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Enhanced Virus Resistance of Transgenic Mice Expressing the Human MxA Protein

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MxA is a GTPase that accumulates to high levels in the cytoplasm of interferon-treated human cells. Expression of MxA cDNA confers to transfected cell lines a high degree of resistance against several RNA viruses, including influenza, measles, vesicular stomatitis, and Thogoto viruses. We have now generated transgenic mice that express MxA cDNA in the brain and other organs under the control of a constitutive promoter. Embryonic fibroblasts derived from the transgenic mice were nonpermissive for Thogoto virus and showed reduced susceptibility for influenza A and vesicular stomatitis viruses. The transgenic animals survived challenges with high doses of Thogoto virus by the intracerebral or intraperitoneal route. Furthermore, the transgenic mice were more resistant than their nontransgenic littermates to intracerebral infections with influenza A and vesicular stomatitis viruses. These results demonstrate that MxA is a powerful antiviral agent in vivo, indicating that it may protect humans from the deleterious effects of infections with certain viral pathogens.

Most components of the mammalian interferon (IFN) system have been discovered, and the genes for the various IFNs, the IFN receptors, the IFN signal transducers, and some IFN-induced effector proteins have been cloned (7, 16, 25, 35, 39, 40, 45). Nonetheless, our knowledge regarding the function of these components during an IFN response is still very limited. A popular new approach that has yielded important information is to create cell lines or animals with targeted gene disruptions and test them for defects in the IFN response (6, 15, 19, 22, 23, 31, 33, 47). Such studies demonstrated, for example, that mice without a functional type I IFN system are extremely susceptible to viral infections, underscoring the importance of virus-induced IFN for host defense at early times after infection. Another experimental approach is to express individual components of the IFN system in suitable cell lines or transgenic animals, which are then tested for acquired functions. Experiments of the latter type have demonstrated that the IFN-induced human proteins MxA (30), 2′,5′-oligoadenylate synthase (5), PKR (21), and ISGF2/IRF-1 (32) possess antiviral activity when expressed in transfected cell lines. However, the important question of whether these proteins have similar functions in vivo has not been answered.

Most inbred mouse strains carry defective Mx genes that no longer code for a functional protein (41, 42, 44). These mice are highly susceptible to infections with influenza A virus (11) and Thogoto virus (12). Mice devoid of functional Mx genes would thus seem to represent an ideal experimental system for testing the antiviral activities of Mx proteins in vivo. The great potential of this system was emphasized by the recent demonstration that transgenic expression of the wild-type form of mouse Mx1 cDNA rendered such mice resistant to challenges with influenza A virus (3, 17) and Thogoto virus (12).

The MxA protein of human cells shows a high degree of sequence similarity to the antivirally active Mx1 protein of mice (1). Both proteins are GTPases (13, 24) that are synthesized in a large number of cell types in response to IFN (28). But unlike Mx1, the human MxA protein accumulates in the cytoplasm. It renders transfected cells resistant to several RNA viruses, including influenza A virus (30), measles virus (36, 37), Thogoto virus (9), and vesicular stomatitis virus (VSV) (30). MxA inhibits viral multiplication steps that follow virus penetration but precede genome amplification (27, 38, 43). We have now produced transgenic mice that lack endogenous Mx proteins but which permanently express the human MxA protein in various organs. These animals were highly resistant to challenges with Thogoto virus and showed reduced susceptibilities to influenza A virus and VSV. To generate MxA-transgenic mice, we microinjected fertilized oocytes with a plasmid construct that directs MxA cDNA expression via the promoter of the ubiquitously expressed mouse hydroxymethylglutaryl coenzyme A reductase gene (10). Similar expression vectors were successfully used to achieve strong constitutive expression of various cDNAs in transgenic mice (4, 20) and transfected cell lines (9, 12, 30). The 8-kb *Not*I restriction fragment was excised from pHMG-MxA (30) and purified by agarose gel electrophoresis. Microinjection of DNA into the pronuclei of fertilized eggs from (C57BL/6 × SJL)F_2 mice was done as described previously (17). Fifteen mice that developed after oviduct transfer were transgenic and transmitted the transgene to their offspring, as revealed by PCR analysis of blood samples. Standard PCRs were carried out with a pair of oligonucleotide primers corresponding to positions 1754 to 1776 and 2218 to 2240 of the MxA cDNA (1). Western blot (immunoblot) analysis showed that MxA cDNA was expressed in two lines of transgenic mice, designated G and L. Animals of the transgenic line G contained easily detectable levels of MxA protein in the brain, heart, kidney, spleen, thymus, and lung (Fig. 1), whereas very little MxA protein was present in liver and muscle. Transgenic mice of line L resembled those of line G with regard to the pattern and levels of MxA transgene expression

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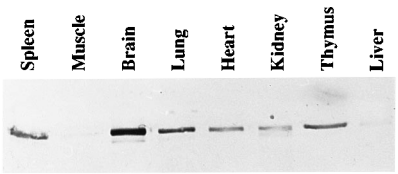

FIG. 1. Expression of MxA in various organs of transgenic mice. The frozen organs were homogenized and suspended in a solution containing 50 mM Tris-HCl (pH 8.3), 50 mM KCl, 5 mM $MgCl_2$, 1 mM EDTA, and 0.1% Triton X-100, and the cells were lysed by sonication. The lysates were cleared by centrifugation and mixed with sodium dodecyl sulfate-gel sample buffer. Samples (100 μg of protein per lane) were electrophoresed through a sodium dodecyl sulfate–10% polyacrylamide gel. Western blot analysis was carried out as described previously (1), using a monoclonal antibody specific for MxA (14).

(data not shown). The other 13 transgenic mouse lines did not contain measurable amounts of MxA mRNA or protein in the spleen and liver. To quantitate transgene expression, we determined the MxA concentrations in various organs of a transgenic animal of line G by enzyme-linked immunosorbent assay (46). Brain, spleen, and lung samples contained 440, 280, and 100 ng, respectively, of MxA per mg of soluble protein. The MxA concentration in the liver could not be determined because of a nonspecific reactivity of the antibodies. Third-passage embryonic fibroblasts from L mice homozygous for the MxA transgene expressed about 2,000 ng of MxA per mg of soluble protein. For comparison, our transfected 3T3 cell lines, which showed a high degree of virus resistance (30), contained about 20,000 ng of MxA per mg of soluble protein. Thus, organs and cultured cells of MxA-transgenic mice contained 10% or less of the amount of MxA protein contained by the cell lines previously used to study the antiviral activity of MxA (9, 30, 36).

To test whether MxA rendered cells from transgenic mice resistant to virus infection, we prepared cultures of embryonic fibroblasts from transgenic L mice and nontransgenic controls and challenged the confluent cell monolayers with several RNA viruses as described previously (30). At various times after infection with a multiplicity of 5 PFU per cell, the supernatant of the MxA-transgenic cell culture contained 10- to 40-fold less influenza A virus than the supernatant of the control culture (Fig. 2A). A similar picture emerged when transgenic and nontransgenic cells were infected with VSV: the culture of MxA-transgenic cells produced about 10-fold fewer infectious virus particles than the nontransgenic control culture (Fig. 2B). When the cells were challenged with Thogoto virus, which is extremely sensitive to the inhibitory effect of MxA in transfected 3T3 and Vero cells (9), the difference in virus susceptibility between transgenic and nontransgenic cells became even more obvious (Fig. 2C). The MxA-transgenic cell cultures showed no cytopathic effect, and no infectious Thogoto virus was released from these cells during the observation period. By contrast, Thogoto virus grew to high titers in cell cultures from nontransgenic mice. Infected embryonic fibroblast cultures from MxA-transgenic and nontransgenic mice produced similar amounts of mengovirus (Fig. 2D), a picornavirus which is not restricted by MxA in transfected 3T3 cells (30). To determine whether MxA could protect the transgenic animals from the deleterious effects of virus infections, animals of line G were first challenged with NWS, a neurotropic strain of influenza A virus (3, 18). Neither the transgenic mice nor the nontransgenic littermates survived infection with NWS by the intracerebral route. However, the transgenic mice died on day 6 on average, whereas the nontransgenic mice had a mean survival time of only 3.9 days (Fig. 3A). When challenged with the pneumotropic influenza A virus strain PR/8/34 by the intranasal route, all 13 nontransgenic mice died, whereas 3 of the 9 infected transgenic littermates survived (Fig. 3B). We also observed enhanced resistance of transgenic mice to infections with VSV: the 12 transgenic mice survived virus infection by the intracerebral route for 2.7 days on average, whereas the mean survival time of their nontransgenic littermates was only 1.5 days (Fig. 3C). L mice heterozygous or homozygous for the MxA transgene exhibited similar degrees of resistance to challenges with influenza virus strain NWS (data not shown). As breeding of G mice did not yield homozygous females, the effect of transgene homozygosity could not be determined for this MxA-transgenic line.

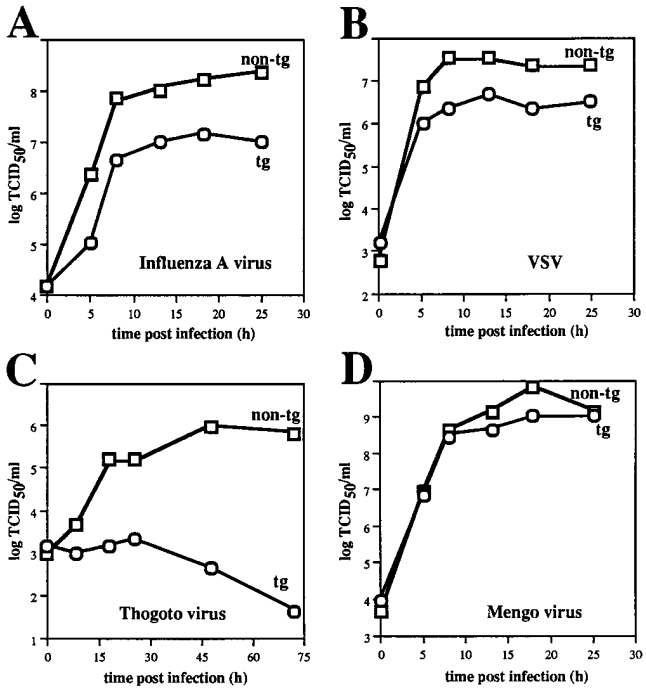

FIG. 2. Enhanced virus resistance of cultured embryonic fibroblasts of transgenic mice. Embryonic fibroblasts were prepared from 14-day embryos and passaged in Dulbecco's modified Eagle's medium containing 10% fetal calf serum as described previously (42). Cells from L mice homozygous for the MxA transgene (tg) and from nontransgenic animals of similar genetic background (non-tg) were infected with, per cell, 5 PFU of the FPV-B strain of influenza A virus (A/FPV/Dopson/34) (A), 4 PFU of VSV serotype Indiana (B), 1 PFU of Thogoto virus (C), and 5 PFU of mengovirus (D). After 45 min at room temperature, nonabsorbed virus was removed and the cultures were incubated at 37°C. Samples of the culture supernatants were collected at various times, and infectious virus was titrated on BHK-21 cells by determining the highest dilutions that destroyed 50% of the infected tissue cultures ($TCID_{50}$).

We next challenged the MxA-transgenic mice with Thogoto virus, which causes rapid viremia in susceptible mice. In a first experiment, L mice homozygous for the MxA transgene and nontransgenic BALB/c control mice were challenged with 10 PFU of Thogoto virus by the intracerebral route. None of the 9 transgenic mice died, whereas 9 of the 10 control mice died between 4 and 5 days postinfection (Fig. 4A). In a second experiment, the virus dose was raised to 100 PFU. Nine of the ten transgenic animals survived the Thogoto virus challenge, whereas all eight BALB/c control mice died (Fig. 4B). In a third experiment, we infected the mice with 10 PFU of Thogoto virus by the intraperitoneal route. All five transgenic L mice survived the virus challenge, whereas three of the five nontransgenic C57BL/6 control mice died (Fig. 4C). In a fourth experiment, we challenged the mice with 1,000 PFU of

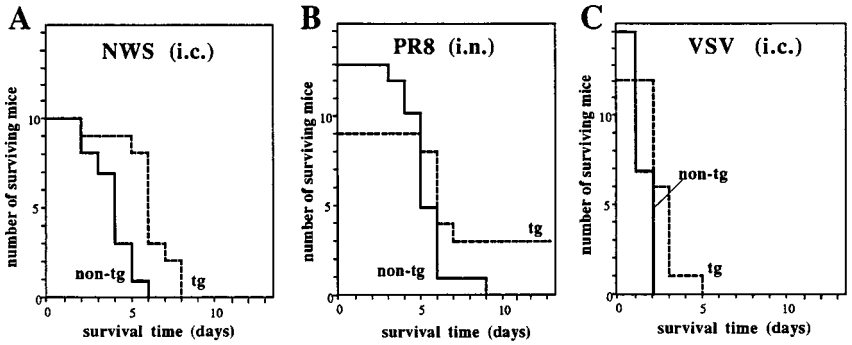

FIG. 3. Enhanced resistance of transgenic mice to infections with influenza A virus and VSV. Groups of heterozygous transgenic animals of line G (tg) and their nontransgenic littermates (non-tg) were infected with 20 50% lethal doses (LD_{50}) of strain NWS (A) or strain PR8 (B) of influenza A virus by the intracerebral (i.c.) or intranasal (i.n.) route, respectively. Other animals (C) were infected with 300 PFU of VSV by the intracerebral route. Survival of the infected animals was monitored at 24-h intervals for 14 days. Stocks of the mouse-adapted influenza virus strains A/NWS (3, 18) (2×10^4 LD_{50}/ml) and A/PR/8/34 (4×10^3 LD_{50}/ml) were prepared from allantoic fluid of infected chicken eggs. The stock of VSV serotype Indiana (3×10^8 PFU/ml) was a supernatant of virus-infected Swiss 3T3 cells.

Thogoto virus by the intraperitoneal route. All four transgenic animals survived, whereas all four nontransgenic C57BL/6 mice died (Fig. 4D). To demonstrate that Thogoto virus resistance of transgenic mice was due to MxA and not to some other genetic traits that might fortuitously be present in our transgenic mice of mixed genetic background, we crossed the homozygous L mice with nontransgenic C57BL/6 mice and analyzed the offspring of the second backcross generation for the MxA transgene and for resistance to intracerebral infections with 100 PFU of Thogoto virus. All 11 mice that inherited the MxA transgene survived the Thogoto virus challenge, whereas all 8 mice without the transgene died from fulminant viral hepatitis within 6 days after infection (Table 1). These experiments confirmed the beneficial effect of the MxA transgene during Thogoto virus infection.

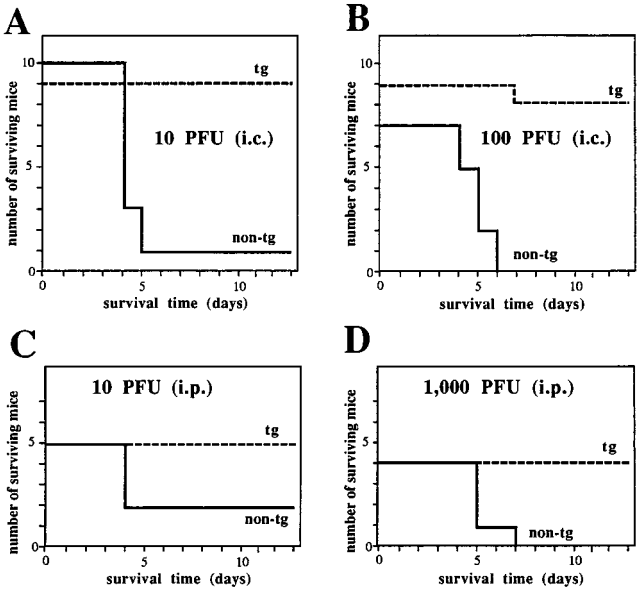

FIG. 4. Resistance of MxA-transgenic mice to intracerebral and intraperitoneal infection with Thogoto virus. Groups of L mice homozygous for the MxA transgene (tg) and nontransgenic BALB/c and C57BL/6 control animals (non-tg) were infected by the intracerebral (i.c.) (A and B) or intraperitoneal (i.p.) (C and D) route with the indicated doses of Thogoto virus. Survival of the infected animals was monitored at 24-h intervals for 14 days. A liver homogenate from a Thogoto virus-infected BALB/c mouse (12) (10^6 LD_{50}/ml; 3×10^6 PFU/ml) served as the stock virus.

Experimental evidence supporting the view that IFN-regulated genes play a prominent role in the antiviral defense of humans has been lacking. Our experiments with MxA-transgenic mice now demonstrate that the human MxA protein indeed exhibits antiviral activity in vivo and can prevent fatal disease after Thogoto virus infection. Thogoto virus is transmitted by ticks and has many features in common with the influenza viruses (26). Standard laboratory mice develop severe disease symptoms and die within days after infection with Thogoto virus (8). By contrast, our infected MxA-transgenic mice remained healthy, demonstrating that a susceptible mouse can be rendered virus resistant by expression of a single human cDNA. The mice survived not for immunological reasons but rather because they were equipped with an antiviral protein that is normally induced by IFN and which operates directly in the host cells by blocking virus genome amplification. We previously showed that MxA, which is present at low concentrations in blood cells of most humans, is induced to high levels during virus infections (34). It thus seems safe to assume that MxA is also protective during natural infections of humans with certain viral pathogens. MxA expression is not inducible in our transgenic mice. It is conceivable that the rather poor protective effect of the MxA transgene against influenza virus and VSV could be improved by expressing it under the control of an IFN-inducible promoter.

We found that only a low percentage of our founder mice expressed the MxA transgene at detectable levels and that expression was not uniform in the various organs. This finding was unexpected because the promoter that we used should

TABLE 1. Cosegregation of the MxA transgene and the Thogoto virus resistance phenotype[a]

Litter	Mortality (no. dead/no. infected)	
	Transgenic	Nontransgenic
A	0/7	3/3
B	0/3	3/3
C	0/1	2/2
Total	0/11	8/8

[a] Heterozygous males of the MxA-transgenic mouse line L were backcrossed with nontransgenic C57BL/6 females. Offspring were tested for the MxA transgene by PCR analysis of blood samples and infected with 100 PFU of Thogoto virus by the intracerebral route. Susceptible mice died within 4 to 8 days after infection. The experiment was terminated at 14 days postinfection.

permit ubiquitous expression of transgenes in mice (10, 20). Our results suggest that constitutive expression of MxA in certain organs is deleterious, as has been suggested for the mouse Mx1 protein (2, 3, 17). It should be noted that a construct that permits MxA synthesis under the control of the IFN-regulated promoter of the mouse Mx1 gene gave no improvement: 8 of the 10 transgenic mice that carried this construct failed to express MxA after treatment with an IFN-inducing substance. The other two lines of transgenic mice contained only very low levels of MxA in the brain and spleen but not in the liver and lung (29). This result was surprising considering the fact that a similar construct was successfully used to create transgenic mice that expressed the mouse Mx1 protein at high levels in various organs (3). Thus, successful expression of MxA in transgenic mice seems to require stringent spatial and temporal control.

Inhibition of VSV and influenza A virus in transfected cells requires much higher levels of MxA than inhibition of Thogoto virus (9). Our transgenic mice, which express rather low levels of MxA, could therefore not be expected to show a very high degree of resistance toward the former viruses. However, they were expected to exhibit a higher degree of resistance toward Thogoto virus. Our experimental findings completely agreed with these expectations. Considering the hepatotropic nature of Thogoto virus and the fact that our transgenic mice contained very little MxA in the liver, it was rather surprising that they resisted intraperitoneal challenges with Thogoto virus. It is possible that low levels of MxA are sufficient for strong inhibition of Thogoto virus in hepatocytes. Alternatively, the liver may not represent the first site of virus multiplication after intraperitoneal infection of mice with Thogoto virus. Possibly, the cell population in the peritoneum which normally supports the initial rounds of Thogoto virus replication expressed MxA at a high level. This might have prevented the spreading of virus to the liver in transgenic mice.

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