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Characterization and Antiviral Effects of Mx Proteins from Various MHC Haplotype Chickens Showing Different Susceptible to Marek's Disease Virus

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Chicken Mx protein (cMx) induced interferon (IFN) is an antiviral protein to inhibit replication of RNA virus, particularly negative stranded RNA virus, through blockage of transfortation of viral RNA and proteins. In order to determine antiviral effects of cMx from different MHC haplotype chicken, we characterized cMx gene by studying on nucleotide sequencing, antiviral effects to Newcastle disease virus, VSV and MDV, and transcription activities. Three types of cMx genes (2,118 bp) were detected from the different MHC haplotype chickens [B19 (N), B15 (F) and B21 (GSP)] chickens, which have showed different susceptible to Marek's disease (MD). Several amino acid substitutions were showed in the cMx. The amino acid 548 and 631 in the cMxs from N and F, chickens susceptible to MD, was Val and Asn which was important on antiviral effects, and showed in resistant cMx. Those in the cMx from GSP, chicken resistant to MD, were same that showed in susceptible cMx. Though every cMx transactivated the expression of the reporter gene, the transcription activation by resistant cMx from N and F was lower compared to that by susceptible cMx from GSP. The decease of the cell growth in the resistant cMx cloned cells was seen in comparison with another cMx clone cells. Replication of NDV and VSV was suppressed in the clones with resistant cMx from N and F. NMx258-transducted cells lack of antiviral effects, and NMx437 or NMx646-transducted cells was showed 60% of antiviral effects compared to NMx705. Mean death time (MDT) and hemaggutination (HA) titer to NDV was long and low in the eggs of N and F lines, but short and high in the egg of GSP line. Interestingly, strong suppression to NDV was observed in the clone with N-Mx and in the eggs of N line. However, the effects of Mx for replication of vvMDV1 have not been. Thus, resistant types of cMx, N- and F-Mx, have showed the anti-viral effects to only RNA virus including NDV and VSV, but not to DNA virus. Antiviral effects of cMx were required whole length of amino acid including Val and Asn in amino acid 548 and 631.

Key Words: Antiviral effects, Chicken Mx genes, Marek's disease virus, Newcastle disease virus, Transcription activities

INTRODUCTION

Interferon (IFN) is very important cellular self-protection protein to virus infection. The antiviral effects of interferon (IFN)-α have been consisted of three major antiviral mechanisms that are degradation of viral RNA by The 2-5A synthetase/Rnase L following activation by double stranded RNA (dsRNA), inhibition of viral mRNA translation by PKR and p56 phosphorylating the translation factor eIF-2α, and blockage of transportation of viral RNA and proteins by Mx. (Staeheli, 1990; Stark et al., 1998; Samuel, 2001).

Mx protein show a broad antiviral effects to negative-stranded RNA viruses. The various Mx proteins differ widely with respect to biologic activities. Mouse Mx1 and rat Mx1 efficiently block the replication of influenza virus and Togoto virus (Sandrock et al., 2001; Dittmann et al., 2008). Rat Mx2 and mouse Mx2 inhibit the multiplication

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of VSV and bunyavirus (Sandrock et al., 2001; Stertz et al., 2007). Human MxA shows broader antiviral activity against influenza virus, Thogoto virus, vesicular stomatitis virus, mealsles virus, and hepatitis B virus in human (Haller et al., 1995; Pavlovic J et al., 1998; Rosmorduc et al., 1999; Gordien et al., 2001). However, human MxB and rat Mx3 are cytoplasmic proteins without recognized antiviral activities. Duck Mx protein seems to lack antiviral activity and is found in the nucleus and in the cytoplasm of induced duck cells (Bazzigher et al., 1993). Chick Mx protein (cMx) is mainly cytoplasmic and had a granular appearance, and lacks antiviral activity (Bernasconi et al., 1995). Recently, polymorphisms and the differential antiviral effects of the chicken Mx gene have been reported (Ko et al., 2002). Furthermore, various Mxs have been discovered in the fish, sheep, swine, horse, and cattle (Trobridge and Leong, 1995; Robertsen et al., 1997; Ellinwood et al., 1998; Jensen et al., 2000; Yankey et al., 2001). Our results show that amino acid 548 and 631 of C-terminal of resistant Mx is the most important for antiviral effects under presence of leucine zupper motif and tripartite GTP binding domain

IFN is able to regulate the major histocompatibility complex class (MHC) I and II proteins which play important roles in immune response to infected cells (Samuel, 2001). It has been shown that resistance to MD is associated with alleles of the B-F region of the major histocompatibility complex (MHC) (Briles et al., 1983). The B21 alleles are more strongly associated with resistance to MD compared to other alleles. The MHC haplotype B21 (B21 B21) chickens are very resistant and the MHC haplotype B19 (B19 B19) chickens are very susceptible. The notable difference among lines of chickens is the level of expression of the MHC class I molecules on the cell surface (Kaufman et al., 1995); much lower expression in B21 lines.

In addition, genetic susceptibility of indigenous chicks to subgroup A Rous sarcoma virus inoculated via the chorioallantoic membrane has been showed differently (Rout et al., 1992). In chicken farms, a disease occurrence was showed differently depending on chicken line. The infection by viruses is occurred in both resistant and susceptible lines of chickens. However, the level of infection is lower in resistant birds, and the disease occurrence and re-emergence of the infection, which occurred in susceptible lines of chickens, are absent in resistant chickens. Although the resistance to viruses seems to be closely related to the genotype of chickens, the in vivo differences among lines of chickens have not been studied yet.

Thus, in this study, we have isolated the Mx genes from four kinds of chicken lines showed different susceptible to MD, determined the sequence, and cloned a vector to express in the mammalian cell. In order to elucidate antiviral effects and transcription activity of isolated chicken Mx genes (cMx), we have compared the antiviral activities against NDV and VSV to the cells transfected with the cMx plasmids, and measured transactivation activities of every cMx using reporter assay. We have measured the antiviral effects of Mx deletion mutants to find the antiviral site in the cMx. We have studied on difference of antiviral effects against NDV among chicken embryo eggs including 3 kinds of cMx, which is N-Mx, F-Mx, or G-Mx.

MATERIALS AND METHODS

Experimental chickens

Embryonated chicken eggs of GSP line (B21), WL-BM line (B15), WL-F line (B15), and WL-N line (B19) were obtained from Nippon Institute for Biological Science (Tokyo, Japan), incubated in our laboratory and used for isolation of CEF and infection of NDV.

Cells and viruses

Chicken embryo fibroblast (CEF), which prepared from 11-day-old embryonated eggs, was maintained with Eagle's Modified Essential Medium (EMEM, Nissuiseiyaku, Tokyo, Japan) supplemented with 0.6% tryptose phosphate broth and 10% calf serum (CS). NIH 3T3 cells, a mouse embryo fibroblast cell line, were obtained from Niken cell bank (Tsukuba, Japan), and maintained in Dulbecco's Modified Eagle Medium (DMEM, GIBCO BRL, Grand Island, NY) supplemented with 10% fetal calf serum (FCS).

Strains of very virulent MDV1, Md5, and virulent MDV1, JM, which is attenuated by serial passages, were obtained from chicken kidney cell culture from experimentally infected chickens. A velogenic strain of Newcastle

disease virus, Sato and Yukaipa, was isolated from chickens infected with NDV. MDV and NDV viruses were propagated in CEF. When cytopathic effects were confluent, the infected cells were harvested and, virus titers were measured by plaque assay. These MDV-infected CEFs and NDV-infected supernatants were used for further experiments. VSV was obtained from Nichen (Tsukuba, Japan), and propagated in BHK cell.

Stimulation of CEF with poly(I) (C)

mRNA (poly(A) +RNA) was extracted by mRNA extraction kit (Pharmacia) from chick embryo fibroblast cells treated with the IFN inducer poly(I) (C). cDNA synthesis was performed with manufacturer's instructions.

Cloning of cMx gene and construction of plasmids to express cMx protein

The cDNA was used as a template for nested PCR amplification to detect the Mx gene. The first round of PCR was performed with a primer set (Ko et al., 2002), Mx-S and Mx-AS, to amplify the 2.1-kb Mx gene fragments. After the amplification, 1 μl of the reaction was used for the second round of PCR. The second round of PCR was performed using Mx1 and Mx8 primer sets, to amplify 2.1-kb fragment. The amplified Mx genes were inserted into pGEM easy T vector (Promega). PGEM-NMx, -FMx, and -GMx plasmids were constructed.

To construct vectors to express the Mx genes in mammalian cells, the constructed pGEM-Mx plasmids digested with *Not*I. The digested fragments containing Mx ORFs were cloned into the *Not*I of the pCI-neo vectors (Promega, Madison, WI), which permits constitutive high-level expression of recombinant proteins in various cell types, to construct pCI-NMx705 -FMx705, and -GMx705 plasmids, respectively. In order to elucidate antiviral sites and transcription site in the cMx, we constructed 3 types of cMx mutants which are pCI-NMx258, -NMx437 and -NMx646 (Fig. 1B).

DNA sequencing

The plasmids were purified by standard mini-prep method, and sequenced by using BigDye terminator cycle sequencing kit (Applied Biosystems, Foster City, CA) and Model 310 genetic analyzer (Applied Biosystems). The nucleotide sequences of the chicken Mx genes have been submitted to GenBank, and addigned for accession No. AB088533 (cMx from WL-N), AB088534 (cMx from WL-F), AB088535 (cMx from WL-BM), and AB088536 (cMx from GSP) (Fig. 2).

Renilla luciferase assay

In order to analyze transactivation of N-, F- or G-Mx plasmids were contransfected with the pRL-CMV plasmids into NIH 3T3 cell lines. Transfection of NIH 3T3 cells (1×10^5/ml cells) were carried out in 12-well plate culture format using the FuGENE™ 6 Transfection Reagent (Roche Diagnostics GmbH, Mannheim, Germany) following manufacturer's protocol. For each well, a total amount of 1.5 μg of vectors were used, including 0.5 μg of the reporter plasmid, and 0.5 μg each of the expression plasmid and control plasmid, pRL-CMV, in which the cytomegalovirus enhancer and immediate-early promoter are inserted into the upstream of the chimeric intron and Renilla luciferase reporter gene. After the transfected cells were incubated at 37℃ for 36 hours, the cells were lysed with Passive Lysis Buffer (PLB), and luciferase activity was measured using Dual-Luciferase reportor assay system (Promega, WI, USA), and a Luminescencer-JNR AB-2100 (ATTO, Tokyo, Japan). The luciferase activities presented were representative of four different experiments.

Expression of chick Mx in NIH 3T3 cell line

NIH 3T3 cells were grown in DMEM containing 10% fetal calf serum (FCS); the chick fibroblast cell line was grown in DMEM containing 7% FCS. The complete coding sequences of cMx cDNA clones were cloned into the *Not*I site of expression vector pCI-neo, which permits constitutive high-level expression of recombinant proteins in various cell types. The resulting constructs were transfected with the FuGene 6 into NIH 3T3. Plasmid pCI-neo encodes resistance to the drug G-418 was used for permanent transfections. Seletive medium contained 500 μg per ml G-418 (GIBCO BRL). For transient transfections, the cells were shocked for 4 min with DMEM containing 10% glycerol at

24 h post-DNA addition. They were incubated for another 24 h in DMEM containing FCS before infection with virus and plaque analysis. To know expression level of Mx protein, mRNA was isolated by mRNA extraction kit (Pharmacia), and amplified by RT-PCR.

Plaque assay

Effects of various cMxs on the production of VSV and NDV were studied using confluent monolayers of cells expressing N-, F- or G-Mx in 12-well plates. The NIH 3T3 cell transducted with pCI-neo served as the control. The cells transducted with cMx were infected with VSV and NDV at multiplicities of infection (MOI) indicated in individual experiments. Culture supernatants were collected at 24 h postinfection, unless otherwise stated, and the infectious virus yield was measured by plaque assay on BHK or Vero cell for VSV, and CEF or MDBK for NDV.

Similarly, the effects of chicken INF alpha on antiviral effects of chicken Mx proteins were measured using NIH 3T3 cells expressing both INF alpha and different chicken Mx in 12-well plates. The cell lines were infected with VSV and NDV at the MOI indicated for individual experiments. At 24 h postinfection, culture supernatants were collected and infectious virus yields were quantitated by plaque assay.

Mean death time (MDT) and HA titers in the embryonated chicken eggs of MHC haplotype chickens infected with NDV

Embryonated chicken eggs were incubated for 9~10 days at 37℃ before use. Each of five or six embryonated eggs was inoculated with 0.2 ml of the Sato or Yukaipa strains of NDV into the allantoic cavity. The eggs were placed at 37℃ and examined regularly. Eggs dead or dying were chilled to 4℃ and the allantoic/amniotic fluids were harvested. The viral titers of fluids were measured by an HA test using 0.5% chicken RBC.

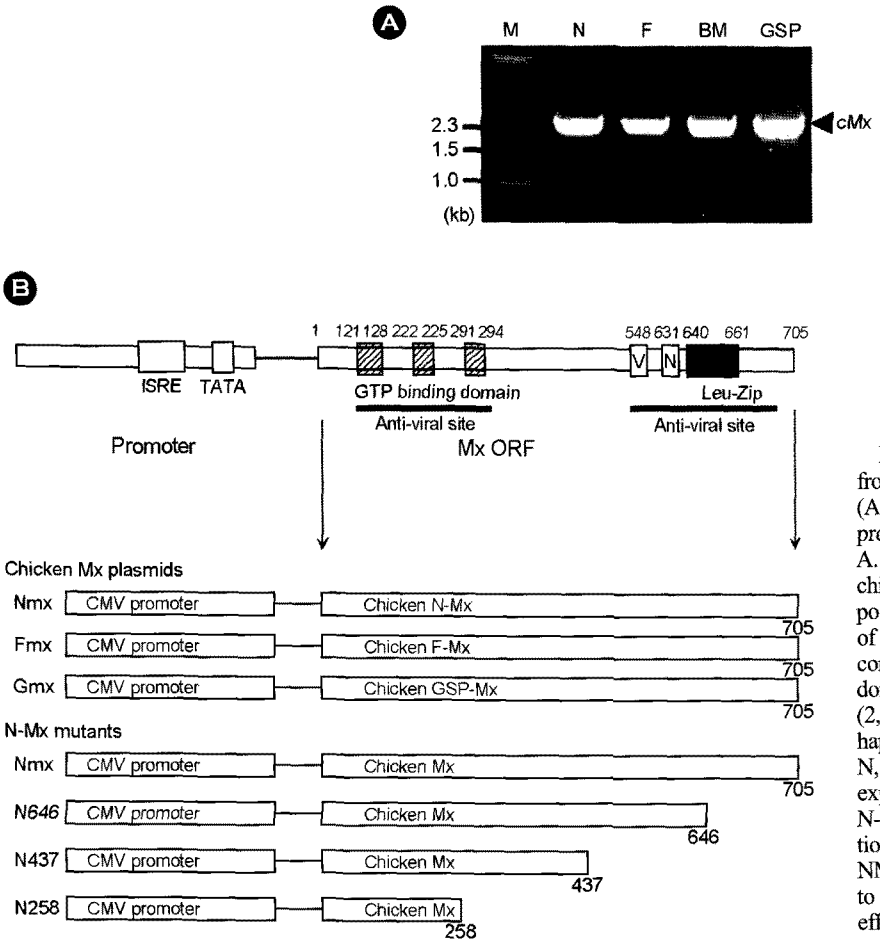

Fig. 1. detection of chicken Mx genes from chicken embryo fibroblast (CEF) cells (A) and Structure of the chicken Mx proteins and construction of plasmids (B). A. Detection of 2,118-bp Mx genes from chick embryo fibroblast cells stimulated by poly(i) (c) using RT-PCR. B. The Mx ORF of chicken encodes a 705 amino-acid protein consisting of an N-terminal GTP binding domain and the leucine zipper motif. cMx (2,118 bp) genes from different MHC haplotype chickens, which are GSP, F and N, were inserted into pCI-neo vector for expression in mammalian cell. G-, F- and N-Mx plasmids were constructed. In addition, various deletion mutants, NMx258, NMx437, and NMx646, were constructed to determine a domain related to antiviral effects of chicken Mx.

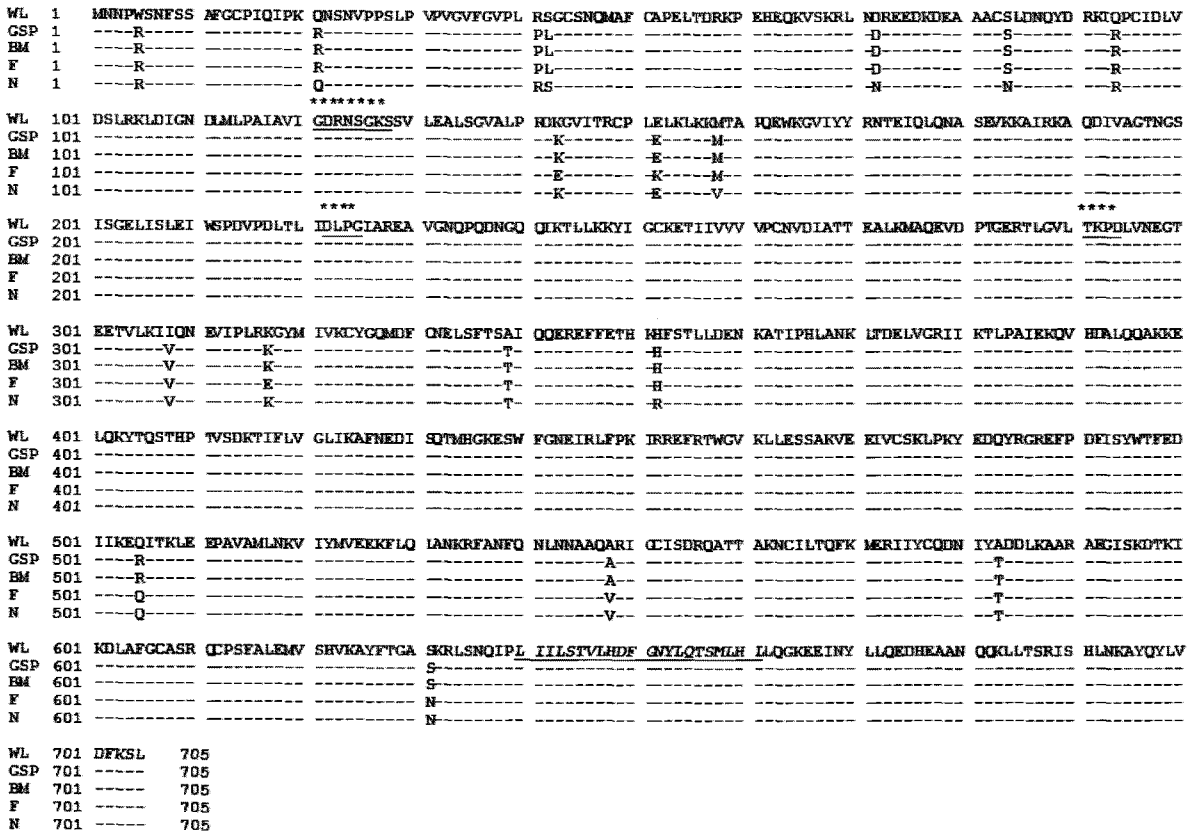

Fig. 2. Comparison of 705 amino acid sequences of chick Mx proteins from reported white leghorn, GSP (B19), BM (B15), F (B15) and N (B21) strains which express different major histocompatibility complex (MHC) and have been showed different susceptibility against MDV. Dashes indicate the presence of identical amino acids at corresponding position in various Mx proteins. Asterisks mark the tripartite GTP binding consensus motif. Critical residue of a leucine zipper motif is underlined. The differences among 2,118-bp sequence and 705 amino acid of open reading frame (ORF) of four cMxs were exchanges of 28 bp and 18 amino acids, respectively, though the homology among the cMx genes was very high. In particular, the reported amino acid 631 (Ser to Asn) in the C-terminal of cMx was important on antiviral effects.

RESULTS

Cloning and sequencing of cMx genes from various lines of chickens

To elucidate the difference among cMx proteins from various MHC haplotype chickens resistant or susceptible against MDV, 4 kinds of chick embryo fibroblast (CEF) cells were treated with poly(I) (C) for 24 h. Mx mRNAs were extracted from the cells, amplified as a length of about 2.1 kb by PCR (Fig. 1A), and cloned into pGEM T easy vector. The sequences of 2,118-bp of Mx cDNA were decided using 4 pairs of primer sets. The deduced 705 amino acid sequence of cMx protein is shown in Fig. 2. Nucleotide substitutions have been occurred in the 28 sites of Mx mRNAs, and amino acid substitution in 18 sites of Mx protein. Homologies of nucleotide and amino acid sequences among Mx mRNAs are about over 98.7% and 97.4%, respectively. Comparing the primary sequence among various cMx proteins yielded some potentially important insights into the Mx protein structure. GTP-binding domain and leucine zipper motif have been conserved in the various Mx proteins. Variable region has been observed in the N-terminal of Mx protein. In addition, we compared the amino acid substitutions in each chicken line (Table 1). We have divided 2 group of cMx depending on amino acids 548 and 631. We selected Mx genes from GSP, F, N line for transcription activity and antiviral effect of cMx.

Cell growth of NIH 3T3 cells expressing cMx protein

In order to investigate the expression of cMx protein in

Table 1. The representative substitution of amino acid sequences in various Mx proteins from chickens expressed differently MHC

Chicken lines	Resistance		Substitution of Mx amino acids								
	MDV	Mx	72	84	143	152	158	317	352	548	631
GSP (B21)	R	?	D	S	K	D	M	K	H	A	S
F (B15)	MR	?	D	S	E	K	M	E	H	V	N
N (B19)	S	?	N	N	K	E	V	K	R	V	N

S: sensitive type, R: resistant type, MR: mild resistant type.

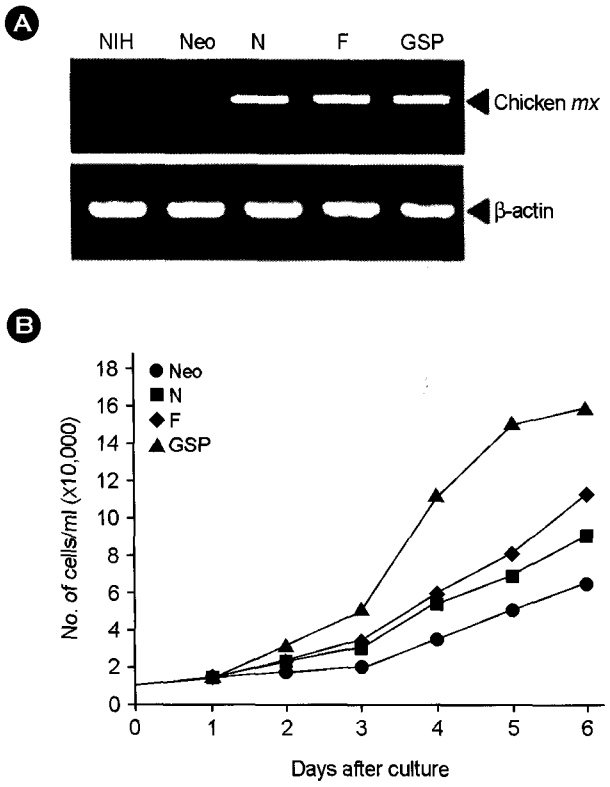

Fig. 3. The expression of Mx mRNA (A) and the effects of chicken Mx for cell growth (B) in each clone. A. Total RNA from transfected NIH 3T3 cells was amplified by RT-PCR using specific chicken Mx (top) and β-actin (bottom) primer sets. NIH 3T3, parental NIH 3T3 cells; neo, control NIH 3T3-pCIneo, N, NIH 3T3 cells expressing N Mx, F, NIH 3T3 cells expressing N Mx, GSP, NIH 3T3 cells expressing N Mx. An aliquot of each PCR product was elecrophoresed on a 1.5% agarose gel and visualized with ethidium bromide. B. Typical curve of cell growth obtained various chick Mx clones from GSP (B19), BM (B15), F (B15) and N (B21) strains. Cells were seeded at a confluence of 10,000 cells per well of twelve-well plates, trypsinized, and counted daily. Experiments were done in duplicate at least triple, and vertical bars represent mean ± SEM.

the NIH 3T3 cells transfected with various Mx plasmids, the cells were tested for their Mx expression by RT-PCR using Mx1 and Mx2 primer sets to amplify a length of about 0.8 kb (Fig. 3A). We selected one clone, which expressed the Mx protein at a high level compared to another clone, from cells transducted with each Mx. A clone expressing the neomycin resistance gene only was also selected as a negative control. The effect of cMx protein on cell growth was observed. No significant influence on the growth rate of the G- and F-Mx clone was seen in comparison with the neo clone. A reduction of cell growth was observed in the N-Mx clone after 2 days of culture: this probably reflected a chicken line related to Mx expression, since modification was seen in the G-Mx clone. In addition, identical expression of beta-actin protein was observed in the different cell clones (Fig. 3B).

Chicken Mx gene showed high transactivation function

In order to determine transactivation activity of Mx, we performed Renilla luciferase assay using mammalian expression vector to express each Mx from N-, F-, or GSP-lines of chicken and reporter vectors under the control of the CMV immediate-early promoter. When these vectors were transfected into the NIH 3T3 cells, Mx mRNA were detected at 2 days after transfection, determined by RT-PCR. In the presence of N-, F-, or GSP-Mx, luciferase expression under the control of the CMV immediate early promoter was increased 2.8, 6.5 and 10.7 times in the NIH 3T3 cells, compared to that in the presence of the negative control, pCI-neo vector without inserts (Fig. 4A). These results show that the transactivation by G-Mx was reacted differently compared to that by N- and F-Mx.

To know the transactivation of defective cMx mutants, we measured transcription activities of constructed NMx258, NMx437 and NMx646 mutants. The defective Mx mutants were showed an increase of transactivation in transducted cells. NMx437 mutant were showed the most transcription activity ($P<0.01$) (Fig. 4B).

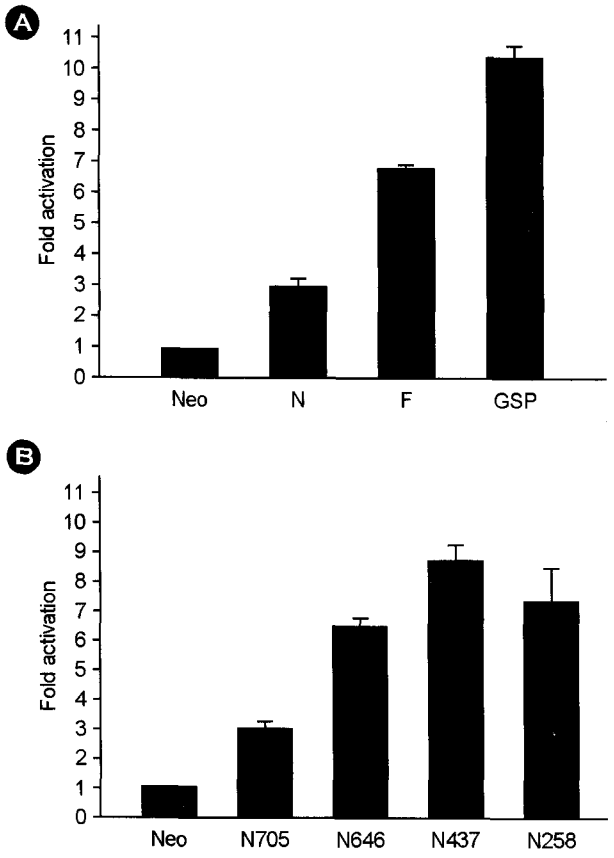

Fig. 4. Transactivation activity of the various chicken Mxs from different MHC haplotype chickens (A) or Mx mutants from N (B19) chicken species (B) in NIH 3T3 cells. NIH 3T3 cells were cotransfected with various pCI-Mx or Mx deletion mutants or control pCIneo and reporter plasmid under the control of CMV, and luciferase activities were determined as described in the Materials and Methods. Results are shown as relative ratios of luciferase activity detected in cells transfected only with respective reporter plasmids. Experiments were done in duplicate at least triple, and vertical bars represent mean ± SEM (P<0.01).

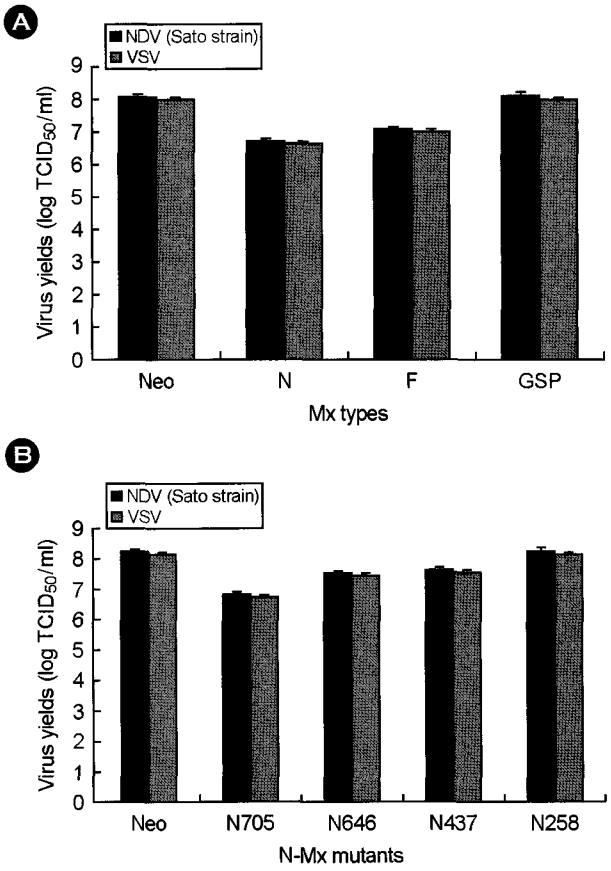

Fig. 5. Inhibition of virus multiplication by various cMxs (A) and Mx deletion mutants (B). PCIneo-transfected NIH 3T3 cell and independent clone lines of NIH-NMx, NIH-FMx, NIH-GMx, or NIH-NMx mutants cells were infected with 1 PFU of VSV or NDV (Sato strain) per cell. The viral titers in the culture supernatants at 24 h postinfection are plotted. $TCID_{50}$, 50% tissue culture infective dose. Experiments were done in duplicate at least triple, and vertical bars represent mean ± SEM (P<0.01).

Chicken N- and F-Mx genes confer enhanced resistance to NDV and VSV

To know the antiviral effects of various chicken Mx, we infected confluent-cell monolayer with viruses representing two different families, namely, Paramyxoviridae (Newcastle disease virus Sato and Yukaipa strains), and Rhabdoviridae (VSV). All viruses that we used were cytopathic and lysed susceptible 3T3-neo cells completely within 24 h after infection at 1 PFU per cell. Samples of individual culture supernatants were removed at 24 h postinfection and assayed for infectivity. For NDV, the titers were maximal at 12 and 24 h postinfection and decreased thereafter. The virus yields of VSV reached a plateau between 12 h and 24 h. Titers of the samples taken at 24 h postinfection are shown in Fig. 5A.

The cells expressing the NMx or FMx showed a high degree of resistance to NDV and VSV. The viral titers at 24 h postinfection were 100- to 200- fold lower than the titers of the control cells transducted with pCI-neo. However, the cells expressing the G-Mx showed no enhanced resistance to NDV or VSV. These results suggest that N- and F-Mx are resistant though G-Mx is sensitive to NDV and VSV (Fig. 5A). The permanently trnasfected NIH 3T3 cell clones expressing N-, F- or GSP-Mx was infected with the MDV. All cells expressing chicken Mx confer no enhanced resistance to MDV. These results suggest that antiviral effects of chicken Mx have been related with RNA viruses

but not with DNA virus like MDV (results not shown).

To know antiviral effects of defective chicken Mx mutants, we measured the antiviral effects of constructed NMx258, NMx437 and NMx646 mutants. NMx258-transducted cells lack of antiviral effects, and NMx437 and NMx646- transducted cells was showed 50- to 60-fold of antiviral effects compared to control cell transducted with pCI-neo. NMx646 lacks of leucine-zipper motif but included amino acid 631. NMx437 laks of leucine-zipper motif and amino acid 548 and 631, but includes 3 sites of GTP-binding domain. NMx258 include only 2 site of GTP-binding domain (Fig. 5B). These results show that requirements for antiviral effects of chicken Mx are amino acid 548 and 631 as well as leucine-zipper motif and 3 sites of GTP-binding domain.

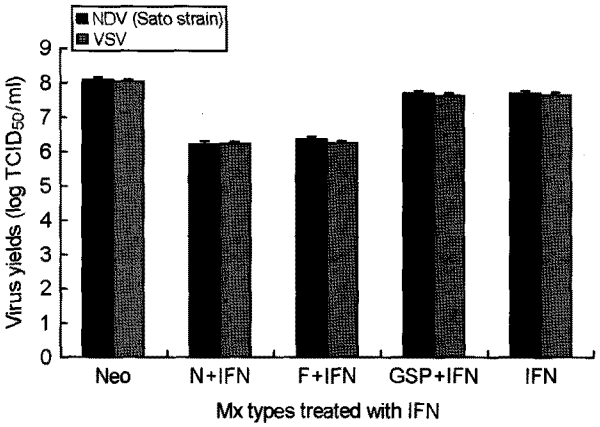

Fig. 6. Effects of IFN-α for inhibition of virus multiplication by various cMxs and Mx deletion mutants. PCIneo-transfected NIH 3T3 cell and independent clone lines of IFN-NMx, IFNFMx, IFN-GMx, or IFN-NMx mutants cells were infected with 1 PFU of VSV or NDV (Sato strain) per cell. The viral titers in the culture supernatants at 24 h postinfection are plotted. $TCID_{50}$, 50% tissue culture infective dose. Experiments were done in duplicate at least triple, and vertical bars represent mean ± SEM (P<0.01).

IFN doesn't enhance the antiviral effects of chicken Mx

To know effects of IFN on antiviral effects of chicken Mx, we measured antiviral effects in cells transducted with both IFN and chicken Mx. IFN increased slightly antiviral effects of N- and F-Mx, but not that of G-Mx (Fig. 6). These results suggest that antiviral effects of cMx are not related with chicken IFN. cMx proteins include at least two type of Mx proteins, which are resistant and sensitive cMx proteins.

Resistant line of chicken against NDV infection

To elucidate the difference among 3 kinds of chicken lines against NDV infection *in vivo*, NDV was infected to allantoic cavity of chick eggs at 9 days old. We measured the mean death time (MDT) of chicken embryo every 12 h pi. MDTs against Sato strain of NDV were 0% (0/6), 67% (4/6), and 83% (5/6) at 3 day pi, and HA titers of the collected allantoic fluids were 16~32, 64~128 and 256~512 HA titers to N, F, and GSP lines of chicken, respectively (Table 2). MDTs against Yukaipa strain of NDV were 17% (1/6), 17% (1/6), and 83% (5/6) at 3 day pi, and HA titers of the collected allantoic fluids were 8~16, 32~64, and 128~256 HA titers to N, F, and GSP lines of chicken, respectively (Table 2). These results showed that N and F lines are resistant type, but GSP line is sensitive type against NDV infection. We supposed that resistant type might be resulted from the effects of Mx genes.

Table 2. The means of dead time of embryo fetus and HA titer of chorioallantoic fluids at 9- day-old eggs infected with NDV

Chicken lines	NDV strains	Hours pi							HA titer
		0	12	24	36	48	60	72	
N	Sato	6*	6	6	6	6	6	6	16~31**
	Yukaipa	6	6	6	6	5	5	5	8~16
F	Sato	6	6	4	2	2	2	2	64~128
	Yukaipa	6	6	6	5	5	5	5	32~64
GSP	Sato	6	6	3	2	1	1	1	256~512
	Yukaipa	6	6	4	3	2	1	1	128~256

N, F or GSP were infected with 256 HA titer of Sato or Yukaipa strains.
*No. of live eggs.
**HA titers of chorioallantoic fluids from GSP, F and N chicken eggs infected with 256 HA titer of NDV at 72 hr pi.

Table 3. Summary of resistance for chicken line against MDV, NDV or VSV

Chicken lines	Chicken strains	MDV	NDV	VSV
B21	GSP (GSP, H, g), FS (FS), G	R	S	S
B15	F (F, WL-F), BM (BM-C)	MR	MR	MR
B19	N (N1, N2, etc)	S	R	R

MR: mild resistant, S: sensitive, R: resistant.

DISCUSSION

The various anti-viral effects of Mx gene have reported in mammalian. However, currently, very little is known about the relationship between disease progression and Mx gene in host chickens though Mx gene appears to play an important role in mammalian.

Only a little information is available for relationship between pathogenesis of infectious diseases and Mx gene in chickens. Mx gene have induced by interferon and double stranded RNA and produced from fibroblast cells. We have detected cMx gene from chicken embryo fibroblast (CEF) cells and PBMC of chicken infected with Md5, which is very virulent MDV1. The cMx could show antiviral effects throughout in the whole body. However, polymorphism of Mx depending on amino acid substitution, particularly, has been observed among the different MHC haplotype chickens. The transcription activities and antiviral effects of various Mx genes have been different following the amino acid substitution.

cMx sequences could be divided two groups which are resistant and sensitive type of cMx. cMxs from N and F are resistant type of cMx, that from GSP is sensitive type of cMx. As shown in Table 3, resistance against MDV and NDV has reacted differently in the different MHC haplotype chickens. These results are very interesting, though the reason has not been known. These discovering can support the development of new race, which resist against disease and show strong immunity. All Mx genes have activated strongly transcription activity. No differences are among 3 kinds of Mx genes on transactivation, though the transactivation activity of the Mx from N line of chicken was lowest among 3 kinds of Mx from different chicken line. Cell growth was related with transactivation activity of Mx. However, antiviral effects were different among 3 kinds of Mx genes. Amino acid of C-terminal was related with antiviral effects. In addition, amino acid of central domain of cMx might be related closely with antiviral effects to RNA viruses. GTP-binding domain is important for transcription activity but not for anti-viral effects.

White race has been showed more strong resistant against ND infection than color race in chicken. The reason has not been elucidated by far. Our results also have showed that white race resist against ND infection compared to another race. The cell transducted with cMx from N and F line has showed more strong resistant than the other transducted with cMx from GSP line. Particularly, cMx from N line has been showed the most resistant against NDV and VSV. These results suggest that resistance of some chicken lines against NDV, VSV, and influenza virus has been related to cMx protein. In our studies, the antiviral effects in the cells transducted with chicken Mx and that in the different MHC haplotype chickens against NDV have been showed similarly. That is, the antiviral effects in the chickens have been closely related with chicken Mx type.

We have supposed that various types of cMx genes have been were existed in the chickens. Specific substitutions of amino acids of resistant-type Mx have been related with antiviral activity against infection by RNA viruses. Our results show that there are no evidences in the relationship between the expression of MHC class and the antiviral effects of cMx in the chickens, though MHC and Mx have been induced by interferon. In present, we are studying on relationship and interaction between Mx and MHC class antigen in the different chicken lines. In addition, we will study whether chicken Mx can suppress the expression of F and HN protein of NDV.

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