Expert Opinion

Cytokines & Chemokines

Role of interferons in the treatment of severe acute respiratory syndrome

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Severe acute respiratory syndrome (SARS) is caused by the SARS coronavirus (SCV). The disease appeared in the Guandong province of southern China in 2002. The epidemic affected > 8422 patients and caused 908 deaths in 29 countries on 5 continents. Several treatment modalities were tried with limited success to treat SARS and a variety of experimental drugs are under development. Type I interferons (IFNs-α/β) were suggested as potential candidates to treat SARS. Several animal and human coronaviruses, including SCV, were shown to be sensitive to IFNs both *in vitro* and *in vivo.* A pilot clinical report showed effectiveness of IFN-α for the treatment of SARS patients. This review summarises antiviral activities of IFNs with special regard to SARS, and reviews the published clinical and experimental data describing the use of IFNs for SARS.

Keywords: interferon, SARS, SARS coronavirus

Expert Opin. Biol. Ther. (2004) 4(6):827-836

1. Introduction

Severe acute respiratory syndrome (SARS) is a recently recognised, febrile, severe lower respiratory illness that is caused by infection with a novel coronavirus, SARS coronavirus (SCV) [101]. Since the first reported outbreak of atypical pneumonia in Guandong province in China in late 2002, successive similar outbreaks were widely reported from March 2003 onward in Hong Kong, Canada and ~ 29 countries around the world, affecting > 8422 patients and causing at least 908 deaths [1,2] (updated information can be found at [102]). The overall mortality rate is ~ 10%, but varies profoundly with age. SARS affects relatively few children and generally appeared to be milder in the paediatric age group. In contrast, the mortality rate in the elderly was as high as 50%.

A number of strategies have been used for treatment and prevention of SARS [3,4]. These have included empirical antibiotic therapy, intravenous and oral ribavirin, corticosteroids, oseltamivir and intravenous immunoglobulin [5-10]. However, the significant *in vivo* toxicity of ribavirin [11] and its limited antiviral activity in Vero cells infected with different SCV strains [12] did not encourage ribavirin treatment of patients infected with SCV. The most recent results show that anti-SCV effects of ribavirin depend on the cell line used, as ribavirin was shown to inhibit SCV replication in fetal rhesus kidney-4 cells [13].

Moreover, the rapid emergence of the epidemic did not permit any controlled studies to be conducted documenting the efficacy of therapies. Hyperimmunoglobulin, neutralising antibodies, protease inhibitors, fusion inhibitors, silencing of SCV genes by RNA interference, and natural products such as glycyrrhizin (a component of liquorice roots) and interferons (IFNs) represent other therapeutic options for treating SARS patients [3,12-15]. IFNs were suggested as potential candidates to treat

 ISSN 1471-2598

Table 1. Characteristics of type I and type II interferons.

Characteristics	Type I	Type II
Inducer	dsRNA virus, cytokine, viral protein	Immunological stimuli
Producing cell type	Most cell types	T cells, NK cells
Receptor	Type I (IFN-α-R1, IFN-α-R2)	Type II (IFN-γ-R1, IFN-γ-R2)
Direct antiviral effects	+	+
Direct antiproliferative effects	+	+
MHC class I stimulation	+	+
MHC class II stimulation	-*	+
NK cell activation	+	+**

*IFN-β slightly stimulated MHC class II; **Delayed and low activation.
dsRNA: Double-stranded RNA; IFN: Interferon; MHC: Major histocompatibility complex; NK: Natural killer.

SARS as they play a critical role in host resistance to viral infection. Experimental and preliminary clinical studies showed that treatment with type I IFNs was beneficial *in vitro*, in experiments with animals, as well as in patients with SARS [6,16-18]. This review presents the general mechanisms of IFN activity, with a special focus on type I IFNs, as well as summarising and discussing published data that describe the effects of IFNs on SCV replication *in vitro*, *in vivo* and in clinical settings.

2. Type I and II interferons

IFNs consist of multiple type I species (IFN-α, IFN-β, IFN-ω and IFN-τ) and one type II species (IFN-γ), both of which have antiviral activity [19-21]. Type I IFNs have functional, but no structural, similarity with IFN-γ (Table 1). Type I IFNs, induced in most cell types by viruses and double-stranded RNA (dsRNA), are clustered on chromosome 9 and consist of several α genes and pseudogenes and one β gene. In contrast, IFN-γ, mainly secreted by T helper (Th)1 lymphocytes and natural killer (NK) cells, is coded by a single gene on chromosome 12. IFNs mediate their effects by binding to cell surface receptors and thus activating members of the Janus kinase (JAK) family [22,23]. Activated JAKs phosphorylate the signal transducer and activator of transcription (STAT) family of transcription factors. As shown in Figure 1, IFN-α/β receptor engagement leads to the activation of the JAK–STAT signalling pathway through the actions of JAK1 and tyrosine kinase (TYK)2 protein kinases (JAK1 and JAK2 for IFN-γ), which catalyse phosphorylation events leading to the activation and heterodimerisation of STAT proteins, mostly including STAT1 and STAT2 (STAT1 reciprocal homodimer for IFN-γ). The STAT1/2 heterocomplex translocates to the nucleus, where it associates with p48/IFN regulatory factor (IRF)-9 to form IFN-stimulated gene factor 3 (ISGF3). ISGF3 binds to the IFN-stimulated response element (ISRE) of cellular genes, known as IFN-stimulated genes (ISGs), leading to their induced expression and synthesis of the ISG products. Sequence motifs within the ISRE also serve as target sites for IRFs, whose action and ISRE-binding properties contribute to define the overall spectrum and duration of ISG expression [21,24].

Gene products regulated by IFNs are the primary effectors of the IFN-mediated biological responses. ISGs represent diverse functional groups, ranging from genes encoding immunomodulatory proteins to those encoding metabolic regulators [19,25]. Oligonucleotide microarray studies in a fibrosarcoma (HT1080) cell line, as well as in mouse embryonic fibroblasts and human dendritic cells treated with IFN, have identified > 300 induced genes [25,26]. However, the exact pattern of induction varies depending on cell type and the type of IFN. This impressive stimulation of transcription has made it difficult to attribute the effects of IFNs to any particular gene product.

Transcription of some ISGs can be induced in an IFN-independent fashion by other agents such as dsRNA or virus infection. STAT and JAK kinases were shown not to be essential for dsRNA-mediated induction of ISGs. Other transcription factors, known as dsRNA-activated factor and virus-activated factor (VAF), have been implicated in dsRNA- and virus-mediated induction of ISGs [27]. They form transcriptional complexes with activated IRF3 and the co-activator CREB binding protein (CBP)/P300 (Figure 1). Although in virally infected cells both IFNs and viral dsRNA can contribute to the induction of ISGs, in some cases mere binding of viral envelope proteins to the cell surface receptors can trigger transcription of specific ISGs. For example, for ISG induction, IFN may recruit additional signalling pathways in a cell, such as mitogen-activated protein kinase (p38 MAPK) and phosphatidylinositol 3 kinase pathways [19].

3. Interferon-induced antiviral pathways

IFNs can induce several parallel antiviral pathways in cells, including four major factors: 2'-5'-oligoadenylate synthetase (OAS) protein family/ribonuclease L (RNase L), dsRNA-dependent protein kinase (PKR), Mx proteins and RNA-specific adenosine deaminase (ADAR)-1 (Figure 2) [19]. Some of these pathways are more specific for a particular group of

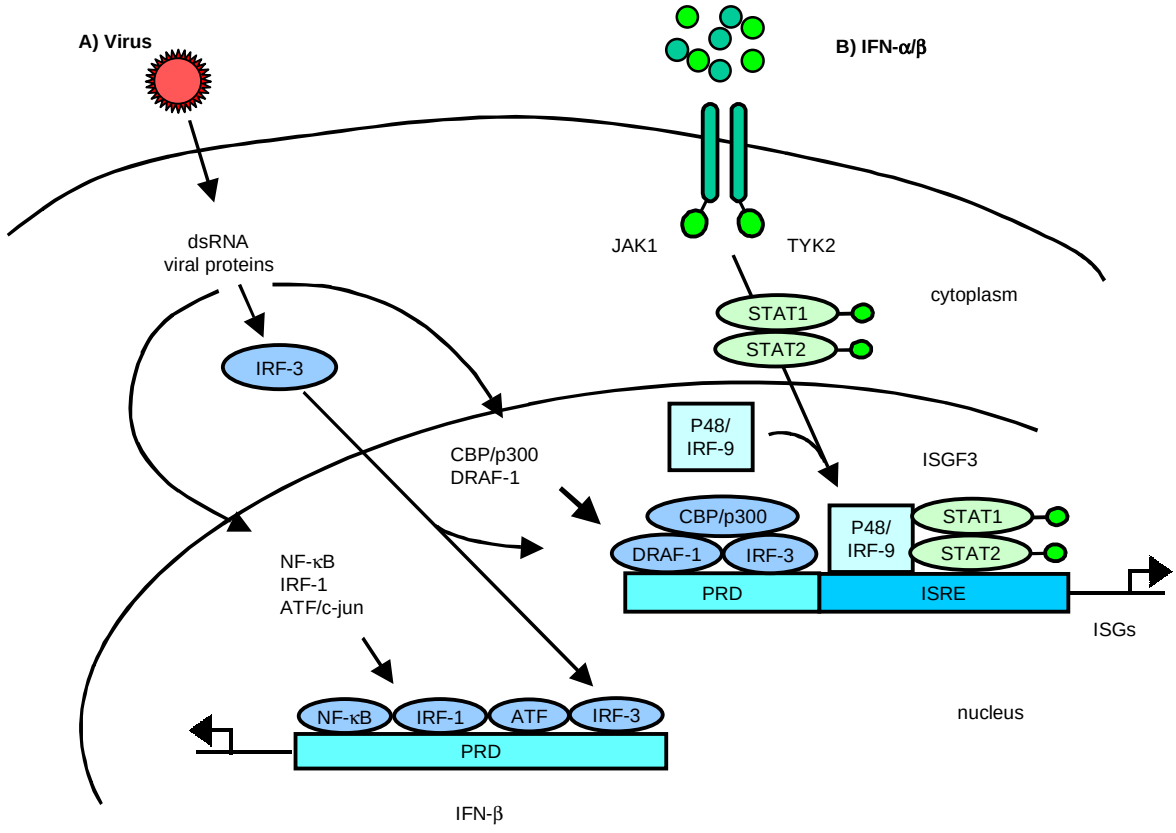

Figure 1. A) Virus infection triggers IFN-independent signalling events in the host cell that involve IRF-3, NF-κB, IRF-1 and ATF/c-jun pathways. These transcription cofactors assemble on the PRD of the IFN-β promoter to drive the expression and production of IFN-β. Alternatively, the PRD of IFN-β may be activated by the transcriptional complex composed of IRF-3, DRAF-1 and CBP/p300 (not shown). **B) Therapeutic application of IFN-α/β and secreted IFN-β (induced by virus dsRNA or proteins) bind to the type I IFN receptor to initiate JAK–STAT signalling and the formation of ISGF3, the latter of which promotes expression of ISGs.** Some ISGs also contain PRDs allowing dual responsiveness to IRF-3 complexed with DRAF-1 and transcription factor CBP/p300.

ATF: Activating transcription factor; CBP: CREB binding protein; CREB: cAMP response element binding protein; DRAF: dsRNA-activated factor; dsRNA: Double-stranded RNA; IFN: Interferon; IRF: IFN regulatory factors; ISG: IFN-stimulated gene; ISGF: IFN-stimulated gene factor; ISRE: IFN-stimulated response element; JAK: Janus kinase; NF-κB: Nuclear factor kappaB; PRD: Positive regulatory domain; STAT: Signal transducer and activator of transcription; TYK: Tyrosine kinase.

viruses, although more than one pathway may control infection with a single virus. A common feature among these antiviral pathways, excluding Mx protein 1, is the requirement for dsRNA as a common activator or substrate to IFN-induced protein factors. Whereas OAS and PKR enzymes require non-specific association with dsRNA for their activation and antiviral effects, ADAR-1 uses dsRNA as a substrate for deamination of adenosines and their conversion into inosines (see below). The existence of considerable residual effects of type I IFNs against encephalomyocarditis virus (EMCV) in mice lacking functional RNase L, PKR and Mx suggests the presence of additional pathways [28].

The IFN-inducible OAS protein family has been shown to be involved in viral and cellular RNA degradation in concert with a latent RNase L. There are three structurally related size classes of OAS enzymes (small, medium, large) encoded by three separate, but clustered, genes [29]. Within each class, multiple isozymes with different carboxyl terminal regions are produced by splicing of the primary transcripts. These enzymes polymerise ATP into 2'-5'-linked oligoadenylates (2-5(A)) of different lengths, but all of them need to be activated by dsRNA. The 2-5(A) molecules activate the latent ribonuclease, RNase L, by inducing its dimerisation. The 2-5(A) synthetase/RNase L system mediates some of the antiviral and anticellular actions of IFNs by degradation of single-stranded RNA (ssRNA). This antiviral pathway has been reported to operate against a number of viruses, including important human pathogens such as hepatitis C virus, vaccinia and HIV [30]. If RNA degradation is targeted to cellular RNA, this leads to programmed cell death (apoptosis). Programmed cell death may then prevent virus replication and spread if it occurs early enough in infection, or promote virus spread if it occurs late. An RNase L-independent action of OAS 9-2 isozyme causes apoptosis by a process that does not require its enzyme activity, but is mediated by binding to antiapoptotic proteins, such as B cell leukaemia/lymphoma (BCL)-2 and BCL-X_L, thereby blocking their actions [31].

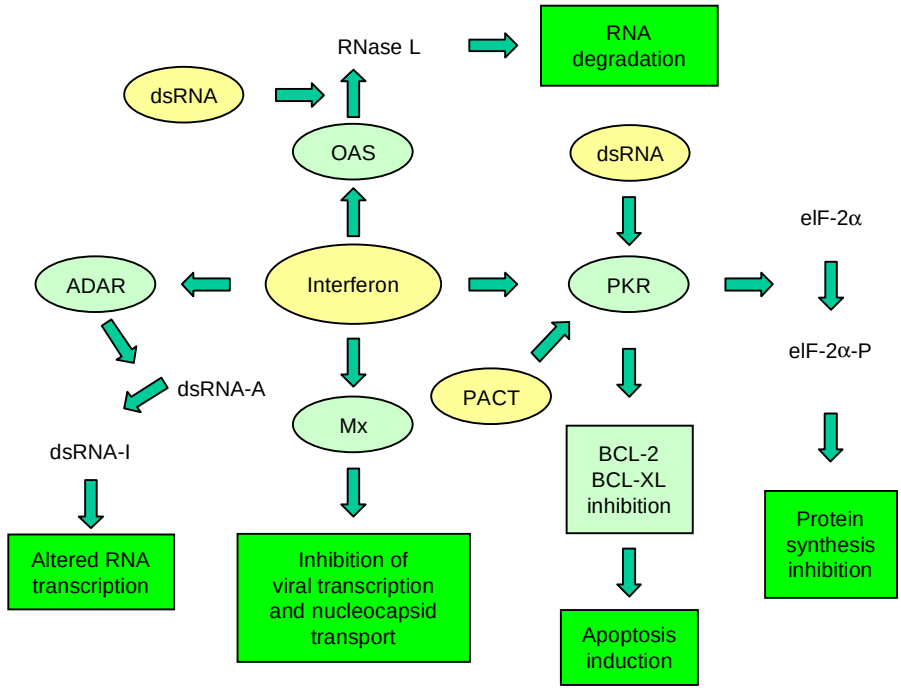

Figure 2. IFN-induced antiviral pathways. Four major pathways in the cells are induced by IFNs, including 2'-5'-OAS catalysing the production of 2'-5'-oligoadenylates [2'5'(A)n] that activate RNase L, PKR catalysing phosphorylation of the α-subunit of eIF-2α, Mx protein (a dynamin-like GTPase) and ADAR catalysing conversion of A into I. dsRNA produced by virus infection induces OAS and PKR. PKR may also be induced by cellular protein PACT.

A: Adenosine; ADAR: RNA-specific adenosine deaminase; BCL: B cell leukaemia/lymphoma; dsRNA: Double-stranded RNA; eIF: Protein synthesis initiation factor; I: Inosine; OAS: Oligoadenylate synthetase; PACT: Protein activator of PKR; PKR: dsRNA-dependent protein kinase; RNase L: Latent ribonuclease.

PKR represents an IFN-induced serine-threonine protein kinase that needs to be activated by autophosphorylation. IFN-induced PKR associates with viral dsRNA and becomes activated. However, other agents, such as heparin, can also activate it. PKR may interact with several cellular proteins, including protein activator of PKR (PACT) [32]. PACT can directly bind to PKR and activate it independently of dsRNA. Once activated, PKR may catalyse phosphorylation of a number of substrates, including the α-subunit of protein synthesis initiation factor 2 (eIF-2α), thus preventing a translation initiation factor recycling and resulting in inhibition of protein synthesis. In addition to its role in translation, PKR participates in several signalling transcription pathways. For example, PKR contributes to the induction of early genes such as c-*fos* and c-*jun* by platelet-derived growth factor and modulates transcription functions of STAT1. Other studies clearly demonstrated a role for PKR in the activation of transcription factor nuclear factor-kappaB (NF-κB). Activation of NF-κB leads to transcription induction of a number of genes, including IFN-β, perpetuating IFN synthesis and induction of parallel antiviral pathways [30]. It is clear that PKR is an important mediator of extracellular stimuli that regulate many aspects of cellular physiology, including regulation of cell growth and differentiation. Moreover, PKR might function as a tumour suppressor and its overexpression leads to apoptosis via BCL-2 and BCL-X_L inhibition. Numerous viruses block PKR activation or action using a variety of biochemical strategies. Although some investigations suggest that PKR is dispensable in defence against virus infection, several experiments underline its crucial role as a mediator of IFN activity. For example, overexpression of PKR leads to inhibition of EMCV replication, and dominant-negative PKR mutant or antisense PKR cDNA suppress the anti-EMCV action of type I IFNs. Mice lacking PKR are predisposed to lethal intranasal infection by usually innocuous vesicular stomatitis virus and display increased susceptibility to influenza virus infection [33,34]. Studies using PKR null mice have also shown that this kinase is a key component of the host early defence system that acts early in innate immunity before activation of the IFN system and acquired immune response [34].

Mx proteins, first identified as the anti-influenza virus proteins, are induced solely by type I IFNs [35,36]. There are both nuclear and cytoplasmic isoforms of Mx, which belong to the dynamin superfamily of GTPases that are involved in endocytosis and vesicle transport [37]. The human MxA protein forms large oligomers. A mutant MxA that fails to form oligomers is devoid of GTPase activity. This mutant, however, retains antiviral activity, although it is rapidly degraded in cells [38]. Mx proteins possess intrinsic antiviral activity and do not require cooperation with any other IFN-induced cellular

protein for their action. Their antiviral activity depends on GTP binding as well as protein–protein interactions with viral proteins, resulting in either disruption of viral RNA synthesis or blocking of viral nucleocapsid transport. A unique property of Mx GTPases is their antiviral activity against a wide range of RNA viruses. A mouse *Mx* gene has been shown to exert inhibitory effects on orthomyxoviruses, paramyxoviruses, rhabdoviruses and togaviruses [27]. No inhibition of picornavirus (Mengo and EMCV) replication was observed with the Mx pathway [27]. Another family of IFN-induced guanylate-binding proteins (GBPs) includes GBP-1 and GBP-2. They constitute the most abundant class of proteins induced by IFN-γ. GBPs have GTPase functions. They bind and hydrolyse GTP and may have mild antiviral activity [39].

The IFN-inducible ADAR family of enzymes are involved in editing RNA by substituting adenosines (A) with inosines (I) in cellular mRNA and viral dsRNA targets [40,41]. The transition of A to I decreases the stability of duplex RNA by conversion of stable AU base pairs into less stable IU base pairs. Subsequently, coding capacities of the target RNA are changed. Such site-specific editing can cause amino acid substitution and, as a result, synthesis of proteins with altered functions [41,42]. ADAR may cause a hypermutability to a number of viruses, including measles, parainfluenza type 3, vesicular stomatitis, polyomavirus and hepatitis delta virus, that may lead to inhibition of virus replication and eventually to persistent infection [27,43].

Given the ability of viruses to induce ISGs with antiviral activity, it is not surprising that viruses developed mechanisms that counteract the stimulation of ISGs. This includes inhibition of almost all aspects of the IFN regulatory pathway, including disruption of dsRNA and IFN/JAK–STAT signalling, inhibition of IRF, inhibition of NF-κB and others [20,30].

4. Treatment of coronaviruses with interferons

Sensitivity of animal and human coronaviruses (unrelated to SCV) to IFNs was demonstrated in both *in vitro* and *in vivo* studies. Coronaviruses, including avian infectious bronchitis virus, murine hepatitis virus (MHV), transmissible gastroenteritis virus and human coronaviruses, were sensitive to IFN treatment [44-48]. Although both types of IFNs were effective against coronavirus infection, some studies suggested that type I IFNs may be more potent than IFN-γ. In one study, IFN-γ even stimulated 100-fold production of infectious virions of human coronavirus OC43 strain in human neuronal cells [49]. IFN-α was more potent against MHV compared with IFN-γ in a mouse model. More recent work showed that IFN-α displayed the strongest inhibitory activity among several potential anti-SARS drugs on human coronavirus 229E replicase [50]. Combined treatment with both IFNs showed synergistic antiviral effects [51]. As discussed in the previous sections, most viruses potentially induced IFN pathways, eventually leading to IFN production by infected cells. The ability to induce cellular IFN production, which may modulate virus infection, was demonstrated both for animal and human coronaviruses [47,52-54]. It has been suggested that the enteropathogenic potential of human coronavirus OC43 (strain Paris) may be due to a lack of IFN induction [53]. Moreover, leukocytes of children with recurrent respiratory infections produced lower yields of IFN after stimulation with coronavirus compared with the control cohort [54].

There are few clinical experiences with treatment of coronavirus infections with IFNs. In an experimental setting, 55 healthy volunteers were treated with intranasal recombinant IFN (IFN-α-$_{2b}$; 2×10^6 IU/day) or placebo for 15 days and were exposed to coronavirus by direct intranasal inoculation on the eighth day of treatment [55]. The therapy with IFN shortened the duration and reduced the severity of coronavirus cold symptoms, suggesting that intranasal recombinant IFN-α may be an effective prophylactic treatment for coronavirus infection in humans. Similarly, intranasal sprays of IFNs given 1 day before and for 3 days after virus challenge protected human volunteers from infection with coronavirus [56].

5. Treatment of SCV with interferons

The published data describing the effect of IFNs on SCV replication and on SARS is listed in Table 2. Two clinical reports described the use of IFN-α for the treatment of SARS patients [6,10]. The first study described treatment of 190 SARS-patients from Guangzhou, the capital of Guangdong [10]. The authors concluded that the best outcome was achieved by the combination of high-dose steroids with quinolone plus azithromycin. In the authors' opinion, the use of IFN-α did not result in an obvious anti-SARS effect. However, IFN-α remained an optional part of the developed protocol.

The second study used IFN alfacon-1, (Infergen®, Intermune, Brisbane, California, USA), a non-naturally occurring synthetic recombinant type I IFN-α that contains in each amino acid position the most commonly observed amino acid from 13 IFN-α non-allelic subtypes [6]. Its specific activity against numerous viruses was higher than that exhibited by other IFN-α agents, indicating its higher antiviral activity on a molar basis [57]. Moreover, IFN alfacon-1 induced NK cell activity more potently compared with IFN-α-$_{2a}$ and IFN-α-$_{2b}$. In the preliminary, uncontrolled study of patients with SARS, 13 patients who received single treatment with corticosteroids were compared with 9 patients who additionally received IFN alfacon-1 (7 patients received 9.0 μg/day, 2 patients received 15.0 μg/day) [6]. Use of IFN alfacon-1 resulted in more rapid resolution of radiographic lung abnormalities and better oxygen saturation levels. Moreover, IFN alfacon-1 patients showed less increases in creatine kinase levels and a more rapid return of lactate dehydrogenase to normal levels. Elevated lactate dehydrogenase and creatine kinase levels are suspected to indicate lung parenchymal damage and are associated with poor prognosis [6]. In

Table 2. Studies describing the examination of interferons on SCV replication *in vitro*.

Conditions		IFN-	Outcome	Reference
Cell culture				
Cell lines	**SCV strain**			
Vero, Caco-2	FFM-1, Hong Kong	IFN-α-$_{2b}$, IFN-β-$_{1b}$, IFN-γ-$_{1b}$	IFN-β is most effective	[16]
Vero E6	Tor2, Tor7, Urbani	IFN-β-$_{1a}$	IFN-β-$_{1a}$ inhibits SCV replication	[18]
Vero	Patient 5688	PEG-IFN-α-$_{2b}$	PEG-IFN-α-$_{2b}$ inhibits SCV replication	[17]
Vero E6	Tor2, Tor3, Tor7, Tor684	IFN-α-$_{2b}$	IFN-α-$_{2b}$ inhibits SCV replication	[62]
Vero E6	SCV 2003VA2774	IFN-α (2a, 2b, n1, n3, human leukocyte) IFN-β (1a, 1b)	IFN-α-$_{n1}$, IFN-α-$_{n3}$ and IFN-β-$_{1b}$ were most effective	[63]
Animal experiments				
Species	**SCV strain**			
Cynomolgous macaques	Patient 5688	PEG-IFN-α-$_{2b}$	PEG-IFN-α-$_{2b}$ inhibits SARS symptoms	[17]
Clinical trials				
Number of IFN-treated patients				
135		IFN-α (3,000,000 IU/d)	No improvement in IFN-α-treated patients detected	[10]
9		IFN-alfacon 1 (9 – 15 µg/d)	Improved outcome of IFN-α-treated patients detected	[6]

IFN: Interferon; PEG-IFN-α-$_{2b}$: Pegylated IFN-α-$_{2b}$; SARS: Severe acute respiratory syndrome; SCV: SARS coronavirus.

addition, treatment with IFN alfacon-1 decreased the median time of peak lung involvement and IFN alfacon-1-treated patients needed supplemental oxygen for shorter periods. In the case of a re-emergence of SARS, Health Canada has already approved a protocol for a trial with alfacon-1 that does not include steroids or ribavirin [58].

The reason for the different activities of IFN-α in these two studies remains speculative. Zhao *et al*. did not explicitly focus on IFN-α and did not report all of the parameters investigated by Loutfy *et al.*, such as lactate dehydrogenase and creatine levels, which are regarded to be indicators of tissue damage in the lungs [10]. In addition, given the specific activity of IFN alfacon-1 at $\geq 3 \times 10^9$ IU/mg [57], doses of 9 or 15 µg/day (i.e., $\geq 2.7 \times 10^7$ or $\geq 4.5 \times 10^7$ IU/mg) used by Loutfy *et al.* [6] are ~ 10-fold higher than the 3,000,000 IU used by Zhao *et al.* [10]. Therefore, differences might be explained by different dosing of IFNs.

Loutfy *et al*. concluded that anti-SARS effects of IFN alfacon-1 may be the result of treatment-induced reduction of viral load and/or synergistic immunosuppression in combination with corticosteroids [6]. The first published *in vitro* study that compared effects of different classes of IFNs on SCV replication, however, demonstrated IFN-β to be the most effective in inhibiting SCV replication [16]. So far, this is the only report that has investigated the influence of IFNs on SCV replication in a human cell line (human colorectal adenocarcinoma cell line, Caco2). All other investigations were exclusively performed in African green monkey kidney cell line Vero. Effects of recombinant IFN-α (IFN-α-$_{2b}$ [Intron® A], Essex Pharma, Munich, Germany), IFN-β (IFN-β-$_{1b}$ [Betaferon®], Schering, Berlin, Germany) and IFN-γ (IFN-γ-$_{1b}$ [Imukin®], Boehringer Ingelheim, Ingelheim, Germany) on two different virus strains (FFM-1, Hong Kong) were compared in Vero and in Caco2 cells. After pretreatment, EC_{50} (concentration of the compound needed to inhibit the cytopathic effect to 50% of the control value) of IFN-β for SCV FFM-1 in Vero cells was 50-fold and 25-fold lower than that of IFN-α and IFN-γ, respectively. Similar results were obtained for the comparison of anti-SCV effects of IFN-α with IFN-β using SCV Hong Kong in Vero cells and for both strains in Caco2 cells. By contrast, IFN-γ did not inhibit SCV replication in Caco2 cells. When added after virus infection, IFN-β was the only IFN that showed anti-SCV effects.

Although IFN-α and IFN-β share the same receptors and primarily induce the same proteins, they may evoke different antiviral activity due to different abilities to activate signalling pathways and different impacts on specific gene induction. Comparison of IFN-α and IFN-β effects on herpes simplex virus type 1 (HSV-1) replication resulted in superior antiviral

activity of IFN-β [59]. By using oligonucleotide arrays with probe sets corresponding to > 6800 human genes, > 300 genes differentially regulated by IFNs were already identified [26]. PKR, which plays an essential role in replication inhibition of numerous viruses, was upregulated by IFN-β, but not by IFN-α, in the human fibrosarcoma cell line HT1080 [26]. Moreover, most viruses developed mechanisms in order to antagonise IFN-induced antiviral effects by different mechanisms, including interference with ISGs [20]. SCV induced robust upregulation of ISGs with antiviral activity, such as MxA in human cells. This upregulation did not abolish virus replication [16], indicating that SCV developed strategies to counteract antiviral activities of IFNs. The nature of those effects possibly influences the sensitivity of SCV to different IFNs. This might help to explain the different sensitivities of SCV to IFN-β and IFN-α.

A second *in vitro* study reported on the use of IFN-β-$_{1a}$ on SCV replication [18]. In this study, effects of IFN-β-$_{1a}$ on replication of three SCV isolates (Tor2, Tor7, Urbani) were investigated in Vero E6 cells because IFN-β-$_{1a}$ was found to have a specific activity 14 times higher than IFN-β-$_{1b}$ [60]. The use of IFN-β-$_{1a}$ required IC_{50} (50% inhibitory concentration) as low as 50 IU/ml after pretreatment and 500 IU/ml after posttreatment. As IFN-β-$_{1b}$ was not assessed by Hensley *et al.* in their system, differences in anti-SCV activity of different IFN-β preparations remained undetermined. Cinatl *et al.* compared different IFN-β preparations and found that they exhibited similar effects when compared at an antiviral units basis, that is, IU/ml of a culture medium [61]. However, the higher specific activity of IFN-β-$_{1a}$ compared with IFN-β-$_{1b}$ may be favourable for the achievement of maximal doses of IFN for patients.

On completion of this manuscript, two further publications that investigated the effect of IFNs on SCV replication *in vitro* appeared. The first report confirms the already published data, showing that IFN-α-$_{2b}$ inhibits replication of SCV strains Tor2, Tor3, Tor7 and Tor684 in Vero E6 cells [62]. The second report describes the screening of clinically approved antivirals on SCV strain SCV 2003VA2774 in Vero E6 cells [63]. Surprisingly, IFN-α-$_{2a}$, IFN-α-$_{2b}$ and IFN-β-$_{1a}$ were found to be ineffective. In contrast to this, IFN-α-$_{n1}$, IFN-α-$_{n3}$ and IFN-β-$_{1b}$ inhibited SCV replication. No explanation for this result was offered.

A recent study considered the use of pegylated recombinant IFN-α-$_{2b}$ (PEG-IFN-α-$_{2b}$, PEG-Intron®, Schering-Plough Corporation [64]), a registered drug for the treatment of chronic hepatitis C, as a candidate drug for SARS therapy [17]. Pegylation is the attachment of an inert polyethlylene glycol polymer to proteins. The absorption of pegylated molecules is slower, the half-life is longer and the rate of clearance from the plasma is lower than that of the native molecule. Therefore, pegylation of IFN increases the duration of its biological activity [65]. Pegylated IFN-α exhibited a dose-dependent inhibitory effect on SCV replication *in vitro*. The results were similar to those obtained for IFN-α by Cinatl *et al.* [16]. Prophylactic treatment of cynomolgous macaques prior to SCV infection substantially protected type 1 pneumocytes, the main target cells for SCV infection in macaques, from SCV infection *in vivo*. Use of pegylated IFN-α-$_{1b}$ postexposure protected type 1 pneumocytes less effectively. Although *in vitro* data suggested direct influence of pegylated IFN-α on SCV replication, the investigators stated that it remains to be determined whether *in vivo* protection by pegylated IFN-α is caused by direct antiviral activity or immunomodulatory effects [17]. Given the superior anti-SCV activity of IFN-β compared with IFN-α *in vitro*, *in vivo* evaluation of pegylated IFN-β [66] would be of high interest.

Severe immunological tissue damage was detected in the lungs of SARS patients, suggesting that immune responses and inflammatory processes exacerbate SARS [65,66]. In accordance with this, the only generally accepted pharmacological clinical intervention in SARS is suppression of local immune responses and inflammatory processes using corticosteroids [68]. Therefore, the combination of antiviral therapy with anti-inflammatory therapy is a promising therapeutic approach for the treatment of SARS [70] and the influence of IFNs on immune response is also important for clinical outcome. Nearly all phases of innate and adaptive immune responses are affected by IFNs. The immunomodulatory action of type I IFNs includes enhanced cytotoxicity of NK cells, enhanced expression of major histocompatibility complex class I proteins and enhanced susceptibility of T cells to apoptosis [19,30,71]. Moreover, IFNs are able to modulate inflammatory processes [25].

No experimental data concerning the influence of IFNs on immune response and inflammatory processes in response to SCV infection are available so far. However, knowledge of the impact of IFNs on the immune response to SCV and on SARS-associated inflammatory processes will be highly important for the rational use of IFNs as SCV replication inhibitors and/or modifiers of immune response and inflammatory processes.

6. Expert opinion

In view of possible further SARS outbreaks, highly efficient therapeutic strategies are needed. Experimental and pilot clinical data strongly suggest that type I IFNs are promising candidates for SARS treatment protocols. Not only may they be used as inhibitors of SCV replication, but they may also be used to improve deregulated immune responses and inflammatory processes that are known to contribute to SARS. Although the majority of studies focus on the evaluation and clinical use of IFN-α, in the authors' hands, IFN-β was superior compared with IFN-α in terms of SCV replication inhibition. The fact that IFN-β elicits its inhibitory potential even after infection has already occurred further underlines the supposed therapeutic value. To be well prepared against possible future SARS outbreaks, the authors strongly recommend the evaluation and improvement of therapeutic strategies involving IFN-β preparations.

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