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Interferon induction by avian reovirus

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ABSTRACT

We have previously shown that the replication of avian reovirus (ARV) in chicken embryo fibroblasts (CEF) is more resistant to the antiviral action of interferon (IFN) than the replication of vesicular stomatitis virus (VSV) or vaccinia virus (VV). In this study we examined the capacity of these three viruses to induce the expression of IFN when infecting avian cells. Efficient expression of both type-α and type-β IFNs, as well as of the double-stranded RNA (dsRNA)-activated protein kinase (PKR), takes place in ARV-infected CEF, but not in cells infected with VSV or VV. PKR expression is not directly induced by ARV infection, but by the IFN secreted by ARV-infected cells. IFN induction in ARV-infected cells requires viral uncoating, but not viral gene expression, a situation similar to that reported for apoptosis induction by ARV-infected cells. However, our results demonstrate that IFN induction by ARV-infected CEF occurs by a caspase-independent mechanism.

Introduction

Interferons (IFNs) comprise a family of multifunctional cytokines that were originally discovered by their strong antiviral activity (Isaacs and Lindenmann, 1957), and which are now recognized as the first barrier that viruses have to overcome to establish a productive infection. Of the three IFN types, type I interferon-α/β displays the highest antiviral activity and its expression is induced in many cell types by viral infection or following contact with double-stranded RNA (dsRNA) (reviewed in Samuel (2001)).

Successful host defense against viruses relies on early detection of intracellular virus particles followed by the rapid production of type I interferons. For this, cells contain a series of endosomal and cytosolic sensors, called pattern recognition receptors (PRRs), which recognize pathogen associated molecular patterns (PAMPs), such as viral nucleic acids or viral intermediate products. When contacting PAMPs, PRRs become activated and transmit intracellular signaling pathways, culminating in the activation of specific transcription factors that translocate to the nucleus to stimulate type I IFN promoters (reviewed in Diebold (2010), Edwards et al. (2007), Jefferies and Fitzgerald (2005), Koyama et al. (2008) and Yoneyama and Fujita (2010)). Newly-synthesized type I IFNs are secreted out of the cell to interact with the ubiquitously expressed IFNAR receptor complex present in neighboring cells. This interaction triggers the activation of a signal transduction pathway that leads to increased expression of the designated IFN-stimulated genes (ISGs), thus creating an antiviral state. Subsequent viral infection of IFN-primed cells induces the activation of ISG-encoded proteins; the antiviral activity of these proteins prevents further dissemination of the virus (reviewed in Doly et al. (1998), Haller et al. (2006), Sadler and Williams (2008), Samuel (2001) and Takaoka and Yanai (2006)).

Despite that IFN was initially discovered as a soluble chicken factor that directly interfered with influenza virus replication in chorioallontoic membranes of chicken embryos (Isaacs and Lindenmann, 1957), our understanding of the host response to pathogens in poultry is very limited, since most efforts were dedicated at characterizing the antiviral response in mammals. However, interest in IFNs of birds has recently emerged from increasing problems with viral diseases in poultry and from the observation that chickens infected with highly pathogenic avian influenza virus strains pose a high threat to human health (Imai et al., 2013; Karpala et al., 2012; Poovorawan et al., 2013). As in mammals, three types of chicken IFN (chIFN) have been identified in virus-infected chicken cells, and all three have been reported to display antiviral activity (reviewed in Goosens et al. (2013)). Type I chIFN, which comprises multiple chIFN-α isoforms and a single chIFN-β, has the strongest antiviral activity, although chIFN-α is the dominant virus-induced IFN subtype produced by virus-infected avian cells (Schultz et al., 1995; Schwarz et al., 2004), a

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situation opposite to the one found in mammalian-infected cells. This, and the observations that chIFN-α exhibits stronger antiviral activity than chIFN-β against several viruses and greater induction potency on several ISGs encoding antiviral proteins (Qu et al., 2013; Schwarz et al., 2004), suggests that chIFN-α is the main defense used by chicken cells to combat viral infections.

Previous studies from different laboratories, including ours, have revealed that the replication of avian reovirus (ARV) in cultured avian cells is much more resistant to the antiviral action of chIFN than vaccinia virus (VV), vesicular stomatitis virus (VSV) or Semliki Forest virus (Ellis et al., 1983; González-López et al., 2003; Martinez-Costas et al., 2000; Sekellick et al., 1994). In this study we have examined the capacity of ARV, VV and VSV to induce IFN expression when infecting avian cells. We found that IFN is only expressed and secreted by ARV-infected cells and that IFN induction requires virus uncoating, but not the expression of the ARV genes.

Results

IFN induction by virus-infected avian cells

In the first part of this study we sought to compare the capacity of ARV, VV and VSV to induce the production and secretion of IFN by infected avian cells. Our previous finding that VSV and VV are very sensitive to priming of CEF cells with IFN (Martinez-Costas et al., 2000) suggests that IFN should not be secreted by avian cells infected with these two viruses, otherwise the IFN present in the viral stocks used to infect the cells would block viral replication. In the case of VV, this suggestion is supported by the results of previous studies that revealed that chIFN activity was not detected upon infection of CEF cells with wild-type VV (Hornemann et al., 2003). In contrast, it has been recently reported that infection of the CEF-derived avian cell line DF1 with VSV induces increasing expression of the mRNAs coding for chIFN-α and chIFN-β (Qu et al., 2013), although the presence of IFN in the cultured medium of VSV-infected cells was not examined in this study.

To determine the capacity of the three viruses to induce chIFN expression, we first analyzed by Western blot the intracellular levels of the IFN-inducible protein PKR in virus-infected CEF cells. Since the possibility existed that IFN is produced and secreted by ARV-infected CEF, and consequently that the ARV stock used to infect the cells contains chIFN, the infection with ARV was carried out with a suspension of purified reovirions devoid of cellular proteins (Grande and Benavente, 2000). VSV and VV viruses did not require purification since these viruses were grown on BHK-21 hamster cells and therefore their stocks should not contain chIFN. The results shown in Fig. 1A revealed that PKR expression was induced when the cells were infected with ARV, but not when infected with VSV or VV, suggesting that IFN is only expressed by ARV-infected cells. To confirm this suggestion, we analyzed the presence of IFN in the cultured medium (supernatant) of virus-infected CEF cells by two different approaches. In the first approach, we examined the capacity of virus-free supernatants to induce PKR expression when incubated with monolayers of uninfected CEF. Viral particles were removed from the supernatants of infected cells by precipitation with perchloric acid at 4 °C, as previously reported (Sekellick and Marcus, 1986), but similar results were obtained when the viral particles were inactivated by incubating the supernatants at 65 °C for 30 min (Liniger et al., 2012). The results shown in Fig. 1B revealed that only the supernatant from ARV-infected cells, but not those from cells infected with VSV or VV, was able to induce the expression of PKR. In the second approach, we determined the capacity of the supernatants to activate the promoter of the chicken Mx gene contained within the reporter pGL3-P-chMx-luc plasmid (Liniger et al., 2012). The supernatants were incubated with plasmid-transfected DF1 cells, since control experiments revealed that the transfection of any plasmid into CEF cells, but not into DF1 cells, already induces PKR expression (not shown). The results shown in Fig. 1C revealed that only the supernatant from ARV-infected CEF was able to activate the Mx promoter. Taken together, these results demonstrate that infection of CEF with ARV, but not with VSV or VV, induces the production and secretion of chIFN.

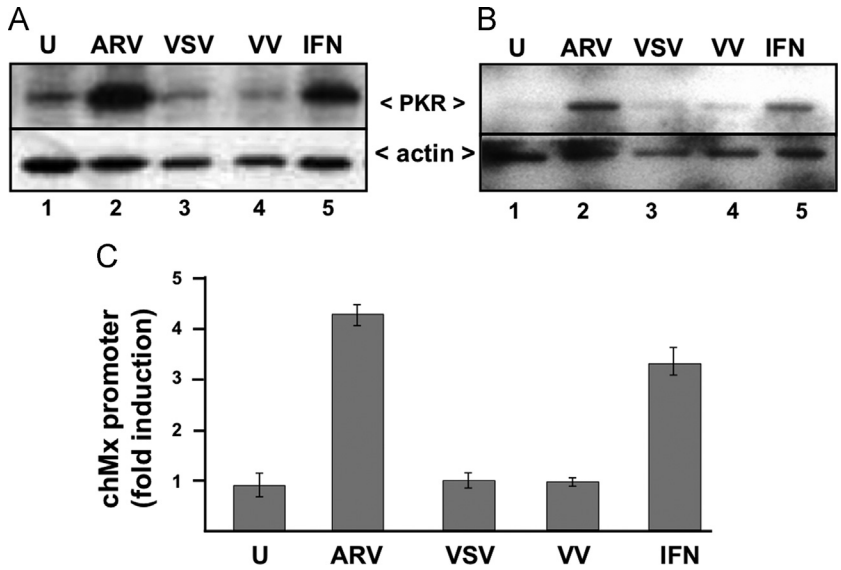

Fig. 1. IFN induction by virus-infected CEF cells. (A) CEF monolayers were mock-infected (U) or infected with 2 PFU/cell of the viruses indicated on top. At 18 hpi the cells were lysed and the resulting extracts were analyzed by Western blot with anti-chPKR (top panel) and anti-actin (bottom panel) antibodies. (B) Virus-free supernatants from the same cells shown in A were added to the culture medium of CEF cell monolayers and 24 h later the cells were lysed and the intracellular PKR and actin levels were compared by Western blotting. A Western blot analysis of extracts from uninfected CEF cells that were incubated with 1000 U/ml of chIFN for 24 h is shown in lanes 5 of A and B. (C) Virus-free supernatants from the same cells shown in A were added to the culture medium of DF1 cells that had been transfected with the pGL3-P-chMx-luc plasmid. These cells, as well as IFN-primed uninfected cells, were lysed 24 h later and the luciferase activity of the extracts was determined with a luminometer. The induction of the Mx promoter-dependent firefly luciferase was expressed as fold induction compared to that of unstimulated cells. The data are representative of three independent experiments. The error bars indicate the standard deviation of three measurements for each experiment.