

## Host Cell–Intrinsic Antiviral Defense Induced by Type I Interferons

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### ABSTRACT

Type I Interferons (IFNs) are potent antiviral cytokines that modulate both innate immunity and adaptive immunity. Type I IFNs are immediately induced by viral infection, and stimulate production of a broad range of gene products such as double-stranded RNA-activated protein kinase (PKR), 2' 5'-oligoadenylate synthetase (OAS)/RNaseL and Mx GTPases. These proteins inhibit viral replication in host cells. Type I IFNs, in turn, lead to antiviral state at early phase of viral infection. We provide an overview of the knowledge of IFN-inducible antiviral proteins conserved in vertebrates.

**Key words** : Interferon, Innate immunity, Mx

### 1. Introduction

Viruses are the most abundant pathogens on earth. Many of them must infect into the host animal and replicate themselves in the host cells for their survival. In contrast, host animal possess defense mechanisms against viruses, which is mediated by the early responses of innate immunity and the later responses of

adaptive immunity. Type I interferons (IFNs) are a key mediator to activate host defense against viruses. Type I IFNs, including IFN- $\alpha$  and IFN- $\beta$ , are a large family of structurally related cytokines. Production of type I IFNs is directly stimulated by viral infection. Viral nucleic acids, which bind to various intracellular receptors, trigger the response of type I IFNs induction (Moore et al., 2008). IFN- $\alpha$  is mainly produced

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by immune cells such as plasmacytoid dendritic cells and mononuclear phagocytes, and IFN- $\beta$  is produced by many cells such as fibroblast. Type I IFNs act in an autocrine and paracrine manner to signal the presence of viral infection. Type I IFNs activate signal transduction through type I IFN receptor on target cells, which triggers induction both innate and adaptive immunity (Stetson and Medzhitov, 2006). For example, type I IFNs enhance cytotoxic functions of natural killer cells and virus-specific CD8<sup>+</sup> T cells, which is important in recognizing and eliminating virus-infected cells. In addition, type I IFN induce expression of hundreds of gene products, which inhibit viral replication in the cells. As a consequence, type I IFNs lead host cells to be in an "antiviral state". Although type I IFN was initially discovered as a factor which interfering with virus replication in host cells 50 years ago (Vilcek, 2006), antiviral functions of many IFN-inducible gene products are still unknown. We have studied the gene structure, expression and function of Mx protein, which is one of interferon-inducible antiviral factor (Asano et al., 2003; Asano, 2002; Jin, 2001; Ko, 2002; Morozumi, 2001; Nakamura, 2005). In this presentation, we show the role of interferon-inducible proteins including Mx, which contribute to host cell-intrinsic antiviral state activated by type I IFNs.

## II. IFN-inducible gene products with antiviral effects

To date, several antiviral pathways induced by type I IFNs have been established. For example, double-stranded RNA (dsRNA)-activated protein kinase PKR is induced by Type I IFNs. PKR is a serine/threonine kinase, which belongs to the eukaryotic initiation factor 2 $\alpha$  (eIF2 $\alpha$ ) family. Although PKR is normally inactive, binding to dsRNA, which is formed during transcription of virus-derived RNA, causes a conformational change. As a results, PKR dimerizes and autophosphorylates and becomes active. Activated PKR phosphorylates eIF2 $\alpha$ , leading to inactivation of this factor and to inhibition of translation of most cellular and viral mRNA (Meurs et al., 1992). In addition, PKR is involved in different cellular pathways such as activation NF- $\kappa$ B and FADD/caspase 8, leading to apoptosis of virus-infected cells (Balachandran et al., 1998; Gil et al., 1999; Gil and Esteban, 2002). 2'-5' oligoadenylate synthetases (OAS) are also type I IFN-inducible gene products. OAS are stimulated by dsRNA, and produce 2'-5'-linked oligoadenylates with the general formula pppA(2' p5'A)<sub>n</sub>, n>1, named 2-5A (Hovanessian, 2007). 2-5A binds to RNase L, resulting in dimerization and activation. Activated RNase L cleaved viral RNA transcripts as well as host RNAs (Hovanessian, 2007). In addition, the OAS/RNase

L system can induce apoptosis of virus-infected cells (Ghosh, et al., 2001; Zhou et al., 1997).

### III. Mx proteins are type I IFN-inducible GTPase

Mx proteins are 70- to 80-kDa GTPases with homology to dynamin and highly conserved in vertebrates including mammals, birds and fish (Haller et al., 2007). Mx proteins contain a consensus GTPase domain at the amino-terminus, and a leucine zipper motif in the carboxyl-terminus, which plays a role in the formation of homo-oligomer (Di Paolo et al., 1999, Melén et al., 1992; Schumacher and Staeheli, 1998). Most of the vertebrates express Mx proteins in the cytoplasm (Horisberger and Gunst, 1991; Nakamura et al., 2005). In contrast, rodents express Mx proteins in both cytoplasm and nucleus (Meier et al., 1990; Staeheli et al., 1986; Zürcher et al., 1992). Mx proteins interfere with replication of various RNA virus. The specificity of antiviral activity of Mx proteins correlates with their intracellular localization. For example, rodent Mx1 protein, which is known to localize in the nucleus, confers to cells resistance to influenza virus, which is replicated in the nucleus, but not to vesicular stomatitis virus (VSV), which is replicated in the cytoplasm (Staeheli et al., 1986). However, cytoplasmic Mx2 proteins of the mouse have antiviral activity against VSV but not against influenza virus (Jin et al.,

2001; Zürcher et al., 1992). In contrast, human MxA, which is localized in the cytoplasm, inhibit both influenza virus and VSV (Pavlovic et al., 1990). It is suggested that MxA protein recognize incoming viral nucleocapsids and inactivates their function by wrapping around the viral structures thereby forming MxA/nucleocapsid oligomers (Haller et al., 2007).

### IV. Genetics of Mx genes: variations in the antiviral activities of the allelic gene products

Mx protein was initially discovered in an inbred strain of laboratory mouse (*Mus musculus domesticus*), A2G, which was highly resistant to influenza virus (Haller et al., 2007). Until now, it is revealed that a deletion or a nonsense mutation was seen at the coding region of *Mx1* mRNA in almost all of the inbred strains of laboratory mouse leading to their susceptibility to influenza virus (Haller et al., 2007). Another murine *Mx* gene, *Mx 2* is also polymorphic. All inbred laboratory mice studied previously have a frame-shift mutation, resulting a defect of functional Mx2 protein (Haller et al., 2007). We have investigated the structure of *Mx* genes in wild-derived inbred strains. Because they are distant from standard inbred laboratory mice in genetic background, it is expected to find allelic variations. As expected, we found that two wild-derived strains, SPR(*Mus spretus*) and NJL(*Mus*

*musculus musculus*), conserve wild-type *Mx1* and *Mx2* alleles (Jin et al., 2001). A congenic strain containing C57BL/6-derived genetic background and *Mx1* and *Mx2* genes derived from SPR was able to express both *Mx1* and *Mx2* genes in various tissues after administration of IFN  $\alpha/\beta$  (Asano et al., 2003). SPR-derived *Mx2* cDNA-transfected cells showed lower infectivity to hantavirus than non-treated cells (Jin et al., 2001). However, these cells showed the same infectivity to Influenza virus as that of non-treated cells (Jin et al., 2001). Furthermore, we found polymorphisms of *Mx* genes in pig and chicken, which are important host of influenza virus (Asano et al., 2002; Ko et al., 2002; Morozumi et al., 2001). Notably, amino acid variations of chicken *Mx* protein was involved in its antiviral activity (Ko et al., 2002). Chicken breeds we examined were classified into two groups: one that contains a serine (S) residue at position 631 of the *Mx* protein, and other that contains an asparagine (N) residue at the same position. *Mx*(631N)-transfected fibroblast cell lines were more resistant against both VSV and influenza virus than *Mx*(631S)-transfected cells.

## V. Conclusion

Innate immunity induced by IFNs is essential for antiviral defense, because Type I IFN receptor-deficient mice are quickly succumb

to viral infections despite having a normal adaptive immunity (Hwang et al., 1995; Müller et al., 1994; van den Broek et al., 1995). IFN-inducible gene products described above are important in host cell-intrinsic antiviral defense. Transgenic mice that express human *MxA* gene and are deficient in type I IFN-signaling are resistant to viral infections (Hefti et al., 1999). Mouse *Oas1b* is identified as a responsible gene for resistance/susceptibility to Flavivirus(West Nile virus)-induced morbidity (Mashimo et al., 2002; Perelygin et al., 2002). These studies and our results indicate that a single IFN-inducible protein is able to control infectivity to viruses. In contrast, a triple knock-out mice lacking PKR, RNaseL and *Mx* still showed a limited antiviral state, indicating another IFN-inducible antiviral pathways (Zürcher et al., 1992). The accumulation of knowledge of IFN-induced genes is necessary to understand antiviral defense induced by IFN, and should be helpful to develop novel preventive strategies against infectious diseases in domestic animals.

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