

Antiviral Strategies: The Present and Beyond

J.D. Burke and E.N. Fish*

Division of Cellular and Molecular Biology, Toronto General Research Institute, University Health Network, Toronto, Canada

Abstract: Historically, vaccine strategies have proven to be most effective at eradicating the targeted virus infections. With the advent of new or re-emerging altered viruses, some of which jump species to infect humans, the threat of viral pandemics exists. The protracted time to develop a vaccine during a pandemic necessitates using antiviral drugs in the intervening months prior to vaccine availability. Antiviral drugs that are pathogen specific, for example Amantadine, Tamiflu® and Relenza®, targeted against influenza viruses, are associated with the emergence of virus strains that are drug resistant. The use of ribavirin, a more broad spectrum antiviral, in combination therapies directed against influenza and hepatitis C virus, has proven effective, albeit to a modest extent. Attention is focused on the potential use of interferons (IFN)- α/β as broad spectrum antivirals in acute infections, to invoke both direct antiviral effects against viruses and activation of specific immune effector cells.

Keywords: Influenza, HCV, pandemic, immunomodulatory, antiviral, interferon, drug resistance.

INTRODUCTION

Newly emerging viral infections represent a major threat to human health. The 3 major influenza pandemics in the last century together killed more people than any other natural or man-made disaster, distinguishing influenza as one of the deadliest acute infectious diseases in human history. An epizootic influenza A virus, H5N1, that is highly pathogenic in humans poses an increasing pandemic threat [1-3]. During the last few decades dengue virus has reemerged in several regions in southeast Asia [4]. Nipah virus is a recently emergent paramyxovirus that is capable of causing severe encephalitic disease in both humans and animals [5]. Enteroviruses are ubiquitous and not normally considered as emerging pathogens, yet several enterovirus serotypes have been associated with the emergence of specific diseases to cause outbreaks of major public health concern: enterovirus 70 associated with acute hemorrhagic conjunctivitis and enterovirus 71 as the recognized cause of epidemic severe central nervous system disease in SE Asia [6]. Severe acute respiratory syndrome (SARS) emerged in southern China in late 2002 and spread in the Spring of 2003 to 30 countries, affecting over 8000 individuals and causing over 800 deaths. The etiologic agent was identified as a novel coronavirus, SARS-CoV [7]. Ebola and Marburg viruses are among the best known examples of emerging and re-emerging pathogens. Although outbreaks have been sporadic and geographically restricted to areas of central Africa, the hemorrhagic fevers caused by these viruses are remarkably severe and are associated with high case fatality rates often exceeding 80 percent [8].

Vaccine development is an extremely effective strategy to protect from *specific* virus infections. An appropriate vaccine cannot be developed before a virus emerges that can be replicated in sufficient quantities to manufacture the vaccine. Notably, newly emerging viruses exhibit genetic drift or reassortment of genes, such that targeted vaccines require the virus strain to be first identified and characterized. Antiviral drugs, that are also pathogen-specific, have a role in pandemic situations. For example, the neuraminidase inhibitor, oseltamivir, constitutes an important treatment option against H5N1 and stockpiling of this drug is part of pandemic preparedness [9]. However, extensive data on its efficacy are not available and recent reports of drug resistance in Vietnamese patients infected with H5N1, raise serious concerns [10]. Indeed, the cost to develop antivirals targeted against single viruses, the concerns about stability of drug and development of drug resistance, let alone affordability, suggest new strategies may be warranted. This review focuses on current treatment modalities for two global virus infections – influenza and hepatitis C (HCV)– and addresses the notion

of alternative immunomodulatory approaches to complement current antiviral strategies.

ANTIVIRAL STRATEGIES TARGETED AGAINST INFLUENZA VIRUSES

Influenza viruses, members of the *Orthomyxoviridae* family, are classified as types A, B or C, based on antigenic differences in their nucleoprotein (NP) and matrix protein (M1). Type A viruses are further subtyped based on the antigenicity of two surface glycoproteins: hemagglutinin (HA) and neuraminidase (NA) [11]. Currently, 16 HA and 9 NA subtypes have been identified among type A viruses [11, 12]. Type A influenza viruses have been isolated from various animals, including humans, pigs, horses, sea mammals, cats, dogs and birds [13].

The epizootic influenza A virus, H5N1, that is highly pathogenic in humans, has crossed the species barrier in Asia and most recently, Turkey, Iraq, Egypt, Djibouti and Nigeria, to cause many human fatalities and poses an increasing pandemic threat [1-3]. As of November 12, 2007, a total of 335 cases of human H5N1 influenza have been reported, with 206 deaths (i.e. 60% case-fatality ratio) [14].

Emerging influenza viral strains exhibit genetic drift or reassortment of genes [15], such that humans are immunologically naive with respect to the HA. In the context of a pandemic influenza strain, the virus may also contain a novel NA subtype. Therefore, a vaccine strategy requires the virus strain to be first identified and characterized, implying that vaccines are not likely to be available at an early stage of a pandemic. Although there may be some benefit in developing a vaccine against an H5 influenza virus, the likelihood of a good match with a pandemic strain may be low. Limited supplies of vaccine against existing H5N1 avian viruses are being manufactured to test for dosing schedules and the immune response that might be expected [16-18]. The possibility that such generic H5 vaccines might be used to prime most vulnerable individuals is under consideration.

During the first months of a pandemic, control measures therefore, would rely mainly on antiviral drugs. The adamantane derivatives, amantadine and rimantadine, as M2 ion channel-blockers were the first antivirals licensed for use against influenza A viruses [19]. Amantadine-resistant influenza A viruses have emerged, including resistant H5N1 influenza variants in Asia [20]. Since 2003, the frequency of adamantane resistance has risen dramatically from less than 5% to greater than 90% of isolated influenza A [21]. This resistance arises within days of treatment in the patient [22, 23], and is predominantly observed in H3N2 isolates, whereas resistant H1N1 strains are also increasingly frequent [24]. It has been shown that the typical mutations at M2 amino acid residues 26, 27, 30, 31 and 34 have not had an abortive effect on the replicative fitness of

*Address correspondence to this author at the Department of Immunology, University of Toronto, Toronto, 67 College Street, Rm. 424, Toronto, Ontario M5G 2M1, Canada; Tel: 416 340-5380; Fax: 416 340-3453; E-mail: en.fish@utoronto.ca

the emerging viruses [25-27]. Thus, resistant strains remain infectious and transmissible. Sequence analysis of 55 avian H5N1 isolates from SE Asia has revealed that close to 90% of the strains bear mutations that are known to confer adamantane resistance [28]. Given the high prevalence of adamantane resistant strains of influenza, the CDC has discouraged the use of adamantanes for the 2007-2008 influenza seasons [29].

Two other drugs have been licensed globally for specific treatment and prevention of influenza, function as NA inhibitors (Fig. 1). NA inhibitors are structural analogues of sialic acid, and prevent the dissociation of budding virions from the cell surface that is normally mediated by NA cleavage of cellular sialic acid. Relenza® (zanamivir) was developed based on knowledge of the 3-dimensional structure of the influenza virus NA complexed with its substrate, sialic acid [30]. Tamiflu® (oseltamivir) was subsequently developed, based on the structure of Relenza® [31]. Relenza® has poor bioavailability, it must be delivered topically and is administered by means of an inhaler which delivers drug to the upper respiratory tract, the primary site of virus replication. Substitution of the glycerol side chain with a hydrophobic side chain enables Tamiflu® to be orally available. Tamiflu® is taken in an encapsulated form as a prodrug, which is activated by liver esterases to form the active drug. Stockpiling of Tamiflu® is part of pandemic preparedness [9]. There is accumulating evidence of emerging resistance towards Tamiflu® and to a lesser extent, zanamivir [32]. Moreover, *in vitro* experiments have demonstrated the evolution of a zanamivir dependent virus, where the drug enhances infection [33]. Several clinical studies have revealed Tamiflu® resistance in 1-2% of adults [10, 34, 35] and a notably higher frequency of 18% in children [36, 37]. A third NA inhibitor, peramivir, has been developed, but failed to show efficacy in clinical trials when administered orally and is currently being tested for intramuscular and intravenous administration efficacy [38]. In 55 avian H5N1 isolates from SE Asia, 2 strains of influenza exhibited *in vitro* resistance to NA inhibitors. One of those strains showed 63-, 11- and 4-fold increased IC₅₀s for

zanamivir, oseltamivir and peramivir, respectively [28]. A novel peptide that inhibits attachment of influenza viruses to the HA protein, the cellular receptor for influenza viruses, has been developed as an antiviral, but clinical data are not available yet and the likelihood of emerging resistant strains exists [39]. Effective inhibition of multiple subtypes of influenza A virus is achievable *in vitro* using peptide conjugated phosphorodiamidate morpholino oligomers, single-stranded-nucleic-acid-like antisense agents that can reduce gene expression by sterically blocking complementary viral RNA sequences [40].

COMBINATION THERAPIES FOR INFLUENZA

Given the emergence of drug resistant strains of influenza, there is an increased interest in moving away from monotherapies to consider combination therapies [41]. Amantadine and ribavirin exhibit synergistic effects in reducing mortality in mice infected with lethal doses of influenza type A [42]. Similarly, combination therapy with oseltamivir and ribavirin revealed that treatment up to 4 days post-infection afforded a 50-80% reduction in mortality from a lethal infection of either influenza A or B [43]. The application of an antioxidant, N-acetylcysteine, has been investigated in combination with oseltamivir [44] or ribavirin [45], conferring 90-100% protection when doses were administered after a lethal infection with influenza. In addition to *in vitro* data that identify synergistic antiviral effects of rimantadine combined with NA inhibitors [46], there is evidence that this combination therapy reduces the emergence of resistant strains of influenza.

HEPATITIS C: ACUTE AND CHRONIC DISEASE

Afflicting an estimated 170 million individuals worldwide, hepatitis C virus (HCV) is more prevalent than HIV [47]. Initially described as a non-A, non-B hepatitis virus, HCV was identified as an RNA virus belonging to the family *Flaviviridae*, separate from

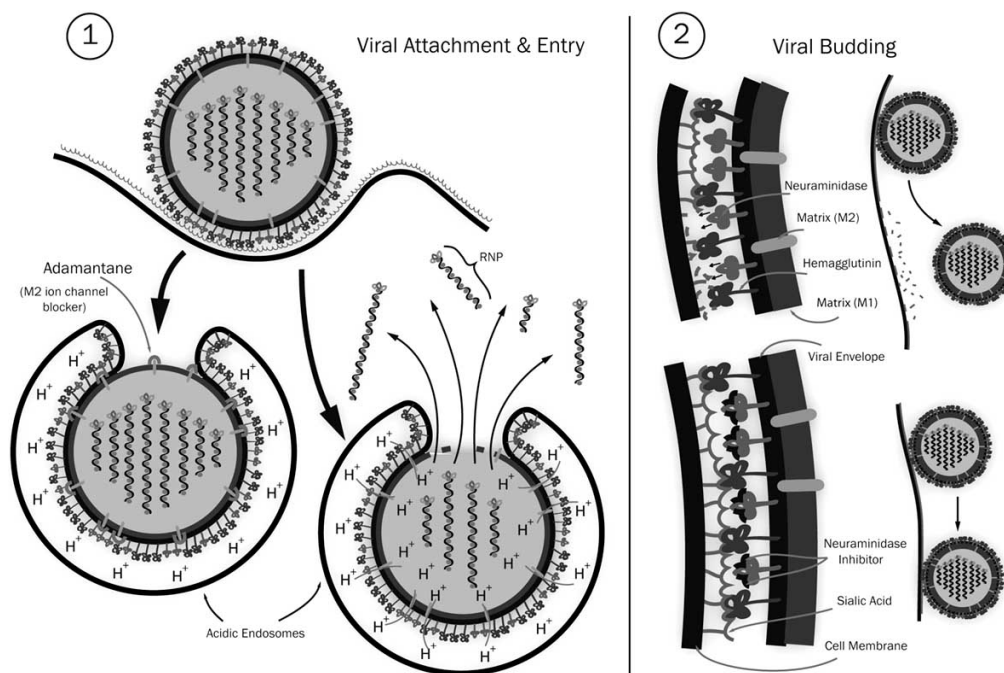


Fig. (1). Inhibition of influenza virus with neuraminidase inhibitors and adamantanes. 1) Entry of the influenza virions is initiated by the binding of neuraminidase to sialic acid residues on the cell surface. Following endocytosis, acidification of the endosomal compartment promotes fusion of the virion and endosomal membranes mediated by hemagglutinin (HA). Further acidification of the virion core allows the release of the viral ribonucleoprotein (RNP) complex into the cytoplasm. Adamantanes block the M2 channel preventing acidification of the virion core, thus inhibiting release of the viral RNP complex. 2) Egress of a newly formed influenza virion is mediated by the viral neuraminidase (NA) which cleaves the link formed between the viral HA and sialic acid in the extracellular matrix. Neuraminidase inhibitors competitively bind the active site of NA, thus preventing the cleavage of sialic acid and release of the virus.

the families of HAV and HBV, belonging to the families *Picornaviridae* and *Hepadnaviridae*, respectively. Two major stages of disease have been delineated: acute and chronic. In the relatively asymptomatic acute phase of the disease, the onset of which occurs within 7-8 weeks of exposure to virus, some patients exhibit jaundice, malaise and nausea [48]. Spontaneous resolution of acute hepatitis has been observed, however most (~85%) untreated cases progress to chronic disease [49]. Although not all patients experience the same level of disease severity, over a period of years chronic HCV leads to fibrosis of the liver, then cirrhosis, end-stage liver disease and in many cases, hepatocellular carcinoma [50].

HCV THERAPY

Since the mid 1990s, the standard treatment for chronic HCV has been a combination treatment of interferon (IFN)- α with ribavirin, to achieve a sustained virological response (SVR) of undetectable viral RNA 6 months after completion of therapy. Pegylation of IFN- α 2a and IFN- α 2b has led to improved pharmacokinetic properties and chemical stability [51, 52]. For chronic infection with HCV, the success rate of combination treatment is clearly linked to the infecting HCV genotype. Of the 6 known genotypes, types 2 and 3 HCV are most responsive to combination therapy, for which SVRs are achieved in 75-90% of cases, whereas only 40-50% of patients with type 1 HCV infections achieve a SVR [53]. The underlying reasons for this difference in sensitivity of the different HCV genotypes to IFN + ribavirin therapy remains unclear. Notably, for those patients with acute HCV genotypes 2/3, if treatment is implemented within 8-12 weeks of the initial onset of symptoms, >90% of patients exhibit a SVR [54, 55].

RIBAVIRIN: MODES OF ACTION

Ribavirin was initially developed as a broad spectrum antiviral effective against DNA and RNA viruses [56]. Ribavirin inhibits inosine monophosphate dehydrogenase (IMPDH), an enzyme required for GTP synthesis, thereby limiting viral replication by de-

pleting available GTP pools. As a nucleoside analogue, ribavirin is converted to ribavirin triphosphate (RTP) and has the potential to act as a mutagen. *In vitro* experiments have shown that the HCV RNA polymerase incorporates RTP into a nascent strand of RNA without chain termination. The incorporated ribavirin residues can then base-pair with cytidine or uridine, contributing further to an already error-prone replication process [57]. Before its widespread use for HCV, ribavirin was clinically effective as an aerosol therapy for respiratory syncytial virus infections in children [58]. As a nucleoside analogue directly inhibiting viral replication, it has been suggested that ribavirin may also influence the host immune response to virus infection (Fig. 2). Specifically, ribavirin will modulate the balance between a Th1 and Th2 immune response, favouring polarization towards Th1 [59, 60]. Individuals chronically infected with HCV exhibit poor CD8⁺ T-cell responses, whereas those who mount a robust CD8⁺ T-cell response during the acute phase of disease clear HCV efficiently [61]. Additionally, in the context of influenza (see above), because of intracellular accumulation of RTP, ribavirin acts as a competitive inhibitor of ATP and GTP for the influenza virus RNA polymerase, thus limiting viral replication [62].

IFNS: CRITICAL FOR AN INNATE IMMUNE RESPONSE TO VIRUS INFECTION

IFNs are a family of biologically active proteins classified as type I (IFN- α s, - β , - ω , - τ , - κ , - ϵ), type II (IFN- γ) and type III (IFN- λ) [63-66]. Type I IFNs mediate diverse biological effects, including the largely cell type-independent antiviral and antiproliferative responses, and several cell type-restricted responses of immunological relevance such as maturation and differentiation of dendritic cells and modulation of B, T and NK cell responses [67]. IFNs have widespread clinical application as therapeutic agents for the treatment of viral infections, especially in the context of hepatitis B and C (HCV) infections (see above). IFNs inhibit viral infection by preventing viral entry into target cells and by blocking different stages of the viral replicative cycle for different viruses [68].

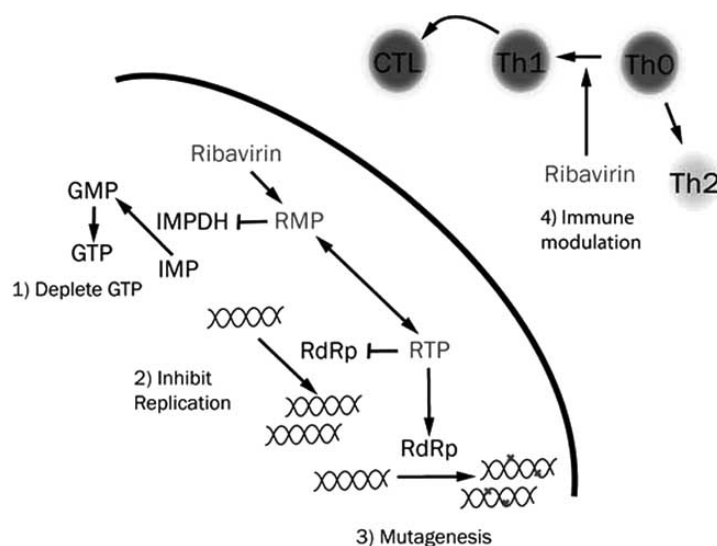


Fig. (2). Cellular and viral targets of ribavirin.

1) The conversion of ribavirin to ribavirin monophosphate (RMP) enables RMP to competitively bind to inosine monophosphate dehydrogenase (IMPDH) and prevent the conversion of inosine monophosphate (IMP) to guanosine monophosphate (GMP). This inhibition results in the downstream depletion of GTP pools, thus blocking *de novo* RNA synthesis. 2) Ribavirin triphosphate (RTP) is able to competitively inhibit the viral RNA dependent RNA polymerase (RdRp) and prevent the polymerization of other nucleotides into the nascent RNA strand. 3) Alternatively, RTP will incorporate into viral RNA thereby creating sequence mutations. 4) Ribavirin exerts immunomodulatory effects by promoting the polarization of T cells towards a Th1 bias, thereby promoting a stronger CD8⁺ T-cell immune response.

IFN- α treatment in hepatitis B and hepatitis C patients, in addition to directly inhibiting viral replication, also suppresses the associated hepatocellular carcinoma and liver fibrosis [69]. Additionally, Type 1 IFNs have a critical role in linking the innate and adaptive immune responses to viral challenge. IFN- α/β expression occurs as an early response to viral challenge, preceding other immune responsive cytokines. IFNs- α/β regulate the activities of other cytokines and their receptors, including IFN- γ , IL-1, IL-1 receptor, IL-2, IL-3, IL-8, TNF- α , IL-18 etc, and chemokines such as MIP-1 α , MIP-1 β , RANTES, and the CC chemokine receptor, CCR5.

The type I IFNs are critical components of the antiviral innate immune response, induced as a consequence of activation of pattern recognition receptors, namely the toll-like receptors (TLRs) and the cytoplasmic RNA helicases, that recognize viral replicative intermediates and viral nucleic acids in a non-pathogen-specific manner [70] (Fig. 3). These sensors are involved in coupling recognition of viruses to the induction of type I IFN genes. Plasmacytoid dendritic cells (pDCs) produce large amounts of type I IFNs after viral activation [71, 72]. pDCs express TLR7 and TLR9 which are restricted to endosomal compartments where they recognize viral nucleic acids, single stranded (ss) RNA and DNA [73, 74]. TLR3 functions as a pattern recognition receptor for double stranded (ds) RNA, a pathogen associated molecular pattern produced by viruses during replication [75]. TLR3 is localized to intracellular vesicles in monocyte-derived DCs, whereas fibroblasts express TLR3 on their cell surface [76]. The RNA helicases, RIG-I and Mda5, detect actively replicating virus in the cell cytoplasm, specifically dsRNA [77, 78].

The high affinity interaction of an IFN- α/β with its cognate cell surface receptor complex initiates multiple signaling cascades that include activation of the JAK-STAT, CrkL, p38 MAPK, and PI3K/mTOR pathways [79, 80] (see inset in Fig. 3). These pathways induce the expression of antiviral proteins such as the

dsRNA-dependent protein kinase R (PKR), 2'-5'-oligoadenylate synthetase (OAS)/endoribonuclease L (RNase L), the myxovirus resistance protein (Mx), RNA-specific adenosine deaminase (ADAR1), the P56 family of proteins, tumor necrosis factor-related apoptosis-inducing ligand (TRAIL), viperin, phospholipids scramblase (PLSCR1), and ISG15. These antiviral effectors coordinately control virus spread by limiting virus replication, assembly and egress and by inducing apoptosis in virally infected cells. PKR senses viral dsRNA, signaling the inhibition of translation *via* phosphorylation of eIF2 α . Activation of 2'-5'OAS by dsRNA initiates the cleavage of ssRNA by an associated protein RNase L. Mx GTPases interfere with viral replication by binding to viral components, e.g. nucleocapsid-like structures, preventing their transportation to appropriate cellular compartments, thereby making them unavailable for the generation of new viral particles [81]. The type III IFN- λ s elicit similar antiviral effects as the IFNs- α/β [64].

IFN- α AS A THERAPEUTIC AGAINST SARS-COV

No effective therapeutic strategy has been developed for patients with SARS. Treatments that were initially used in the last outbreak of SARS included ribavirin and corticosteroids but recent reports have shown that these are largely ineffective [82-84]. Subsequently, hyper immune globulin, protease inhibitors, fusion inhibitors and IFNs represented alternative therapeutic options for treating SARS patients [85-88]. In an open-label study, our group demonstrated the potential efficacy of IFN- α in SARS patients. Specifically, in this study of 22 patients diagnosed with SARS in Toronto, 13 patients were treated with corticosteroids alone and 9 with corticosteroids plus IFN- α . Our data revealed that IFN- α was well tolerated clinically, that the IFN treatment group had a shorter time to 50% resolution of lung radiographic abnormalities compared to the controls and that IFN treatment was associated with significantly better oxygen saturation. Additionally, IFN-treated

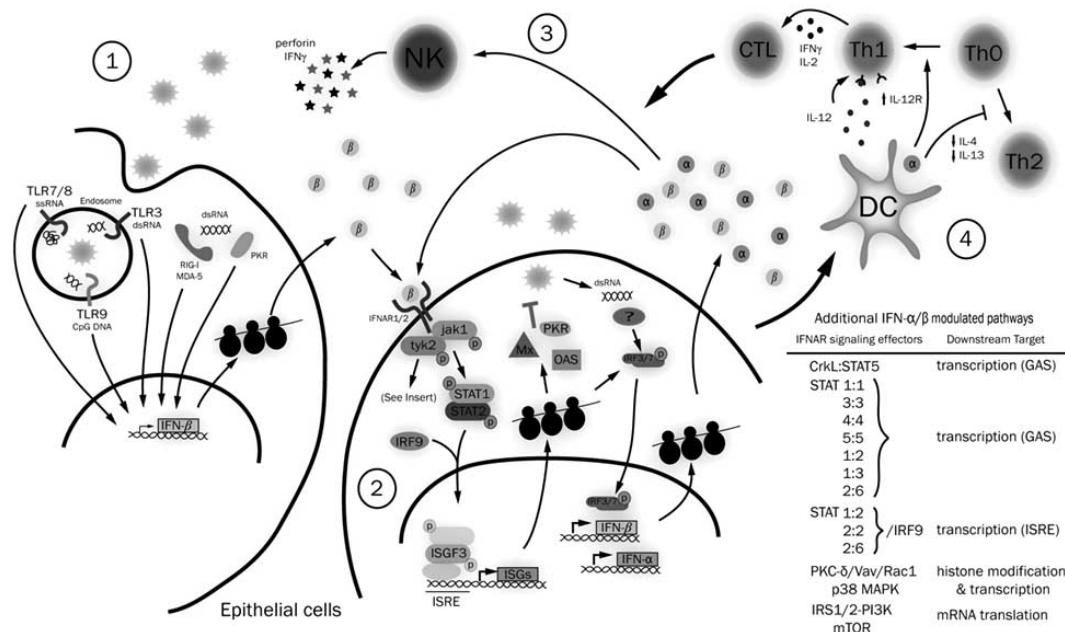


Fig. (3). The virus-inducible IFN α/β response.

1) Upon viral infection various pattern recognition receptors (PRRs) (ie TLRs, RIG1/MDA5, PKR) detect the presence of viral DNA/RNA in the cytoplasmic or endosomal compartments. Signaling cascades are rapidly activated to initiate transcription of IFN- β as well as other proinflammatory cytokines. 2) Release of IFN- β from an infected cell acts in paracrine to alert surrounding cells of a virus, and to induce an antiviral state. Several pathways including the Jak/STAT, CrkL, p38 MAPK and PI3K/mTOR upregulate the expression of a number of antiviral proteins (Mx GTPases, 2'-5'OAS, PKR, IRF3). If viral dsRNA is detected in the cell, IRF3/7 triggers the expression of IFN α/β which then acts to enhance the antiviral response. 3) IFN α present in the extracellular matrix activates natural killer (NK) cells by stimulating perforin and IFN γ mediated cytotoxicity. 4) In the draining lymph nodes, IFN α produced by activated dendritic cells (DC) effects the polarization of CD4⁺ T-lymphocytes: through increased expression of IL-12R and reduced IL-4 and IL-13 expression, Th0 cells are polarized to differentiate towards a Th1 lineage, specifically triggering CD8⁺ cytotoxic T lymphocytes (CTLs) to clear virus.

patients resolved their need for supplemental oxygen significantly faster than controls [89].

Broad Spectrum Antivirals

Given the broad-spectrum antiviral activities of type I IFNs, antiviral drug development strategies under consideration now include TLR stimulatory ligands and activators of IFN-inducible antiviral effectors as drug targets. Intranasal prophylactic administration of a TLR3 agonist, poly ICLC, a synthetic dsRNA, completely protected mice challenged with lethal doses of either influenza A or western equine encephalitis virus [90]. The prophylactic effects of a liposomal preparation of poly ICLC were sustained for a period of 21 days prior to infection [91]. Similarly, prophylactic administration of a TLR4 agonist, a lipid A mimetic, produced a protective effect against influenza infection [92]. TLR9 agonists, CpG oligodeoxynucleotides, likewise exhibit prophylactic effects in a number of respiratory infections [93]. More recently, TLR7/8 agonists mimicking dsRNA have been used successfully in the prophylaxis and treatment of influenza A infection in rats [94]. Notably, each of these TLR agonists induces IFN- α/β expression to effect antiviral activity.

dsRNA generated during viral infections activates IFN-inducible synthetases that produce 5'-phosphorylated 2',5'-oligoadenylates (2-5A) from ATP. 2-5A activates RNase L thereby blocking viral replication. Screening for low molecular weight activators of RNase L that bind to the 2-5A-binding domain of RNase L led to the identification of two lead compounds exhibiting broad-spectrum antiviral activity against diverse types of RNA viruses [95].

Distinct from IFNs and the broad spectrum antivirals that induce IFN production or activate IFN-inducible proteins that interfere with viral replication, are those antiviral agents that interfere with virus-cell interactions. An example of the latter is arbidol (ethyl-6-bromo-4-[(dimethylamino)methyl]-5-hydroxy-1-methyl-2-[(phenylthio)methyl]-indole-3-carboxylate hydrochloride monohydrate), that interferes with virus-membrane interactions for a number of viruses: influenza A and B viruses, parainfluenza virus 3, HCV, HBV and rhinovirus 14 [96-99].

CONCLUSIONS

Antiviral drug resistance is a major concern when proposing widespread use of pathogen-specific antiviral medications. Amantadine resistance has been found in human and avian H5N1 strains in China, Thailand, Vietnam and Cambodia and seasonal A/H3N2 and A/H1N1 strains are becoming increasingly resistant to the adamantanes. Although H5N1 is susceptible *in vitro* to NA inhibitors, oseltamivir resistant strains of H5N1 have been identified and recent studies demonstrate that treatment of human H5N1 infection may require higher doses and longer administration of oseltamivir to be effective. The paucity of information about the safety and efficacy of NA inhibitors against H5N1 is a current cause of concern in pandemic influenza planning. Vaccine development for pandemic preparedness requires prior knowledge of related strains of the anticipated pandemic virus, yet correspondence with the pandemic strain may be low. Moreover, current pandemic preparedness assumes that the most likely pandemic virus will be an avian influenza virus. Given the recent global outbreak of SARS, the potential for a new or re-emerging virus pandemic unrelated to influenza exists.

Accordingly, a complementary strategy that focuses on host factors that mediate broad spectrum antiviral activity is warranted. IFNs present as ideal candidates, based on their ability to interfere with multiple stages in viral replicative cycles: viral entry, uncoating, replication of viral genetic material, viral protein assembly and egress of viral progeny. Moreover, IFNs are implicated in enhanc-

ing the innate immune response to virus infection, activating T cells, B cells, DCs and NK cells. Drug development focused on enhancing IFN production or activating IFN-inducible antiviral effectors is ongoing. Additionally, the development of antiviral agents that interfere with essential virus-host interactions that are common to many viruses – broad spectrum antivirals – is underway. Viewed altogether, a multi-faceted approach to acquire a panel of safe, effective and non-cross reactive antiviral agents is both feasible and appropriate given the potential risk of a viral pandemic.

ACKNOWLEDGEMENTS

The authors gratefully acknowledge the Canadian Institutes of Health Research for grant MOP 15094 to ENF.

ABBREVIATIONS

ADAR1	=	RNA-specific Adenosine Deaminase-1
CCR	=	Chemokine Receptor
CD	=	Cluster of Differentiation
DC	=	Dendritic Cell
eIF2a	=	Eukaryotic Initiation Factor-2a
HA	=	Hemagglutinin
HAV	=	Hepatitis A Virus
HBV	=	Hepatitis B Virus
HCV	=	Hepatitis C Virus
HIV	=	Human Immunodeficiency Virus
ICLC	=	Polyriboinosinic and Polyribocytidylic Acids Stabilized in Poly-L-lysine and Carboxymethylcellulose
IFN	=	Interferon
IL	=	Interleukin
IMPDH	=	Inosine Monophosphate Dehydrogenase
ISG15	=	Interferon Stimulated Gene-15
JAK	=	Janus Kinase
Mda5	=	Melanoma Differentiation Associated Protein 5
MIP	=	Macrophage Inflammatory Protein
mTOR	=	Mammalian Target of Rapamycin
Mx	=	Myxovirus
NA	=	Neuraminidase
NK	=	Natural Killer
NP	=	Nucleoprotein
OAS	=	2'5' Oligoadenylate Synthetase
p38 MAPK	=	p38 Mitogen Activated Protein Kinase
pDC	=	Plasmacytoid Dendritic Cell
PI3K	=	Phosphoinositide 3 Kinase
PKR	=	Double-stranded RNA Activated Protein Kinase
PLSCR1	=	Phospholipid Scramblase-1
RANTES	=	Regulated Upon Activation, Normal T Cell Expressed and Secreted
RIG-I	=	Retinoid Inducible Gene-I
RNaseL	=	Endoribonuclease L
RTP	=	Ribavirin Triphosphate
SARS	=	Severe Acute Respiratory Syndrome

SARS-CoV	=	Severe Acute Respiratory Syndrome Coronavirus
STAT	=	Signal Transducer and Activator of Transcription
SVR	=	Sustained Virological Response
Th	=	T Helper
TLR	=	Toll Like Receptor
TNF	=	Tumour Necrosis Factor
TRAIL	=	Tumour Necrosis Factor-related Apoptosis-inducing Ligand

REFERENCES

- Tiensin, T.; Chaitaweeseub, P.; Songserm, T.; Chaisingh, A.; Hoonsuwan, W.; Buranathai, C.; Parakamawongsa, T.; Premasathira, S.; Amonsin, A.; Gilbert, M.; Nielen, M.; Stegeman, A. Highly pathogenic avian influenza H5N1, Thailand, 2004. *Emerg. Infect. Dis.* **2005**, *11*, 1664-1672.
- Writing Committee of the World Health Organization (WHO) Consultation on Human Influenza A/H5. Avian influenza A (H5N1) infection in humans. *N. Engl. J. Med.* **2005**, *353*, 1374-1385.
- Perez, D. R.; Sorrell, E. M.; Donis, R. O. Avian influenza: an omnipresent pandemic threat. *Pediatr. Infect. Dis. J.* **2005**, *24*, S208-16.
- Dash, P. K.; Saxena, P.; Abhyankar, A.; Bhargava, R.; Jana, A. M. Emergence of dengue virus type-3 in northern India. *Southeast Asian J. Trop. Med. Public Health* **2005**, *36*, 370-377.
- Bellini, W. J.; Harcourt, B. H.; Bowden, N.; Rota, P. A. Nipah virus: an emergent paramyxovirus causing severe encephalitis in humans. *J. Neurovirol.* **2005**, *11*, 481-487.
- Palacios, G.; Oberste, M. S. Enteroviruses as agents of emerging infectious diseases. *J. Neurovirol.* **2005**, *11*, 424-433.
- Wong, S. S.; Yuen, K. Y. The severe acute respiratory syndrome (SARS). *J. Neurovirol.* **2005**, *11*, 455-468.
- Hensley, L. E.; Jones, S. M.; Feldmann, H.; Jahrling, P. B.; Geisbert, T. W. Ebola and Marburg viruses: pathogenesis and development of countermeasures. *Curr. Mol. Med.* **2005**, *5*, 761-772.
- Moscona, A. Neuraminidase inhibitors for influenza. *N. Engl. J. Med.* **2005**, *353*, 1363-1373.
- de Jong, M. D.; Tran, T. T.; Truong, H. K.; Vo, M. H.; Smith, G. J.; Nguyen, V. C.; Bach, V. C.; Phan, T. Q.; Do, Q. H.; Guan, Y.; Peiris, J. S.; Tran, T. H.; Farrar, J. Oseltamivir resistance during treatment of influenza A (H5N1) infection. *N. Engl. J. Med.* **2005**, *353*, 2667-2672.
- Lamb, R. A.; Krug, R. M. Orthomyxoviridae: The Viruses and Their Replication. In *Fields Virology*; (4th ed), Knipe, D. M.; Fields, B. N.; Griffin, D. E.; Howley, P. M., Eds.; Lippincott Williams & Wilkins: Philadelphia, **2001** pp. 1487.
- Fouchier, R. A.; Munster, V.; Wallensten, A.; Bestebroer, T. M.; Herfst, S.; Smith, D.; Rimmelzwaan, G. F.; Olsen, B.; Osterhaus, A. D. Characterization of a novel influenza A virus hemagglutinin subtype (H16) obtained from black-headed gulls. *J. Virol.* **2005**, *79*, 2814-2822.
- Horimoto, T.; Kawaoka, Y. Pandemic threat posed by avian influenza A viruses. *Clin. Microbiol. Rev.* **2001**, *14*, 129-149.
- World Health Organization. (Nov 12, 2007) Cumulative Number of Confirmed Human Cases of Avian Influenza A(H5N1) Reported to WHO. http://www.who.int/csr/disease/avian_influenza/country/cases_table_2007_1_1_12/en/index.html (accessed Nov 15, 2007).
- Rabadan, R.; Levine, A. J.; Robins, H. Comparison of avian and human influenza A viruses reveals a mutational bias on the viral genomes. *J. Virol.* **2006**, *80*, 11887-11891.
- Horimoto, T.; Kawaoka, Y. Strategies for developing vaccines against H5N1 influenza A viruses. *Trends Mol. Med.* **2006**, *12*, 506-514.
- Lin, J.; Zhang, J.; Dong, X.; Fang, H.; Chen, J.; Su, N.; Gao, Q.; Zhang, Z.; Liu, Y.; Wang, Z.; Yang, M.; Sun, R.; Li, C.; Lin, S.; Ji, M.; Liu, Y.; Wang, X.; Wood, J.; Feng, Z.; Wang, Y.; Yin, W. Safety and immunogenicity of an inactivated adjuvanted whole-virion influenza A (H5N1) vaccine: a phase I randomised controlled trial. *Lancet* **2006**, *368*, 991-997.
- Suguitan, A. L., Jr; McAuliffe, J.; Mills, K. L.; Jin, H.; Duke, G.; Lu, B.; Luke, C. J.; Murphy, B.; Swayne, D. E.; Kemble, G.; Subbarao, K. Live, attenuated influenza A H5N1 candidate vaccines provide broad cross-protection in mice and ferrets. *PLoS Med.* **2006**, *3*, e360.
- Jefferson, T.; Deeks, J. J.; Demicheli, V.; Rivetti, D.; Rudin, M. Amantadine and rimantadine for preventing and treating influenza A in adults. *Cochrane Database Syst. Rev.* **2004**, *3*, CD001169.
- Cheung, C. L.; Rayner, J. M.; Smith, G. J.; Wang, P.; Naipospos, T. S.; Zhang, J.; Yuen, K. Y.; Webster, R. G.; Peiris, J. S.; Guan, Y.; Chen, H. Distribution of amantadine-resistant H5N1 avian influenza variants in Asia. *J. Infect. Dis.* **2006**, *193*, 1626-1629.
- Simonsen, L.; Viboud, C.; Grenfell, B. T.; Dushoff, J.; Jennings, L.; Smit, M.; Macken, C.; Hata, M.; Gog, J.; Miller, M. A.; Holmes, E. C. The genesis and spread of reassortment human influenza A/H3N2 viruses conferring adamantane resistance. *Mol. Biol. Evol.* **2007**, *24*, 1811-1820.
- Hayden, F. G. Perspectives on antiviral use during pandemic influenza. *Philos. Trans. R. Soc. Lond., B, Biol. Sci.* **2001**, *356*, 1877-1884.
- Shiraishi, K.; Mitamura, K.; Sakai-Tagawa, Y.; Goto, H.; Sugaya, N.; Kawaoka, Y. High frequency of resistant viruses harboring different mutations in amantadine-treated children with influenza. *J. Infect. Dis.* **2003**, *188*, 57-61.
- Barr, I. G.; Hurt, A. C.; Iannello, P.; Tomasov, C.; Deed, N.; Komadina, N. Increased adamantane resistance in influenza A(H3) viruses in Australia and neighbouring countries in 2005. *Antiviral Res.* **2007**, *73*, 112-117.
- Abed, Y.; Goyette, N.; Boivin, G. Generation and characterization of recombinant influenza A (H1N1) viruses harboring amantadine resistance mutations. *Antimicrob. Agents Chemother.* **2005**, *49*, 556-559.
- Hay, A. J.; Wolstenholme, A. J.; Skehel, J. J.; Smith, M. H. The molecular basis of the specific anti-influenza action of amantadine. *EMBO J.* **1985**, *4*, 3021-3024.
- Pinto, L. H.; Holsinger, L. J.; Lamb, R. A. Influenza virus M2 protein has ion channel activity. *Cell* **1992**, *69*, 517-528.
- Hurt, A. C.; Selleck, P.; Komadina, N.; Shaw, R.; Brown, L.; Barr, I. G. Susceptibility of highly pathogenic A(H5N1) avian influenza viruses to the neuraminidase inhibitors and adamantanes. *Antiviral Res.* **2007**, *73*, 228-231.
- Fiore, A. E.; Shay, D. K.; Haber, P.; Iskander, J. K.; Uyeki, T. M.; Mootrey, G.; Bresee, J. S.; Cox, N. J.; Advisory Committee on Immunization Practices (ACIP), Centers for Disease Control and Prevention (CDC). Prevention and control of influenza. Recommendations of the Advisory Committee on Immunization Practices (ACIP), 2007. *MMWR Recomm. Rep.* **2007**, *56*, 1-54.
- von Itzstein, M.; Wu, W. Y.; Kok, G. B.; Pegg, M. S.; Dyason, J. C.; Jin, B.; Van Phan, T.; Smythe, M. L.; White, H. F.; Oliver, S. W. Rational design of potent sialidase-based inhibitors of influenza virus replication. *Nature* **1993**, *363*, 418-423.
- Kim, C. U.; Lew, W.; Williams, M. A.; Liu, H.; Zhang, L.; Swaminathan, S.; Bischofberger, N.; Chen, M. S.; Mendel, D. B.; Tai, C. Y.; Laver, W. G.; Stevens, R. C. Influenza neuraminidase inhibitors possessing a novel hydrophobic interaction in the enzyme active site: design, synthesis, and structural analysis of carbocyclic sialic acid analogues with potent anti-influenza activity. *J. Am. Chem. Soc.* **1997**, *119*, 681-690.
- Reece, P. A. Neuraminidase inhibitor resistance in influenza viruses. *J. Med. Virol.* **2007**, *79*, 1577-1586.
- Barnett, J. M.; Cadman, A.; Burrell, F. M.; Madar, S. H.; Lewis, A. P.; Tisdale, M.; Bethell, R. *In vitro* selection and characterisation of influenza B/Beijing/1/87 isolates with altered susceptibility to zanamivir. *Virology* **1999**, *265*, 286-295.
- Hurt, A. C.; McKimm-Breschkin, J. L.; McDonald, M.; Barr, I. G.; Komadina, N.; Hampson, A. W. Identification of a human influenza type B strain with reduced sensitivity to neuraminidase inhibitor drugs. *Virus Res.* **2004**, *103*, 205-211.
- Jackson, H. C.; Roberts, N.; Wang, Z. M.; Belshe, R. Management of influenza: Use of new antivirals and resistance in perspective. *Clin. Drug Investig.* **2000**, *20*, 447-454.
- Kiso, M.; Mitamura, K.; Sakai-Tagawa, Y.; Shiraishi, K.; Kawakami, C.; Kimura, K.; Hayden, F. G.; Sugaya, N.; Kawaoka, Y. Resistant influenza A viruses in children treated with oseltamivir: Descriptive study. *Lancet* **2004**, *364*, 759-765.
- Ward, P.; Small, I.; Smith, J.; Suter, P.; Dutkowski, R. Oseltamivir (Tami-flu®) and its potential for use in the event of an influenza pandemic. *J. Antimicrob. Chemother.* **2005**, *55*, i5-i21.
- Babu, Y. S.; Chand, P.; Bantia, S.; Kotian, P.; Dehghani, A.; El-Kattan, Y.; Lin, T. H.; Hutchison, T. L.; Elliott, A. J.; Parker, C. D.; Ananth, S. L.; Horn, L. L.; Laver, G. W.; Montgomery, J. A. BCX-1812 (RWJ-270201): discovery of a novel, highly potent, orally active, and selective influenza neuraminidase inhibitor through structure-based drug design. *J. Med. Chem.* **2000**, *43*, 3482-3486.
- Jones, J. C.; Turpin, E. A.; Bultmann, H.; Brandt, C. R.; Schultz-Cherry, S. Inhibition of influenza virus infection by a novel antiviral peptide that targets viral attachment to cells. *J. Virol.* **2006**, *80*, 11960-11967.
- Ge, Q.; Pasty, M.; Kobasa, D.; Puthavathana, P.; Lupfer, C.; Bestwick, R. K.; Iversen, P. L.; Chen, J.; Stein, D. A. Inhibition of multiple subtypes of influenza A virus in cell cultures with morpholino oligomers. *Antimicrob. Agents Chemother.* **2006**, *50*, 3724-3733.
- Ilyushina, N. A.; Bovin, N. V.; Webster, R. G.; Govorkova, E. A. Combination chemotherapy, a potential strategy for reducing the emergence of drug-resistant influenza A variants. *Antiviral Res.* **2006**, *70*, 121-131.
- Wilson, S. Z.; Knight, V.; Wyde, P. R. Amantadine and ribavirin aerosol treatment of influenza A and B infection in mice. *Antimicrob. Agents Chemother.* **1980**, *17*, 642-648.
- Smee, D. F.; Wong, M.; Bailey, K. W.; Sidwell, R. W. Activities of oseltamivir and ribavirin used alone and in combination against infections in mice with recent isolates of influenza A (H1N1) and B viruses. *Antivir. Chem. Chemother.* **2006**, *17*, 185-192.
- Garozzo, A.; Tempera, G.; Ungheri, D.; Timpanaro, R.; Castro, A. N-acetylcysteine synergizes with oseltamivir in protecting mice from lethal influenza infection. *Int. J. Immunopathol. Pharmacol.* **2007**, *20*, 349-354.
- Ghezzi, P.; Ungheri, D. Synergistic combination of n-acetylcysteine and ribavirin to protect from lethal influenza viral infection in a mouse model. *Int. J. Immunopathol. Pharmacol.* **2004**, *17*, 99-102.

- [46] Govorkova, E. A.; Fang, H.; Tan, M.; Webster, R. G. Neuraminidase inhibitor-rimantadine combinations exert additive and synergistic anti-influenza virus effects in MDCK cells. *Antimicrob. Agents Chemother.* **2004**, *48*, 4855-4863.
- [47] Jaeckel, E.; Cornberg, M.; Wedemeyer, H.; Santantonio, T.; Mayer, J.; Zankel, M.; Pastore, G.; Dietrich, M.; Trautwein, C.; Manns, M. P. Treatment of acute hepatitis C with interferon alpha-2b. *N. Engl. J. Med.* **2001**, *345*, 1452-1457.
- [48] Hoofnagle, J. H. Hepatitis C: The clinical spectrum of disease. *Hepatology* **1997**, *26*, S15-20.
- [49] Hoofnagle, J. H. Course and outcome of hepatitis C. *Hepatology* **2002**, *36*, S21-29.
- [50] Lauer, G. M.; Walker, B. D. Hepatitis C virus infection. *N. Engl. J. Med.* **2001**, *345*, 41-52.
- [51] Fried, M. W.; Shiffman, M. L.; Rajender Reddy, K.; Smith, C.; Marinos, G.; Gonc ales Jr, F. L.; Ha ussinger, D.; Diago, M.; Carosi, G.; Dhumeaux, D.; Craxi, A.; Lin, A.; Hoffman, J.; Yu, J. Peginterferon alpha-2a plus ribavirin for chronic hepatitis C virus infection. *N. Engl. J. Med.* **2002**, *347*, 975-982.
- [52] Manns, M. P.; McHutchison, J. G.; Gordon, S. C.; Rustgi, V. K.; Shiffman, M.; Reindollar, R.; Goodman, Z. D.; Koury, K.; Ling, M.; Albrecht, J. K. Peginterferon alpha-2b plus ribavirin compared with interferon alpha-2b plus ribavirin for initial treatment of chronic hepatitis C: A randomised trial. *Lancet* **2001**, *358*, 958-965.
- [53] Pawlotsky, J. Therapy of hepatitis C: From empiricism to eradication. *Hepatology* **2006**, *43*, S207-220.
- [54] Nomura, H.; Sou, S.; Tanimoto, H.; Nagahama, T.; Kimura, Y.; Hayashi, J.; Ishibashi, H.; Kashiwagi, S. Short-term Interferon- α Therapy for Acute Hepatitis C: A Randomized Controlled Trial. *Hepatology* **2004**, *39*, 1213-1219.
- [55] Santantonio, T.; Fasano, M.; Sinisi, E.; Guastadisegni, A.; Casalino, C.; Mazzola, M.; Francavilla, R.; Pastore, G. Efficacy of a 24-week course of PEG-interferon α -2b monotherapy in patients with acute hepatitis C after failure of spontaneous clearance. *J. Hepatol.* **2005**, *42*, 329-333.
- [56] Sidwell, R. W.; Huffman, J. H.; Khare, G. P.; Allen, L. B.; Witkowski, J. T.; Robins, R. K. Broad-spectrum antiviral activity of Virazole: 1- β -D-ribofuranosyl-1,2,4-triazole-3-carboxamide. *Science* **1972**, *177*, 705-706.
- [57] Crotty, S.; Maag, D.; Arnold, J. J.; Zhong, W.; Lau, J. Y. N.; Hong, Z.; Andino, R.; Cameron, C.E. The broad-spectrum antiviral ribonucleoside ribavirin is an RNA virus mutagen. *Nat. Med.* **2000**, *6*, 1375-1379.
- [58] Englund, J. A.; Piedra, P. A.; Jefferson, L. S.; Wilson, S. Z.; Taber, L. H.; Gilbert, B. E. High-dose, short-duration ribavirin aerosol therapy in children with suspected respiratory syncytial virus infection. *J. Pediatr.* **1990**, *117*, 313-320.
- [59] Ning, Q.; Brown, D.; Parodo, J.; Cattral, M.; Gorczynski, R.; Cole, E.; Fung, L.; Ding, J.W.; Liu, M. F.; Rotstein, O.; James Phillips, M.; Levy, G. Ribavirin inhibits viral-induced macrophage production of TNF, IL-1, the procoagulant fg12 prothrombinase and preserves Th1 cytokine production but inhibits Th2 cytokine response. *J. Immunology* **1998**, *160*, 3487-3493.
- [60] Hultgren, C.; Milich, D. R.; Weiland, O.; Sa llberg, M. The antiviral compound ribavirin modulates the T helper (Th)1/Th2 subset balance in hepatitis B and C virus-specific immune responses. *J. Gen. Virol.* **1998**, *79*, 2381-2391.
- [61] Neumann-Haefelin, C.; Spangenberg, H. C.; Blum, H. E.; Thimme, R. Host and viral factors contributing to CD8+ T cell failure in hepatitis C virus infection. *World J. Gastroenterol.* **2007**, *13*, 4839-4847.
- [62] Eriksson, B.; Helgstrand, E.; Johansson, N. G. Inhibition of influenza virus ribonucleic acid polymerase by ribavirin triphosphate. *Antimicrob. Agents Chemother.* **1977**, *11*, 946-951.
- [63] Samuel, C. E. Antiviral actions of interferons. *Clin. Microbiol. Rev.* **2001**, *14*, 778-809.
- [64] Zhou, Z.; Hamming, O. J.; Ank, N.; Paludan, S. R.; Nielsen, A. L.; Hartmann, R. Type III interferon (IFN) induces a type I IFN-like response in a restricted subset of cells through signaling pathways involving both the Jak-STAT pathway and the mitogen-activated protein kinases. *J. Virol.* **2007**, *81*, 7749-7758.
- [65] Valente, G.; Ozmen, L.; Novelli, F.; Geuna, M.; Palestro, G.; Forni, G.; Garotta, G. Distribution of interferon- γ receptor in human tissues. *Eur. J. Immunol.* **1992**, *22*, 2403-2412.
- [66] De Weerd, N. A.; Samarajiwa, S. A.; Hertzog, P. J. Type I interferon receptors: Biochemistry and biological functions. *J. Biol. Chem.* **2007**, *282*, 20053-20057.
- [67] Brierley, M. M.; Fish, E. N. Review: IFN- α /beta receptor interactions to biologic outcomes: understanding the circuitry. *J. Interferon Cytokine Res.* **2002**, *22*, 835-845.
- [68] Williams, B. R. G.; Fish, E. N. Interferon and viruses: *in vitro* studies. In *Interferons: Their Impact in Biology and Medicine*; Taylor-Papadimitriou, J., Ed.; Oxford University Press: New York, **1985** pp. 40-60.
- [69] Nishiguchi, S.; Shiomi, S.; Nakatani, S.; Takeda, T.; Fukuda, K.; Tamori, A.; Habu, D.; Tanaka, T. Prevention of hepatocellular carcinoma in patients with chronic active hepatitis C and cirrhosis. *Lancet* **2001**, *357*, 196-197.
- [70] Trinchieri, G.; Sher, A. Cooperation of Toll-like receptor signals in innate immune defence. *Nat. Rev. Immunol.* **2007**, *7*, 179-190.
- [71] Liu, Y. J. IPC: professional type 1 interferon-producing cells and plasmacytoid dendritic cell precursors. *Annu. Rev. Immunol.* **2005**, *23*, 275-306.
- [72] Akira, S. TLR signaling. *Curr. Top. Microbiol. Immunol.* **2006**, *311*, 1-16.
- [73] Diebold, S. S.; Kaisho, T.; Hemmi, H.; Akira, S.; Reis e Sousa, C. Innate antiviral responses by means of TLR7-mediated recognition of single-stranded RNA. *Science* **2004**, *303*, 1529-1531.
- [74] Hochrein, H.; Schlatter, B.; O'Keefe, M.; Wagner, C.; Schmitz, F.; Schiemann, M.; Bauer, S.; Suter, M.; Wagner, H. Herpes simplex virus type-1 induces IFN- α production via Toll-like receptor 9-dependent and -independent pathways. *Proc. Natl. Acad. Sci. USA* **2004**, *101*, 11416-11421.
- [75] Alexopoulou, L.; Holt, A. C.; Medzhitov, R.; Flavell, R. A. Recognition of double-stranded RNA and activation of NF- κ B by Toll-like receptor 3. *Nature* **2001**, *413*, 732-738.
- [76] Matsumoto, M.; Funami, K.; Tanabe, M.; Oshiumi, H.; Shingai, M.; Seto, Y.; Yamamoto, A.; Seya, T. Subcellular localization of Toll-like receptor 3 in human dendritic cells. *J. Immunol.* **2003**, *171*, 3154-3162.
- [77] Yoneyama, M.; Kikuchi, M.; Matsumoto, K.; Imaizumi, T.; Miyagishi, M.; Taira, K.; Foy, E.; Loo, Y. M.; Gale, M., Jr; Akira, S.; Yonehara, S.; Kato, A.; Fujita, T. Shared and unique functions of the DEXD/H-box helicases RIG-I, MDA5, and LGP2 in antiviral innate immunity. *J. Immunol.* **2005**, *175*, 2851-2858.
- [78] Kawai, T.; Takahashi, K.; Sato, S.; Coban, C.; Kumar, H.; Kato, H.; Ishii, K.J.; Takeuchi, O.; Akira, S. IPS-1, an adaptor triggering RIG-I- and Mda5-mediated type I interferon induction. *Nat. Immunol.* **2005**, *6*, 981-988.
- [79] Platanias, L. C. Mechanisms of type-I- and type-II-interferon-mediated signalling. *Nat. Rev. Immunol.* **2005**, *5*, 375-386.
- [80] Galligan, C. L.; Murooka, T. T.; Rahbar, R.; Baig, E.; Majchrzak-Kita, B.; Fish, E. N. Interferons and viruses: signaling for supremacy. *Immunol. Res.* **2006**, *35*, 27-40.
- [81] Haller, O.; Kochs, G.; Weber, F. Interferon, Mx, and viral countermeasures. *Cytokine Growth Factor Rev.* **2007**, *18*, 425-433.
- [82] Auyeung, T. W.; Lee, J. S. W.; Lai, W. K.; Choi, C. H.; Lee, H. K.; Lee, J. S.; Li, P. C.; Lok, K. H.; Ng, Y. Y.; Wong, W. M.; Yeung, Y. M. The use of corticosteroid as treatment in SARS was associated with adverse outcomes: A retrospective cohort study. *J. Infect.* **2005**, *51*, 98-102.
- [83] MacKay, I. F.; Garrah, J. M.; Tabah, B. M.; Freeman, L.; Maher, M.; Macdonald, C. L. Adverse drug reactions associated with the use of ribavirin in the treatment of severe acute respiratory syndrome (SARS). *Can. J. Clin. Pharmacol.* **2005**, *12*, e165-e179.
- [84] Muller, M. P.; Dressler, L.; Raboud, J.; McGeer, A.; Rea, E.; Richardson, S. E.; Mazzulli, T.; Loeb, M.; Louie, M. Adverse events associated with high-dose ribavirin: Evidence from the Toronto outbreak of severe acute respiratory syndrome. *Pharmacotherapy* **2007**, *27*, 494-503.
- [85] Plemper, R. K.; Hammond, A. L. Inhibition of membrane fusion as a target for antiviral therapy. *Anti-Infect. Agents Med. Chem.* **2007**, *6*, 248-262.
- [86] Ghosh, A. K.; Xi, K.; Grum-Tokars, V.; Xu, X.; Ratia, K.; Fu, W.; Houser, K. V.; Baker, S. C.; Johnson, M. E.; Mesecar, A. D. Structure-based design, synthesis, and biological evaluation of peptidomimetic SARS-CoV 3CLpro inhibitors. *Bioorg. Med. Chem. Lett.* **2007**, *17*, 5876-5880.
- [87] Barnard, D. L.; Day, C. W.; Bailey, K.; Heiner, M.; Montgomery, R.; Lauridsen, L.; Chan, P. K. S.; Sidwell, R. W. Evaluation of immunomodulators, interferons and known *in vitro* SARS-CoV inhibitors for inhibition of SARS-CoV replication in BALB/c mice. *Antiviral Chem. Chemother.* **2006**, *17*, 275-284.
- [88] Zhang, Z.; Xie, Y.; Hong, J.; Zhang, X.; Kwok, S. Y.; Huang, X.; Wong, S. W.; Wong, B. Purification of severe acute respiratory syndrome hyperimmune globulins for intravenous injection from convalescent plasma. *Transfusion* **2005**, *45*, 1160-1164.
- [89] Loutfy, M. R.; Blatt, L. M.; Siminovitch, K. A.; Ward, S.; Wolff, B.; Lho, H.; Pham, D. H.; Deif, H.; LaMere, E. A.; Chang, M.; Kain, K. C.; Farcas, G. A.; Ferguson, P.; Latchford, M.; Levy, G.; Dennis, J. W.; Lai, E. K. Y.; Fish, E. N. Interferon Alfacon-1 Plus Corticosteroids in Severe Acute Respiratory Syndrome: A Preliminary Study. *JAMA* **2003**, *290*, 3222-3228.
- [90] Wong, J. P.; Nagata, L. P.; Christopher, M. E.; Salazar, A. M.; Dale, R. M. K. Prophylaxis of acute respiratory virus infections using nucleic acid-based drugs. *Vaccine* **2005**, *23*, 2266-2268.
- [91] Wong, J. P.; Yang, H.; Nagata, L.; Kende, M.; Levy, H.; Schnell, G.; Blasetti, K. Liposome-mediated immunotherapy against respiratory influenza virus infection using double-stranded RNA poly ICLC. *Vaccine* **1999**, *17*, 1788-1795.
- [92] Cluff, C. W.; Baldrige, J. R.; Stover, A. G.; Evans, J. T.; Johnson, D. A.; Lacy, M. J.; Clawson, V. G.; Yorgensen, V. M.; Johnson, C. L.; Livesay, M. T.; Hershberg, R. M.; Persing, D. H. Synthetic toll-like receptor 4 agonists stimulate innate resistance to infectious challenge. *Infect. Immun.* **2005**, *73*, 3044-3052.
- [93] Krieg, A. M. Antiinfective applications of toll-like receptor 9 agonists. *Proc. Am. Thorac. Soc.* **2007**, *4*, 289-294.
- [94] Hammerbeck, D. M.; Burleson, G. R.; Schuller, C. J.; Vasilakos, J. P.; Tomai, M.; Egging, E.; Cochran, F. R.; Woulfe, S.; Miller, R. L. Administration of a dual toll-like receptor 7 and toll-like receptor 8 agonist protects against influenza in rats. *Antiviral Res.* **2007**, *73*, 1-11.
- [95] Thakur, C. S.; Jha, B. K.; Dong, B.; Das Gupta, J.; Silverman, K. M.; Mao, H.; Sawai, H.; Nakamura, A. O.; Banerjee, A. K.; Gudkov, A.; Silverman, R. H. Small-molecule activators of RNase L with broad-spectrum antiviral activity. *Proc. Natl. Acad. Sci. USA* **2007**, *104*, 9585-9590.

- [96] White, J.; Kartenbeck, J.; Helenius, A. Membrane fusion activity of influenza virus. *EMBO J.* **1982**, *1*, 217-222.
- [97] Zhao, C.; Zhao, Y.; Chai, H.; Gong, P. Synthesis and *in vitro* anti-hepatitis B virus activities of some ethyl 5-hydroxy-1H-indole-3-carboxylates. *Bioorg. Med. Chem.* **2006**, *14*, 2552-2558.
- [98] Brooks, M. J.; Sasadeusz, J. J.; Tannock, G. A. Antiviral chemotherapeutic agents against respiratory viruses: where are we now and what's in the pipeline? *Curr. Opin. Pulm. Med.* **2004**, *10*, 197-203.
- [99] Boriskin, Y. S.; Pecheur, E. I.; Polyak, S. J. Arbidol: a broad-spectrum antiviral that inhibits acute and chronic HCV infection. *Viol. J.* **2006**, *3*, 56.

Received: December 17, 2007

Revised: April 03, 2008

Accepted: April 11, 2008