibit a broader range of restrictions. This antiviral landscape has been shaped by interactions with myriad viral pathogens over evolutionary time. Viral

Editor Felicia Goodrum, The University of Arizona, Tucson, Arizona, USA

Address correspondence to Adam P. Geballe, ageballe@fhcrc.org.

The authors declare no conflict of interest.

See the funding table on p. 15.

Received 1 June 2023
Accepted 23 August 2023
Published 5 October 2023

Copyright © 2023 American Society for Microbiology. All Rights Reserved.

adaptations that circumvent cellular defenses exert pressure on the host genes to evolve and refine their antiviral potency. This ongoing reciprocal evolution of the host and virus is consistent with the Red Queen hypothesis of genetic conflict (2, 3) and leads to an increased rate of non-synonymous (amino acid altering) compared to synonymous (amino acid preserving) nucleotide substitutions among host restriction factor homologs in related host species.

One such rapidly evolving interferon-induced antiviral factor is myxovirus resistance protein B (MxB; also known as Mx2) (4–6). MxB is one of two dynamin-like GTPase antiviral ISG proteins. Previous studies have shown that primate MxB is an important restriction factor for HIV-1 (7, 8). However, the MxB residues identified as rapidly evolving in MxB do not correspond to those that were identified as being important for lentiviral restriction (6). Thus, the recent finding that MxB is also a pan-herpesvirus restriction factor (9–11) raised the possibility that the rapid evolution of MxB might result from pressure to evade herpesviruses. To test this possibility, Schilling et al. tested a few substitutions at positively selected residues but found none that impacted the ability of human MxB to inhibit herpes simplex virus (HSV-1) replication (10). However, it remained possible that the primate MxB orthologs might differ in their ability to restrict HSV-1 due to variants that were not tested or to epistatic interactions between multiple residues that differ between the orthologs. Moreover, even if HSV-1 and related herpesviruses did not drive the evolution of MxB, they would nevertheless have been under pressure to evade MxB restriction.

Here, we investigated whether the evolution of MxB has affected its potential for inhibiting herpesviruses. We compared the ability of humans, chimpanzees, and representative Old World and New World monkey MxB orthologs to restrict herpesvirus replication. We found that all the tested primate MxB orthologs could inhibit human cytomegalovirus (HCMV), but only human MxB restricted HSV-1. By generating and testing human-chimp MxB chimeras, we were able to identify a single residue, M83, as the critical determinant of HSV-1 sensitivity. While most human MxB alleles encode a methionine at residue 83, chimps and most other primates encode a lysine at this residue. In fact, a K83M substitution in chimp MxB was sufficient to confer HSV-1 restriction. We also found that although M83 is unique to and highly prevalent in human MxB alleles, a minor fraction of human alleles encode a threonine at position 83, and this variant loses the ability to restrict HSV-1. Thus, our work identifies a single residue, M83, that is critical for human MxB to restrict HSV-1, as well as a minor polymorphism at this site that abolishes HSV-1 restriction, potentially affecting HSV-1 disease pathogenesis.

RESULTS

MxB expression is necessary for HSV-1 inhibition in fibroblasts

Previous studies showed that HSV-1 titers increased upon MxB knockdown in interferon (IFN)-treated cells (9, 10). We recapitulated these results in IMR90 cells by transducing them with a lentiviral vector expressing Cas9 and an MxB-targeting guide RNA or a non-targeting control (NTC). We either pretreated these cells with IFN for 24 h or left them untreated and assessed MxB expression by immunoblotting (Fig. 1A). These results confirmed a lack of MxB expression in the KO cells.

We next treated the KO and NTC cells ± IFN and then infected them with HSV-1 (strain KOS) at a multiplicity of infection (MOI) of 0.1. As expected, IFN treatment reduced HSV-1 replication in the control NTC cells, yet we observed a >10-fold restoration of viral titers in the MxB KO cells (Fig. 1B). These results suggest that MxB is a major component of the ISG response to HSV-1 in IMR90 cells. When the same cells were infected with HSV-2 (strain MS; MOI = 0.1), equivalent levels of restriction were observed in the control and KO cells (Fig. 1C). These results confirm that the IFN system is functional in this system and reveal that MxB is necessary for restriction of HSV-1 but not HSV-2 replication in interferon-treated IMR90 fibroblasts.

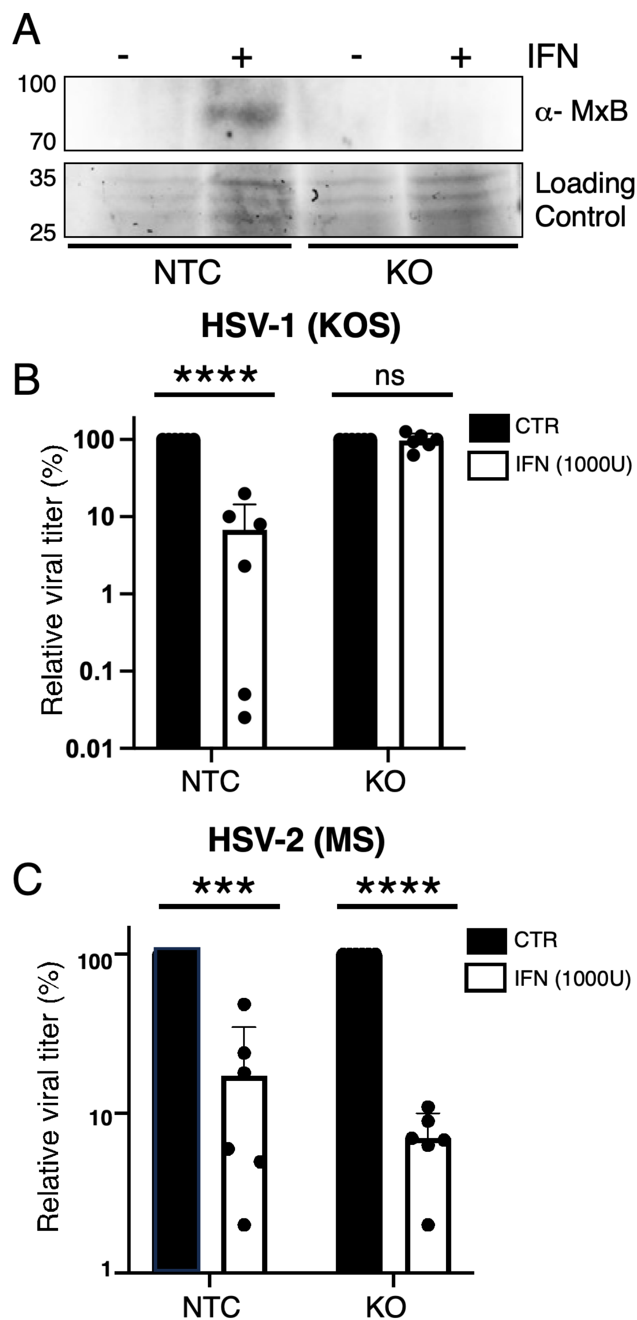

FIG 1 MxB expression is necessary for HSV-1 inhibition in fibroblasts. (A) MxB knockout IMR90 fibroblasts (KO) or NTC cells were left untreated or treated with 1,000 units/mL IFN-β for 24 h. Cell lysates were then collected, and MxB expression was analyzed by immunoblot assay. The loading control bands are cellular proteins detected by trichloroethanol staining (12). (B) The same cells described in A were left untreated or treated with IFN-β and infected with HSV-1 (strain KOS; MOI = 0.1) for 48 h, and the amount of virus present in the medium was determined by plaque assay. (C) Cells treated as in B were infected with HSV-2 (strain MS; MOI = 0.1) for 48 h and the virus in the medium was titered as in B. Titers were normalized to those in the untreated controls. B and C were repeated in duplicate three independent times. Statistical significance for replication in control vs KO cells was determined using a Student's *t*-test (ns, $P > 0.05$; ***, $P < 0.001$; ****, $P < 0.0001$).

The restriction of HSV-1 by MxB is species-specific

MxB has been evolving under diversifying selection throughout primate evolution (and likely in all mammals), which suggests that it has been engaged in arms races with a changing repertoire of viruses. We tested whether, as a consequence of this lineage-specific exposure to different viruses, MxB orthologs from different primates might have evolved to exhibit variable abilities to inhibit herpesviruses. We transduced our MxB-KO IMR90 cell line with lentiviruses containing either a doxycycline (dox)-inducible human *MxB* or *MxB* orthologs from representative simian primates: *Pan troglodytes* [chimpanzee (hominid)], *Chlorocebus tantalus* [African green monkey (Old World monkey)], and *Aotus trivirgatus* [owl monkey (New World monkey)]. Each *MxB* ortholog was designed with synonymous mutations in the KO cell guide RNA target sequence so that the transgenes would not be targeted by the guide. The MxB constructs also each contained a C-terminal flag tag.

We confirmed that doxycycline treatment induced expression of the *MxB* orthologs (Fig. 2A), although expression levels of the transgenes were somewhat variable. To test whether HSV-1 was sensitive to the different MxB orthologs, we infected dox-treated or untreated cells with HSV-1 (strain KOS; MOI = 0.1), and progeny virus was harvested at 48 hpi and titered. Surprisingly, we found that only human MxB restricted HSV-1 (Fig. 2B). We confirmed that the restriction was not strain-specific, as HSV-1 strain 17 demonstrated a similar phenotype to HSV-1 KOS (Fig. 2C).

We also infected these cells with HCMV (strain AD169; MOI = 0.1) and quantified progeny production at 6 days pi (Fig. 2D). In contrast to HSV-1, we observed that all MxB orthologs restricted HCMV, despite low levels of MxB expression in some cases. We observed no restriction of HCMV in control IMR90 cells transduced with a lentivirus expressing $IRS1^{263}$, a short product of the HCMV IRS gene (Fig. 2E and F) (13), demonstrating that HCMV restriction was not an artifact of the lentiviral expression system. Thus, the *MxB* restriction of HSV-1 but not HCMV appears to be species-specific under these conditions.

The species specificity of non-human primate MxBs is not due to poor expression or mislocalization

Although the observed differences in expression of the MxB orthologs in the IMR90 cells did not eliminate their ability to inhibit HCMV (Fig. 2A and D), it remained possible that this variability might contribute to differences in their impact on HSV-1. To eliminate integration site variation, a potential contributor to variability in transgene expression, we introduced the *MxB* orthologs into U2OS cells containing a landing pad into which genes are introduced by Cre-Lox recombination (14). Expression of the *MxB* orthologs was less variable in this system (Fig. 3A). Infection of these U2OS cells confirmed our previous finding in IMR90 cells that restriction of HSV-1 by *MxB* is species-specific; cells expressing human *MxB*, but none of the other orthologs, restricted HSV-1 replication (Fig. 3B).

Prior studies have demonstrated that MxB localizes to the cytoplasmic face of the nuclear membrane, where it associates with nuclear pore complex components such as NUP358 (15, 16). Indeed, this subcellular localization of MxB, which is dependent on an N-terminal nuclear localization signal, has been shown to be critical for restriction of both lentiviruses and herpesviruses (10, 17, 18). We therefore evaluated whether the non-human MxB orthologs also localized to the nuclear membrane or if their inability to counteract HSV-1 could be attributed to mislocalization. We treated the MxB variant-expressing U2OS cells with dox (24 h) to induce MxB expression, fixed the cells, and stained them with antiflag antibody (see Materials and Methods). To evaluate the localization of the orthologs, we collected confocal images and combined those from the middle region of the cells. These images revealed a predominantly perinuclear expression pattern for all MxB orthologs (Fig. 3C), consistent with previous reports (5). We also detected MxB in cytoplasmic puncta (e.g., Fig. 3C, owl monkey), as noted in a recent report demonstrating the dynamic formation of MxB into cytoplasmic condensates that

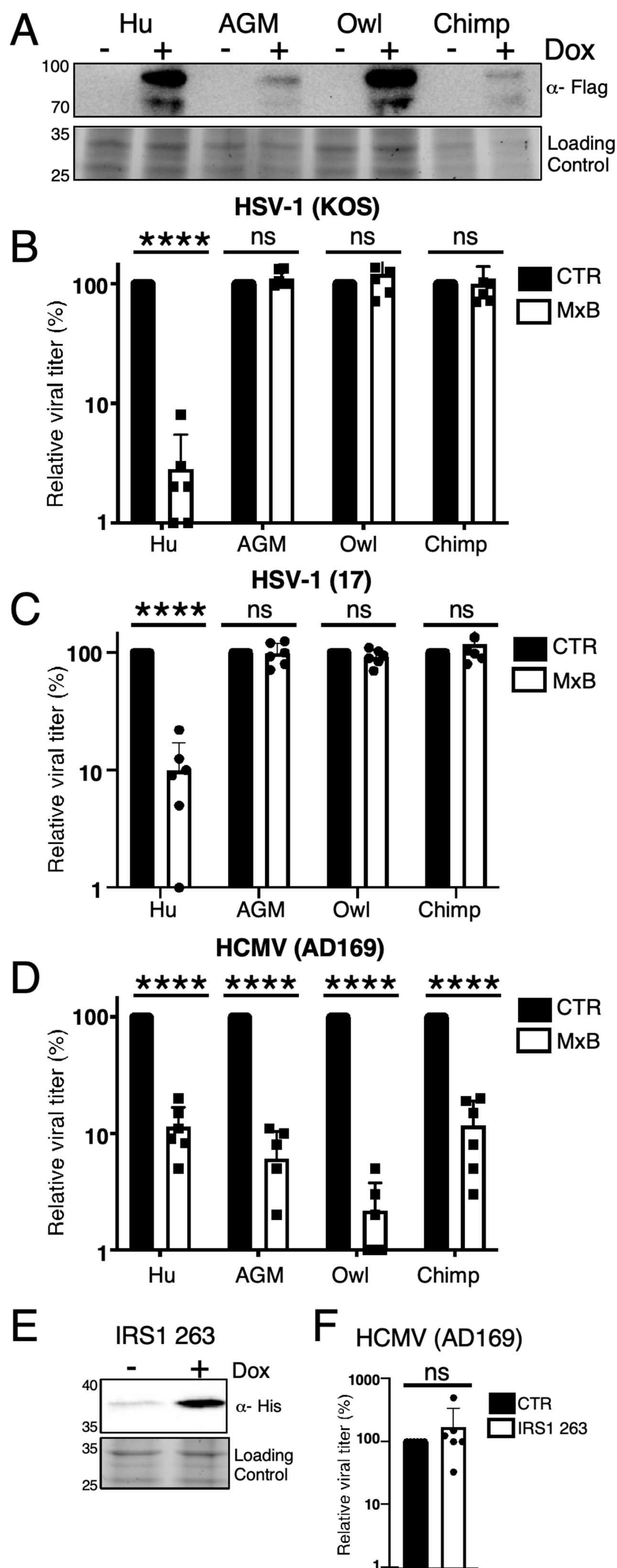

FIG 2 The restriction of HSV-1 by MxB is species-specific. (A) Immunoblot analysis of cell lysates from IMR90s expressing the MxB orthologs (human, chimpanzee, African green monkey, or owl monkey) that were untreated or treated with dox (1 µg/mL) for 24 h to determine MxB expression levels. The

(Continued on next page)

FIG 2 (Continued)

ortholog-expressing IMR90s treated as in A were infected with HSV-1 strain KOS (B) or HSV-1 strain 17 (C) for 48 h (MOI = 0.1). The virus present in the medium was then titered, and the titers were normalized to those of the untreated controls. (D) The cells were treated as in A-C and then infected with HCMV (strain AD169; MOI = 0.1) for 6 days following which the virus in the medium was titered and the titers were normalized as above. (E) Immunoblot analysis of IMR90 cells expressing a non-MxB control protein ($IRS1^{263}$) that were either untreated (control) or treated with doxycycline. (F) IRS^{263}-expressing IMR90 cells were treated or untreated with dox for 24 h and then infected with HCMV (AD169; MOI = 0.1) for 6 days, following which the virus in the medium was titered and the titers were normalized to control without dox. B, C, D, and F were done in duplicate three independent times. (ns, $P > 0.05$; ****, $P < 0.0001$).

might act as decoy nuclear pores (16). Thus, the observed species specificity of HSV-1 restriction is not likely due to variation in expression or localization. Instead, amino acid variation among the MxB orthologs must account for their different abilities to restrict HSV-1 replication.

Chimpanzee MxB can restrict HSV-2 but not HSV-1

To further explore the anti-herpesviral spectrum of the hominoid MxB orthologs, we tested whether chimp MxB could restrict HSV-2. Humans are the only primate species to be infected with two different simplex viruses; HSV-2 likely evolved following the transfer of chimp simplex virus into humans ~1.6 million years ago (19, 20). The MxB-expressing U2OS cells were treated ±dox for 24 h and infected with either HSV-1 (strain KOS) or HSV-2 (strain MS) at an MOI of 0.1. The supernatant virus was collected 48 h post-infection, and viral replication was measured by titering. As before, we observed that chimp MxB did not restrict HSV-1. However, chimp MxB was able to restrict HSV-2 replication by approximately fivefold (Fig. 3D). Thus, both human and chimp MxB inhibit HCMV and HSV-2 (9, 10), whereas only human MxB is capable of inhibiting HSV-1, further suggesting that sequence variation among these MxB orthologs confers species-specific differences in their antiviral spectra.

Codon 83 is a critical determinant of HSV-1 restriction

Human and chimp MxB share 98% sequence identity, differing by 17 nucleotides, only 11 of which encode non-synonymous substitutions. Thus, we were particularly intrigued by the observation that human, but not chimp, MxB is able to restrict HSV-1 (Fig. 2B and C; Fig. 3B and D). To map the determinants responsible for the differential effect of human and chimp MxB on HSV-1, we made six chimeric human-chimp constructs (Fig. 4A), introduced them into the U2OS landing pad cells, and then tested them for restriction of HSV-1. All chimeras containing the N-terminal portion of human MxB were capable of restricting HSV-1 (Fig. 4B, chimeras B, C, and E), whereas those containing the chimp N-terminal region were not (Fig. 4B, chimeras A, D, and F).

Although different MxB chimeras varied in their expression levels (Fig. 4C), the variation did not correlate with HSV-1 restriction. For example, chimeras C and E restricted HSV-1 replication despite being expressed poorly relative to chimera D, which could not restrict HSV-1. Instead, differences in the N-terminal domain of MxB likely account for the differences in the abilities of the human and chimp genes to block HSV-1 replication.

This N-terminal portion of MxB contains only two non-synonymous substitutions between the human and chimp alleles (at codons 48 and 83). We therefore engineered point mutations to test whether single human MxB amino acids would enable chimp MxB to inhibit HSV-1. Introduction of the human variant at position 48 (asparagine) into chimp MxB did not endow chimp MxB with the ability to restrict HSV-1 (Fig. 4D and E). However, replacing lysine at residue 83 in chimp MxB with methionine from human MxB was sufficient to enable chimp MxB to inhibit HSV-1 with an efficacy comparable to that of human MxB. Conversely, when methionine 83 in human MxB was replaced with the chimp codon (lysine), human MxB lost the ability to restrict HSV-1 (Fig. 4D). These results

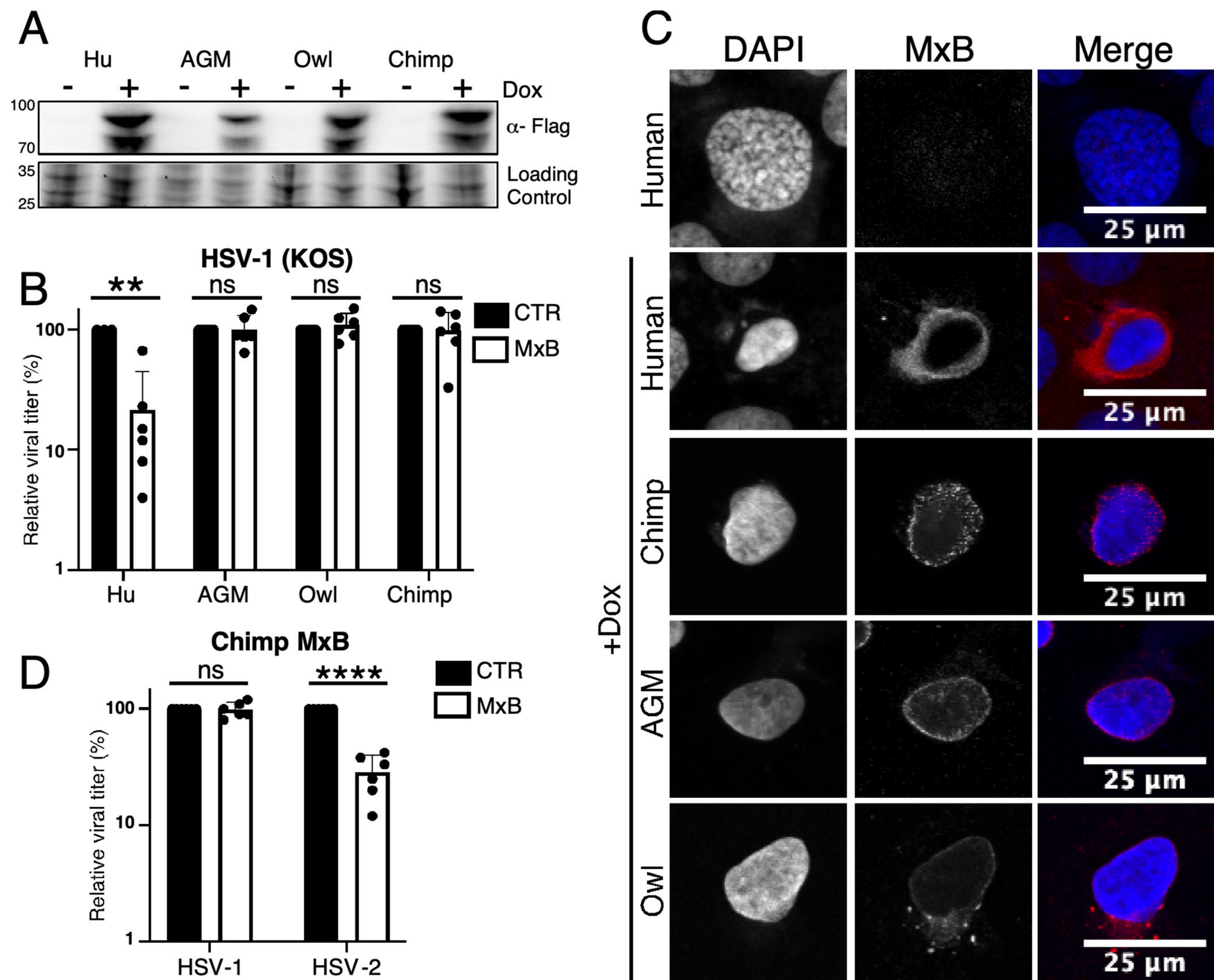

FIG 3 The species specificity of the non-human primate MxB orthologs is not due to poor expression or mislocalization. (A) Immunoblot analysis of cell lysates from U2OS cells expressing the different MxB orthologs [untreated or treated with dox (1 μg/mL) for 24 h to induce MxB expression] to determine the expression level of each ortholog. (B) These U2OS cells, either untreated (CTR) or treated with dox (MxB), were infected with HSV-1 (strain KOS; MOI = 0.1) for 48 h, and titers were quantified and normalized to the untreated controls as described above. (C) The MxB-expressing cells were fixed with 4% paraformaldehyde and stained to detect flag-tagged MxB (red) or DNA (blue), and confocal microscopic images were combined into a z-stack. (D) The chimp MxB-expressing U2OS cells (treated with dox or untreated) were infected with either HSV-1 (KOS; MOI = 0.1) or HSV-2 (MS; MOI = 0.1), and the supernatant virus was collected and quantified as in the preceding figures. B and D were done in duplicate three independent times. (ns, $P > 0.05$; **, $P \leq 0.01$; ****, $P < 0.0001$).

suggest that a methionine at position 83 is critical for inhibition of HSV-1 by hominoid MxBs.

HSV-1 infection does not impact MxB abundance or localization

We wondered whether the inability of lysine 83 variants of MxB to restrict HSV-1 may be related to virally induced degradation by HSV-1, as has been reported for other HSV-1 restriction factors (21). However, upon comparing the levels of human and chimp MxB in mock- or HSV-1-infected cells, we observed that infection did not affect the accumulation of either MxB protein (Fig. 5A). We next examined whether MxB localizes differentially during infection. Cells expressing human MxB were mock-infected or infected with a GFP-tagged HSV-1 (strain 17) for 24 h before being fixed and stained. We observed no difference in localization between the mock and infected cells (Fig. 5B), suggesting that HSV-1 infection does not affect MxB localization under these conditions.

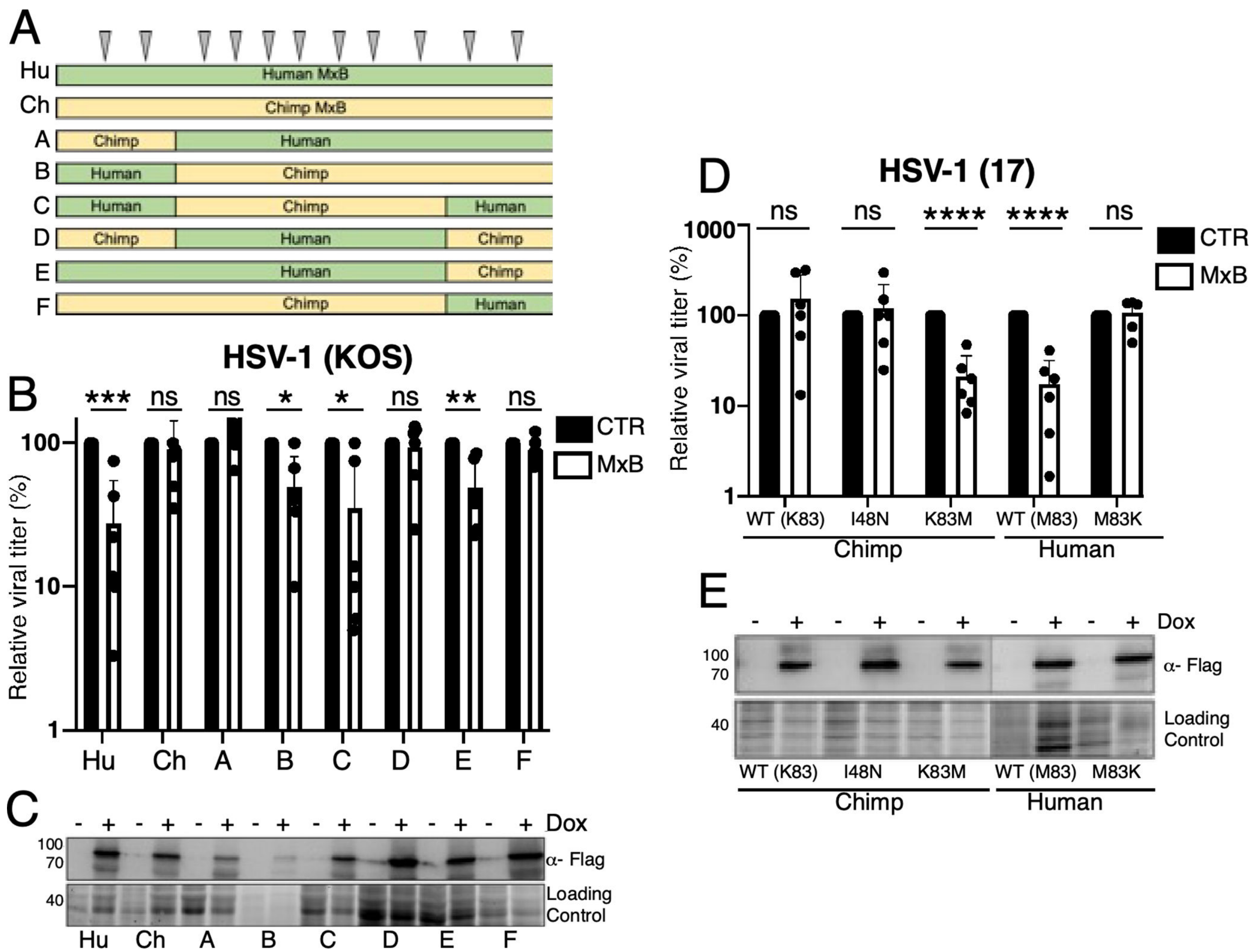

FIG 4 Codon 83 is a critical determinant of HSV-1 restriction. (A) Schematic of human MxB, chimp MxB, and the human-chimp chimeras. Gray arrows indicate non-synonymous substitutions between human and chimp MxB. (B) Titers of HSV-1 (KOS; MOI = 0.1) present in the medium at 48 h after infection of U2OS cells expressing human, chimp, or the chimeric MxBs were determined as described in previous figures (ns, *, $P \le 0.05$; **, $P \le 0.01$; ***, $P < 0.001$). (C) Immunoblot analysis to determine the expression levels of each of the MxBs shown in A in the presence (+) or absence (−) of dox. (D) Replication of HSV-1 (17; MOI = 0.1) was measured at 48 h post-infection in cells containing wild-type chimp or human MxB, point mutants I48N and K83M in the chimp gene, or point mutant M83K in the human background following a 24-h incubation with (MxB) or without (CTR) dox. Titering and normalization were performed as described in previous figures (ns, $P > 0.05$; ****, $P < 0.0001$). (E) Immunoblot analysis to demonstrate expression levels of the MxBs described in D. B and D were done in duplicate three independent times.

Human-specific evolution of MxB variants

Our results indicate that a single non-synonymous mutation that converted an ancestral lysine codon to a methionine codon is responsible for the acquisition of HSV-1 restriction. This variant predates the separation of human, Neanderthal, and Denisovan lineages; all of them encode methionine at residue 83.

However, this position is polymorphic within extant humans, with a M83T (methionine→threonine) variant present at a minor allele frequency of 2.5% in all humans but at an elevated allele frequency of ~18% in African and African American populations (21-41377154-T-C variant, gnomAD browser) (22, 23). We tested whether this non-synonymous polymorphism affected MxB localization, accumulation, or restriction of HSV-1. The T83 variant displayed a similar perinuclear localization (Fig. 6A) and was expressed at a similar level as wild-type MxB (Fig. 6B). The T83 variant was able to restrict HSV-2 (Fig. 6C) but was unable to restrict HSV-1 infection (Fig. 6D). These data confirm that although a recently arising methionine residue at position 83 of full-length MxB is critical for the

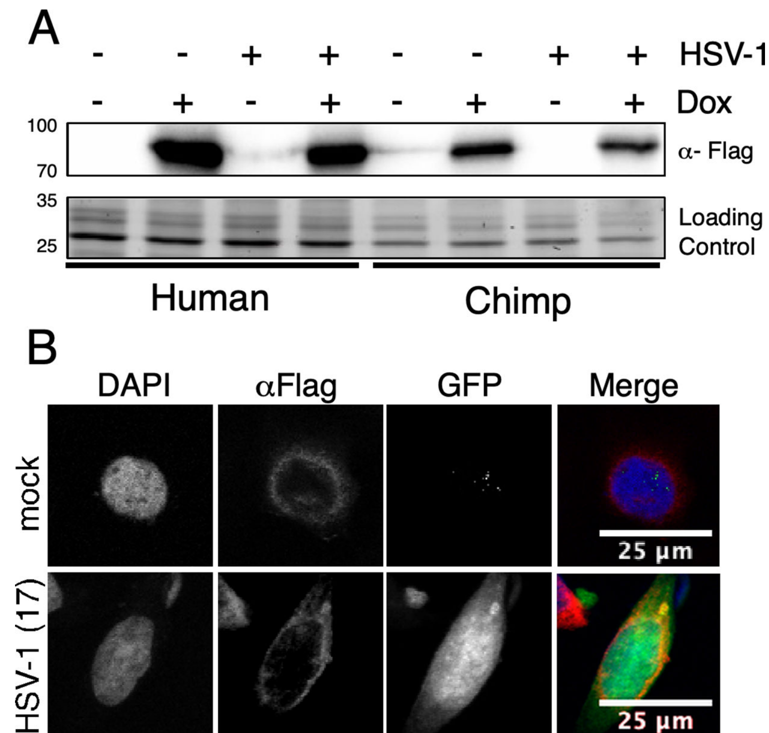

FIG 5 Infection does not affect MxB accumulation or localization. (A) Measurement of MxB expression levels from human or chimp MxB-expressing cells that were untreated or pretreated with dox, then mock-infected or infected with HSV-1 (MOI = 5) for 24 h. (B) The MxB-expressing cells were either treated or untreated with dox and then left uninfected or infected with GFP-tagged HSV-1 at an MOI of 1. Cells were fixed with 4% paraformaldehyde for 24 hpi and stained to detect flag-tagged MxB (red), DNA (blue), or HSV-1 (green), and confocal microscopic images were combined into a z-stack.

anti-HSV-1 activity of human MxB, a significant fraction of humans have subsequently lost this restriction.

DISCUSSION

Our work extends previous findings implicating the important role of MxB as a herpesvirus restriction factor in primates (9–11). Our knockout experiments further reveal that MxB is a major ISG affecting HSV-1 in IMR90 cells (Fig. 1), similar to previous findings in T98G fibroblast-like cells (10).

MxB is among the set of broadly acting ISGs that target multiple viruses (24). Previous computational analyses revealed that six residues in MxB have undergone recurrent positive selection during simian primate evolution, presumably in response to one or more viral infections (5, 6). However, these sites did not overlap with the sites that have been demonstrated to be critical for HIV-1 restriction, suggesting that the rapid evolution of MxB was likely driven by pathogens other than lentiviruses. In response to the finding that MxB can inhibit herpesviruses (9–11), Schilling et al. tested a few point mutations at rapidly evolving residues but found that they did not alter the restriction of HSV-1 by human MxB, suggesting that HSV-1 is not affected by evolution at those sites (10).

Recurrent positive selection may not represent all modes of adaptive evolution driven by host-virus arms races since it requires multiple, independent episodes of adaptation and counter-adaptation at the same interaction interface. For instance, a single lineage-specific episode of adaptation would not lead to a signature of recurrent positive selection. Therefore, we undertook a more agnostic approach to test whether there is any variability in herpesviral restriction by primate MxB orthologs. We found that

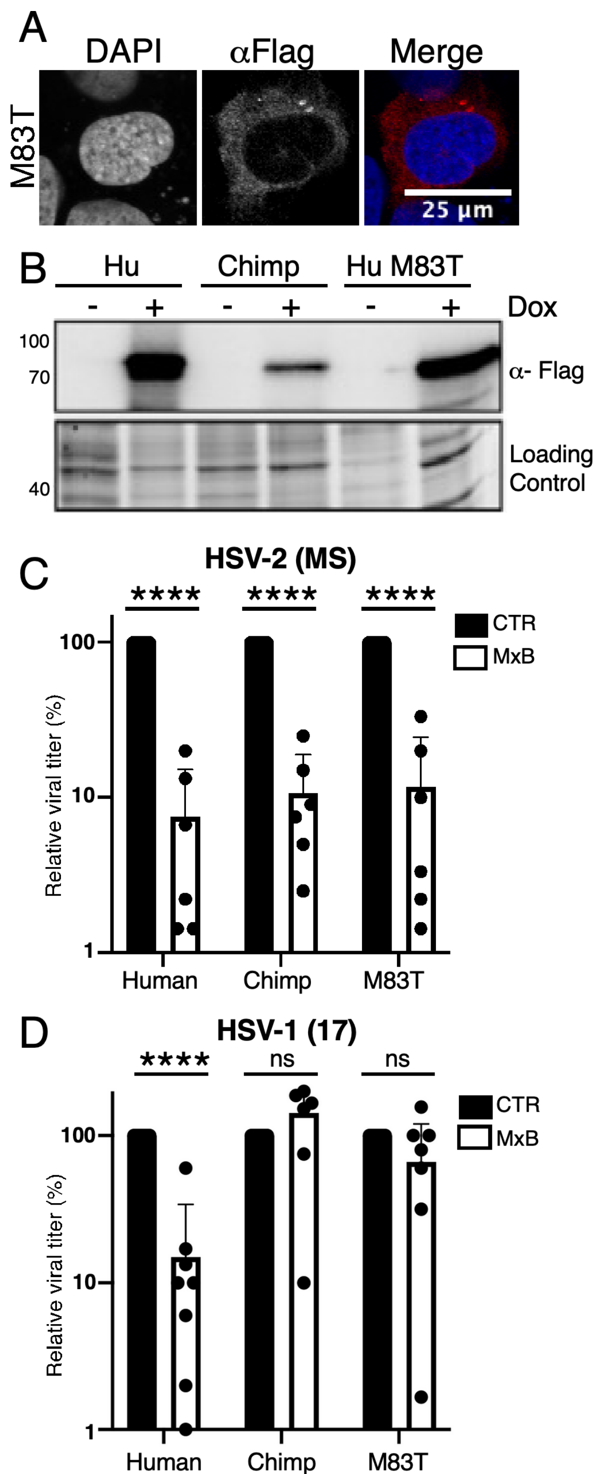

FIG 6 Human-specific evolution of MxB variants. (A) U2OS cells expressing the human M83T MxB variant were fixed with 4% paraformaldehyde and stained to detect flag-tagged MxB (red) or DNA (blue), and confocal microscopic images were combined into a z-stack. (B) Immunoblot demonstrating the expression levels of human, chimp, or the human MxB M83T point mutant ±dox in U20S cells. The untreated or dox-treated cells described in B were infected with (C) HSV-2 (MS; MOI = 0.1) or (D) HSV-1 (strain 17; MOI = 0.1) for 48 h, following which titers were quantified and normalized as before (ns, $P > 0.05$; ****, $P < 0.0001$). C and D were done in duplicate three independent times.

representative MxB orthologs from non-human primates (chimpanzee, African green monkey, owl monkey) were all able to restrict HCMV despite being variable at rapidly evolving residues. However, only human MxB was able to restrict HSV-1. This unique HSV-1 restriction ability of human MxB could be attributed to acquiring a methionine at residue 83, which has not undergone recurrent positive selection (4–6). Thus, although selection for an ability to antagonize HSV-1 could have driven the single K83M change in MxB, our data do not provide any support for herpesviruses driving the recurrent positive selection of primate MxB.

Since the K83M mutation occurred in the common ancestor of humans, Neanderthals, and Denisovans, there is not enough standing variation to test whether this change was driven by an adaptive sweep at this locus. Nevertheless, the methionine at residue 83 is not observed in any other primate MxBs, where a lysine residue is highly conserved. Our finding that a single residue is critical for species-specific antagonism by MxB is consistent with past work reporting that very few residues can confer species-specific lentiviral restriction by MxB. For example, human MxB but not African green monkey MxB restricts clade O HIV-1, with residues 37 and 39 identified as the determinants of these phenotypic differences (5). Similarly, residue 37 accounts for the restriction of an HIV-1 capsid mutant by Rhesus macaque but not African green monkey MxB.

Our data corroborate previous reports that human MxB restricts HSV-1, HSV-2, and HCMV. However, we find that the ability to restrict HSV-1 is unique to human MxB among primate orthologs. For example, while the chimp ortholog restricts HCMV and HSV-2, it does not restrict HSV-1. Humans are the only primate known to be infected with two herpes simplex viruses. HSV-2 seems to have crossed from the chimpanzee ancestral lineage into the human ancestral lineage about 1.6 million years ago and is more closely related to the extant chimpanzee simplex virus than to HSV-1 (19). Therefore, chimp MxB may be better adapted to restricting HSV-2 because of its co-evolution with the chimpanzee simplex virus, but not with HSV-1. Nevertheless, it is intriguing that the apparent selection of M83 in the human ancestral MxB resulted in it acquiring the ability to inhibit HSV-1 without affecting its ability to counteract at least two other herpesviruses (HCMV and HSV-2).

What is the mechanism underlying the different HSV-1 restriction abilities of M83 vs K83 or T83 in human MxB? We first considered the possibility that M83 might function as an alternative translational start site, yielding an N-terminally truncated protein that is uniquely capable of inhibiting HSV-1. Previous studies have reported one such down-stream initiation codon at methionine 26 in human MxB, which produces a shorter isoform of MxB (17). However, this short form does not inhibit HIV-1 or HSV-1, suggesting the N-terminus is necessary for the antiviral function of MxB, possibly because it is needed for perinuclear localization (7, 10, 25). Our studies also did not reveal a clear band corresponding to the expected size of a protein produced by translational initiation at codon 83 on our immunoblots, although we cannot rule out the possibility that this alternative form of MxB could contribute to inhibition of HSV-1 even at low abundance. Future insight into the contribution of M83 may emerge by testing whether M83 is unique in its ability to confer HSV-1 restriction or if other amino acid substitutions also retain anti-HSV-1 activity. It will also be of interest to evaluate whether the K83 or T83 variants in human MxB retain the ability to "punch" holes in HSV-1 capsids, as was recently reported (26).

Second, we considered the possibility of differential expression of the M83 vs K83 variants of MxB following infection. The immediate early ICP0 protein of HSV-1 is known to facilitate the degradation of PML and ND10 via its E3 ligase activity (27). K83, which is found in almost all primate MxBs, could similarly make MxB a target for ubiquitination, leading to its degradation during HSV-1 infection. However, upon analysis of human and chimp MxB expression in uninfected vs infected cells, we found that the levels of both orthologs remained comparable regardless of infection status (Fig. 5), arguing against HSV-1-driven degradation of MxB. Nevertheless, our results suggest the unusual scenario

that the majority of humans appear to be "winning" the arms race against HSV-1 by virtue of the acquisition of the MxB M83 variant in the ancestral human lineage.

Interestingly, codon 83 also represents the most polymorphic non-synonymous polymorphism in human MxB. Approximately 2.5% of over 280,000 sequenced human MxB alleles have a M83T polymorphism, and the frequency of this polymorphism is as high as ~18% in populations of African ancestry (data from gnomeAD) (4, 22), with a significant proportion of individuals being homozygous for the T83 variant. Although this geographical distribution is intriguing, previous F_{st} analysis (a measure of population genetic differentiation) of human MxB alleles did not find evidence of selection-driven differentiation at this site, rs56680307 (4). Our finding that the major M83 variant, but not the minor T83 variant, can restrict HSV-1 could be medically significant. However, we are not aware of any clinical data evaluating whether the pathogenesis of HSV-1 infections in humans with codon 83 polymorphisms is altered. To understand the impact of polymorphisms at codon 83, it will be important to translate the insights gained in cell culture systems to the role of MxB in restricting HSV-1 replication and spread in nature.

MATERIALS AND METHODS

Cells

IMR90, Vero, human fibroblasts (HF), and U2OS cells were maintained in Dulbecco's modified Eagle's medium supplemented with 10% NuSerum (BD Biosciences). U2OS osteosarcoma cells containing a loxP landing pad were obtained from Daphne Avgousti (Fred Hutchinson Cancer Center) (14). IMR90 MxB KO cells were constructed by transducing the cells with a lentiviral vector (lentiCRISPR v2; Addgene #52961) containing a gRNA targeting the genomic MxB target sequence (5′- GGTGGTGGTTCCCTG-TAACG-3′) as previously described (28). Following selection with puromycin (1 µg/mL), these cells were transduced with lentiviral vectors derived from pEQ1607 (29) and containing various MxB transgenes with silent mutations in the gRNA target region (described below) or with a control $IRS1^{263}$ transgene (13). Ortholog-expressing cells and IRS^{263} cells were selected with hygromycin B (100 µg/mL). U2OS cells expressing MxB orthologs under the control of a dox-inducible promoter were constructed by transfecting plasmid pDA0173 containing the different MxB orthologs along with Cre recombinase and selecting with puromycin (1 µg/mL).

Viruses, infections, and plaque assays

HCMV (strain AD169; ATCC VR-538) was propagated in HFs. HSV-1 strains KOS and 17 were obtained from Daphne Avgousti (Fred Hutchinson Cancer Center). HSV-2 strain MS was obtained from ATCC (VR-540). Both HSV-1 and HSV2 were propagated and titered on Vero cells. For MxB restriction assays, cells were either pretreated with dox for 24 h (1 µg/mL) or left untreated and then infected with the indicated viruses at an MOI of 0.1 for 48 h for HSV-1 and HSV2 and 6 days for HCMV. Supernatants were then collected, and viral replication was quantified by plaque assay. HSV-1 and HSV-2 plaque assays were performed on Vero cells by incubating dilutions of each sample for 48 h and then staining with crystal violet. HCMV titers were determined on HFs by incubating dilutions of each sample for 6 days prior to staining with crystal violet.

Plasmids

MxB orthologs from humans, chimpanzees, African green monkeys, and owl monkeys, all containing a C-terminal 3× flag tag, were previously cloned into the pQCXIP vector (6). Using these plasmids as templates, we introduced silent mutations into each ortholog at the guide RNA target site using primers #2607 and #2608 in stitch PCR reactions. The outside primers were #2534 and #2543 for human and chimp MxB, #2536 and #2543 for AGM MxB, and #2561 and #2543 for owl monkey MxB. The resulting amplicons were cloned into the lentiviral vector pEQ1607 digested with BstEII (29) by Gibson assembly. The resulting constructs, pEQ1779 (human), pEQ1819 (AGM), pEQ1823 (owl monkey),

and pEQ1817 (chimp) express MxB under the control of a dox-inducible promoter. A lentiviral vector containing IRS1[263] was made by first cloning an amplicon made by PCR amplification of IRS1 from pEQ1007 (30) using primers #2497 and #2498, followed by Gibson assembly into pEQ1607 digested with BstEII, generating pEQ1746. An amplicon made by PCR amplification of pEQ1746 using primers #2498 and #2865 was cloned into pEQ1607 as described for pEQ1746, resulting in pEQ1807.

To generate MxB ortholog-expressing U2OS cell lines, MxB orthologs were PCR amplified from the pEQ1607-based vectors using primers #2654 and #2655 (human and chimp), #2653 and #2655 (AGM), and #2652 and #2655 (owl monkey). The products were then introduced by Gibson assembly into HiLo vector [DA0173 (14), provided by Daphne Avgousti] digested with Ippo-1 and BstX1. The resulting constructs were pEQ1774 (human MxB), pEQ1782 (chimp MxB), pEQ1780 (AGM MxB), and pEQ1778 (owl monkey MxB). Plasmid sequences were verified by long-read sequencing (Plasmidsaurus, Eugene, OR, USA). The resulting sequences revealed that some of the sequences originally deposited in GenBank were missing 42 nt from the 3′ end of MxB. In addition, the chimp MxB ortholog was found to have two point mutations, one at codon 48 resulting in an asparagine to isoleucine substitution that is not present in other chimp sequences (great ape genome project) (31).

MxB chimeras were generated by restriction digestion of the human and chimp MxB constructs (pEQ1774 and pEQ1782) using combinations of restriction enzymes BsrG1, Sph1, and Not1 (NEB). These fragments were then swapped into each parental vector using T4 DNA ligase (Invitrogen). Chimeras A and B (pEQ1785 and pEQ1784) were generated by cutting pEQ1774 and pEQ1782 with BsrG1 and Not1 and swapping the N-terminal segments. Chimeras C and D (pEQ1787 and pEQ1786) were generated by cutting pEQ1774 and pEQ1782 with BsrG1 and Sph1 and swapping the internal segments. Chimeras E and F (pEQ1791 and pEQ1788) were generated by cutting pEQ1774 and pEQ1782 with Sph1 and Not1 and swapping the C-terminal segments.

Point mutants were generated by PCR amplification of either pEQ1774 or pEQ1782 using additional internal primers to introduce the mutations. The resulting amplicons were then joined by overlap extension PCR. Chimp MxB I48N (pEQ1794) was generated by amplifying pEQ1782 with primers #2654 and #2882 (5′ end) and primers #2883 and #2655 (3′ end). Chimp MxB K83M (pEQ1795) was generated by amplifying pEQ1782 with primers #2654 and #2886 (5′ end) and primers #2887 and #2655 (3′ end). Human MxB M83K (pEQ1793) was generated by amplifying pEQ1774 with primers #2654 and #2888 (5′ end) and primers #2889 and #2655 (3′ end). Human MxB M83T (pEQ1804) was generated by amplifying pEQ1774 with primers #2654 and #2877 (5′ end) and primers #2878 and #2655 (3′ end). The amplicons were then Gibson-cloned into the DAO173 HiLo vector and introduced into U2OS cells as described above. Table 1 contains a full list of the sequences of the primers used here.

Immunoblot analyses

Samples for immunoblotting were prepared by washing cells with phosphate buffered saline (PBS) and then lysing them in 2% SDS. The protein lysates were separated by 10% SDS-polyacrylamide gel electrophoresis on gels containing 0.5% 2,2,2-trichloroethanol to allow for stain-free visualization of total proteins (12) and then transferred to polyvinylidene difluoride membranes (Millipore). Blots were probed with the indicated antibodies using the Western Star chemiluminescence detection system (Applied Biosciences) according to the manufacturer's recommendations. The antibodies used in these experiments include antiMxB (Abcam 196833; diluted 1:5,000), alkaline phosphatase-conjugated flag M2 (A9469; diluted 1:10,000), flag (F1804; diluted 1:10,000) (all from Sigma), and antiHIS antibody (1:5,000; 34610 Qiagen). Antibodies were used as per the manufacturer's recommendations. Immunoblot images were captured and quantified with a ChemiDoc Touch imaging system and Image Lab software (Bio-Rad Laboratories, Hercules, CA, USA).

TABLE 1 Primers used

Primer #	Sequence
2534 (hominid MxB forward)	TGATCACTAGTGGTAACCATGTCTAAGGCCCACAAGCC
2543 (FLAG reverse)	AAAGCTGGGTCTAGAGCTTGTCATCGTCATCCTTGTAATC
2607 (silent mutation forward)	GGTGGCAATGTCCACGTTACATGGTACGACTACCAAGGTGATCGTCTGCT
2608 (silent mutation reverse)	AGCAGACGATCAACTTGGTAGTCGTACCATGTAACGTGGACATTGCCACC
2536 (Old World forward)	TGATCACTAGTGGTAACCATGTCTAAGGCCCACAAGTCT
2561 (New World reverse)	TGATCACTAGTGGTAACCATGTCTAAGGCCCACAGG
2497 (IRS1 forward)	TAGGCGTCTGATCACTAGTGACCATGGCCCAGCGCAAC
2498 (IRS1 reverse)	AAGAAAGCTGGGTCTAGAGGTCAATGGTGATGGTGATGATGACCG
2865 (IRS1 263 forward)	TAGGCGTCTGATCACTAGTGACCATGACAGAGCGTCAAAGTCAATTGCC
2654 (hominid HiLo forward)	GCACCGGGTGACTCTCTTAATGTCTAAGGCCCACAAGCCT
2655 (Flag Hilo reverse)	GGTATTTGTGAGCCAGGGCATAGAGCTTGTCATCGTCATCCTTGTAAT
2653 (Old World HiLo forward)	GCACCGGGTGACTCTCTTAAATGTCTAAGGCCCACAAGTCTT
2652 (New World HiLo forward)	GCACCGGGTGACTCTCTTAAATGTCTAAGGCCCGCAAGAC
2882 (I48N forward)	TCCTCCAAACTGGCAGGGGG
2883 (I48N reverse)	CCCCCTGCCAGTTTGGAGGA
2888 (M83K forward)	AAAGGCAAGGGGCCCGAGA
2889 (M83K reverse)	TCTCGGGCCCCTTTGCCCTT
2886 (K83M forward)	AAGGGCAATGGGGCCCGAGA
2887 (K83M reverse)	TCTCGGGCCCCATTGCCCTT
2877 (M83T forward)	AAGGGCAACGGGGCCCGAGA
2878 (M83T reverse)	TCTCGGGCCCCGTTGCCCTT

Immunofluorescence

Cells were grown in eight well chamber slides (LAB-TEK II) and either untreated or treated with doxycycline for 24 h. Cells were then fixed with 0.4% paraformaldehyde and permeabilized with 0.25% Triton X-100. The slides were incubated in blocking agent (I-block; Tropix) for 1 h, with flag antibody (1:10,000) for 1 h, rinsed, and then incubated with an antimouse 555 fluorophore (Santa Cruz). Slides were mounted with Vectashield containing DAPI (Vector Laboratories; H-1200). Images were captured on a high-resolution Leica Stellaris confocal microscope using a 63× oil objective and analyzed using ImageJ software.

Statistical analysis

All experiments were repeated in duplicate a minimum of three independent times. Statistical analyses were performed using a Student's *t*-test to calculate statistical significance. ns, $P > 0.05$; *, $P \le 0.05$; **, $P \le 0.01$; ***, $P < 0.001$; ****, $P < 0.0001$. Statistical analyses were performed using Prism 7 software (GraphPad).

ACKNOWLEDGMENTS

We thank Daphne Avgousti and Michael Emerman (both at Fred Hutchinson Cancer Center) for reagents and helpful comments. We also thank the Fred Hutchinson Cancer Center Genomics Shared Resource for technical assistance.

This work was supported by NIH RO1 AI145945 (to A.P.G.), NIH T32 CA080416 (to A.B.), a subaward from NIH grant U54 AI170792 (PI: Nevan Krogan) (to H.S.M.), an Investigator Award from Howard Hughes Medical Institute (HHMI, to H.S.M.), and the Fred Hutch/University of Washington Cancer Consortium (P30 CA015704). The content is solely the responsibility of the authors and does not necessarily represent the official views of the National Institutes of Health.

The funders had no role in the study design, data collection and analysis, or preparation of the manuscript.

AUTHOR AFFILIATIONS

[1]Division of Human Biology, Fred Hutchinson Cancer Center, Seattle, Washington, USA
[2]Basic Sciences Division, Fred Hutchinson Cancer Center, Seattle, Washington, USA
[3]Howard Hughes Medical Institute, Fred Hutchinson Cancer Center, Seattle, Washington, USA
[4]Clinical Research Division, Fred Hutchinson Cancer Center, Seattle, Washington, USA
[5]Department of Medicine, University of Washington, Seattle, Washington, USA
[6]Department of Microbiology, University of Washington, Seattle, Washington, USA

AUTHOR ORCIDs

Avraham Bayer http://orcid.org/0000-0001-8801-5378

Adam P. Geballe http://orcid.org/0000-0002-9279-6653

FUNDING

Funder	Grant(s)	Author(s)
HHS | NIH | National Institute of Allergy and Infectious Diseases (NIAID)	AI45945	Adam P. Geballe
HHS | NIH | National Cancer Institute (NCI)	CA080416	Avraham Bayer
Howard Hughes Medical Institute (HHMI)		Harmit S. Malik
HHS | NIH | National Cancer Institute (NCI)	CA015704	Harmit S. Malik Adam P. Geballe
HHS | NIH | National Institute of Allergy and Infectious Diseases (NIAID)	AI170792	Harmit S. Malik

AUTHOR CONTRIBUTIONS

Avraham Bayer, Conceptualization, Data curation, Formal analysis, Funding acquisition, Writing – original draft, Writing – review and editing | Stephanie J. Child, Data curation, Writing – review and editing | Harmit S. Malik, Conceptualization, Methodology, Supervision, Writing – review and editing | Adam P. Geballe, Conceptualization, Funding acquisition, Project administration, Supervision, Writing – original draft, Writing – review and editing

REFERENCES

1. Schoggins JW. 2019. Interferon-stimulated genes: what do they all do? Annu Rev Virol 6:567–584. https://doi.org/10.1146/annurev-virology-092818-015756
2. VanValen L. 1973. A new evolutionary law, p 1–30. University of Chicago: Evolutionary Theory.
3. Daugherty MD, Malik HS. 2012. Rules of engagement: molecular insights from host-virus arms races. Annu Rev Genet 46:677–700. https://doi.org/10.1146/annurev-genet-110711-155522
4. Sironi M, Biasin M, Cagliani R, Gnudi F, Saulle I, Ibba S, Filippi G, Yahyaei S, Tresoldi C, Riva S, Trabattoni D, De Gioia L, Lo Caputo S, Mazzotta F, Forni D, Pontremoli C, Pineda JA, Pozzoli U, Rivero-Juarez A, Caruz A, Clerici M. 2014. Evolutionary analysis identifies an Mx2 haplotype associated with natural resistance to HIV-1 infection. Mol Biol Evol 31:2402–2414. https://doi.org/10.1093/molbev/msu193
5. Busnadiego I, Kane M, Rihn SJ, Preugschas HF, Hughes J, Blanco-Melo D, Strouvelle VP, Zang TM, Willett BJ, Boutell C, Bieniasz PD, Wilson SJ. 2014. Host and viral determinants of Mx2 antiretroviral activity. J Virol 88:7738–7752. https://doi.org/10.1128/JVI.00214-14
6. Mitchell PS, Young JM, Emerman M, Malik HS. 2015. Evolutionary analyses suggest a function of MxB immunity proteins beyond lentivirus restriction. PLoS Pathog 11:e1005304. https://doi.org/10.1371/journal.ppat.1005304
7. Matreyek KA, Wang W, Serrao E, Singh PK, Levin HL, Engelman A. 2014. Host and viral determinants for MxB restriction of HIV-1 infection. Retrovirology 11:90. https://doi.org/10.1186/s12977-014-0090-z
8. Liu Z, Pan Q, Ding S, Qian J, Xu F, Zhou J, Cen S, Guo F, Liang C. 2013. The interferon-inducible MxB protein inhibits HIV-1 infection. Cell Host Microbe 14:398–410. https://doi.org/10.1016/j.chom.2013.08.015
9. Crameri M, Bauer M, Caduff N, Walker R, Steiner F, Franzoso FD, Gujer C, Boucke K, Kucera T, Zbinden A, Münz C, Fraefel C, Greber UF, Pavlovic J. 2018. MxB is an interferon-induced restriction factor of human herpesviruses. Nat Commun 9:1980. https://doi.org/10.1038/s41467-018-04379-2
10. Schilling M, Bulli L, Weigang S, Graf L, Naumann S, Patzina C, Wagner V, Bauersfeld L, Goujon C, Hengel H, Halenius A, Ruzsics Z, Schaller T, Kochs G. 2018. Human MxB protein is a pan-herpesvirus restriction factor. J Virol 92:e01056-18. https://doi.org/10.1128/JVI.01056-18
11. Jaguva Vasudevan AA, Bähr A, Grothmann R, Singer A, Häussinger D, Zimmermann A, Münk C. 2018. MxB inhibits murine cytomegalovirus. Virology 522:158–167. https://doi.org/10.1016/j.virol.2018.07.017
12. Ladner CL, Yang J, Turner RJ, Edwards RA. 2004. Visible fluorescent detection of proteins in polyacrylamide gels without staining. Anal Biochem 326:13–20. https://doi.org/10.1016/j.ab.2003.10.047
13. Romanowski MJ, Shenk T. 1997. Characterization of the human cytomegalovirus irs1 and trs1 genes: a second immediate-early transcription unit within irs1 whose product antagonizes transcriptional

activation. J Virol 71:1485–1496. https://doi.org/10.1128/JVI.71.2.1485-1496.1997

14. Khandelia P, Yap K, Makeyev EV. 2011. Streamlined platform for short hairpin RNA interference and transgenesis in cultured mammalian cells. Proc Natl Acad Sci U S A 108:12799–12804. https://doi.org/10.1073/pnas.1103532108
15. Kane M, Rebensburg SV, Takata MA, Zang TM, Yamashita M, Kvaratskhelia M, Bieniasz PD. 2018. Nuclear pore heterogeneity influences HIV-1 infection and the antiviral activity of Mx2. Elife 7:e35738. https://doi.org/10.7554/eLife.35738
16. Moschonas GD, Delhaye L, Cooreman R, Bhat A, Sutter DD, Parthoens E, Desmet A-S, Maciejczuk A, Grzesik H, Lippens S, Debyser Z, Eyckerman S, Saelens X. 2023. Mx2 restricts HIV-1 and herpes simplex virus-1 by forming cytoplasmic biomolecular condensates that mimic nuclear pore complexes. bioRxiv. https://doi.org/10.1101/2023.06.22.545931
17. Melén K, Julkunen I. 1997. Nuclear cotransport mechanism of cytoplasmic human MxB protein. J Biol Chem 272:32353–32359. https://doi.org/10.1074/jbc.272.51.32353
18. Fribourgh JL, Nguyen HC, Matreyek KA, Alvarez FJD, Summers BJ, Dewdney TG, Aiken C, Zhang P, Engelman A, Xiong Y. 2014. Structural insight into HIV-1 restriction by MxB. Cell Host Microbe 16:627–638. https://doi.org/10.1016/j.chom.2014.09.021
19. Wertheim JO, Smith MD, Smith DM, Scheffler K, Kosakovsky Pond SL. 2014. Evolutionary origins of human herpes simplex viruses 1 and 2. Mol Biol Evol 31:2356–2364. https://doi.org/10.1093/molbev/msu185
20. Underdown SJ, Kumar K, Houldcroft C. 2017. Network analysis of the hominin origin of herpes simplex virus 2 from fossil data. Virus Evol 3:vex026. https://doi.org/10.1093/ve/vex026
21. Boutell C, Davido DJ. 2015. A quantitative assay to monitor HSV-1 ICP0 ubiquitin ligase activity *in vitro*. Methods 90:3–7. https://doi.org/10.1016/j.ymeth.2015.04.004
22. Karczewski KJ, Francioli LC, Tiao G, Cummings BB, Alföldi J, Wang Q, Collins RL, Laricchia KM, Ganna A, Birnbaum DP, Gauthier LD, Brand H, Solomonson M, Watts NA, Rhodes D, Singer-Berk M, England EM, Seaby EG, Kosmicki JA, Walters RK, Tashman K, Farjoun Y, Banks E, Poterba T, Wang A, Seed C, Whiffin N, Chong JX, Samocha KE, Pierce-Hoffman E, Zappala Z, O'Donnell-Luria AH, Minikel EV, Weisburd B, Lek M, Ware JS, Vittal C, Armean IM, Bergelson L, Cibulskis K, Connolly KM, Covarrubias M, Donnelly S, Ferriera S, Gabriel S, Gentry J, Gupta N, Jeandet T, Kaplan D, Llanwarne C, Munshi R, Novod S, Petrillo N, Roazen D, Ruano-Rubio V, Saltzman A, Schleicher M, Soto J, Tibbetts K, Tolonen C, Wade G, Talkowski ME, Genome Aggregation Database Consortium, Neale BM, Daly MJ, MacArthur DG. 2020. The mutational constraint spectrum quantified from variation in 141,456 humans. Nature 581:434–443. https://doi.org/10.1038/s41586-020-2308-7
23. Collins RL, Brand H, Karczewski KJ, Zhao X, Alföldi J, Francioli LC, Khera AV, Lowther C, Gauthier LD, Wang H, Watts NA, Solomonson M, O'Donnell-Luria A, Baumann A, Munshi R, Walker M, Whelan CW, Huang Y, Brookings T, Sharpe T, Stone MR, Valkanas E, Fu J, Tiao G, Laricchia KM, Ruano-Rubio V, Stevens C, Gupta N, Cusick C, Margolin L, Genome Aggregation Database Production Team, Genome Aggregation Database Consortium, Taylor KD, Lin HJ, Rich SS, Post WS, Chen Y-D, Rotter JI, Nusbaum C, Philippakis A, Lander E, Gabriel S, Neale BM, Kathiresan S, Daly MJ, Banks E, MacArthur DG, Talkowski ME. 2020. A structural variation reference for medical and population genetics. Nature 581:444–451. https://doi.org/10.1038/s41586-020-2287-8
24. Betancor G. 2023. You shall not pass: Mx2 proteins are versatile viral inhibitors. Vaccines (Basel) 11:930. https://doi.org/10.3390/vaccines11050930
25. Goujon C, Moncorgé O, Bauby H, Doyle T, Barclay WS, Malim MH. 2014. Transfer of the amino-terminal nuclear envelope targeting domain of human Mx2 converts Mx1 into an HIV-1 resistance factor. J Virol 88:9017–9026. https://doi.org/10.1128/JVI.01269-14
26. Serrero MC, Girault V, Weigang S, Greco TM, Ramos-Nascimento A, Anderson F, Piras A, Hickford Martinez A, Hertzog J, Binz A, Pohlmann A, Prank U, Rehwinkel J, Bauerfeind R, Cristea IM, Pichlmair A, Kochs G, Sodeik B. 2022. The interferon-inducible GTPase MxB promotes capsid disassembly and genome release of herpesviruses. Elife 11:e76804. https://doi.org/10.7554/eLife.76804
27. Boutell C, Cuchet-Lourenço D, Vanni E, Orr A, Glass M, McFarlane S, Everett RD. 2011. A viral ubiquitin ligase has substrate preferential SUMO targeted ubiquitin ligase activity that counteracts intrinsic antiviral defence. PLoS Pathog 7:e1002245. https://doi.org/10.1371/journal.ppat.1002245
28. OhAinle M, Helms L, Vermeire J, Roesch F, Humes D, Basom R, Delrow JJ, Overbaugh J, Emerman M. 2018. A virus-packageable CRISPR screen identifies host factors mediating interferon inhibition of HIV. Elife 7:e39823. https://doi.org/10.7554/eLife.39823
29. Carpentier KS, Esparo NM, Child SJ, Geballe AP. 2016. A single amino acid dictates protein kinase R susceptibility to unrelated viral antagonists. PLoS Pathog 12:e1005966. https://doi.org/10.1371/journal.ppat.1005966
30. Hakki M, Marshall EE, De Niro KL, Geballe AP. 2006. Binding and nuclear relocalization of protein kinase R by human cytomegalovirus TRS1. J Virol 80:11817–11826. https://doi.org/10.1128/JVI.00957-06
31. Prado-Martinez J, Sudmant PH, Kidd JM, Li H, Kelley JL, Lorente-Galdos B, Veeramah KR, Woerner AE, O'Connor TD, Santpere G, Cagan A, Theunert C, Casals F, Laayouni H, Munch K, Hobolth A, Halager AE, Malig M, Hernandez-Rodriguez J, Hernando-Herraez I, Prüfer K, Pybus M, Johnstone L, Lachmann M, Alkan C, Twigg D, Petit N, Baker C, Hormozdiari F, Fernandez-Callejo M, Dabad M, Wilson ML, Stevison L, Camprubí C, Carvalho T, Ruiz-Herrera A, Vives L, Mele M, Abello T, Kondova I, Bontrop RE, Pusey A, Lankester F, Kiyang JA, Bergl RA, Lonsdorf E, Myers S, Ventura M, Gagneux P, Comas D, Siegismund H, Blanc J, Agueda-Calpena L, Gut M, Fulton L, Tishkoff SA, Mullikin JC, Wilson RK, Gut IG, Gonder MK, Ryder OA, Hahn BH, Navarro A, Akey JM, Bertranpetit J, Reich D, Mailund T, Schierup MH, Hvilsom C, Andrés AM, Wall JD, Bustamante CD, Hammer MF, Eichler EE, Marques-Bonet T. 2013. Great ape genetic diversity and population history. Nature 499:471–475. https://doi.org/10.1038/nature12228