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Interferon-Inducible Murine Mx Homologs in Swine

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SUMMARY

The interferon (IFN) inducible mouse Mx system confers selective resistance to influenza virus. We show here the existence of at least one Mx homologous gene in the porcine genome. As its murine counterpart the porcine Mx system is IFN dependent. Transcriptional activation of the porcine Mx gene by IFN results in a 2.8 kb mRNA. Protein analysis by immunoprecipitation with anti-mouse Mx antibodies gives rise to a prominent signal in the molecular weight range of 75 kDa.

RESUMEN

El sistema Mx de ratón inducible por interferón (IFN) confiere resistencia selectiva al virus de la influenza. Nosotros demostramos aquí la existencia de al menos un gen homólogo al Mx en el genoma porcino. Tal como sucede con su contraparte murina, el sistema porcino Mx es dependiente del IFN. La activación transcripcional del gen Mx porcino por IFN resulta en un mRNA de 2,8 kb. El análisis de proteína por radioinmunoprecipitación con anticuerpos anti-Mx de ratón da una señal prominente en el rango molecular de 75 kDa.

INTRODUCTION

Alleles at the murine Mx+ locus determine the susceptibility of mice to infection with influenza virus (for review, see Haller, 1981).

Expression of the Mx function is regulated by type I (α/β) interferons. The product of the dominant Mx+ resistance allele is a 3.4 kb mRNA encoding a 72 kDA nuclear protein (for review, see Staeheli and Haller, 1987).

Chromosomal DNA analysis has shown that the Mx+ gene is at least 55 kb in length and consists of 14 exons (Hug *et al.*, 1988).

Influenza susceptible mouse strains have mutations in the Mx gene which abolish Mx protein synthesis. Most of these so called Mx⁻gene are deletion mutants (Exon 9 - 11 is lacking), one known Mx⁻ phenotype is due to a nonsense mutation in exon 10 (Staeheli *et al.*, 1988).

Mx similar proteins or genomic sequences were found in humans (Staeheli and Haller, 1985) and in all examined mammals (Mortier and Haller, 1987).

Hitherto nothing is known about the Mx system of pigs. For this reason Mx similar porcine structures were investigated on DNA, RNA and protein level.

MATERIAL AND METHODS

Pigs and mouse cells

Tissue samples and peripheral blood lymphocytes (PBLs) were taken from pigs of Deutsche Landrasse.

The Mx+ mouse embryofibroblast cell line (BALB.A2G) was established at the Institute of Immunology and Virology, University of Zurich, Switzerland.

Interferons and anti-mouseMx antibodies

Recombinant murine type I interferon (MuIFN I, Stratech Scientific Ltd., Log. No. 82011) was a gift from O. Haller, Institute of Immunology and Virology, University of Zurich, Switzerland.

Porcine type I/II interferon (pIFN I/II) induced by human erythromyeloma cells (K 562) in porcine peripheral blood lymphocytes was a gift from M. Büttner, Institute of Medical Microbiology and Infectious Diseases, LMU Munich, FRG.

Polyclonal anti-mouseMx antibodies were established by Staeheli *et al.*, (1985) and made available by O. Haller.

DNA and Southern blot analysis

Genomic DNA samples were prepared from ectodermal tissue or from blood cells by standard methods (Ausubel *et al.*, 1987).

30 μg of DNA was digested to completion with different restriction endonucleases. After electrophoresis on a 0.6% agarose gel, the DNA was transferred to nylon membranes (Hybond^{TM}-N, Amersham) as described elsewhere (Ausubel *et al.*, 1987).

Hybridization and washing of filters was done as described by Church and Gilbert (1984). A 1.8 kb Bam HI/Hind III Mx cDNA fragment (Staeheli *et al.*, 1986a), labeled with ^{32}P-deoxynucleotides by random oligoprimer extension (Feinberg and Vogelstein, 1984), was used as hybridization probe.

RNA and Northern blot analysis

Mouse embryofibroblasts were grown as described by Arnheiter and Staeheli (1983). Porcine PBLs were isolated by Ficoll gradient and cultured in RPMI containing 10% FCs.

10^6 - 10^7 cells were incubated with 1000 U of the appropriate IFNs per mL medium for 2.5 h. Cells were lysed and RNA was isolated (Ausubel *et al.*, 1987).

For Northern analysis 25 μg total RNA was electrophoresed on a 1.4% agarose/formaldehyde gel and transferred to nitrocellulose filters (Fourney *et al.*, 1988). Hybridization was done in 50 % formamide by using standard protocols (Ausubel *et al.*, 1987). The hybridization probe is described above.

^{35}S-methionine labeling of proteins and immunoprecipitation

Cells were cultured and IFN stimulated as described above. Protein labeling was done in methionine-free medium containing 50 mCi/mL ^{35}S-methionine for 1 h. Labeled cells were washed and then extracted in lysis buffer as described (Staeheli *et al.*, 1985). Clarified cell extracts were incubated for 30 min at 4°C with polyclonal anti-mouseMx antibodies. Immune complexes were collected on protein A-Sepharose.

Analysis of labeled and immunoprecipitated proteins

Precipitated immune complexes were boiled for 2 min in SDS dissociation buffer. Released radioactive proteins were separated by discontinuous buffer electrophoresis as described (Laemmli, 1970), and visualized by fluorography.

RESULTS

Southern blot analysis reveals murine Mx homologous structures in the porcine genome (Figure 1)

The position of molecular weight markers are seen on the right. The time of exposure was 18 h.

Southern blot analysis of chromosomal porcine DNA with a murine Mx cDNA probe reveals a pattern of bands with nearly every restriction enzyme tested. Comparison of the signal intensity suggests relative high homology between murine and porcine Mx sequences. Restriction with Eco RI results in two signals, both over 20 kb in length. This corresponds to data obtained from the murine Mx gene (Hug *et al.*, 1988). Digestion of porcine DNA with Bam HI, Hind III and Pst I shows some signals of reduced intensity. Only small variations in the washing temperature of the Southern blots result in this difference between the intensity of the signals. This implies the existence of more than one Mx-related gene.

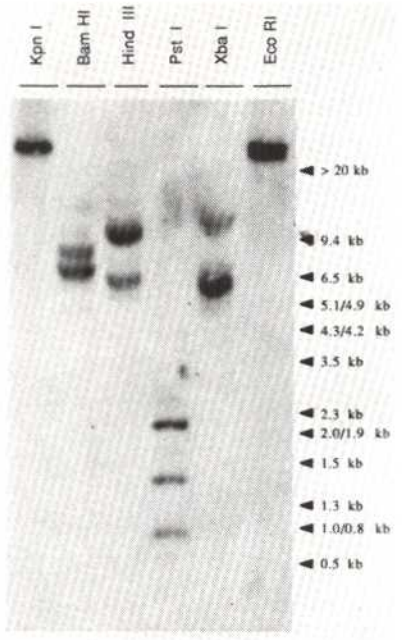

FIG. 1. Southern analysis of porcine genomic DNA.

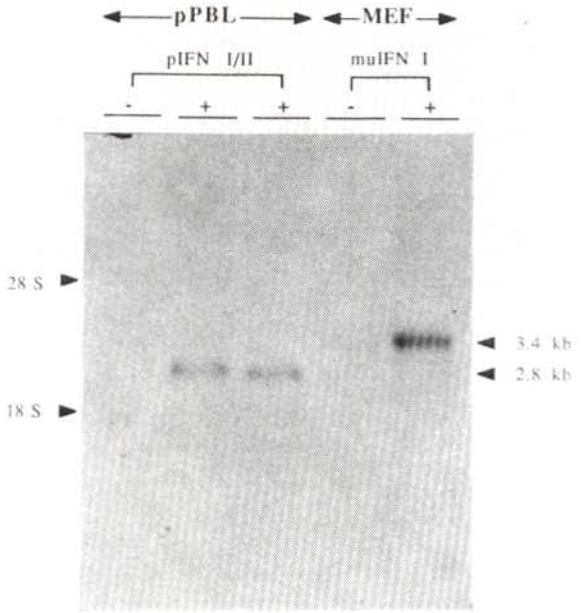

FIG. 2. Interferon inducible Mx - RNA in porcine peripheral blood lymphocytes (pPBLs) and in murine Mx+ embryofibroblasts (MEFs).

Interferon inducible transcriptional activation of the porcine Mx gene (Figure 2)

Interferon stimulated PBLs show significant increase of RNA detectable with the murine Mx cDNA probe. The length of the porcine Mx mRNA is about 2.8 kb. This means that it is about 600 nucleotides shorter than its murine counterpart. About 1 h after IFN induction detectable amounts of RNA appear in pPBLs with a maximum synthesis rate of RNA around 2.5 h after stimulation. This situation is quite similar to the mRNA induction of the mouse Mx gene (Staeheli *et al.*, 1986b).

The position of the 28S and 18S ribosomal RNA is indicated on the left. The time of exposure was 18 h.

Interferon-induced porcine protein(s) with homology to Mx protein of mice (Figure 3)

To detect porcine homologs of murine Mx protein, we exposed porcine peripheral blood lymphocytes (pPBLs) from healthy donors to IFN and assayed newly synthesized proteins by immunoprecipitation for crossreactivity with polyclonal anti-mouseMx antibodies. After 2.5 h of IFN induction and 1 h of protein labeling a signal appears in the molecular weight range of 75 kDa. In Mx+ mouse cells the Mx protein is visualized by immunoprecipitation with the same antibody. Its molecular weight is comparable with the porcine protein. Data from other experiments suggest

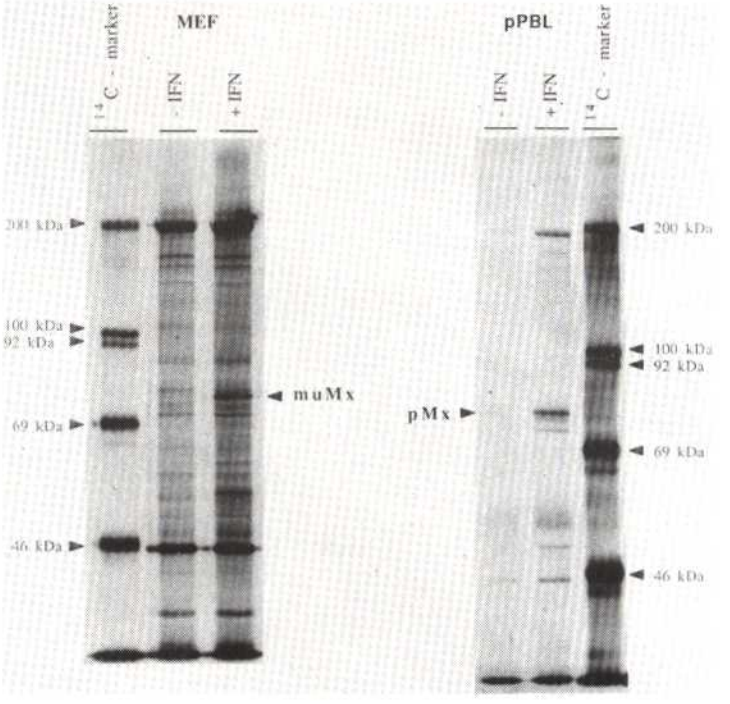

FIG. 3. IFN-induced murine Mx protein and porcine IFN dependent protein crossreacting with polyclonal anti-mouseMx antibody.

the existence of three porcine proteins crossreacting with the antibody. In rats Mx-related mRNAs encode also three proteins (Meier *et al.*, 1988).

Immunoprecipitated proteins were analyzed by SDS/8%-polyacrylamide gelelectrophoresis and visualized by fluorography. The position of the molecular weight markers is indicated. The time of exposure was 1 day (MEF) and 2 days (pPBLs).

DISCUSSION AND CONCLUSIONS

Southern blots with genomic porcine DNA probed with murine Mx cDNA show a high conservation of Mx-related sequences. Hybridization data suggest the existence of more than one porcine Mx gene. Staeheli and Sutcliffe (1988) identified a second murine Mx gene. Small families of Mx-related structures appear to exist in several other species; two Mx-related genes have been found in humans (Aebi *et al.*, 1987), and two Mx-related proteins are detectable in bovines (Horisberger, 1988). In rats, there is evidence for three Mx-related genes (Meier *et al.*, 1988).

Data from Northern blots and immunoprecipitation show the distinct IFN dependence of the porcine Mx system. There are no Mx-related signals detectable in non-stimulated pPBLs.

Little is known about the function of the Mx proteins in the different species. Besides the well characterized murine Mx system, only bovine and rat cells show an Mx-related IFN inducible resistance to experimental influenza virus infections (Horisberger, 1988; Meier *et al.*, 1988).

The significance of the porcine Mx protein(s) for host defence against influenza viruses is not yet clear and is under investigation. Such studies include cloning of the porcine Mx cDNA(s), experimental infections with influenza viruses, and -- if possible -- division in porcine Mx^+ and Mx^- phenotypes.

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