LEADING ARTICLE

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Drugs in Development for Influenza

David A. Boltz, Jerry R. Aldridge Jr, Robert G. Webster and *Elena A. Govorkova*

Division of Virology, Department of Infectious Diseases, St. Jude Children's Research Hospital, Memphis, Tennessee, USA

Abstract

The emergence and global spread of the 2009 pandemic H1N1 influenza virus reminds us that we are limited in the strategies available to control influenza infection. Vaccines are the best option for the prophylaxis and control of a pandemic; however, the lag time between virus identification and vaccine distribution exceeds 6 months and concerns regarding vaccine safety are a growing issue leading to vaccination refusal. In the short-term, antiviral therapy is vital to control the spread of influenza. However, we are currently limited to four licensed anti-influenza drugs: the neuraminidase inhibitors oseltamivir and zanamivir, and the M2 ion-channel inhibitors amantadine and rimantadine. The value of neuraminidase inhibitors was clearly established during the initial phases of the 2009 pandemic when vaccines were not available, i.e. stockpiles of antivirals are valuable. Unfortunately, as drug-resistant variants continue to emerge naturally and through selective pressure applied by use of antiviral drugs, the efficacy of these drugs declines. Because we cannot predict the strain of influenza virus that will cause the next epidemic or pandemic, it is important that we develop novel anti-influenza drugs with broad reactivity against all strains and subtypes, and consider moving to multiple drug therapy in the future. In this article we review the experimental data on investigational antiviral agents undergoing clinical trials (parenteral zanamivir and peramivir, long-acting neuraminidase inhibitors and the polymerase inhibitor favipiravir [T-705]) and experimental antiviral agents that target either the virus (the haemagglutinin inhibitor cyanovirin-N and thiazolides) or the host (fusion protein inhibitors [DAS181], cyclo-oxygenase-2 inhibitors and peroxisome proliferator-activated receptor agonists).

The influenza virus is a member of the Orthomyxoviridae family, which is divided into three types: A, B and C. Influenza A viruses are responsible for causing seasonal epidemics and caused the three pandemics that occurred in the 20th century (1918, 1957 and 1968) as well as the 2009 H1N1 pandemic, the first pandemic of the 21st century. Human influenza is predominantly an upper respiratory tract infection that is typically mild and self-limiting; however, in rare cases severe complications and death can occur. The onset of complications can arise as a result of viral characteristics or predisposition of the host. Severe complications attributed to the host response commonly occur in the very young or elderly after infection with seasonal influenza viruses,[1] whereas life-threatening illness associated with infection by the 1918 pandemic virus or the highly pathogenic (HP) H5N1 viruses is often found in healthy, immunocompetent adults.[2-4]

Annual immunization is our best option for prophylaxis and to control an influenza pandemic; however, limitations on vaccine efficacy, design and delay in strain-specific vaccine production have stimulated the search for antiviral drugs. Two classes of antiviral drugs are currently approved for both prophylaxis and therapeutic treatment of influenza virus infection: M2-ion channel inhibitors (the adamantanes, amantadine and its derivative rimantadine) and neuraminidase inhibitors (zanamivir and oseltamivir). These drug classes differ significantly in their mechanisms of action, pharmacokinetics, tolerability profiles and resistance patterns.[5-11] Amantadine and rimantadine inhibit viral replication by blocking the ion channel at the stage of virus entry into cells[12] and prevent release of the virus by altering the conformation of the haemagglutinin protein.[13,14] On the other hand, the neuraminidase inhibitors zanamivir and oseltamivir, approved by the US FDA in 1999, block the activity of the neuraminidase enzyme and interrupt an established infection at a later stage of virus replication by inhibiting the release of virions from infected cells (figure 1).[15,16] Neuraminidase inhibitors also cause aggregation of the released virions, thus inhibiting viral penetration into mucous secretions and, ultimately, spread to neighbouring cells.[17]

A major concern regarding the use of antiviral compounds to control influenza is the emergence of drug-resistant variants. The use of amantadine or rimantadine has driven the rapid emergence of viruses that are resistant to M2 inhibitors yet maintain full transmissibility.[11] The incidence of naturally occurring amantadine-resistant influenza A (H1N1 and H3N2) variants has increased significantly throughout the world. By 2003, these resistant viruses were found to have spread to all countries surveyed, including some where the use of M2 inhibitors was minimal.[18] In contrast, surveillance data from 1999 to 2007 revealed a low prevalence of resistance to the neuraminidase inhibitor oseltamivir (<1%).[19,20] During 2007–8, there was an increase in the number of H1N1 viruses carrying the H275Y neuraminidase mutation (molecular marker of oseltamivir resistance, N1 numbering) and data from the 2008–9 influenza season show that the prevalence of oseltamivir-resistant viruses exceeds 90%.[21-24] Of the pandemic 2009 H1N1 influenza viruses tested, all were resistant to the M2 inhibitors amantadine and rimantadine.[25,26] Although still rare, the emergence of the H275Y neuraminidase mutation in 2009 H1N1 viruses has been reported.[25,27-29] Therefore, the US Centers for Disease Control and Prevention has discouraged the use of M2 inhibitors and recommended the use of neuraminidase inhibitors to treat the 2009 H1N1 pandemic virus.

The high incidence of influenza viruses developing resistance to both M2 and neuraminidase inhibitors, the only drugs currently licensed for treatment of influenza virus infection, underscores the need for the development of novel antiviral drugs (table I). In this article, we discuss (i) antiviral drugs that are at the early stages of clinical trials; (ii) new antiviral agents in development that target other virus genes and/or host pathways; and (iii) immunomodulators that show potential in limiting the immune-induced pathology associated with HP influenza infections.

1. Investigational Antiviral Agents for Influenza in Clinical Trails

1.1 Intravenous Peramivir

Peramivir (BCX-1812, RWJ-270201), generated using a structure-based drug design, is a neuraminidase inhibitor that is currently being developed. Unlike existing neuraminidase inhibitors, peramivir is a cyclopentane derivative with a negatively charged carboxylate group, a positively charged guanidino group and lipophilic side chains.[30] Peramivir has proven efficacious in reducing replication of influenza A and B viruses *in vitro* and when used as an oral formulation in experimental animal models.[30-35] Although peramivir has been well tolerated in humans when given at an oral dose of 800 mg/day, findings from a phase III clinical trial demonstrated no statistically significant difference in the time required for resolution of symptoms between treatment and placebo groups.[36] These findings are likely to be due to the low (<3%) bioavailability of peramivir when administered orally in humans.

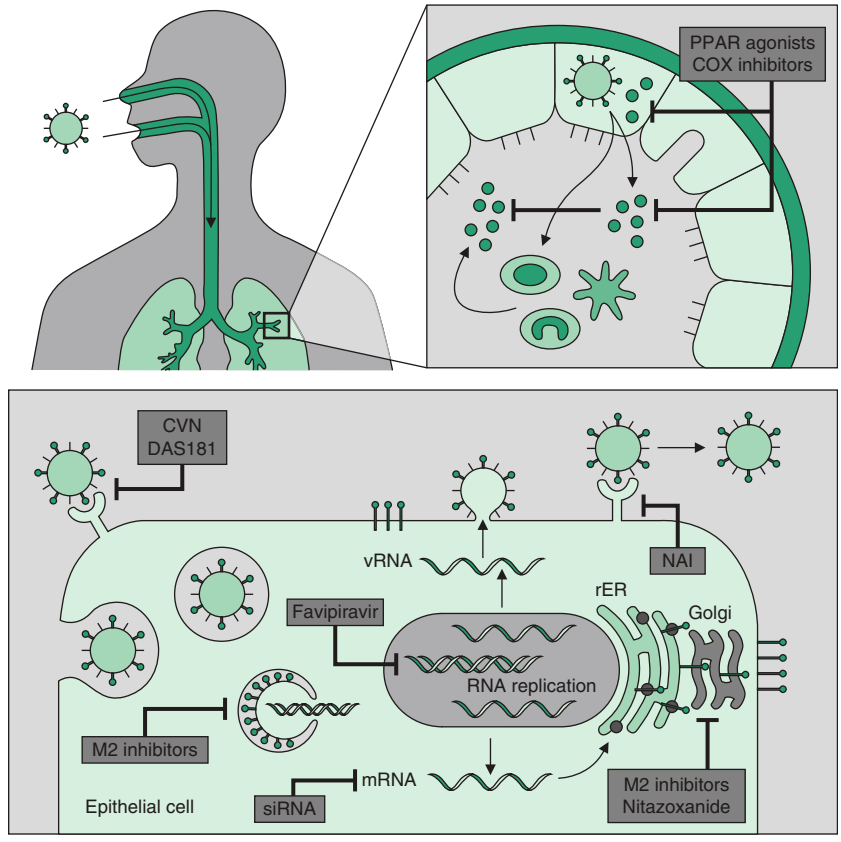

Fig. 1. Sites of action of immunnomodulators and antiviral agents for the control of influenza infection. Influenza infects the epithelial cells of the respiratory tract eliciting an immune response characterized by the release of cytokines/chemokines and infiltration of leukocytes. Cyclooxygenase (COX) inhibitors and peroxisome proliferator-activated receptor (PPAR) agonists reduce leukocyte infiltration, presumably by reducing cytokine/chemokine production. Infection is initiated when the virus attaches to sialic acid receptors on the cell surface. This attachment can be inhibited by sialidase inhibitor DAS181 and haemagglutinin (HA) inhibitor cyanovirin-N (CVN). After endocytosis, the pH of the endosome decreases resulting in HA-mediated fusion of the virus and endosomal membranes. Prior to membrane fusion, the M2 ion channel reduces the pH within the virion to allow for release of viral RNA (vRNA). M2 ion channel inhibitors inhibit the decrease in pH within the virion, thus blocking the release of vRNA into the cytoplasm. vRNA replication occurs in the nucleus and can be blocked by the polymerase inhibitor favipiravir (T-705). Newly made viral messenger RNA (mRNA) is transported to the cytoplasm where translation of viral proteins occurs. Silencing of specific viral genes can be achieved using small interfering RNAs (siRNAs). The maturation of viral proteins such as the viral HA, which occurs in the Golgi, can be disrupted by M2 inhibitors and nitazoxanide. Release of mature virions at the cell surface occurs through enzymatic cleavage of sialic acid receptors by the neuraminidase (NA) enzyme. NA inhibitors (NAIs) block NA activity, thereby preventing the release of virions from the cell. **rER** = rough endoplasmatic reticulum.

To address the limited bioavailability of orally administered peramivir, parenteral formulations are currently being evaluated in preclinical studies and clinical trials for the treatment of seasonal influenza A infection. In healthy volunteers, parenterally administered peramivir was well tolerated and resulted in high drug concentrations in the blood.[37,38] Peramivir has a half-life of 22 hours within human plasma and remains bound to the influenza neuraminidase for an extended period of time.[39] One benefit of the protracted interaction between peramivir and the influenza neuraminidase is that the required frequency of dosing is diminished. Preclinical studies in mice showed that intramuscularly injected peramivir is effective in treating infection with the contemporary H1N1 and H3N2 influenza A viruses,[39] as well as for treating both sub-lethal and

Table I. Drugs and interventions in development for influenza

Agent	Investigational phase[a]	Target	References
Peramivir IV	Phase II–III	Influenza neuraminidase	30-49
Zanamivir IV	Phase II	Influenza neuraminidase	8,17,28,50-53
Laninamivir (CS-8958, R-118958)	Phase III	Influenza neuraminidase	54-60
Favipiravir (T-705)	Phase III	Influenza polymerase	61-67
Cyanovirin-N	Experimental	Influenza haemagglutinin	68-71
DAS181	Phase I	Host sialic acid residues	72-77
Nitazoxanide	Experimental	Influenza haemagglutinin	78
siRNA	Experimental	Host or viral genes	79-85
Combination therapy	Phase I and II	Influenza neuraminidase and polymerase	86-89
COX inhibitors	Experimental	COX-2 enzyme	90-92
PPAR agonists	Experimental	PPAR	86-89

a Phase of drug evaluation for use against influenza.

COX = cyclo-oxygenase; **IV** = intravenous; **PPAR** = peroxisome proliferator-activated receptor; **siRNA** = small interfering RNA.

lethal H5N1 infections.[40,41] Recently, studies were conducted in Asia to measure the efficacy of intravenously administered peramivir in humans, showing generally similar efficacy[42] or non-inferiority[43] when compared with oseltamivir. In a phase II clinical study, intravenous administration of peramivir (200 or 400 mg) to treat patients hospitalized within 72 hours of the onset of symptoms proved to be clinically beneficial.[42] In phase III clinical studies, a single intravenous administration of peramivir 300 or 600 mg was found to reduce the time to alleviation of symptoms and fever.[43] Moreover, treatment with peramivir 600 mg resulted in a significant reduction in viral titres. In a second phase III study, conducted by Shionogi & Co., Ltd, peramivir administered intravenously over multiple days at 300 or 600 mg/day alleviated symptoms (median time 68.6 hours) in all treated patients.[44]

The overall safety of peramivir and the need for a parenterally administered antiviral drug prompted the FDA to issue an emergency use authorization (EUA) of intravenous peramivir as treatment for the 2009 H1N1 influenza in patients not responding to the currently approved antiviral therapies.[45] As directed by the EUA, intravenous peramivir may be administered at 600 mg once daily for 5–10 days in patients infected with the 2009 H1N1 influenza. Although the use of intravenous peramivir is approved for pandemic 2009 H1N1-infected patients, its success in clinical trials provides support for the potential of parenterally administered antiviral drugs. The decreased susceptibility of oseltamivir-resistant viruses that have the H275Y neuraminidase mutation to peramivir has prevented its use in patients infected with a virus that is highly suspected or documented to be oseltamivir resistant.[46,47] In a phase II clinical trial during the 2007–8 season, H1N1 influenza A viruses isolated from 79% of study subjects carried the H275Y neuraminidase mutation, which may have contributed to the unfavourable results of the study.[48] Clinical resistance to intravenous peramivir treatment was recently described in an immunocompromised patient infected with 2009 H1N1 influenza virus carrying the H275Y neuraminidase mutation.[49]

1.2 Intravenous Zanamivir

Intravenous zanamivir is being evaluated as a potential therapy for severe influenza infections. Zanamivir is a potent inhibitor of neuraminidases of influenza A and B viruses, including oseltamivir-resistant viruses possessing the H275Y mutation.[8,17] The high incidence of the H275Y mutation makes zanamivir an excellent alternative for the treatment of infection with oseltamivir-resistant viruses. The combined prophylactic and therapeutic treatment with intravenous zanamivir (10 mg/kg) has been shown to reduce viral

load and lung pathology in macaques infected with HP H5N1 influenza virus.[50] In a clinical study wherein participants received intravenous zanamivir 600 mg twice daily beginning 4 hours prior to inoculation, the drug was well tolerated, decreased viral shedding and alleviated clinical symptoms.[51] Intravenous zanamivir is available for compassionate use from GlaxoSmithKline via an emergency Investigational New Drug application to the FDA. There have been two reports of the emergency use of intravenous zanamivir to treat pandemic 2009 H1N1 influenza virus. In the first case, an immunocompromised child infected with oseltamivir-resistant 2009 H1N1 influenza was successfully treated with intravenous zanamivir. The child failed to respond to oseltamivir treatment and the presence of H275Y neuraminidase mutation was confirmed by polymerase chain reaction.[28] The patient received intravenous zanamivir 600 mg every 12 hours for 15 days, and there was an observed decrease in viral load during therapy. In the second case, a woman with neutropenia following chemotherapy did not respond to oseltamivir treatment and required mechanical ventilation.[52] Intravenous zanamivir 600 mg twice daily was initiated and her condition improved within 48 hours. Oseltamivir resistance was not confirmed but was assumed because of the failure of oseltamivir treatment to alleviate symptoms. These two cases provide promise for the use of intravenous zanamivir. A phase II clinical trial is planned to evaluate the safety and tolerability of intravenous zanamivir 600 mg twice daily for 5 days in hospitalized patients with laboratory-confirmed influenza infection.[53]

1.3 Long-Acting Neuraminidase Inhibitors

For optimal efficacy, the neuraminidase inhibitors described thus far must be administered twice daily. Because of this, the amount of drug needed for pandemic stockpiling is tremendous; thus, the development of high-potency drugs requiring less frequent administration is desirable. To this end, Biota Holdings of Australia and Sankyo Pharmaceuticals of Japan are working in conjunction to develop a multimeric compound of zanamivir, laninamivir (CS-8958 or R-118958), which maintains longer-acting neuraminidase inhibition to allow for once-weekly administration. This multimetric compound has proven more potent than zanamivir and has provided the desired long-acting neuraminidase inhibition when tested in an influenza-infected murine model.[54,55] The active metabolite of the esterified prodrug laninamivir, R-125489, has broad reactivity and has proven effective in inhibiting the neuraminidase activities of influenza A subtypes N1 to N9 *in vitro*, including oseltamivir-resistant viruses.[56] In a lethal mouse model, survival was comparable between animals given a single dose of R-125489 or zanamivir; however, laninamivir significantly increased survival when treatment was given prophylactically as early as 10 days before infection.[56] The increased survival correlated with a significant reduction in viral titres within the infected lung when laninamivir was given 7 days prior to infection. In ferrets, administration of laninamivir 4 hours after infection caused a reduction in viral titres in the nasal wash, thus confirming the findings from the murine model.[57] In a clinical trial in healthy male volunteers, laninamivir, when administered by inhalation, was found to be well tolerated at doses of 1, 2, 5 or 10 mg, with a long retention of the active metabolite.[58] In a phase III trial using adult subjects, inhaled laninamivir administered once daily was found to be as effective as a 75 mg dose of oseltamivir administered twice daily for 5 consecutive days.[59] Phase III clinical trials are currently underway in Japan to evaluate the safety of laninamivir and to measure influenza transmission in participants receiving laninamivir or a placebo.[60]

1.4 Polymerase Inhibitors

Presently, ribavirin is the only polymerase inhibitor available to treat influenza infection; however, its approved use is limited to a few countries. In 2002, Furuta et al.[61] reported that a novel pyrazine molecule, favipiravir (T-705), inhibited influenza virus infections in cell culture and in mice. Favipiravir inhibits replication of both influenza A and B viruses.[61-63] Similar to ribavirin,

favipiravir is an inhibitor of the influenza RNA polymerase (figure 1); however, unlike ribavirin, favipiravir does not interfere with host DNA or RNA synthesis and is only weakly inhibitory to the host inosine monophosphate dehydrogenase, thus rendering favipiravir less cytotoxic.[62,64] These properties make favipiravir an attractive candidate for the treatment of influenza virus infections in humans. High doses of favipiravir resulted in little or no cytotoxicity, and repeated passage of influenza virus in cell culture in the presence of the drug did not result in the development of resistance.[61] Influenza viruses resistant to ribavirin have yet to be described; however, the generation in cell culture of ribavirin-resistant polio virus and ribavirin-resistant hepatitis C virus replicon-containing cell lines has been described.[93,94] Ribavirin resistance has not been identified in the clinical setting; therefore, it is possible to hypothesize that the possibility of emergence of influenza viruses resistant to favipiravir is low.

Although favipiravir was found to reduce influenza viral replication less than that of oseltamivir when measured *in vitro*, it was more protective than oseltamivir *in vivo*. Mice given favipiravir 200 mg/kg/day orally beginning either 1 or 13 hours after challenge with a lethal dose of H1N1 virus displayed increased survival and reduced viral titres from the infected lungs as compared with control animals.[61,65] Phase I/II studies of favipiravir are currently underway in the US and Japan, and it is entering phase III trials in Japan.[66,67]

2. Experimental Antiviral Agents for Influenza

2.1 Cyanovirin-N

Cyanovirin-N is a carbohydrate-binding protein that inhibits the entry of viruses into cells by specifically binding to high-mannose oligosaccharides on the surface glycoproteins of enveloped viruses (figure 1). Initially developed as an antiviral drug to treat HIV infection, cyanovirin-N was found to also have potent antiviral activity against most strains of influenza A and B viruses.[68] The binding of cyanovirin-N to influenza viruses and the resulting antiviral activity was limited by the degree of glycosylation of the haemagglutinin. In the mouse and ferret models, prophylaxis and early initiation of treatment with cyanovirin-N caused a significant, dose-dependent reduction of viral titres in the lungs and nasal washes.[69] Resistance to cyanovirin-N has been reported for influenza viruses isolated in the 1930s, as well as after mouse adaption in the absence of drug or after passage in cell culture in the presence of drug.[70] Resistance to cyanovirin-N may be explained by the lack or loss of glycosylation on the haemagglutinin. Glycosylation patterns of human influenza viruses suggest that cyanovirin-N may be effective against seasonal influenza viruses; however, the pandemic 2009 H1N1 influenza viruses lack glycosylation on the haemagglutinin similar to influenza viruses isolated from the 1930s.[71] The effectiveness of cyanovirin-N across all strains and haemagglutinin subtypes of influenza may therefore be limited.

2.2 Conjugated Sialidase (DAS181)

Influenza A and B viruses bind to saccharides containing terminal sialic acids (SA)-α2,3-galactose (SAα2,3Gal) or (SA)-α2,6-galactose (SAα2,6Gal) on the surface of host cells. Both forms of sialic acids are expressed on the surface of the human respiratory epithelial cells;[95] therefore, enzymatic removal of these sialic acids via a sialidase delivered as an inhalant may reduce or eliminate influenza infection (figure 1). DAS181 is a sialidase fusion construct, consisting of a sialidase from *Actinomyces viscosus* fused with a respiratory epithelium-anchoring domain to increase its retention within the respiratory tract.[72] DAS181 has exhibited potent antiviral activity against a range of seasonal influenza A and B viruses when grown in Madin-Darby canine kidney cells, with concentration that produces a 50% effectiveness response (EC_{50}) values ranging from 0.1 to 13.7 nmol/L.[72] This efficacy *in vitro* has been confirmed *in vivo* through a reduction in viral titres from the lungs of mice and in nasal washes from ferrets subsequent to influenza infection. When tested for efficacy against the potentially

pandemic H5N1 influenza A virus and the pandemic 2009 H1N1 influenza A virus, DAS181 inhibited viral replication in human airway epithelial (HAE) cultures and enhanced the survival of infected mice.[73,74] This protection was found to be elicited through the desialylation of the epithelial cell surface within the respiratory tract. When DAS181 was applied to HAE cultures and *ex vivo* bronchial tissues, desialylation occurred immediately, resulting in an inhibitory effect lasting for at least 2 days.[75] One concern when considering new antiviral drugs is whether existing mutations would confer cross-resistance. However, DAS181 displayed antiviral activity against known oseltamivir-resistant viruses.[76] The demonstrated broad antiviral activity against various influenza subtypes, including oseltamivir-resistant viruses, suggests that DAS181 may represent a future therapeutic and prophylactic treatment for influenza. Thus far, DAS181 was shown to be well tolerated with no serious adverse events in phase I trials, and phase II trials have been initiated.[77]

2.3 Thiazolides

Thiazolides are a class of novel broad-spectrum drugs initially designed for the treatment of parasitic infections. Nitazoxanide is a licensed thiazolide for enteritis and has been found to have antiviral activity against DNA and RNA viruses, including hepatitis B and C viruses.[96,97] The mechanism of action of nitazoxanide remains unclear for hepatitis B and C viruses; however, studies using influenza viruses suggest that thiazolides block the maturation of influenza glycoproteins (figure 1). When tested against influenza infection *in vitro*, nitazoxanide blocked terminal glycosylation of the haemagglutinin. This impaired the trafficking of the haemagglutinin glycoprotein between the endoplasmic reticulum and the Golgi complex, thereby preventing its transport to the cell surface.[78] Nitazoxanide has been found to be safe and effective, and is currently in clinical trails for the treatment of rotavirus gastroenteritis, and chronic hepatitis B and C infection.[97-99] Thus far, the emergence of drug-resistant variants has not been reported.[100] Data obtained from influenza infection *in vitro* data support the potential of thiazolides as anti-influenza agents; however, their efficacy *in vivo* remains to be studied.

2.4 Small Interfering RNA

Small interfering RNAs (siRNAs) are nucleic acid-based molecules designed to inhibit gene expression. These short (21–26 nt) sequences are double-stranded RNAs that selectively reduce specific RNA molecules, thus inhibiting the expression of a target gene (figure 1).[101] siRNAs specific for influenza messenger RNA have effectively inhibited replication of multiple influenza subtypes in culture and provide protection against lethal challenge in mice.[79-82] Protection *in vivo* was elicited through the delivery of siRNA via inhalation and intravenous administration. The high mutation rate of influenza could result in resistance to selected siRNAs; therefore, the addition of host targets could minimize the likelihood of antiviral resistance. siRNA has been used to identify host genes required for the replication of influenza viruses through genome-wide RNA interference (RNAi) screening.[83-85] Silencing the host genes that were identified in these studies effectively reduced viral replication *in vitro*. Although siRNA antiviral therapy for influenza has yet to enter clinical trials, RNAi is currently in phase II clinical trials for respiratory syncytial virus. The siRNAs delivered via inhalation are safe and well tolerated, and have demonstrated significant antiviral efficacy.[102,103] siRNAs show promising antiviral potential to provide a potent therapy for the control of influenza by targeting viral or host genes.

3. Combination Chemotherapy

Each of the previously described antivirals is effective in inhibiting the influenza viral replication alone; however, when used in combination, an additive or synergistic effect can be observed. The combinations of oseltamivir, adamantanes and ribavirin against influenza infection are under investigation. Initial studies of combination chemotherapy included evaluating combination

of oseltamivir and adamantanes. This therapy was found to exert an additive and synergistic antiviral effect against multiple subtypes of influenza *in vitro* and *in vivo*, thus providing an advantage over single drug treatment.[104-107] An oseltamivir and ribavirin combination therapy was tested against H5N1 influenza virus in a mouse model and was shown to be beneficial; however, the results were dependent on the H5N1 influenza strain used.[108] More recent studies have shown a triple combination of amantadine, oseltamivir and ribavirin to be efficacious against multiple influenza virus strains *in vitro*, including drug-resistant strains.[109,110] This triple combination was effective against amantadine- and oseltamivir-resistant viruses, with a synergy greater than any double combination tested. An additional advantage to the use of combination chemotherapy may be the potential for reducing the emergence of drug-resistant influenza variants; however, this requires further investigation.[111] As novel therapeutics are identified, they may prove to be most effective when used in combination.

4. Immunomodulators

Until recently, most efforts have been focused on development of therapeutic strategies that target the virus.[112] However, the pathogenic effects of an exuberant immune response, otherwise known as a cytokine storm, following infection with HP influenza viruses are undeniable.[2,4,113,114] This, along with the growing number of reports that demonstrate viral replication and host survival are not always correlated,[115] suggests that new strategies centred around modulating influenza-induced inflammation are sorely needed. Therapeutic agents known to dampen inflammation initiated by pathogens other than influenza or autoimmune disorders, which may also positively influence the outcome of a HP influenza infection, were recently reviewed in detail.[115] In this article, we focus on therapies that are beginning to be evaluated for efficacy during an experimental influenza infection.

4.1 Cyclo-Oxygenase Inhibitors

The cyclo-oxygenase (COX) pathway, and in particular COX-2, is known for modulating inflammation (figure 1). Indeed, COX-2 has been found to be elevated in H5N1-infected macrophages *in vitro* and in lung tissue obtained from patients who died from H5N1 infection, leading to speculation that targeting COX-2 signalling may improve the outcome of an HP influenza infection.[90] Further supporting this idea, a study by Carey et al.[91] demonstrated that a COX-1 deficiency was detrimental, while a COX-2 deficiency proved beneficial for the murine host response to influenza infection. While lung viral titres were elevated in COX-$2^{-/-}$ animals, both mortality and inflammation was markedly reduced compared with COX-$1^{-/-}$ or wild-type animals following infection with H3N2 influenza.

A more recent study tested the hypothesis that combination therapy using the neuraminidase inhibitor zanamivir along with the COX-2 inhibitors celecoxib and mesalazine would decrease H5N1 virus-induced mortality.[92] Administration of zanamivir 4 hours after infection provided complete protection following H5N1 virus infection in mice. However, in real clinical situations, H5N1 virus-infected patients often fail to seek medical advice until 2–4 days after the onset of symptoms. Consistent with previous findings, when zanamivir treatment was delayed for 48 hours, the survival rate decreased from 100% to 13%. However, when celecoxib and mesalazine were administered 48 hours after infection in addition to zanamivir, the survival rate increased to 53%. Concomitant with increased survival was a decrease in proinflammatory cytokine production and, ultimately, decreased pulmonary damage. In this study, the improved survival realised by the combination therapy was attributed to inhibition of COX-2, but it is likely that there were other contributing factors. In particular, the anti-inflammatory effects of mesalazine are known to be dependent on activation of the peroxisome proliferator-activated receptor (PPAR)-γ,[116] which leads to the next potential therapeutic target to dampen the excessive influenza-induced inflammation, the PPAR axis.

4.2 Peroxisome Proliferator-Activated Receptor Agonists

PPARs are a family of lipid-activated transcription factors that are key regulators of lipid and glucose metabolism as well as inflammation.[117] There are three distinct members of the PPAR family: PPAR-α, PPAR-δ (sometimes referred to as PPAR-β) and PPAR-γ. It has been speculated that the use of fibric acid derivatives (PPAR-α agonists) and/or glitazones (PPAR-γ agonists) may be sufficient to decrease immune-mediated pulmonary damage during H5N1 virus infection.[86] To determine whether PPAR-α activation was a viable method to moderate morbidity and decrease mortality during influenza infection, Budd et al.[87] treated mice with the PPAR-α agonist gemfibrozil on days 4–10 after infection with an H2N2 virus. The authors found that treatment with gemfibrozil (60 mg/kg) increased survival from 26% to 52%. Unfortunately, inflammation was not measured in this study, nor were viral titres, making it difficult to conclude the mechanism of protection. Considering the known anti-inflammatory effects of PPAR-α agonists, it is reasonable to hypothesize that protection was conferred through reduced inflammation.

Recently, a specific subset of chemokine (C-C motif) receptor 2 (CCR2+) inflammatory monocytes were found to be significantly elevated during HP influenza infections.[88,89] Although elimination of these inflammatory cells was an appealing approach to combat lethal infection, it was discovered that they are required for the full realisation of antigen-specific CD8+ T-cell-mediated immunity.[88] The blunted CD8+ T-cell response delayed viral clearance; thus, there was no therapeutic benefit realised. It was hypothesized that reducing, without eliminating, the accumulation of these monocytes may prove beneficial to the host. Chemokine (C-C motif) ligand 2 is the preferred ligand for CCR2 and is known to be suppressed by the PPAR-γ agonist pioglitazone.[118] Aldridge et al.[88] demonstrated that treatment with pioglitazone (60 mg/kg) results in increased survival of mice from 8% to 50% after a HP influenza virus challenge, effectively reducing the accumulation of CCR2+ monocytes without compromising viral clearance. The effects of pioglitazone are therefore believed to be directly related to decreased inflammation (figure 1), as there was no decrease in viral replication when tested either *in vivo* or *in vitro*. Because the study was designed to determine whether moderating the numbers of CCR2+ monocytes would prove beneficial during a HP influenza infection, pioglitazone was given prophylactically. Therefore, further studies are necessary to determine whether it is an effective therapy for an established infection. Of the immunomodulators that have been tested during influenza infection thus far, the potential for PPAR agonists appears to be the most exciting. The potential benefits are also supported by the limited adverse effects of pioglitazone (diarrhoea, nausea and upset stomach) reported in clinical trials.

5. Conclusion

Novel antiviral drugs that are currently being developed for the prophylaxis and treatment of influenza hold great promise. Future management strategies for influenza infection include developing therapies targeting viral genes other than those for neuraminidase, using new delivery methods to improve the efficacy of previously approved drugs, identifying drugs for other pathogens that have antiviral activity against influenza and finding approaches to regulate host immunity. It is likely that the influenza virus will become resistant to monotherapy using antiviral drugs currently in development, as it has for those already licensed for use. The rapid emergence and spread of oseltamivir resistance among seasonal H1N1 influenza viruses in 2007–8 demonstrates that we cannot be complacent about the ability of influenza viruses to circumvent any devised control strategy. One of the attractive options is combination chemotherapy with the drugs affecting different viral proteins or different factors of viral pathogenicity. As more information about the pathogenesis of the influenza infection becomes available, novel antiviral drugs and

immunomodulators will be discovered to provide additional treatment options.

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This work was carried out while DAB was affiliated with St. Jude Children's Hospital. His present affiliation is the Microbiology and Molecular Biology Division, IIT Research Institute, Chicago, IL, USA.

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Correspondence: Dr *David A. Boltz*, Microbiology and Molecular Biology Division, IIT Research Institute, 10 West 35th Street, Chicago, IL 60616, USA.
E-mail: dboltz@iitri.org