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Influenza A virus replication is inhibited in IFN-λ2 and IFN-λ3 transfected or stimulated cells

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ABSTRACT

Interferons lambda (IFN-λ) are the most recently defined members of the class III cytokine family. To investigate whether IFN-λ2 and IFN-λ3 displayed antiviral activity against influenza A virus (IAV), a number of cell lines induced with IFNs – as well as two established cell lines (A549-IFN-λ2 and A549-IFN-λ3) – were infected with IAV. Our results indicate that IFN-λ2 has statistically significant antiviral activity in A549-IFN-λ2 ($P = 0.0028$) although less so than IFN-λ3, which reduced viral titer to 10% ($P < 0.0001$). The reverse was observed for cells treated with IFNs, with IFN-λ2-treated A549 cells inhibiting IAV infection more efficiently than IFN-λ3-treated A549 cells. The antiviral effect on IFN-stimulated cells was most apparent on Vero cells (compared with MDCK and HeLa). Both IFNs significantly inhibited IAV replication and inhibition was observed in a dose-dependent manner, with an optimal IFN concentration of 20 ng/ml. IFN-λ2 was more potent than IFN-λ3 on Vero cells while IFN-λ3 appeared more efficient than IFN-λ2 on MDCK and HeLa cells.

1. Introduction

Influenza A virus (IAV) is a common respiratory pathogen that can cause severe viral pneumonia with alveolar inflammation and infiltration of neutrophils and mononuclear cells (Yeldandi and Colby, 1994). In some cases, primary cellular targets are the epithelial cells of the upper respiratory tract, although the virus is also able to infect other cell types such as T cells, monocytes/macrophages and dendritic cells (DC). Each cell type demonstrates a unique response to viral infection (Ronni et al., 1995; Sareneva et al., 1998; Cella et al., 1999; Pirhonen et al., 1999; Julkunen et al., 2000). Interferons (IFNs) play a critical role in innate as well as adaptive immune responses against viral infections (Alexopoulou et al., 2001; Au et al., 2001). IAV-infected epithelial cells produce limited amounts of α/β interferons (IFN-α/β), proinflammatory cytokines (interleukin 1 [IL-1], IL-6, and tumor necrosis factor alpha [TNF-α]), and chemokines (RANTES, MCP, and IL-8) (Julkunen et al., 2000). Macrophages produce significant levels of IFN-α/β, IL-1β, TNF-α, chemokines and IL-18 (Sareneva et al., 1998; Pirhonen et al., 1999, 2002; Matikainen et al., 2000). IAV-infected DCs (and particularly plasmacytoid DCs) produce high levels of IFN-α/β (Cella et al., 1999, 2000; Coccia et al., 2004). IFN-α/β is involved in the development of Th1 immunity by stimulating natural killer and T-cell Th1 cytokine receptor gene expression as well as IFN-γ production (Sareneva et al., 1998, 2000; Matikainen et al., 2001).

The main role of IFNs is to restrict virus replication in virus-infected cells and to protect uninfected cells from becoming infected. Recently, several novel cytokines have been identified. The type III IFNs (IFN-λ), identified in 2003, include IFN-λ1 (IL-29), IFN-λ2 (IL-28A) and IFN-λ3 (IL-28B). They belong to the interleukin-10 related cytokine family, and are evolutionary only distantly related to the type I IFNs (Sheppard et al., 2003; Kotenko et al., 2003; Pestka et al., 2004). IFN-λs interact with cell surface receptors composed of interleukin-10R2 and interleukin-28R1, distinct from those receptors for types I and II IFNs. The binding of a type III IFN to its receptor results in the intracellular activation of Janus kinase I, together with signalling transducers and activators of transcription (STAT) factors 1 and 2, resulting in formation of the IFN-stimulated gene factor 3 complex. Like other IFNs, expression of IFN-λs is induced by viral infection as well as by treatment with poly (I·C) or lipopolysacharide (Hemmi et al., 2002), although the regulatory mechanisms involved are not well understood.

IAVs can induce expression of IFN-λ2 and 3 as well as IFN-λ1 genes whose gene products are likely to contribute to the host antiviral response. However, it has been shown that only IFN-λ1 exerts an antiviral effect during influenza A infection through activation of antiviral genes and this antiviral effect is independent of IFN-β (Osterlund et al., 2005; Wang et al., 2009).

Abbreviations: IAV, influenza A virus; IFN, interferon; IL, interleukin.

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Table 1
Primers used for cloning of interferons lambda ORFs.

mRNA (GenBank Acc. No.)	Sense primer (5′ → 3′)	Antisense primer (5′ → 3′)	T_m (°C)	Product size
IFN-λ2 + 3 (NM_172138, NM_172139)	CAGCCTCAGAGTGTTTCTTCTGCT	AGGGTCAGACACACAGGTCCC	63	652
IFN-λ3 specific (NM_172139)	GACATGACCGGGGACTGCAT	AGGGTCAGACACACAGGTCCC	65	598

IFNs-λ activate similar transduction signal pathways to type I IFNs. They induce tyrosine phosphorylation of STAT factors, including STAT2, which is typical of type I IFN responses (Ihle and Kerr, 1995; Kotenko et al., 2003; Sheppard et al., 2003; Dumoutier et al., 2004). It is likely, then, that these new proteins also share the biological activities ascribed to type I IFNs. While previous investigations using antiviral assays have shown that IFN-λ2 and -λ3 induce protective effects in a number of cell lines following viral infection (Kotenko et al., 2003; Sheppard et al., 2003), we set out to improve the biological characterization of these IFNs by examining their antiviral effect when expressed in A549 cells and comparing that to the antiviral effects of different cell lines induced by these IFNs following IAV infection.

2. Materials and methods

2.1. Cells and viruses

MDCK, HeLa, A549 and Vero cells (all ATCC CCL) were grown in Eagle's minimum medium (MEM) containing 10% fetal calf serum (FCS). Human peripheral leukocytes (a kind gift from Dr. Miroslav Kubes, Institute of Virology, Slovak Academy of Sciences, Bratislava, Slovakia) were grown in Dulbecco modified Eagle medium (DMEM) containing 10% FCS. Reassortant virus, Bro2a (Hay et al., 1985), comprising the M and HA genes of influenza strain A/chicken/Germany/34 (H7N1 'Rostock' strain) and the remaining genes of influenza strain A/Bel/42 (H1N1) (kindly provided by Dr. A.J. Hay) was grown in 10-day-old fertile hen's eggs.

2.2. Plasmid construction

Expression of IFN-λ2 and -λ3 was induced by cultivation of 25×10^6 leukocytes cells in DMEM (Bio Whittaker) supplemented with 10% FCS and 100 μg/ml poly(I) × poly(C) for 20 h at 37° in a 5% CO_2 atmosphere. Total RNA was extracted using the RNA Insta-Pure System (Eurogentec). Random hexa-nucleotide primers (Sigma–Aldrich) and MuLV reverse transcriptase (Finnzyme) were used for preparation of cDNA.

PCR products obtained using specific primers for IFN-λ2 and IFN-λ3 (Table 1) were cloned into pCR2.1 or pCR-Blunt plasmids (Invitrogen). Fragments were then cloned into the EcoRI site of vector pcDNA 3.1(+) (Invitrogen). Correct orientation of inserted fragments was verified by PCR using the primers specified in Table 1 and vector-specific primer T7 (5′ TAATACGACTCACTATAGGG 3′). Nucleotide sequences of all constructs were verified by DNA sequencing.

2.3. Generation of A549 cells expressing interferons lambda

Human alveolar basal epithelial (A549) cells plated in 35 mm Petri dishes were transfected with 2 μg of either pcDNA3.1-IFN-λ2, pcDNA3.1-IFN-λ3 plasmids or with empty pcDNA3.1(+) vector using the GenePorter transfection reagent (Genlantis). 48 h later, cells were split (into ten 60 mm Petri dishes) and subjected to two-week selection in medium containing 600 μg/ml Geneticin G418 (Invitrogen). Resistant colonies were isolated and expanded. Ectopic expression of IFNsλ was tested by RT-PCR using the primers shown in Table 2, together with primers for β-actin (serving as an internal standard).

2.4. Antiviral activity

Confluent monolayers of A549, Vero, MDCK and Hela cells (in 24-well plates) were preincubated for 24 h with 0, 10, 20, and 40 ng/ml of IFN-λ2 (recombinant mouse IL-28A/IFN-λ2, Biomedica) or IFN-λ3 (recombinant mouse IL-28B/IFN-λ3, Biomedica). Control cells (without IFN and untransfected), preincubated cells or stably transfected interferon-expressing A549 cells were washed once with phosphate buffered saline (PBS) and then infected with influenza A/Bro2a virus at a multiplicity of infection (MOI) of 5 plaque forming units (PFU) per cell for 1 h at room temperature. After adsorption, cells were washed three times with PBS and then cultured in serum-free MEM at 37 °C. At 48 h post-infection, cells were scraped and centrifuged at 500 × *g* for 2 min. Viral titers in supernatants were determined by plaque assay.

2.5. Plaque assay

Confluent MDCK monolayers propagated in 24-well plates were infected with a serial 5-fold dilution of supernatant from scraped cells. Following adsorbtion, cells were washed with PBS and overlaid with 0.5% carboxymethyl-cellulose in MEM. After 72 h, cells were fixed in 10% PBS-buffered formalin and plaques were visualized by staining with crystal violet.

2.6. Statistical analyses

Significant differences of the virus titers between the controls group (untreated cells) and stimulated or transfected cells were calculated using the unpaired Student *t*-test. *P* values < 0.05 were considered significant. Statistical analysis was performed with GraphPad Prism (http://www.graphpad.com/quickcalcs/ttest1.cfm).

3. Results

3.1. Cloning and expression of IFN-λs

Reference sequences for mRNA IFN-λ2 and IFN-λ3 (NM_172138 and NM_172189, respectively) are not complete in the nucleotide database. The sequence of the first exon as well as the 3' nonoverlapping regions of IFN-λ2 and IFN-λ3 were found by analyzing the genomic sequence using ClustalW

Table 2
Primers used for mRNA expression analysis of interferons lambda.

mRNA (GenBank Acc. No.)	Sense primer (5′ → 3′)	Antisense primer (5′ → 3′)	T_m (°C)	Product size
IFN-λ2 (NM_172138)	CTGCACCATATCCTCTCCCAGT	ACTGGCAACACAATTCAGGTCTC	60	198
IFN-λ3 (NM_172139)	CTGCACCATATCCTCTCCCAGC	GCTGGCAACACAATTCAGGTCTC	60	198
β-Actin (NM_001101)	CCAACCGCGAGAAGATGACC	AGGATCTTCATGAGGTAGTCAGTC	60	238

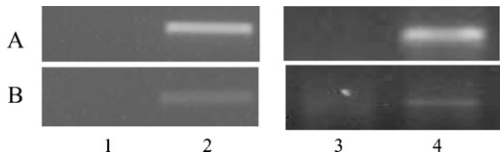

Fig. 1. Expression of mRNA in transfected A549 cell lines. PCR was performed with specific primers to β/actin (A) and IFN-λ2 (B, lines 1 and 2) and IFN-λ3 (B, lines 3 and 4). Negative controls are in lanes 1 and 3.

(http://www.ebi.ac.uk/clustalw). IFN-λ2 and IFN-λ3 are very similar to each other (96.9% identity) (Sheppard et al., 2003) such that we could design primers universal for both interferons (Table 1).

All IFN-λ2 and -λ3 inserts cloned into pCR-Blunt were in the opposite orientation due to the polylinker of this vector, so both genes were re-cloned into the EcoRI site of pcDNA3.1(+). Correct orientation of inserts was verified by PCR using primers specific for interferon and T7 (as described in Section 2). Prepared plasmids pcDNA3.1-λ2, pcDNA3.1-λ3 and pcDNA3.1(+) were used in the preparation of stably transfected A549 cells. Production of IFN mRNA was tested by RT-PCR using the primers shown in Table 2. Primers for β-actin served as an internal control (Fig. 1). Positive clones of A549 producing IFN mRNA were tested for anti-viral activity, using A549 transfected with empty vector pcDNA3.1(+) (A549-neo) as a positive control.

3.2. Replication of AIV in A549-IFN-λ2 and A549-IFN-λ3

A549-IFN-λ2 and A549-IFN-λ3 were found to express their respective interferon mRNAs (Fig. 1). We assumed that translation of proteins was successful but did not compare the amount of expressed proteins. The antiviral activity of IFNs λ2 and λ3 against influenza A was determined by infecting these cells with Bro2a virus. Viral titers obtained were compared with the viral titer yielded by the same infection of A549-neo cells carrying empty pcDNA3.1 plasmid. IFN-λ2 demonstrated statistically significant antiviral activity ($P = 0.0028$) but appeared less efficient than IFN-λ3, which reduced viral titer to just 10%, a statistically significant drop ($P < 0.0001$) (Fig. 2).

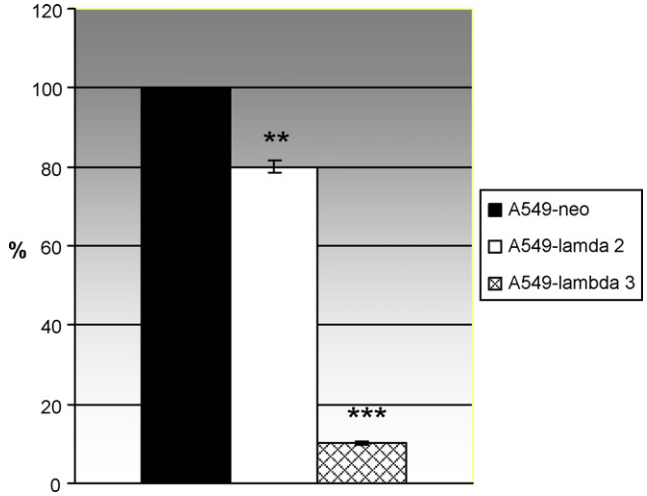

Fig. 2. Antiviral activity of IFN-λ in transfected A549 cells. The column bars represent the average results with standard deviations from two experiments performed on different occasions. 100% represent 10^3 PFU. *Statistical significance (**$P < 0.02$ and ***$P < 0.01$ by unpaired Student t-test).

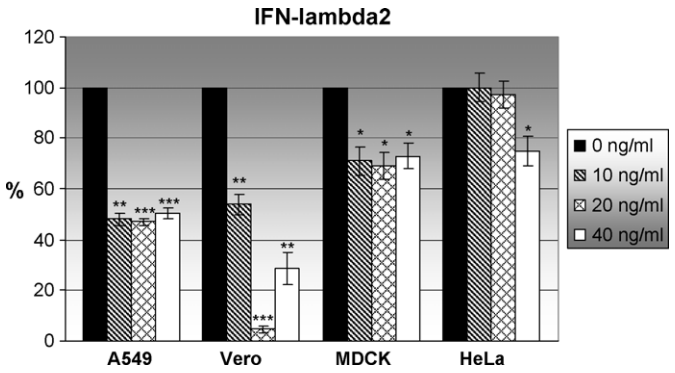

Fig. 3. Antiviral activity of IFN-λ2 in induced A549, Vero, MDCK, and HeLa cells. The column bars represent the average results with standard deviations from two experiments performed on different occasions. 100% represent 10^3 PFU (A549); 10^4 PFU (Vero and HeLa); and 10^6 PFU (MDCK). *Statistical significance (*$P < 0.05$; **$P < 0.02$; ***$P < 0.01$ by unpaired Student t-test).

3.3. Inhibition of AIV replication on the cells pre-incubated with interferons.

Vero cells were stimulated with different concentrations of both IFN-λ2 and IFN-λ3, ranging from 0 to 40 ng/ml. Subsequently, cells were infected with Bro2a virus and infected cells were scraped 24 h, 48 h or 72 h later. Viral titer estimations were determined by plaque assay, as described in materials and methods. Viral titers obtained were compared with the viral titer yielded by the same infection of untreated Vero cells. Both IFNs exerted antiviral activity against virus in a dose-dependent manner (from 2.5 to 40 ng/ml of IFNs), with the optimal concentration of IFNs ranging from 10 to 40 ng/ml (data not shown). We found that IFNs were generally less effective 24 h post-infection compared to 48 h post-infection. After 72 h of infection the inhibition effect did not increase any further (data not shown). For this reason, all tested cells lines were stimulated with concentrations of IFNs ranging from 10 to 40 ng/ml and incubated for 48 h after infection.

Both IFNs substantially inhibited replication of Bro2a virus on Vero cells, with IFN-λ2 more potent than IFN-λ3 (Figs. 3 and 4). The inhibitory effect observed with 20 ng/ml of IFN-λ2 and IFN-λ3 was statistically significant in unpaired t-tests ($P < 0.0001$), with antiviral activity statistically less effective with further increasing doses of IFN.

A statistically significant decrease in IAV replication was observed on A549 cells pre-treated with IFN-λ2 and IFN-λ3 (P varied from 0.004 to 0.0011) compared with untreated A549 cells. The

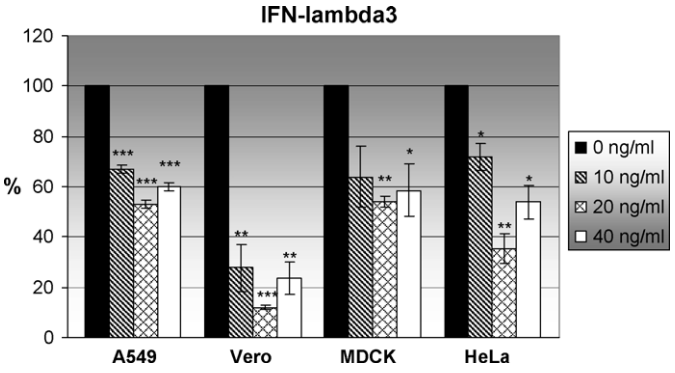

Fig. 4. Antiviral activity of IFN-λ3 in induced A549, Vero, MDCK, and HeLa cells. The column bars represent the average results with standard deviations from two experiments performed on different occasions. 100% represent 10^3 PFU (A549); 10^4 PFU (Vero and HeLa); and 10^6 PFU (MDCK). *Statistical significance (*$P < 0.05$; **$P < 0.02$; ***$P < 0.01$ by unpaired Student t-test).

inhibitory effect of both IFNs on A549 cells was not as strongly dose-dependent as that seen on Vero cells. In a reverse of that what was observed for A549 cells stably expressing IFNs, A549 cells that were induced with IFN-λ2 inhibited IAV infection more efficiently than those induced with IFN-λ3 (Figs. 3 and 4).

IFN-λ3 appeared more efficient at inhibiting viral replication than IFN-λ2 on MDCK and HeLa cells. These effects was statistically significant (P varied from 0.0106 to 0.0252) with the exception of these cells treated with 20 ng/ml of IFN-λ3 when inhibition effect was statistically more significant ($P = 0.0053$) (Figs. 3 and 4). Viral titers obtained were compared with the viral titer yielded by the same infection of MDCK or HeLa cells, respectively.

4. Discussion

We showed that several cell lines responded to the antiviral activity of IFN-λ2 and IFN-λ3 in a manner similar to that induced by type I IFNs.

The A549 lung epithelial cell line is a useful cell substrate for antiviral assays and also produces a high yield of MxA protein in response to IFN; other cell lines produce much less MxA (Files et al., 1998). IAV replication on A549-λ3 cells was remarkably reduced compared to A549-neo cells, which did not express IFN-λ3. The antiviral effect was much weaker on A549-λ2 cells. However, when A549 cell were induced with IFNs and infected with IAV, the antiviral effect of both IFNs was very similar, although IFN-λ2 was slightly more effective than IFN-λ3.

As we have already mentioned, expression of both IFNs in stable A549-IFN cells was not assessed by any quantitative test, such that we are unable to compare the ability of these IFNs to inhibit replication of IAV in transfected cells. Both transfected cell lines produced mRNA, and we assume that A549-IFN-λ3 cells produced more IFN than A549-IFN-λ2 cells, explaining why the antiviral effect was more evident on those cells.

Influenza A and Influenza B viruses induce only a weak cytokine response in human A549 lung epithelial cells and this chemokine response can be greatly enhanced by pre-treating cells with TNF-α or IFN-α prior to virus infection (Veckman et al., 2006). It has previously been shown that A549 cells are responsive to IFN-λ1 and IFN-λ2 in CPER AVA, but in antiviral assays with encepaholomyocardis virus, the effectiveness of IFN-λ2 was far weaker than that of IFN α/β (Meager et al., 2005). Not much is currently known about IFN-λ3. Cytokine pre-treatment resulted in enhanced expression of RIG-1, IKKε, interferon regulatory factor (IRF)1, IRF7 and p50 proteins. IAV induced DNA binding of IRF1, IRF3, IRF7, and NF-κB onto CXCL10 ISRE and NF-κB elements, respectively, was markedly enhanced in cytokine pre-treated cells (Veckman et al., 2006). IAV infection resulted in weak or no binding of IRF3 and IRF7 onto the CXCL10 ISRE element and DNA binding of p50 and p65 onto the CXCL10 NF-κB element was only detectable at late stages of infection. However, TNF-α and IFN-α treatment prior to infection resulted in significantly increased activation and DNA binding of IRFs and NF-κB (Veckman et al., 2006). The antiviral effect of IFN-λ2 and IFN-λ3 was very similar, although IFN-λ2 was slightly more effective than IFN-λ3. We assume that IFN-λ3 is able to activate similar cascades to those activated by IFN-λ1 and IFN-λ2. The mechanism of the pre-treatment effect is likely to be complex and to function at multiple levels.

The antiviral effect on IFN-stimulated cells was most apparent on Vero cells (compared with MDCK and HeLa). Both IFNs significantly inhibited replication of IAV and inhibition was observed in a dose-dependent manner, with an optimal IFN concentration of 20 ng/ml. Since the gene for interferon is defective or absent in Vero cells, such that these cells lack the capacity to produce their own IFN (Emeny and Morgan, 1979a), Vero cells thus represent a very useful tool in the analysis of interferon induction and action (Emeny and Morgan, 1979b).

IAV can inhibit activation of the host cell immune system. NS1 protein-mediated inhibition of IFN synthesis (Noah et al., 2003; Talon et al., 2000) interferes with post-transcriptional processing and stability of the host cell and all TNA polymerase II synthesized pre-mRNAs (Noah et al., 2003). It has been suggested that NS1 protein can inhibit activation and translocation of IRF3 (Talon et al., 2000). Although cytokine pre-treatment has only a minor effect on viral gene expression at the mRNA level, NS1 protein is delayed and reduced in TNF-α and IFN-α pre-treated cells (Veckman et al., 2006). Cytokine-mediated reduction of NS1 protein production, together with the enhanced expression of host cell signalling molecules, may contribute to the greatly enhanced chemokine gene expression seen in cytokine-pre-treated cells.

In comparison with representative type I interferon family members, the responsiveness of cell lines to IFN-λs appears to be weaker or more moderate. The anti-viral effect of IFN-λ2 and IFN-λ3 was dose-dependent and maximal inhibition of IAV replication was achieved with 20 ng/ml of IFN-λ2 or IFN-λ3 while only 1.25 ng/ml of IFN-α was needed to achieve the same degree of inhibition in our experiments (data not shown). Some human lines preincubated with less then 1.25 ng/ml of IFN-α2a showed antiviral protection against the encephalomyocarditis virus (Meager et al., 2005). Dentritic cells preincubated with 100 IU/ml (0.606 ng/ml) of IFN-α showed significant antiviral activity against influenza A virus as the same cells pretreated with 10 ng/ml IFN-λ (Osterlund et al., 2005). Previous studies have shown that purified recombinant IFN-λ2, such as IFN-α2a, was able to protect HepG2 cells from the cytopathogenic effects of encephalomyocarditis virus. Half-maximal protection was achieved with 30 ng/ml of IFN-λ2 compared to 0.5 ng/ml of IFN-α2a (Sheppard et al., 2003). Although IFN-λ2 and IFN-λ3 bind to distinct receptors to those used by type I IFNs, these cytokines activate similar signal transduction pathways to those activated by the type I IFNs (Ihle and Kerr, 1995; Kotenko et al., 2003; Sheppard et al., 2003).

5. Conclusion

Both IFN-λ2 and IFN-λ3 manifest antiviral effects similar to those observed for IFN-α, but maximal inhibition of IAV replication was achieved with 20 ng/ml of IFN-λ2 or IFN-λ3 while only 1.25 ng/ml of IFN-α was needed to achieve the same degree of inhibition. Both IFN-λs show similar activity depending on cell lines. The molecular mechanism of the antiviral effect demonstrated by IFN-λ2 and IFN-λ3, and the signalling pathway involved in IFN-λs induced antiviral response, require further investigation.

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References

Alexopoulou, L., Holt, A.C., Medzhitov, R., Flavell, R.A., 2001. Recognition of double-stranded RNA and activation of NF-kappaB by Toll-like receptor 3. Nature 413, 732–738.

Au, W.C., Yeow, W.S., Pitha, P.M., 2001. Analysis of functional domains of interferon regulatory factor 7 and its association with IRF-3. Virology 280, 273–282.

Cella, M., Salio, M., Sakakibara, Y., Langen, H., Julkunen, I., Lanzavecchia, A., 1999. Maturation, activation and protection of dendritic cells induced by double-stranded RNA. J. Exp. Med. 189, 821–829.

Cella, M., Facchetti, F., Lanzavecchia, A., Colonna, M., 2000. Plasmacytoid dendritic cells activated by influenza virus and CD40L drive a potent TH1 polarization. Nat. Immunol. 1, 305–310.

Coccia, E.M., Severa, M., Giacomini, E., Monneron, D., Remoli, M.E., Julkunen, I., Cella, M., Lande, R., Uzé, G., 2004. Viral infection and Toll-like receptor agonists induce a differential expression of type I and lambda interferons in human plasmacytoid and monocyte-derived dendritic cells. Eur. J. Immunol. 34, 796–805.

Dumoutier, L., Tounsi, A., Michiels, T., Sommereyns, C., Kotenko, S.V., Renauld, J.C., 2004. Role of the interleukin (IL)-28 receptor tyrosine residues for antiviral and antiproliferative activity of IL-29/interferon-lambda 1: similarities with type I interferon signalling. J. Biol. Chem. 279, 32269–32274.

Emeny, J.M., Morgan, M.J., 1979a. Regulation of the interferon system: evidence that Vero cells have a genetic defect in interferon production. J. Gen. Virol. 43, 247–252.

Emeny, J.M., Morgan, M.J., 1979b. Susceptibility of various cells treated with interferon to the toxic effect of poly(rI).poly(rC) treatment. J. Gen. Virol. 43, 253–255.

Files, J.G., Gray, J.L., Do, L.T., Foley, W.P., Gabe, J.D., Nestaas, E., Pungor Jr., E., 1998. A novel sensitive and selective bioassay for human type I interferons. J. Interferon Cytokine Res. 18, 1019–1024.

Hay, A.J., Wolstenholme, A.J., Skehel, J.J., Smith, M.H., 1985. The molecular basis of the specific anti-influenza action of amantadine. EMBO J 4, 3021–3024.

Hemmi, H., Kaisho, T., Takeuchi, O., Sato, S., Sanjo, H., Hoshino, K., Horiuchi, T., Tomizawa, H., Takeda, K., Akira, S., 2002. Small anti-viral compounds activate immune cells via the TLR7 MyD88-dependent signaling pathway. Nat. Immunol. 3, 196–200.

Ihle, J., Kerr, I., 1995. JAKS and STATs in signaling by the cytokine superfamily. Trends Genet. 11, 69–74.

Julkunen, I., Melén, K., Nyqvist, M., Pirhonen, J., Sareneva, T., Matikainen, S., 2000. Inflammatory responses in influenza A virus infection. Vaccine 19, S32–S37.

Kotenko, S.V., Gallagher, G., Baurin, V.V., Lewis-Antes, A., Shen, M., Shah, N.K., Langer, J.A., Sheikh, F., Dickensheets, H., Donnelly, R.P., 2003. IFN-lambdas mediate antiviral protection through a distinct class II cytokine receptor complex. Nat. Immunol. 4, 69–77.

Matikainen, S., Pirhonen, J., Miettinen, M., Lehtonen, A., Govenius-Vintola, C., Sareneva, T., Julkunen, I., 2000. Influenza A and sendai viruses induce differential chemokine gene expression and transcription factor activation in human macrophages. Virology 276, 138–147.

Matikainen, S., Paananen, A., Miettinen, M., Kurimoto, M., Timonen, T., Julkunen, I., Sareneva, T., 2001. IFN-alpha and IL-18 synergistically enhance IFN-gamma production in human NK cells: differential regulation of Stat4 activation and IFN-gamma gene expression by IFN-alpha and IL-12. Eur. J. Immunol. 31, 2236–2245.

Meager, A., Visvalingam, K., Dilger, P., Bryan, D., Wadhwa, M., 2005. Biological activity of interleukins-28 and -29: comparison with type I interferons. Cytokine 31, 109–118.

Noah, D.L., Twu, K.Y., Krug, R.M., 2003. Cellular antiviral responses against influenza A virus are countered at the posttranscriptional level by the viral NS1A protein via its binding to a cellular protein required for the 3' end processing of cellular pre-mRNAS. Virology 307, 386–395.

Osterlund, P., Veckman, V., Sirén, J., Klucher, K.M., Hiscott, J., Matikainen, S., Julkunen, I., 2005. Gene expression and antiviral activity of alpha/beta interferons and interleukin-29 in virus-infected human myeloid dendritic cells. J. Virol. 79, 9608–9617.

Pestka, S., Krause, C.D., Walter, M.R., 2004. Interferons, interferon-like cytokines, and their receptors. Immunol. Rev. 202, 8–32.

Pirhonen, J., Sareneva, T., Kurimoto, M., Julkunen, I., Matikainen, S., 1999. Virus infection activates IL-1 beta and IL-18 production in human macrophages by a caspase-1-dependent pathway. J. Immunol. 162, 7322–7329.

Pirhonen, J., Matikainen, S., Julkunen, I., 2002. Regulation of virus-induced IL-12 and IL-23 expression in human macrophages. J. Immunol. 169, 5673–5678.

Ronni, T., Sareneva, T., Pirhonen, J., Julkunen, I., 1995. Activation of IFN-alpha, IFN-gamma, MxA, and IFN regulatory factor 1 genes in influenza A virus-infected human peripheral blood mononuclear cells. J. Immunol. 154, 2764–2774.

Sareneva, T., Matikainen, S., Kurimoto, M., Julkunen, I., 1998. Influenza A virus-induced IFN-alpha/beta and IL-18 synergistically enhance IFN-gamma gene expression in human T cells. J. Immunol. 160, 6032–6038.

Sareneva, T., Julkunen, I., Matikainen, S., 2000. IFN-alpha and IL-12 induce IL-18 receptor gene expression in human NK and T cells. J. Immunol. 165, 1933–1938.

Sheppard, P., Kindsvogel, W., Xu, W., Henderson, K., Schlutsmeyer, S., Whitmore, T.E., Kuestner, R., Garrigues, U., Birks, C., Roraback, J., Ostrander, C., Dong, D., Shin, J., Presnell, S., Fox, B., Haldeman, B., Cooper, E., Taft, D., Gilbert, T., Grant, F.J., Tackett, M., Krivan, W., McKnight, G., Clegg, C., Foster, D., Klucher, K.M., 2003. IL-28, IL-29 and their class II cytokine receptor IL-28R. Nat. Immunol. 4, 63–68.

Talon, J., Horvath, C.M., Polley, R., Basler, C.F., Muster, T., Palese, P., García-Sastre, A., 2000. Activation of interferon regulatory factor 3 is inhibited by the influenza A virus NS1 protein. J. Virol. 74, 7989–7996.

Veckman, V., Osterlund, P., Fagerlund, R., Melén, K., Matikainen, S., Julkunen, I., 2006. TNF-alpha and IFN-alpha enhance influenza-A-virus-induced chemokine gene expression in human A549 lung epithelial cells. Virology 345, 96–104.

Wang, J., Oberley-Deegan, R., Wang, S., Nikrad, M., Funk, C.J., Hartshorn, K.L., Mason, R.J., 2009. Differentiated human alveolar type II cells secrete antiviral IL-29 (IFN-lambda 1) in response to influenza A infection. J. Immunol. 182, 1296–1304.

Yeldandi, A.V., Colby, T.V., 1994. Pathologic features of lung biopsy specimens from influenza pneumonia cases. Hum. Pathol. 25, 47–53.