MxB Is Not Responsible for the Blocking of HIV-1 Infection Observed in Alpha Interferon-Treated Cells

Silvana Opp,^a Daniel A. S. A. Vieira,^a Bianca Schulte,^a Sumit K. Chanda,^b Felipe Diaz-Griffero^a

Department of Microbiology and Immunology, Albert Einstein College of Medicine, Bronx, New York, USA^a; Immunity and Pathogenesis Program, Sanford Burnham Prebys Medical Discovery Institute, La Jolla, California, USA^b

ABSTRACT

MxB restricts HIV-1 infection by directly interacting with the HIV-1 core, which is made of viral capsid; however, the contribution of MxB to the HIV-1 restriction observed in alpha interferon (IFN- α)-treated human cells is unknown. To understand this contribution, we used HIV-1 bearing the G208R capsid mutant (HIV-1-G208R), which overcomes the restriction imposed by cells expressing MxB. Here we showed that the reason why MxB does not block HIV-1-G208R is that MxB does not interact with HIV-1 cores bearing the mutation G208R. To understand whether MxB contributes to the HIV-1 restriction imposed by IFN- α -treated human cells, we challenged IFN- α -treated cells with HIV-G208R and found that MxB does not contribute to the restriction imposed by IFN- α -treated cells. To more directly test the contribution of MxB, we challenged IFN- α -treated human cells that are knocked out for the expression of MxB with HIV-1. These experiments suggested that MxB does not contribute to the HIV-1 restriction observed in IFN- α -treated human cells.

IMPORTANCE

MxB is a restriction factor that blocks HIV-1 infection in human cells. Although it has been postulated that MxB is the factor that blocks HIV-1 infection in IFN- α -treated cells, this is a hard concept to grasp due to the great number of genes that are induced by IFN- α in cells from the immune system. The work presented here elegantly demonstrates that MxB has minimal or no contribution to the ability of IFN- α -treated human cells to block HIV-1 infection. Furthermore, this work suggests the presence of novel restriction factors in IFN- α -treated human cells that block HIV-1 infection.

Myxovirus resistance proteins represent a family of interferon-inducible factors with a wide range of antiviral activities (1–3). The myxovirus B (MxB) gene was originally cloned from a human glioblastoma cell line treated with alpha interferon (IFN- α) (4, 5). MxB as well as the related protein MxA belong to the dynamin-like family of proteins, which have diverse functions ranging from vesicle transport to antiviral activity (1, 6–11). The most studied dynamin-like protein that exhibits antiviral activity is MxA (1, 2). Contrary to MxB, the antiviral role of MxA has been extensively studied for viruses including influenza virus (1, 12–15), tick-borne Thogoto virus (16), African swine fever virus (17), hepatitis B virus (18), and La Crosse virus (19, 20). The antiviral activity of the long form of MxB was recently described (9, 21–23); these investigations led to the discovery that the IFN- α -inducible protein MxB blocks HIV-1 infection.

Genetic evidence suggested that the HIV-1 capsid is the determinant for the ability of MxB to block HIV-1 infection (9, 22, 23). In agreement with these findings, we recently demonstrated that MxB binds to the HIV-1 capsid and correlated the ability of MxB to block HIV-1 infection with inhibition of uncoating (24). We also showed that the ability of MxB to block infection requires a capsid binding domain and an oligomerization domain provided by the 90 N-terminal and the 143 C-terminal amino acids of MxB, respectively (24). In addition, the work of others and our work showed that the 90 N-terminal amino acids of MxB are important for its ability to bind capsid and restrict HIV-1 infection (24–26).

MxB contains a previously described putative nuclear localization signal on its N-terminal 25 amino acids (4). Deletion of the N-terminal 25 amino acids annihilates the ability of MxB to block HIV-1 infection and to bind to the HIV-1 core (23, 24, 27). Mu-

tagenic studies have revealed that the N-terminal 25 amino acids of MxB exhibit a triple-arginine motif (¹¹RRR¹³) that is important for restriction and the ability of MxB to bind to the HIV-1 core (28, 29).

MATERIALS AND METHODS

Cell lines and plasmids. Human U87 MG cells (ATCC HTB-14), HT-1080 cells (ATCC CCL-121), HEK 293T cells, and dog Cf2Th cells (ATCC CRL-1430) were grown in Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% (vol/vol) fetal bovine serum and 1% (vol/vol) penicillin-streptomycin. Human THP-1 cells (ATCC TIB-202) were grown in RPMI supplemented with 10% (vol/vol) fetal bovine serum and 1% (vol/vol) penicillin-streptomycin.

HIV-1 CA-NC expression and purification. The CA-NC proteins of HIV-1 and HIV-1 bearing the G208R capsid mutant (HIV-1-G208R) were expressed, purified, and assembled as previously described (30).

MxB binding to *in vitro*-assembled HIV-1 and HIV-1-G208R CA-NC complexes. HEK 293T cells were transfected with a plasmid expressing the wild-type MxB protein. Forty-eight hours after transfection, cell lysates were prepared as follows. Cells were harvested, washed, and resuspended in hypotonic lysis buffer (10 mM Tris [pH 7.4], 1.5 mM

Received 19 December 2015 Accepted 23 December 2015

Accepted manuscript posted online 30 December 2015

Citation Opp S, Vieira DASA, Schulte B, Chanda SK, Diaz-Griffero F. 2016. MxB is not responsible for the blocking of HIV-1 infection observed in alpha interferon-treated cells. *J Virol* 90:3056–3064. doi:10.1128/JVI.03146-15.

Editor: S. R. Ross

Address correspondence to Felipe Diaz-Griffero, Felipe.Diaz-Griffero@einstein.yu.edu.

Copyright © 2016, American Society for Microbiology. All Rights Reserved.

FIG 1 HIV-1 bearing the capsid mutation G208R overcomes MxB restriction by a deficiency in the ability of MxB to interact with the HIV-1 core. (A) MxB expression was analyzed in Cf2Th cells stably expressing MxB or containing the empty vector LPCX by Western blotting using anti-FLAG antibodies. As a loading control, expression of GAPDH was analyzed by Western blotting using anti-GAPDH antibodies. (B) Cf2Th cells stably expressing MxB or containing the empty vector LPCX were challenged with increasing amounts of HIV-1 or HIV-1-G208R expressing GFP as a reporter of infection. Viruses used for these infections were normalized for p24. The percentage of GFP-positive cells was measured by flow cytometry at 48 h postinfection. Data from three independent experiments are shown. (C) The ability of MxB to interact with *in vitro*-assembled HIV-1 CA-NC complexes bearing the mutation G208R was measured. Briefly, cellular extracts containing MxB were incubated with wild-type or mutant (G208R) *in vitro*-assembled HIV-1 CA-NC complexes at room temperature for 1 h. The mixtures were applied onto a 70% sucrose cushion and centrifuged, as described previously (36). INPUT represents mixtures analyzed by Western blotting before being applied onto the 70% cushion. The pellet was subjected to Western blotting using anti-FLAG antibodies. The pellet from the 70% cushion (BOUND) was analyzed by Western blotting using anti-FLAG and anticapsid antibodies. The results from three experiments were similar, and the standard deviation is shown.

MgCl₂, 10 mM KCl, 0.5 mM dithiothreitol [DTT]). The cell suspension was incubated on ice for 10 min. Afterwards, the lysate was centrifuged at maximum speed in a refrigerated Eppendorf microcentrifuge ($\sim 14,000 \times g$) for 5 min. Cell lysates were incubated with *in vitro*-assembled HIV-1 CA-NC complexes for 1 h at room temperature (28, 29). A fraction of this mixture was stored (input). The mixture was spun through a sucrose cushion (70% sucrose, 1 $\times$ phosphate-buffered saline [PBS], 0.5 mM DTT) at $100,000 \times g$ in an SW55 rotor (Beckman) for 1 h at 4°C. After centrifugation, the supernatant was carefully removed, and the pellet was resuspended in 1 $\times$ SDS-PAGE loading buffer (pellet). The level of MxB protein was determined by Western blotting using anti-FLAG antibodies. The levels of HIV-1 CA-NC protein in the pellet were assessed by Western blotting using anti-p24 CA antibodies.

Creation of cells stably expressing MxB protein. A retroviral construct encoding the wild-type MxB protein was created by using the LPCX vector (Clontech). The MxB protein contained a FLAG epitope tag at the

C terminus. Recombinant viruses were produced in HEK 293T cells by cotransfecting the LPCX-MxB-FLAG plasmid or empty LPCX vector with the pVPack-GP and pVPack-VSV-G packaging plasmids (Stratagene). The pVPack-VSV-G plasmid encodes the vesicular stomatitis virus G (VSV-G) envelope glycoprotein, allowing efficient entry into a wide range of vertebrate cells. Cf2Th canine thymocytes were transduced and selected in puromycin (Sigma).

Infection with HIV-1 expressing GFP. Recombinant HIV-1 and the HIV-1-G208R mutant expressing green fluorescent protein (GFP) were prepared as described previously (31). Recombinant viruses were pseudotyped with the VSV-G glycoprotein. For infections, 24-well plates were seeded with 25,000 Cf2Th cells stably expressing MxB and incubated at 37°C with virus for 24 h. A total of 250 μ l of medium was added, and cells were returned to culture for another 24 h. In the case of experiments using IFN- α , cells were initially treated with 1,000 U/ml IFN- α (Millipore) for 24 h before infection. Subsequently, GFP-

THP-1

FIG 2 Treatment of human THP-1 cells with IFN- α induces a block to HIV-1 and HIV-1-G208R. IFN- α -treated monocytic THP-1 cells were challenged for 24 h with increasing amounts of HIV-1 or HIV-1-G208R expressing GFP as a reporter of infection. Viruses used for these infections were normalized for p24. At 48 h postinfection, the percentage of GFP-positive cells was measured by flow cytometry. Data from three independent experiments are shown.

positive cells were analyzed by using a flow cytometer (Becton Dickinson).

Western blot analysis. Cellular proteins were lysed in lysis buffer (50 mM Tris [pH 8.0], 280 mM NaCl, 0.5% Igepal 40, 10% glycerol, 5 mM MgCl₂). Detection of proteins by Western blotting was performed by using anti-FLAG (1:1,000 dilution; Sigma), anti-MxB (1:500; Novus Biologicals), anti-glyceraldehyde-3-phosphate dehydrogenase (GAPDH) (1:5,000; Ambion), or anti-p24 (1:1,000, catalog number 183-H12-5C; NIH) antibodies. Secondary antibodies against rabbit and mouse conjugated to IRDye 680LT or IRDye 800CW were obtained from Li-Cor (1:10,000 diluted). Bands were detected by scanning blots using the Li-Cor Odyssey imaging system in the 700-nm or 800-nm channel.

Real-time PCR to detect HIV-1 late reverse transcripts. Total cellular DNA from infected cells was isolated by using the QIAamp DNA microkit (Qiagen) at 7 h postinfection. Before infection, viruses were treated for 30 min at 37°C with 1,000 U of DNase I (Roche). As a control for the experiment, we performed infection in the presence of 10 μ M nevirapine and heat-inactivated viruses for 30 min at 100°C. We measured late reverse transcripts (LRTs) using the following primers and probe: forward primer GACGTAACGGCCACAAG, reverse primer GGTCTTGTAGTTGCCGTCGT, and probe 5'-56-FAM (6-carboxyfluorescein)-CCTACGGCAAGCTGACCC-36-TAMRA (5-carboxytetramethylrhodamine)-3'. A standard curve was created by using the GFP sequence of the HIV-1 reporter. β -Actin amplification was used for normalization, using the following primers and probe for actin: forward primer 50-AACACCCAGCCATGTACGT, reverse primer 50-CGGTGAGGATCTTCATGAGGTAGT, and a SYBR green probe. Reaction mixtures contained 1 $\times$ Fast SYBR green master mix (Applied Biosystems), 300 nM forward primer, 300 nM reverse primer, 100 nM probe primer, and template DNA. Denaturation steps (95°C for 17 min) with 40 cycles of amplification were carried out (95°C for 15 s, 58°C for 30 s, and 72°C for 30 s).

Generation of MxB knockout human cells. MxB knockout (KO) cell lines were generated by using the clustered regularly interspaced short palindromic repeat (CRISPR)-Cas9 gene system (Edit-R CRISPR-Cas9 gene engineering with SMARTCas9 nucleases; GE Healthcare) (32). The CRISPR genomic guide RNA sequences for human MxB in THP-1 and HT-1080 cells were designed to target exon 2 near the start codon. The MxB guide RNA target sequences used for THP-1 and HT-1080 cells were CCGCCATTCGGCACAGTGCC (complementary strand) and CACAAGCCTTGGCCCTACCGG (complementary strand), respectively.

The guide RNA constructs, human cytomegalovirus (hCMV)-Casp9, and CMV-GFP were cotransfected into THP-1 or HT-1080 cells. After 48 h of transfection, GFP-positive cells were single-cell sorted by using the FACSaria system (BD Biosciences, Franklin Lakes, NJ, USA). Clonal THP-1 and HT-1080 colonies were expanded and characterized for the loss of MxB protein expression by Western blot analysis using anti-MxB antibodies. Exon 2 of *MxB* was amplified and sequenced from genomic DNA isolated from wild-type and knockout cells.

RESULTS

MxB does not bind to the HIV-1 core bearing the capsid mutation G208R. Although MxB is a restriction factor with the ability to block HIV-1 infection, the contribution of MxB to the HIV-1 restriction observed in IFN- α -treated cells has not been addressed using capsid mutations that overcome the HIV-1 restriction imposed by MxB. To pursue this question, we initially took advantage of the HIV-1-G208R capsid mutant previously described to overcome MxB restriction (26). In agreement with previous observations, HIV-1-G208R fully overcomes the restriction imposed by MxB (Fig. 1A and B). As shown in Fig. 1B, HIV-1-G208R overcomes the restriction imposed by MxB. Because previous obser-

HT-1080

FIG 3 Treatment of human HT-1080 cells with IFN- α induces a block to HIV-1 and HIV-1-G208R. IFN- α -treated fibrosarcoma HT-1080 cells were challenged for 24 h with increasing amounts of HIV-1 or HIV-1-G208R expressing GFP as a reporter of infection. Viruses used for these infections were normalized for p24. At 48 h postinfection, the percentage of GFP-positive cells was measured by flow cytometry. Data from three independent experiments are shown.

U87 MG

FIG 4 Treatment of human U87 MG cells with IFN- α induces a block to HIV-1 and HIV-1-G208R. IFN- α -treated glioblastoma U87 MG cells for 24 h were challenged with increasing amounts of HIV-1 or HIV-1-G208R expressing GFP as a reporter of infection. Viruses used for these infections were normalized for p24. At 48 h postinfection, the percentage of GFP-positive cells was measured by flow cytometry. Data from three independent experiments are shown.

FIG 5 IFN-α treatment of THP-1 cells prevents the formation of late reverse transcripts (LRTs) by HIV-1 and HIV-1-G208R. THP-1 cells were treated for 24 h with IFN-α and subsequently challenged with either DNase-pretreated HIV-1 or HIV-1-G208R expressing GFP as a reporter. Subsequently, cells were harvested at 7 and 48 h postinfection to measure HIV-1 LRTs (right) and infection (left), respectively. As a control, we performed experiments in the presence of the reverse transcriptase inhibitor nevirapine (Nev). Levels of late reverse transcripts were determined by real-time PCR using specific primers against GFP, as described in Materials and Methods. Infection was determined at 48 h postinfection by measuring the percentage of GFP-positive cells by flow cytometry. Three independent experiments were performed, and standard deviations are shown.

FIG 6 Construction of THP-1 cells that do not express MxB. (A) THP-1 cells were transiently transfected with the CRISPR-Cas9 system using a guide RNA that targets exon 2 of *MxB*. Single-cell clones were analyzed for the integrity of exon 2 by sequencing of genomic DNA. The THP-1 cell line used for these studies contained a 2-bp deletion on exon 2. The 2-bp deletion creates a series of stop codons in the open reading frame of *MxB*. The wild-type *MxB* allele was not detected in the knockout clone. (B) The levels of MxB expression were measured by Western blotting using anti-MxB antibodies in extracts from cells that were treated with IFN-α for 24 h. As a loading control, cell lysates were subjected to Western blotting using anti-GAPDH antibodies.

FIG 7 IFN- α -treated THP-1 cells that do not express MxB potentially block HIV-1 infection. IFN- α -treated THP-1 cells that do not express MxB (THP-1-MxB KO) were challenged for 24 h with increasing amounts of either HIV-1 or HIV-1-G208R expressing GFP as a reporter of infection. Viruses used for these infections were normalized for p24. At 48 h postinfection, the percentage of GFP-positive cells was measured by flow cytometry. Data from three independent experiments are shown.

FIG 8 Construction of HT-1080 cells that do not express MxB. (A) HT-1080 cells were transiently transfected with the CRISPR-Cas9 system using a guide RNA that targets exon 2 of *MxB*. Single-cell clones were analyzed for the integrity of exon 2 by sequencing of genomic DNA. The HT-1080-MxB-KO clone 3 cells used for these studies contained a 69-bp deletion in exon 2. The wild-type *MxB* allele was not detected in HT-1080-MxB-KO clone 3. (B) The levels of MxB expression were measured by Western blotting using anti-MxB antibodies in extracts from cells that were treated with IFN- α for 24 h. As a loading control, cell lysates were subjected to Western blotting using anti-GAPDH antibodies.

FIG 9 IFN- α -treated HT-1080 cells that do not express MxB potentially block HIV-1 infection. (A and B) HT-1080 (A) and HT1080-MxB-KO clone 3 (B) cells were challenged with increasing amounts of either HIV-1 or HIV-1-G208R expressing GFP as a reporter of infection. Viruses used for these infections were normalized for p24. At 48 h postinfection, the percentage of GFP-positive cells was measured by flow cytometry. Data from three independent experiments are shown. (C) Similar infections were performed by using HT-1080-MxB-KO clone 5, which also lost expression of MxB. (D) To control for how the protocol of obtaining MxB knockout cells affects HIV-1 infection, we performed infections with an HT-1080 clone that did not lose expression of MxB (clone 1).

vations suggested that the ability of MxB to bind capsid is necessary for restriction (24, 29, 33, 34), we tested the ability of MxB to bind to *in vitro*-assembled HIV-1 CA-NC complexes bearing the mutation G208R. As shown in Fig. 1C, MxB was impaired in its ability to bind *in vitro*-assembled HIV-1 CA-NC complexes bearing the mutation G208R compared to the wild type. These results suggested that MxB does not block HIV-1-G208R, because MxB poorly interacts with an HIV-1 core bearing the capsid mutation G208R. The mutation G208R provides a useful tool to study the contribution of MxB to the HIV-1 restriction observed in IFN- α -treated cells.

HIV-1 bearing the capsid mutation G208R does not overcome the restriction imposed by IFN- α -treated human cells. Next, we evaluated the ability of HIV-1-G208R to overcome the restriction imposed by the treatment of different human cells with IFN- α . To this end, we treated the indicated cells with 1,000 U/ml of IFN- α for 24 h and subsequently challenged them with increasing amounts of HIV-1-G208R. Although treatment with IFN- α induces the expression of MxB in the different cell types (data not shown), HIV-1-G208R was not able to overcome the restriction imposed by IFN- α -treated human THP-1 (Fig. 2), HT-1080 (Fig. 3), or U87 MG (Fig. 4) cells. These experiments suggested that MxB does not contribute to the ability of IFN- α -treated cells to block HIV-1 infection. This is in agreement with the following reported findings suggesting that MxB is not the restriction factor responsible for the block to HIV-1 observed in IFN- α -treated human cells: (i) MxB is an HIV-1 restriction factor that blocks HIV-1 infection after reverse transcription (9, 22, 23, 27, 29), and (ii) treatment of human cells with IFN- α imposes a restriction on HIV-1 before the occurrence of reverse transcription (35). Next,

we investigated the HIV-1 life cycle stage at which the block imposed by IFN- α -treated THP-1 cells occurs. As shown in Fig. 5, IFN- α -treated THP-1 cells block HIV-1 and HIV-1-G208R before reverse transcription, as determined by measuring late reverse transcripts (LRTs) at 7 h postinfection. Altogether, these experiments suggested that MxB is either minimally or not contributing to the HIV-1 restriction observed in human cells treated with IFN- α .

IFN- α -treated THP-1 cells knocked out for expression of MxB potentially block HIV-1 infection. To genetically address this question, we knocked out the expression of MxB using the CRISPR-Cas9 technology in the human cell line THP-1 and tested whether MxB contributes to the restriction imposed on HIV-1 by treating THP-1 cells with IFN- α . Transfection of the CRISPR-Cas9 constructs by using a specific guide RNA that targets exon 2 of *MxB* resulted in the isolation of a single clone that contained a 2-bp deletion in the open reading frame of *MxB* (Fig. 6A). Although the expression of endogenous MxB is not detectable in the THP-1-MxB knockout (KO) cell line (Fig. 6B), the restriction imposed by IFN- α -treated THP-1-MxB-KO cells did not change compared to that of wild-type cells (Fig. 7). As a control, we also infected cells with HIV-1-G208R (Fig. 7). These experiments suggested that MxB does not contribute to the HIV-1 restriction imposed by IFN- α -treated THP-1 cells.

IFN- α -treated HT-1080 cells knocked out for expression of MxB potentially block HIV-1 infection. Using methods similar to those described above, we knocked out the expression of MxB in HT-1080 cells using the CRISPR-Cas9 system, using a guide RNA that targets exon 2, and obtained a single clone that exhibits a 69-bp deletion in the open reading frame of *MxB* (Fig. 8A). As

shown in Fig. 8B, we generated two clones that lost the expression of MxB (HT-1080-MxB-KO clones 3 and 5). Next, we tested the ability of HIV-1-GFP to infect IFN- α -treated HT-1080-MxB-KO cells. As shown in Fig. 9A and B, HIV-1-GFP did not infect IFN- α -treated HT-1080-MxB-KO cells (clone 3). As a control, we also challenged cells with HIV-1-G208R. These experiments suggested that MxB does not contribute to the HIV-1 restriction observed in IFN- α -treated HT-1080 cells. Similar results were observed by using a different clone that did not express MxB (HT-1080-MxB-KO clone 5) (Fig. 9C). As a control, we also studied HIV-1 infection of a clone that underwent the same treatment to obtain knockout clones but did not lose MxB restriction (clone 1) (Fig. 9D). Overall, these investigations led us to conclude that HIV-1 infection is blocked in IFN- α -treated human cells by a factor(s) different from MxB.

DISCUSSION

This work explored the contribution of MxB to the HIV-1 restriction imposed by IFN- α -treated human cells using two different approaches: (i) we challenged IFN- α -treated human cells with HIV-1 bearing a capsid mutation that allows the virus to overcome MxB restriction, and (ii) we challenged IFN- α -treated human cells that were genetically modified to not express MxB.

HIV-1 bearing the capsid mutation G208R overcomes the restriction imposed by cells expressing MxB (26). Our work demonstrated that MxB does not interact with a viral core containing the mutation G208R. This result makes HIV-1-G208R a very important and reliable tool to study MxB restriction in different types of cells. Although we have tested several capsid mutants (24), HIV-1-G208R is the only mutant that fully overcomes the restriction imposed by cells overexpressing MxB.

To understand the contribution of MxB to the restriction imposed by IFN- α -treated human cells on HIV-1, we challenged IFN- α -treated THP-1, HT-1080, and U87 MG cells with HIV-1-G208R. These results showed that MxB does not contribute to the HIV-1 restriction observed in IFN- α -treated human cells.

To genetically investigate this restriction, we tested HIV-1 infection in THP-1 and HT-1080 cells where the expression of MxB was knocked out. These experiments also suggested that MxB has minimal or no contribution to the ability of IFN- α -treated human cells to block HIV-1.

The results presented here are not in agreement with data from experiments in which short hairpin RNAs (shRNAs) were used to knock down the expression of MxB in IFN- α -treated cells (9, 22, 23). The difference might be that in the above-mentioned studies, the shRNA used to target MxB/Mx2 is off-targeting other mRNAs relieving the block imposed on HIV-1 by IFN- α -treated cells. An alternative explanation is that the disparity in results is due to the different sources of IFN used on our experiments.

Overall, these results suggested the existence of a novel restriction to HIV-1 in IFN- α -treated human cells. The ability of interferon to induce the expression of a large number of genes suggests the possibility that IFN- α -treated human cells induce a variety of restrictions against HIV-1. Future experiments will investigate the nature of these blocks to HIV-1 infection. We strongly believe that the human cell lines that are knocked out for the expression of MxB represent a valuable tool for the study of these novel restrictions.

ACKNOWLEDGMENTS

We are thankful to the NIH/AIDS repository program for providing valuable reagents such as antibodies and drugs.

FUNDING INFORMATION

HHS | National Institutes of Health (NIH) provided funding to Felipe Diaz-Griffero under grant numbers R01 AI087390, R21 AI102824, and R56 AI108432.

REFERENCES

- Mitchell PS, Emerman M, Malik HS. 2013. An evolutionary perspective on the broad antiviral specificity of MxA. *Curr Opin Microbiol* 16:493–499. <http://dx.doi.org/10.1016/j.mib.2013.04.005>.
- Haller O, Kochs G. 2011. Human MxA protein: an interferon-induced dynamin-like GTPase with broad antiviral activity. *J Interferon Cytokine Res* 31:79–87. <http://dx.doi.org/10.1089/jir.2010.0076>.
- Haller O, Staeheli P, Schwemmle M, Kochs G. 2015. Mx GTPases: dynamin-like antiviral machines of innate immunity. *Trends Microbiol* 23:154–163. <http://dx.doi.org/10.1016/j.tim.2014.12.003>.
- Melén K, Keskinen P, Ronni T, Sareneva T, Lounatmaa K, Julkunen I. 1996. Human MxB protein, an interferon- α -inducible GTPase, contains a nuclear targeting signal and is localized in the heterochromatin region beneath the nuclear envelope. *J Biol Chem* 271:23478–23486. <http://dx.doi.org/10.1074/jbc.271.38.23478>.
- Aebi M, Fäh J, Hurt N, Samuel CE, Thomis D, Bazzigher L, Pavlovic J, Haller O, Staeheli P. 1989. cDNA structures and regulation of two interferon-induced human Mx proteins. *Mol Cell Biol* 9:5062–5072. <http://dx.doi.org/10.1128/MCB.9.11.5062>.
- Haller O. 2013. Dynamins are forever: MxB inhibits HIV-1. *Cell Host Microbe* 14:371–373. <http://dx.doi.org/10.1016/j.chom.2013.10.002>.
- Haller O, Gao S, von der Malsburg A, Daumke O, Kochs G. 2010. Dynamin-like MxA GTPase: structural insights into oligomerization and implications for antiviral activity. *J Biol Chem* 285:28419–28424. <http://dx.doi.org/10.1074/jbc.R110.145839>.
- Faelber K, Gao S, Held M, Posor Y, Haucke V, Noé F, Daumke O. 2013. Oligomerization of dynamin superfamily proteins in health and disease. *Prog Mol Biol Transl Sci* 117:411–443. <http://dx.doi.org/10.1016/B978-0-12-386931-9.00015-5>.
- 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. <http://dx.doi.org/10.1016/j.chom.2013.08.015>.
- Morlot S, Roux A. 2013. Mechanics of dynamin-mediated membrane fission. *Annu Rev Biophys* 42:629–649. <http://dx.doi.org/10.1146/annurev-biophys-050511-102247>.
- McNew JA, Sondermann H, Lee T, Stern M, Brandizzi F. 2013. GTP-dependent membrane fusion. *Annu Rev Cell Dev Biol* 29:529–550. <http://dx.doi.org/10.1146/annurev-cellbio-101512-122328>.
- Gao S, von der Malsburg A, Paeschke S, Behlke J, Haller O, Kochs G, Daumke O. 2010. Structural basis of oligomerization in the stalk region of dynamin-like MxA. *Nature* 465:502–506. <http://dx.doi.org/10.1038/nature08972>.
- Pavlovic J, Zürcher T, Haller O, Staeheli P. 1990. Resistance to influenza virus and vesicular stomatitis virus conferred by expression of human MxA protein. *J Virol* 64:3370–3375.
- Xiao H, Killip MJ, Staeheli P, Randall RE, Jackson D. 2013. The human interferon-induced MxA protein inhibits early stages of influenza A virus infection by retaining the incoming viral genome in the cytoplasm. *J Virol* 87:13053–13058. <http://dx.doi.org/10.1128/JVI.02220-13>.
- Matzinger SR, Carroll TD, Dutra JC, Ma Z-M, Miller CJ. 2013. Myxovirus resistance gene A (MxA) expression suppresses influenza A virus replication in alpha interferon-treated primate cells. *J Virol* 87:1150–1158. <http://dx.doi.org/10.1128/JVI.02271-12>.
- Frese M, Kochs G, Meier-Dieter U, Siebler J, Haller O. 1995. Human MxA protein inhibits tick-borne Thogoto virus but not Dhori virus. *J Virol* 69:3904–3909.
- Netherton CL, Simpson J, Haller O, Wileman TE, Takamatsu H-H, Monaghan P, Taylor G. 2009. Inhibition of a large double-stranded DNA virus by MxA protein. *J Virol* 83:2310–2320. <http://dx.doi.org/10.1128/JVI.00781-08>.
- Li N, Zhang L, Chen L, Feng W, Xu Y, Chen F, Liu X, Chen Z, Liu W.

2012. MxA inhibits hepatitis B virus replication by interaction with hepatitis B core antigen. *Hepatology* 56:803–811. <http://dx.doi.org/10.1002/hep.25608>.
19. Kochs G, Janzen C, Hohenberg H, Haller O. 2002. Antivirally active MxA protein sequesters La Crosse virus nucleocapsid protein into perinuclear complexes. *Proc Natl Acad Sci U S A* 99:3153–3158. <http://dx.doi.org/10.1073/pnas.052430399>.
20. Reichelt M, Stertz S, Krijnse-Locker J, Haller O, Kochs G. 2004. Mis-sorting of LaCrosse virus nucleocapsid protein by the interferon-induced MxA GTPase involves smooth ER membranes. *Traffic* 5:772–784. <http://dx.doi.org/10.1111/j.1600-0854.2004.00219.x>.
21. Schoggins JW, Wilson SJ, Panis M, Murphy MY, Jones CT, Bieniasz P, Rice CM. 2011. A diverse range of gene products are effectors of the type I interferon antiviral response. *Nature* 472:481–485. <http://dx.doi.org/10.1038/nature09907>.
22. Goujon C, Moncorgé O, Bauby H, Doyle T, Ward CC, Schaller T, Hué S, Barclay WS, Schulz R, Malim MH. 2013. Human MX2 is an interferon-induced post-entry inhibitor of HIV-1 infection. *Nature* 502:559–562. <http://dx.doi.org/10.1038/nature12542>.
23. Kane M, Yadav SS, Bitzegeio J, Kutluay SB, Zang T, Wilson SJ, Schoggins JW, Rice CM, Yamashita M, Hatzioannou T, Bieniasz PD. 2013. MX2 is an interferon-induced inhibitor of HIV-1 infection. *Nature* 502:563–566. <http://dx.doi.org/10.1038/nature12653>.
24. Fricke T, White TE, Schulte B, de Souza Aranha Vieira DA, Dharan A, Campbell EM, Brandariz-Núñez A, Diaz-Griffero F. 2014. MxB binds to the HIV-1 core and prevents the uncoating process of HIV-1. *Retrovirology* 11:68. <http://dx.doi.org/10.1186/s12977-014-0068-x>.
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. <http://dx.doi.org/10.1128/JVI.01269-14>.
26. Busnadiego I, Kane M, Rihn SJ, Preugschas HF, Hughes J, Blanco-Melo D, Strouelle 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. <http://dx.doi.org/10.1128/JVI.00214-14>.
27. Matreyek KA, Wang W, Serrao E, Singh P, Levin HL, Engelman A. 2014. Host and viral determinants for MxB restriction of HIV-1 infection. *Retrovirology* 11:90. <http://dx.doi.org/10.1186/s12977-014-0090-z>.
28. Goujon C, Greenbury RA, Papaioannou S, Doyle T, Malim MH. 2015. A triple-arginine motif in the amino-terminal domain and oligomerization are required for HIV-1 inhibition by human MX2. *J Virol* 89:4676–4680. <http://dx.doi.org/10.1128/JVI.00169-15>.
29. Schulte B, Buffone C, Opp S, Di Nunzio F, De Souza Aranha Vieira DA, Brandariz-Núñez A, Diaz-Griffero F. 2015. Restriction of HIV-1 requires the N-terminal region of MxB as a capsid-binding motif but not as a nuclear localization signal. *J Virol* 89:8599–8610. <http://dx.doi.org/10.1128/JVI.00753-15>.
30. Lienlaf M, Hayashi F, Di Nunzio F, Tochio N, Kigawa T, Yokoyama S, Diaz-Griffero F. 2011. Contribution of E3-ubiquitin ligase activity to HIV-1 restriction by TRIM5 rh: structure of the RING domain of TRIM5. *J Virol* 85:8725–8737. <http://dx.doi.org/10.1128/JVI.00497-11>.
31. Diaz-Griffero F, Perron M, McGee-Estrada K, Hanna R, Maillard PV, Trono D, Sodroski J. 2008. A human TRIM5α B30.2/SPRY domain mutant gains the ability to restrict and prematurely uncoat B-tropic murine leukemia virus. *Virology* 378:233–242. <http://dx.doi.org/10.1016/j.virol.2008.05.008>.
32. Dominguez AA, Lim WA, Qi LS. 2016. Beyond editing: repurposing CRISPR-Cas9 for precision genome regulation and interrogation. *Nat Rev Mol Cell Biol* 17:5–15. <http://dx.doi.org/10.1038/nrm.2015.2>.
33. 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. <http://dx.doi.org/10.1016/j.chom.2014.09.021>.
34. Buffone C, Schulte B, Opp S, Diaz-Griffero F. 2015. Contribution of MxB oligomerization to HIV-1 capsid binding and restriction. *J Virol* 89:3285–3294. <http://dx.doi.org/10.1128/JVI.03730-14>.
35. Goujon C, Malim MH. 2010. Characterization of the alpha interferon-induced postentry block to HIV-1 infection in primary human macrophages and T cells. *J Virol* 84:9254–9266. <http://dx.doi.org/10.1128/JVI.00854-10>.
36. Diaz-Griffero F, Qin XR, Hayashi F, Kigawa T, Finzi A, Sarnak Z, Lienlaf M, Yokoyama S, Sodroski J. 2009. A B-box 2 surface patch important for TRIM5 self-association, capsid binding avidity, and retrovirus restriction. *J Virol* 83:10737–10751. <http://dx.doi.org/10.1128/JVI.01307-09>.