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Interferon-Induced Rat Mx Proteins Confer Resistance to Rift Valley Fever Virus and Other Arthropod-Borne Viruses

MARKUS SANDROCK, MICHAEL FRESE, OTTO HALLER, and GEORG KOCHS

ABSTRACT

Mx proteins belong to the interferon (IFN)-induced antiviral defense. The rat genome contains three *Mx* genes, *ratMx1*, *ratMx2*, and *ratMx3*. The *Mx* gene products differ in their subcellular localization and antiviral specificity. The nuclear ratMx1 protein confers resistance to influenza A virus, and the cytoplasmic ratMx2 is active against vesicular stomatitis virus (VSV), whereas the cytoplasmic ratMx3 protein is antivirally inactive. To investigate the antiviral potential of the rat Mx proteins against arboviruses, a phylogenetically diverse group of viruses that frequently infect rodents, we studied the replication of LaCrosse virus (LACV). Rift Valley fever virus (RVFV) (both family *Bunyaviridae*), and Thogoto virus (THOV) (family *Orthomyxoviridae*). To that end, we used transfected Vero cells constitutively expressing one of the rat Mx proteins. We observed that the antiviral activity of rat Mx proteins against these arboviruses correlates with their intracellular localization: ratMx1 is active against THOV, which replicates in the nucleus, whereas ratMx2 inhibits bunyaviruses that replicate in the cytoplasm. The results indicate that rats have evolved two Mx proteins to efficiently control viruses with different replication strategies.

INTRODUCTION

Mx PROTEINS ARE KEY COMPONENTS of the interferon (IFN)-induced antiviral defense.[1] They are large, antivirally active GTPases that have been found in all vertebrate species analyzed to date. The antiviral effect was first described as an inborn resistance of laboratory mice (*Mus musculus*) against orthomyxovirus infections.[2] The murine Mx1 protein (MuMx1) accumulates in the nucleus and blocks the primary transcription of the influenza A virus (FLUAV) RNA genome.[3,4] Mice have also a cytoplasmic Mx protein, MuMx2, that inhibits the replication of Hantaan virus, Seoul virus, and vesicular stomatitis virus (VSV).[5–7] However, this protein is not functional in most inbred strains because of mutations that disrupt the *MuMx2* open reading frame (ORF).[8] In rats (*Rattus norvegicus*), three different *Mx* genes have been identified, *ratMx1*, *ratMx2*, and *ratMx3*.[9] The ratMx1 protein accumulates in the nucleus and inhibits the replication of FLUAV.[10] The cytoplasmic proteins ratMx2 and ratMx3 are almost identical, but only ratMx2 shows antiviral activity against VSV.[10]

Small rodents serve as hosts for a variety of arthropod-borne viruses, including some important human pathogens. LaCrosse virus (LACV) belongs to the California serogroup within the genus *Bunyavirus* (family *Bunyaviridae*) and is transmitted by mosquitoes to woodland rodents and rabbits.[11] In laboratory mice, LACV infection provokes a lethal encephalitis.[12] LACV infections also can lead to an acute encephalitis in children and, therefore, represent a severe health problem in the midwestern United States.[13–15] Rift Valley fever virus (RVFV), a member of the genus *Phlebovirus* in the *Bunyaviridae* family, is also transmitted by mosquitoes and causes fulminant hepatitis and encephalitis in infected rats and mice.[16,17] RVFV causes epizootic outbreaks in sub-Saharan Africa, Egypt, and most recently in the Kingdom of Saudi Arabia and Yemen.[18,19] During such outbreaks, sheep and goats frequently become infected and develop a severe disease, with high mortality.[11] RVFV also infects humans. In the 1977–1978 epidemic in Egypt, over 200,000 people developed Rift Valley fever, but most of them showed only mild symptoms of the disease. However, in at least 600 cases, the disease was more severe, and patients died from encephalitis, fatal hepatitis, or hemorrhagic fever.[20,21]

Rodents are also a reservoir for tick-borne orthomyxoviruses. Serologic data indicate that Thogoto virus (THOV) and Dhori virus (DHOV) frequently infect mice and rats, which may represent a reservoir for these viruses.[22,23] Experimental infection of laboratory mice leads to severe hepatitis and death of the animals that do not express the MuMx1 protein.[24–27] THOV-specific antibodies have been detected in a wide range of animals and humans.[23,28] Reports about human THOV infections are rare, but there are two case reports stating that the

Abteilung Virologie, Institut für Medizinische Mikrobiologie und Hygiene, Universität Freiburg, D-79104 Freiburg, Germany.

virus has been isolated from patients with neurologic disorders.[29] Furthermore, accidental laboratory infections of humans with DHOV, resulting in febrile illness and encephalitis, have been reported.[30]

The IFN system plays a critical role in the early defense against viruses. In the present study, we describe the ability of rat Mx proteins to inhibit the replication of LACV, RVFV, and THOV. Our results are important for a better understanding of the mechanisms that protect rats against arboviruses.

MATERIAL AND METHODS

Cells

African green monkey (Vero) cells[31] were cultured in Dulbecco's modified Eagle's medium (DMEM) supplemented with 200 U/ml penicillin, 200 μg/ml streptomycin, and 10% fetal bovine serum (FBS).

Viruses

The original strain of LACV[32] was grown in BHK-21 cells. Stock virus contained 1.6×10^8 50% tissue infective dose ($TCID_{50}$)/ml. The attenuated MP12 strain of RVFV[33] was propagated in Vero cells. Stock virus contained 2×10^7 $TCID_{50}$/ml. The Sicilian SiAr126 strain of THOV[34] was grown in BHK-21 cells. Stock virus contained 8.3×10^7 plaque-forming units (PFU)/ml. A mammalian cell-adapted variant of influenza A/FPVDobson/34 (H7N7), called FPV-B,[35] was propagated in Swiss mouse 3T3 cells. Stock virus contained 6.0×10^7 $TCID_{50}$/ml. VSV (serotype Indiana) was produced in Vero cells. Stock virus contained 1.3×10^8 $TCID_{50}$/ml.

Plasmids

The rat Mx expression vectors pSVrMx1, pSVrMx2, pSVrMx2-N, pSVrMx3, and pSVrMx3-N[10,36] were kindly provided by Ellen Meier (National Institute of Neurological Disorders and Stroke, Bethesda, MD).

Antibodies

Mx proteins were labeled using the monoclonal mouse antibody 2C12 that binds to rat Mx proteins[37] or a polyclonal rabbit antibody (kindly provided by Ellen Meier) that is directed against ratMx3 but also recognizes ratMx2.[36] A mixture of three monoclonal antibodies (mAb) (807-25, 807-33, and 807-35) directed against the G_1 envelope glycoprotein[38] was used to detect LACV antigens (the antibody mixture was kindly provided by Francisco González-Scarano, University of Pennsylvania, Philadelphia, PA). RVFV-infected cells were labeled using the monoclonal mouse antibody 4D4 that is directed against the G_2 envelope glycoprotein (kindly provided by Jonathan F. Smith, USAMRIID, Fort Detrick, Frederick, MD). A hyperimmunized guinea pig antiserum (a gift from Patricia A. Nuttall, NERC Institute of Virology and Environmental Microbiology, Oxford, U.K.) was used to label THOV-infected cells.[39] A rabbit antiserum directed against influenza A/FPVDobson/34 was used to detect FLUAV proteins. VSV antigens were labeled using a rabbit antiserum directed against the P protein of VSV (obtained from Peter Staeheli, Universität Freiburg, Freiburg, Germany). Bound primary antibodies were stained using fluorescein (DTAF)-conjugated, rhodamine (TRITC)-conjugated, or Cy3™-conjugated goat secondary antibodies against mouse, rabbit, or guinea pig immunoglobulins. All secondary antibodies were purchased from Dianova (Hamburg, Germany).

Analysis of antiviral activity

Vero cells were transfected with expression vectors coding for the various Mx proteins using the calcium phosphate coprecipitation method.[40] About 24 h after transfection, cells were trypsinized, seeded on glass coverslips, and grown for another 24 h. Cells were then infected with LACV, RVFV, THOV, FLUAV, or VSV at a high multiplicity of infection (moi) (see legends of Figs. 1 and 2 for details). Subsequently, cells were fixed with 3% paraformaldehyde in phosphate-buffered saline (PBS), permeabilized with 0.5% Triton X-100 in PBS, and finally stained for Mx proteins and viral antigens using a combination of the antibodies described. Immunofluorescence analysis was performed using a Reichert-Jung Polyvar microscope equipped with epifluorescence. Transfected cells that express Mx proteins and accumulate viral antigens as well as cells that express Mx proteins but lack viral protein expression were counted. The percentage of virus-infected cells that express Mx protein with respect to the total number of Mx-expressing cells was taken as a value for the antiviral activity of the analyzed Mx protein.

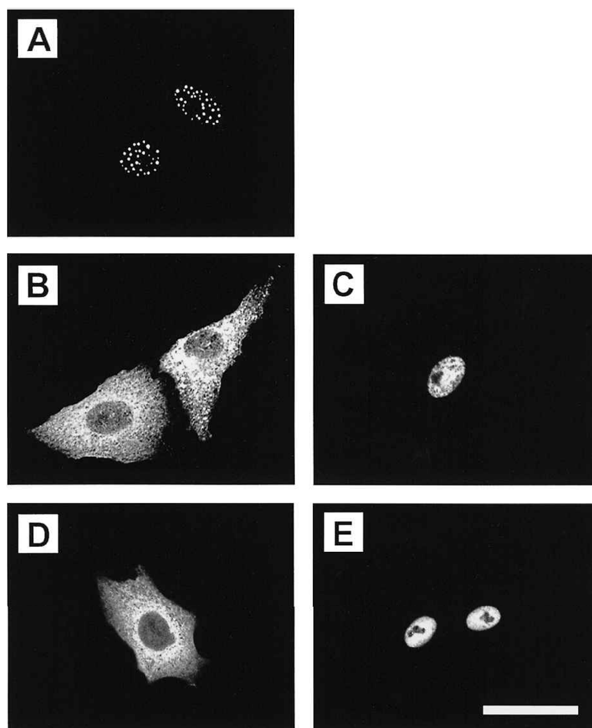

FIG. 1. Expression of rat Mx proteins in transfected Vero cells. Indirect immunofluorescence analysis of transiently transfected Vero cells that express either ratMx1 (**A**), ratMx2 (**B**), ratMx2-N (**C**), ratMx3 (**D**), or ratMx3-N (**E**). Cells were transfected with expression plasmids coding for one of the various Mx proteins, fixed 24 h after transfection, and stained for Mx proteins using the mAb 2C12. Bar = 50 μm.

RESULTS

Subcellular localization of Mx proteins in transfected Vero cells

To investigate the antiviral potential of the rat Mx proteins, we constitutively expressed ratMx1, ratMx2, and ratMx3 in Vero cells. Analysis of the intracellular localization of the recombinant proteins by indirect immunofluorescence revealed that ratMx1 accumulated in distinct dots within the nucleus, whereas ratMx2 and ratMx3 accumulated in the cytoplasm of transfected cells (Fig. 1A, B, D). These results are in line with the intracellular localization of rat Mx proteins in transfected

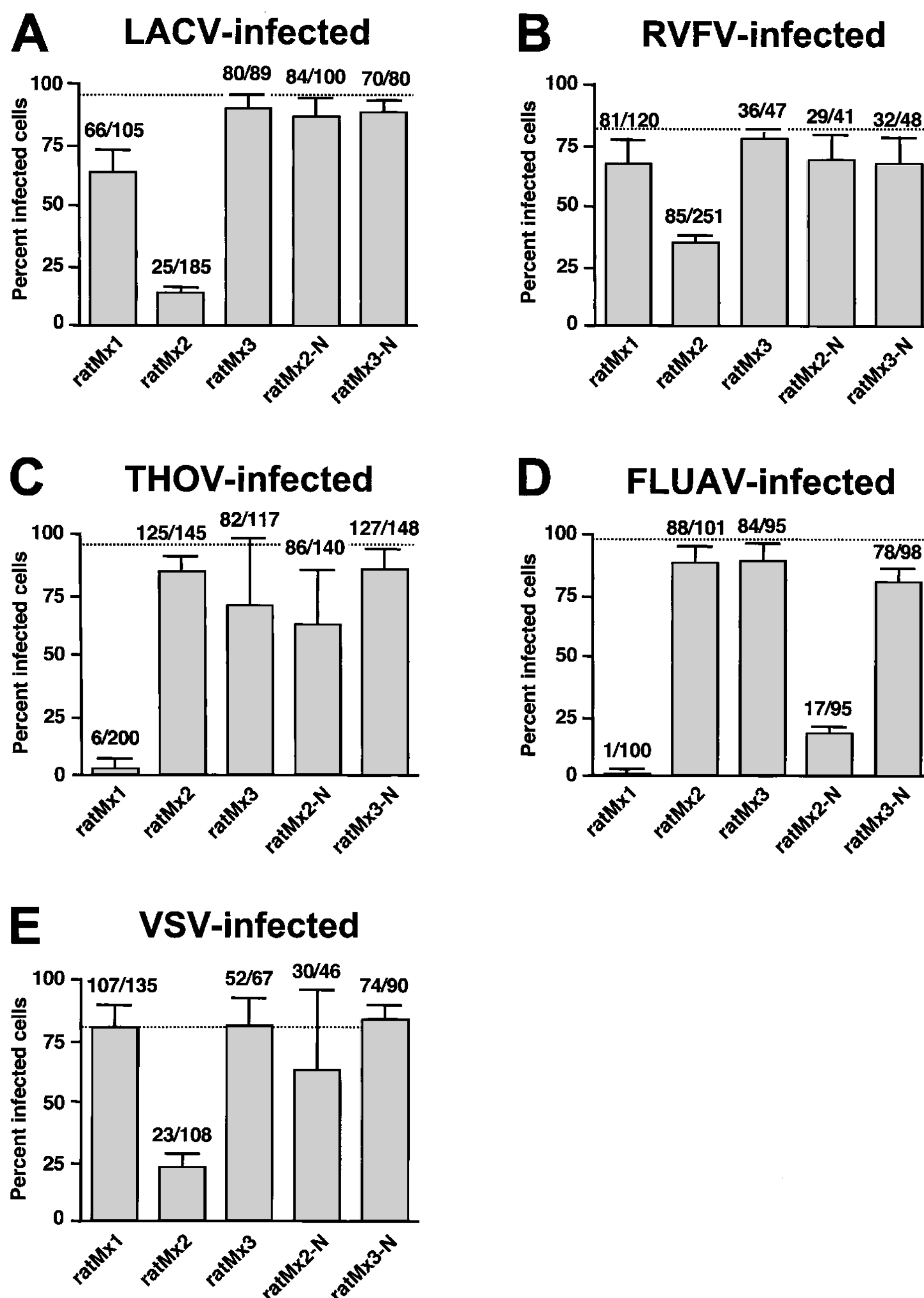

FIG. 2. Antiviral activity of rat Mx proteins. Vero cells were transfected with expression plasmids coding for either ratMx1, ratMx2, ratMx3, ratMx2-N, or ratMx3-N. About 48 h after transfection, cells were infected with LACV (**A**) or RVFV (**B**) at an moi of 15 for 10 h, THOV at an moi of 5 for 16 h (**C**), FLUAV at an moi of 10 for 5 h (**D**), or VSV at an moi of 20 for 3 h (**E**). The cells were then fixed and double-immunostained for Mx and viral proteins using specific antibodies (see Material and Methods). The percentage of cells expressing both viral proteins and Mx proteins is given in relation to the total number of Mx-expressing cells. Columns and error bars represent mean values of two (FLUAV), three (LACV RVFV, and VSV), or five (THOV) independent experiments and 95% confidence intervals, respectively. The number of cells expressing viral proteins and Mx proteins per total number of cells expressing Mx is indicated above each column. Viral proteins were detected in about 97% (LACV), 82% (RVFV), 97% (THOV), 98% (FLUAV), and 80% (VSV) of nontransfected control cells (dotted lines).

mouse 3T3 cells and in IFN-treated rat cells.[9,10] To investigate whether the intracellular localization has an effect on the antiviral specificity of the rat Mx proteins, we also expressed nuclear forms of ratMx2 and ratMx3, called ratMx2-N and ratMx3-N, which carry the nuclear translocation signal of the SV40 large T antigen at the N-terminus.[36] As expected, ratMx2-N and ratMx3-N accumulated in the nucleus of transfected Vero cells (Fig. 1C, E).

Antiviral activity of rat Mx proteins

The antiviral activities of rat Mx proteins have been assessed for VSV and FLUAV.[10] To determine the capacity of rat Mx proteins to inhibit arthropod-borne members of the families *Bunyaviridae* and *Orthomyxoviridae*, we challenged rat Mx-expressing cells with LACV, RVFV, and THOV. Cells were transfected with expression plasmids coding for one of the rat Mx proteins and infected 24 h later. The cells were incubated to allow viral replication and protein accumulation, fixed and double-immunostained for rat Mx and viral proteins. We compared the number of Mx-expressing cells that also accumulate viral proteins with the total number of Mx-expressing cells. Figure 2 shows that multiplication of LACV and RVFV was sensitive to the presence of ratMx2 (Fig. 2A, B). Production of viral proteins in LACV-infected and RVFV-infected cells was suppressed to 14% and 41%, respectively, in ratMx2-expressing cells compared to untransfected control cells. The antiviral activity of ratMx2 against the bunyaviruses was comparable to that against VSV, which was reduced to 27% (Fig. 2E), whereas expression of ratMx2 did not interfere with viral protein synthesis in THOV-infected or FLUAV-infected cells (Fig. 2C, D). In contrast, the nuclear ratMx1 clearly inhibited viral replication and, subsequently, protein synthesis of THOV and FLUAV (Fig. 2C, D) to 3% and 1% of the control cells, respectively. As expected, expression of the nuclear ratMx1 protein had no effect on the replication of LACV, RVFV, and VSV (Fig. 2A, B, E). Furthermore, there was no detectable reduction of viral protein synthesis in ratMx3-expressing cells for all viruses tested in this study.

Antiviral activity of nuclear variants of ratMx2 and ratMx3

The subcellular localization of Mx proteins has been reported to have a strong influence on their antiviral specificity.[36,41,42] Therefore, we tested the effect of ratMx2 and ratMx3 on viral replication by translocation of these proteins into the nucleus with the help of an N-terminal nuclear localization signal. Not surprisingly, the nuclear form of ratMx2 (ratMx2-N) showed no antiviral activity against RVFV as well as VSV, and its activity against LACV was significantly reduced compared with that of the wild-type protein (Fig. 2A, D, E). In contrast, ratMx2-N inhibited the replication of FLUAV and, to a lesser extent, that of THOV (Fig. 2C, D). Furthermore, the expression of ratMx3-N did not affect the replication of any of the viruses tested (Fig. 2A–E), confirming that ratMx3 is an antivirally inactive protein.

DISCUSSION

Rat cells express three different Mx proteins on IFN-α treatment.[9] Here we demonstrate that ratMx1 and ratMx2 but not ratMx3 possess antiviral activity against several arthropod-borne viruses of the families *Orthomyxoviridae*, *Bunyaviridae*, and *Rhabdoviridae* (Table 1). We show that the nuclear ratMx1 protein blocks the replication of THOV and FLUAV (both of which replicate their genome in the nucleus), whereas the cytoplasmic ratMx2 protein inhibits LACV, RVFV, and VSV (all of which replicate in the cytoplasm). The results demonstrate that rat Mx proteins have to be at the intracellular site of viral replication in order to inhibit viral multiplication, as previously proposed.[10,36] The observation that a considerable number of cells express RVFV proteins even in the presence of ratMx2 (85 of 251 ratMx2-expressing cells also accumulate RVFV proteins) is in line with previous findings. We and others have shown that the extent of viral inhibition correlates with the amount of human MxA protein expressed in infected cells.[43–45] It is, therefore, not unexpected that some viral protein synthesis may also occur in cells expressing low amounts of rat Mx proteins.

The murine Mx system (consisting of MuMx1 and MuMx2) closely resembles that of rats.[1] Sequence analysis, intracellular localization, and antiviral profile indicate that MuMx1 is a homolog to ratMx1. Similarly, MuMx2 is homologous to ratMx2. Thus, both rodent species express highly specialized Mx proteins to fight viruses with different replication strategies. These findings are remarkable because Mx proteins of other species have the ability to inhibit viruses irrespective of

TABLE 1. SUBCELLULAR LOCALIZATION AND ANTIVIRAL ACTIVITIES OF MX PROTEINS

Species	Mx protein	Subcellular localization	*Antiviral activity against*				
			VSV	*FLUAV*	*THOV*	*LACV*	*RVFV*
Rat	ratMx1	Nuclear	−	+	+	±	−
	ratMx2	Cytoplasmic	+	−	−	+	+
	ratMx3	Cytoplasmic	−	−	−	−	−
	ratMx2-N[a]	Nuclear	−	+	±	−	−
	ratMx3-N[a]	Nuclear	−	−	−	−	−
Mouse	MuMx1	Nuclear	−	+	+	n.d.[b]	n.d.
	MuMx2	Cytoplasmic	+	−	n.d.	n.d.	n.d.

[a]ratMx2-N and ratMx3-N contain the nuclear translocation signal of the SV40 large T antigen at its amino-terminus.
[b]n.d., not determined.

the intracellular site of their replication. The human MxA protein, for example, is localized in the cytoplasm but interferes with the multiplication of FLUAV,[46] influenza C virus,[47] and THOV.[43] The mechanism by which MxA blocks the replication of THOV has been described recently.[48,49] MxA binds to incoming viral ribonucleoprotein complexes (vRNP) and thereby prevents their nuclear import. This results in an early and efficient block of viral multiplication. In the case of FLUAV, MxA allows the transport of incoming vRNP into the nucleus, and primary transcription takes place but subsequent viral multiplication steps are inhibited.[3,4] Besides orthomyxoviruses, MxA is able to inhibit the multiplication of many other RNA viruses that replicate in the cytoplasm. Among these are members of the families *Bunyaviridae*,[31,45] *Paramyxoviridae*,[50,51] *Rhabdoviridae*,[46] and *Togaviridae*.[52,53] Obviously, humans and rodents have evolved different strategies to inhibit RNA viruses. Human cells express a single Mx protein that inhibits a wide range of viruses, whereas rodent cells express two different Mx proteins with more specific antiviral activities.

The artificial translocation of antivirally active Mx proteins from one cellular compartment to another often changes their activities.[36,41,42] In line with these observations, we have found that ratMx2-N shows only a residual antiviral activity against LACV and does not inhibit the replication of RVFV and VSV but is active against FLUAV and moderately active against THOV. Most likely, ratMx2-N is unable to interact with viral targets in the cytoplasm because of its nuclear localization. It is, however, less obvious why the multiplication of FLUAV and THOV is not affected in cells expressing ratMx2, whereas the replication of these viruses is inhibited by ratMx2-N. One might speculate that the nucleus contains a cellular factor enabling ratMx2-N to recognize its target (probably the vRNP) and to inhibit viral transcription/replication. Interestingly, in a yeast two-hybrid screen, MuMx1 has been shown recently to interact with several nuclear body-associated proteins, including protein kinase interacting with Mx proteins (PKM), Fas death domain-associated protein (Daxx), and 100 kDa nuclear protein antigen (Sp100) (O. Engelhardt, G. Kochs, and O. Haller, unpublished observations). It remains to be seen whether one of these proteins is needed by ratMx2-N to execute its antiviral function. Alternatively, the activity of the viral polymerase in the nucleus leads to conformational changes that expose target structures to ratMx2-N. This might also explain why ratMx2-N but not ratMx2 inhibits the multiplication of orthomyxoviruses.

The most recent outbreak of RVFV in the Kingdom of Saudi Arabia and in Yemen[18,19] shows that trade and traffic of the modern world allows viruses to spread quickly outside their original endemic areas. A better understanding of the innate antiviral defense of rodents might help us to develop new strategies to control emerging viral infections.

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REFERENCES

1. HALLER, O., FRESE, M., and KOCHS, G. (1998). Mx proteins: mediators of innate resistance to RNA viruses. Rev. Sci. Tech. Off. Int. Epiz. **17,** 220–230.
2. LINDENMANN, J., LANE, C.A., and HOBSON, D. (1963). The resistance of A2G mice to myxoviruses. J. Immunol. **90,** 942–951.
3. PAVLOVIC, J., HALLER, O., and STAEHELI, P. (1992). Human and mouse Mx proteins inhibit different steps of the influenza virus multiplication cycle. J. Virol. **66,** 2564–2569.
4. KRUG, R.M., SHAW, M., BRONI, B., SHAPIRO, G., and HALLER, O. (1985). Inhibition of influenza viral mRNA synthesis in cells expressing the interferon-induced Mx gene product. J. Virol. **56,** 201–206.
5. ZÜRCHER, T., PAVLOVIC, J., and STAEHELI, P. (1992). Mouse Mx2 protein inhibits vesicular stomatitis virus but not influenza virus. Virology **187,** 796–800.
6. JIN, H.K., TAKADA, A., KON, Y., HALLER, O., and WATANABE, T. (1999). Identification of the murine *Mx2* gene: interferon-induced expression of the Mx2 protein from the feral mouse gene confers resistance to vesicular stomatitis virus. J. Virol. **73,** 4925–4930.
7. JIN, H.K., YOSHIMATSU, K., TAKADA, A., OGINO, M., ASANO, A., ARIKAWA, J., and WATANABE, T. (2001). Mouse Mx2 protein inhibits hantavirus but not influenza virus replication. Arch. Virol. **146,** 41–49.
8. STAEHELI, P., and SUTCLIFFE, J.G. (1988). Identification of a second interferon-regulated murine *Mx* gene. Mol. Cell. Biol. **8,** 4524–4528.
9. MEIER, E., FÄH, J., GROB, M.S., END, R., STAEHELI, P., and HALLER, O. (1988). A family of interferon-induced Mx-related mRNAs encodes cytoplasmic and nuclear proteins in rat cells. J. Virol. **62,** 2386–2393.
10. MEIER, E., KUNZ, G., HALLER, O., and ARNHEITER, H. (1990). Activity of rat Mx proteins against a rhabdovirus. J. Virol. **64,** 6263–6269.
11. GONZALEZ-SCARANO, F., and NATHANSON, N. (1996). Bunyaviridae. In: *Virology.* 3rd ed. B.N. Fields (ed.) Philadelphia: Lippincott-Raven Publishers, pp. 1475–1504.
12. JOHNSON, K.P., and JOHNSON, R.T. (1968). California encephalitis. II. Studies of experimental infection in the mouse. J. Neuropathol. Exp. Neurol. **27,** 390–400.
13. KALFAYAN, B. (1983). Pathology of La Crosse virus infections in humans. In: *California Serogroup Viruses*. C.H. Calisher and W.H. Thompson (eds.) New York: A.R. Liss, pp. 179–186.
14. GRIOT, C., GONZALEZ-SCARANO, F., and NATHANSON, N. (1994). Molecular determinants of the virulence and infectivity of California serogroup bunyaviruses. Annu. Rev. Microbiol. **47,** 117–138.
15. CENTERS FOR DISEASE CONTROL AND PREVENTION. (1998). Arboviral infections of the central nervous system—United States, 1996–1997. MMWR **47,** 517–522.
16. McGAVRAN, M., and EASTERDAY, B.C. (1963). Rift Valley fever virus hepatitis. Am. J. Pathol. **42,** 587–607.
17. PETERS, C.J., and ANDERSON, G.W., Jr. (1981) Pathogenesis of Rift Valley fever. Contrib. Epidemiol. Biostatist. **3,** 21–41.
18. WORLD HEALTH ORGANIZATION. (2000). Outbreak of Rift Valley fever, Saudi Arabia, August–October 2000. Weekly Epidemiol. Rec. **75,** 370–371.
19. WORLD HEALTH ORGANIZATION. (2000). Outbreak of Rift Valley fever, Yemen, August–October 2000. Weekly Epidemiol. Rec. **75,** 392–395.
20. LAUGHLIN, L.W., MEEGAN, J.M., STRAUSBAUGH, L.H., MORENS, D.M., and WATTEN, R.H. (1979). Epidemic Rift Valley fever in Egypt: observations of the spectrum of human illness. Trans. R. Soc. Trop. Med. Hyg. **73,** 630–633.

21. MEEGAN, J.M. (1979). The Rift Valley fever epizootic in Egypt 1977–78. 1. Description of the epizootic and virological studies. Trans. R. Soc. Trop. Med. Hyg. **73,** 618–623.
22. DARWISH, M.A., HOOGSTRAAL, H., and OMAR, F.M. (1979). A serological survey for Thogoto virus in human, domestic animals and rats in Egypt. J. Egypt. Public Health Assoc. **54,** 1–8.
23. NUTTALL, P.A., MORSE, M.A., JONES, L.D., and PORTELA, A. (1995). Orthoacariviruses. In: *Molecular Evolution of Viruses*. A.J. Gibbs and C.M. Calisher (eds.) Cambridge: Cambridge University Press, pp. 416–425.
24. FILIPE, A.R., PELETEIRO, M.C., MONATH, T.M., and CALISHER, E.H. (1986). Pathological lesions in mice infected with Thogoto virus, a tick-borne orthomyxovirus. Acta Virol. (Praha) **30,** 337–340.
25. HALLER, O., FRESE, M., ROST, D., NUTTALL, P.A., and KOCHS, G. (1995). Tick-borne Thogoto virus infection in mice is inhibited by the orthomyxovirus resistance gene product Mx1. J. Virol. **69,** 2596–2601.
26. THIMME, R., FRESE, M., KOCHS, G., and HALLER, O. (1995). Mx1 but not MxA confers resistance against tick-borne Dhori virus in mice. Virology **210,** 296–301.
27. FRESE, M., WEEBER, M., WEBER, F., SPETH, V., and HALLER, O. (1997). Mx1 sensitivity: Batken virus is an orthomyxovirus closely related to Dhori virus. J. Gen. Virol. **78,** 2453–2458.
28. DARWISH, M.A., and HOOGSTRAAL, H. (1981). Arboviruses infecting humans and lower animals in Egypt: a review of thirty years of research. J. Egypt Public Health Assoc. **56,** 1–112.
29. MOORE, D.L., CAUSEY, O.R., CAREY, D.E., REDDY, S., COOKE, A.R., AKINKUGBE, F.M., DAVID-WEST, T.S., and KEMP, G.E. (1975). Arthropod-borne viral infections of man in Nigeria, 1964–1970. Ann. Trop. Med. Parasitol. **69,** 49–64.
30. BUTENKO, A.M., LESCHINSKAJA, E.V., SEMASHKO, I.V., DONETS, M.A., MARTIJANOWA, L.I., MARTYNENKO, I.N., RUBIN, S.G., and CHUMAKOW, M.P. (1987). Dhori virus, a causative agent of human disease: five cases of laboratory infections. Vopr. Virusol. **32,** 724–729.
31. FRESE, M., KOCHS, G., FELDMANN, H., HERTKORN, C., and HALLER, O. (1996). Inhibition of bunyaviruses, phleboviruses, and hantaviruses by human MxA protein. J. Virol. **70,** 915–923.
32. THOMPSON, W.H., KALFAYAN, B., and ANSLOW, R.O. (1965). Isolation of California encephalitis group virus from a fatal human illness. Am. J. Epidemiol. **81,** 245–253.
33. CAPLEN, H., PETERS, C.J., and BISHOP, D.H.L. (1985). Mutagen-directed attenuation of Rift Valley fever virus as a method for vaccine development. J. Gen. Virol. **66,** 2271–2277.
34. ALBANESE, M., BRUNO-SMIRAGLIA, C., DI CUONZO, G., LAVAGNINO, A., and SRIHONGSE, S. (1972). Isolation of Thogoto virus from *Rhipicephalus bursa* ticks in western Sicily. Acta Virol. **16,** 267.
35. ISRAEL, A. (1979). Preliminary characterization of the particles from productive and abortive infections of L cells by fowl plague virus. Ann. Microbiol. (Paris) **130B,** 85–100.
36. JOHANNES, L., ARNHEITER, H., and MEIER, E. (1993). Switch in antiviral specificity of a GTPase upon translocation from the cytoplasm to the nucleus. J. Virol. **67,** 1653–1657.
37. STAEHELI, P., and HALLER, O. (1985). Interferon-induced human protein with homology to protein Mx of influenza virus-resistant mice. Mol. Cell. Biol. **5,** 2150–2153.
38. GONZALEZ-SCARANO, F., SHOPE, R.E., CALISHER, C.E., and NATHANSON, N. (1982). Characterization of monoclonal antibodies against the G_1 and N proteins of LaCrosse and Tahyna, two California serogroup bunyaviruses. Virology **120,** 42–53.
39. JONES, J.D., and NUTTALL, P.A. (1989). The effect of virus-immune hosts on Thogoto virus infection of the tick, *Rhipicephalus appendiculatus.* Virus Res. **14,** 129–140.
40. STAEHELI, P., HALLER, O., BOLL, W., LINDENMANN, J., and WEISSMANN, C. (1986). Mx protein: constitutive expression in 3T3 cells transformed with cloned Mx cDNA confers selective resistance to influenza virus. Cell **44,** 147–158.
41. ZÜRCHER, T., PAVLOVIC, J., and STAEHELI, P. (1992). Mechanism of human MxA protein action: variants with changed antiviral properties. EMBO J. **11,** 1657–1661.
42. ZÜRCHER, T., PAVLOVIC, J., and STAEHELI, P. (1992). Nuclear localization of mouse Mx1 protein is necessary for inhibition of influenza virus. J. Virol. **66,** 5059–5066.
43. FRESE, M., KOCHS, G., MEIER-DIETER, U., SIEBLER, J., and HALLER, O. (1995). Human MxA protein inhibits tick-borne Thogoto virus but not Dhori virus. J. Virol. **69,** 3904–3909.
44. WEBER, F., KOCHS, G., and HALLER, O. (2000). MxA GTPase blocks reporter gene expression of reconstituted Thogoto virus ribonucleoprotein complexes. J. Virol. **74,** 560–563.
45. KANERVA, M., MELEN, K., VAHERI, A., and JULKUNEN, I. (1996). Inhibition of puumala and tula hantaviruses in Vero cells by MxA protein. Virology **224,** 55–62.
46. PAVLOVIC, J., ZÜRCHER, T., HALLER, O., and STAEHELI, P. (1990). Resistance to influenza virus and vesicular stomatitis virus conferred by expression of human MxA protein. J. Virol. **64,** 3370–3375.
47. MARSCHALL, M., ZACH, A., HECHTFISCHER, A., FOERST, G., MEIER-EWERT, H., and HALLER, O. (2000). Inhibition of influenza C viruses by human MxA protein. Virus Res. **67,** 179–188.
48. KOCHS, G., and HALLER, O. (1999). GTP-bound human MxA protein interacts with the nucleocapsids of Thogoto virus (Orthomyxoviridae). J. Biol. Chem. **274,** 4370–4376.
49. KOCHS, G., and HALLER, O. (1999). Interferon-induced human MxA GTPase blocks nuclear import of Thogoto virus nucleocapsids. Proc. Natl. Acad. Sci. USA **96,** 2082–2086.
50. SCHNEIDER-SCHAULIES, S., SCHNEIDER-SCHAULIES, J., SCHUSTER, A., BAYER, M., PAVLOVIC, J., and TER MEULEN, V. (1994). Cell type-specific MxA mediated inhibition of measles virus transcription in human brain cells. J. Virol. **68,** 6910–6917.
51. ZHAO, H., DE, B.P., and BANERJEE, A.K. (1996). Inhibition of human parainfluenza virus-3 replication by interferon and human MxA. Virology **220,** 330–338.
52. LANDIS, H., SIMON-JÖDICKE, A., KLOTI, A., DI PAOLO, C., SCHNORR, J.J., SCHNEIDER-SCHAULIES, S., HEFTI, H.P., and PAVLOVIC, J. (1998). Human MxA protein confers resistance to Semliki Forest virus and inhibits the amplification of a Semliki Forest virus-based replicon in the absence of viral structural proteins. J. Virol. **72,** 1516–1522.
53. HEFTI, H.P., FRESE, M., LANDIS, H., DI PAOLO, C., AGUZZI, A., HALLER, O., and PAVLOVIC, J. (1999). Human MxA protein protects mice lacking a functional alpha/beta interferon system against La Crosse virus and other lethal viral infections. J. Virol. **73,** 6984–6991.

Address reprint requests to:
Dr. Georg Kochs
Abteilung Virologie
Institut für Medizinische Mikrobiologie und Hygiene
Universität Freiburg
Hermann-Herder-Str, 11
D-79104 Freiburg
Germany

Tel: +49-761-203-6623
Fax: +49-761-203-6562
E-mail: kochs@ukl.uni-freiburg.de

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