Influenza pathogenesis: Lessons earned from animal studies with H5N1, H1N1 Spanish, and pandemic H1N1 2009 influenza

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Because cases of highly pathogenic influenza are rare, no systematic clinical studies have evaluated different therapeutic approaches. Instead, treatment recommendations are aimed at the alleviation of clinical signs and symptoms, especially the restoration of respiratory function, and at the inhibition of virus replication, assuming viral load is responsible for disease phenotype. Studies of highly pathogenic influenza in different animal models, especially nonhuman primates and ferrets, reproduce many of the key observations from clinical cases. Host-response kinetics reveal a delayed but broad activation of genes involved in the innate and acquired immune responses (innate responses produce inflammatory responses), which continue after the virus has been cleared and may contribute importantly to the clinical signs observed.

Experimental animal models point to an important role for immune dysregulation in the pathogenesis of highly pathogenic influenza. The use of these models to develop and validate therapeutic approaches is just beginning, but published studies reveal the importance of early treatment with antivirals and show the potential and limitations of approaches aimed at the host response. (Crit Care Med 2010; 38[Suppl.]:S000–S000)

Key Words: ●●●

Tropism

In animal models, the different H5N1 strains are characterized by a wide range of infectivity, tropism, clinical signs, and mortality rate (6–11). Generally, the virulence of a given isolate correlates with its ability to replicate efficiently in the lower respiratory tract of the respective species, including mice, in which these viruses cause lethal disease without the previous adaptation generally required for human influenza A viruses (7, 8, 12). In mice, ferrets, and nonhuman primates, severe disease is associated with spread beyond the respiratory tract, especially to the gastrointestinal tract and the central nervous system (7, 9–12). The gastrointestinal tropism and the ability to infect mice and ferrets via the digestive system suggest a potential for fecal–oral transmission of these viruses (13), although human epidemiologic studies do not support an important role for fecal–oral transmission in influenza epidemics (Table).

Pathogenesis

On infection with clinical isolates, ferrets closely reproduce the disease severity and clinical signs observed in the similarly infected patient, including high fever, weight loss, anorexia, extreme lethargy, diarrhea, and, in some cases, neurologic signs. The morbidity of avian isolates, however, varies from highly pathogenic to asymptomatic (9, 10, 14). Anorexia, fever (>40°C), depression, coughing, signs of acute respiratory distress syndrome, and diarrhea also have been observed in macaques infected with highly pathogenic H5N1 viruses (6, 11). As observed in other animal models, pathogenesis and lethality in mice are strongly strain-dependent and, to a lesser extent, dose-dependent (7, 10). Mice infected with a lethal dose begin to lose weight within 2 days, showing signs of illness (such as ruffled fur and listlessness) during the first week of infection, and they die after 7 to 9 days (10).

Histopathology

Regardless of the animal model, histopathological changes in the lung are characterized by extensive bronchiolitis and alveolitis, edema, and focal hemorrhage starting as early as 24 hrs after infection (6). Type II pneumocytes are the primary target of infection, and antigen-positive epithelial cells in the lung are generally found in close proximity to damaged, necrotic bronchi, either lining the bronchi or extracellularly within the bronchiolar lumen in association with necrotic debris (7, 9, 15). At later disease stages, focal immunostaining of inflammatory cells, mainly mononuclear cells, is found in subepithelial tissues in the pulmonary interstitium and in association with hemorrhage (7, 9).
was not critical for virus replication and also was detected (7) and, although MIP-1/H9251 sustained expression of MCP-1 and MIP-1(IFN)-IL-6, tumor necrosis factor-mokines in the lung, especially high levels of associated with a strong transcriptional induc-infiltration of macrophages and neutrophils H5N1 strains cause a massive and sustained degeneration and neuronophagia associ-

In all animal models, highly pathogenic viruses cause multi-organ failure with a high mortality rate, especially in ferrets and non-human primates (NHPs). The respiratory system is the primary target, with bronchopneumonia and alveolitis being common findings. Systemic infection also occurs, with viral RNA detected in multiple organs including the liver, spleen, and brain. The immune response is characterized by a cytokine storm, indicating an excessive immune activation.

### Immune Response

In all animal models, highly pathogenic H5N1 strains cause a massive and sustained infiltration of macrophages and neutrophils associated with a strong transcriptional induction of pro-inflammatory cytokines and chemokines in the lung, especially high levels of IL-6, tumor necrosis factor-α, interferon (IFN)-γ, and CXCL-10. In mice, sustained expression of MCP-1 and MIP-1α was detected (7) and, although MIP-1α was not critical for virus replication and spread in this animal model (18), it has been associated with fatal outcomes in human infections (4). In addition to the increase of local cytokine and chemokine expression in the lung, high cytokine levels are also detected in the blood, indicative of a general and possibly excessive immune activation (6).

All influenza infections cause a transient lymphopenia, but the extent is more pronounced in animals infected with highly pathogenic strains (6, 10, 19). In macaques, circulating CD4⁺ and CD8⁺ T cells decrease within the first 2 days after infection (6), and cell suspension analysis reveals a reduction of cellularity and an alteration of the relative proportion of CD4⁺ and CD8⁺ cells in the thymus of mice infected with highly pathogenic H5N1 strains (6, 19).

### Table. Overview of the different animal models and viruses

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Spanish Flu H1N1 1918</th>
<th>H5N1 HPAI</th>
<th>Pandemic H1N1 2009</th>
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</thead>
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<tr>
<td>High replication in higher and lower respiratory tract</td>
<td>Mouse (24), ferret (25), guinea pig (28), pig (27), NHP (26)</td>
<td>Mouse (7, 8), ferret (9, 12), NHP (6, 11)</td>
<td>Mouse (35, 36), ferret (34–36), pig (35), NHP (35)</td>
</tr>
<tr>
<td>Clinical signs</td>
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<td></td>
<td>Ferret (36)</td>
</tr>
<tr>
<td>Sneezing, coughing, and respiratory signs</td>
<td>Ferret (31), NHP (26)</td>
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<td>Mouse: weight loss (35, 36)</td>
</tr>
<tr>
<td>Fever, diarrhea, and weight loss</td>
<td>Mouse (24), ferret (31), NHP (26), pig, only fever (27)</td>
<td>Mouse (7, 9, 10, 12), NHP (6, 11)</td>
<td>Ferret: strongly dependent on the isolate (34–36)</td>
</tr>
<tr>
<td>Lethal</td>
<td>Mouse (24), ferret (31), NHP (26)</td>
<td>Mouse (7, 8, 10), ferret (9, 10), NHP (6)</td>
<td>Mouse (35)</td>
</tr>
<tr>
<td>Severe lung injuries</td>
<td></td>
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<td>Ferret: strongly dependent on the isolate (34–36)</td>
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<tr>
<td>Necrotizing bronchiolitis</td>
<td>Mouse (15, 24), ferret (25)</td>
<td>Mouse (7), ferret (9)</td>
<td>More severe than seasonal strain but less severe than observed with Spanish flu 1918 or HP H5N1, mouse (35)</td>
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<td>Alveolitis–peribronchitis</td>
<td>NHP (26), pig (27)</td>
<td>NHP (6, 128), Pig, mild-to-moderate broncholitis and multifocal alveolitis (14)</td>
<td>Ferret: dependent on the isolate (34–36)</td>
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<td>Hemorrhage</td>
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<td>Bronchopneumonia</td>
<td>Mouse (15, 17, 32), ferret (25), pig (27)</td>
<td>Mouse (7, 17), ferret (9, 12), NHP (6, 128), pig (14, 129), mouse (17, 18)</td>
<td>Pig (35), NHP (35)</td>
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<tr>
<td>Cytokine storm (lungs)</td>
<td>Mouse (15, 17), NHP (26), NHP: heart and spleen (26)</td>
<td>Mouse (7, 17), ferret (9, 12), NHP (6, 128), pig (14, 129), mouse (17, 18)</td>
<td>NHP (35), mouse (35), NHP (35)</td>
</tr>
<tr>
<td>Systemic infection</td>
<td></td>
<td>Mouse: brain, thymus, spleen, heart (7, 10); ferret: spleen, brain, intestine, and pathology in the liver (9, 10, 12) NHP: trabecular lymph nodes, spleen, meninges, and blood (6, 11)</td>
<td>Viral RNA and low amount of infectious virus in rectal swab and intestinal tissues in ferret (36)</td>
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<td>Leukopenia</td>
<td>NHP (26)</td>
<td>Mouse (10), ferret (10, 12), NHP (6)</td>
<td></td>
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<tr>
<td>Cytokine storm (serum)</td>
<td>NHP (26)</td>
<td>NHP (6)</td>
<td></td>
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</table>

NHP, nonhuman primate.
Pathogenesis

In mice, ferrets, and nonhuman primates, infection with H1N1 1918/ rec results in the onset of severe clinical signs within 1 to 2 days after infection and mortality rates ranging from 50% to 100% (24, 29, 30). Clinical signs in ferrets and nonhuman primates include lethargy, anorexia, rhinorrhea, sneezing, severe weight loss, high fever, and, ultimately, respiratory distress syndrome leading to death in 50% to 75% of the animals within 2 wks (26, 31). In primates, an increased respiratory rate and a decrease in blood oxygen saturation of as much as 36%, compared to preinfection levels, are also observed (26).

Histopathology

Infection with the H1N1 1918/rec virus results in widespread lung lesions, including necrotizing bronchitis, moderate-to-severe peribronchial and alveolar edema, alveolar hemorrhage, bronchiolitis, and moderate-to-severe alveolitis in all animal models (25–28, 32). The distribution of the alveolitis varies from peribronchial to diffuse, and it is composed of neutrophils and macrophages (32). Neutrophils are the predominant inflammatory cells, but alveolar macrophages are also frequently found (32). In macaques, widespread detection of antigen in plump alveolar cells and desquamation of these cells into the alveolar space is a prominent characteristic of the H1N1 1918/rec virus-infected lung tissue (26). However, in contrast to H5N1, no viral antigen is detected outside the respiratory tract for any of the animal models.

Although they are a natural host and may play an important role in influenza epidemiology, pigs infected with H1N1 1918/rec did not have severe respiratory signs or die (27), indicating that the relevance of this animal model for human influenza pathogenesis may be limited.

Immune Responses

The infiltration of inflammatory cells in the lungs is accompanied by a significant increase of pro-inflammatory cytokines and chemokines, which correlates with the disease severity observed in the different animal models (15, 17). The global gene expression profiles in bronchi of infected macaques revealed that both a seasonal H1N1 strain and the highly pathogenic H1N1 1918/rec virus activate the transcription of several inflammatory chemokine and cytokine genes, including IL-6 (26); however, the IL-6 messenger RNA was expressed at a high level until day 8 only in the H1N1 1918/rec-infected macaques. Higher levels of IL-6 also were observed in the serum of the H1N1 1918/rec-infected animals, which reflects a systemic alteration of the immune response (26), suggesting a prominent activation of cells of the monocyte/macrophage lineage. Comparison of the H1N1 1918/rec virus with a highly pathogenic H5N1 strain in mice demonstrated higher levels of cytokines and chemokines in the lungs of the H5N1 group, whereas overall lung cellularity, virus growth, and patterns of immune cell subpopulation dynamics over time were very similar for both viruses (17), suggesting a common immunopathogenic mechanism.

DISCUSSION

Pandemic H1N1 2009 Influenza

The ongoing H1N1 pandemic started in the Mexican town of La Gloria, Veracruz, in mid February 2009. This new strain is thought to be a reassortment of recent North American H3N2 and H1N2 swine viruses with Eurasian avian-like swine viruses (33).

So far, most human infections with pandemic H1N1 2009 viruses result in a self-limiting, uncomplicated disease with a clinical course similar to that observed for seasonal influenza. Clinical signs atypical for seasonal influenza have been reported, however, including vomiting and diarrhea in a relatively large proportion of cases (33). Furthermore, some patients have required hospitalization because of severe pneumonia and respiratory failure, with a fatal outcome occurring in 0.5% of laboratory-confirmed cases. In contrast to seasonal influenza, a substantial proportion of the cases of severe illness and death have occurred among previously healthy adults aged 18 to 50 yrs, as well as among adults with underlying disease and pregnant women (34). Additionally, the current H1N1 pandemic virus is spreading rapidly as seasonal influenza in the Northern Hemisphere disappears, suggesting a greater transmission efficacy (Table).
subsequent release of the virus into the cytoplasm. The viral M2 protein, which forms an ion channel in the viral envelope, plays an essential role in this process by enabling the influx of H^+ ions from the endosome into the virus particle. The class of M2 ion channel inhibitors includes the amantadanes, amantadine, and rimantadine, which sterically block the ion channel, thereby interfering with virus entry into the cell (37) (Fig. 1.). Amantadine and rimantadine have been licensed in the US as antiviral drugs since 1966 and 1993, respectively. Historically, they have been mainly used for prophylaxis during outbreaks or to reduce the duration of uncomplicated influenza infections. Both drugs confer 80% to 90% protection against illness and lowered transmission (38–41). Furthermore, amantadines decrease the duration of the clinical course even if given 48 hrs after infection (41). Today, rimantadine is the drug of first choice because of the gastro-intestinal and central nervous system side effects of amantadine (38).

Because of widespread resistance in circulating strains, amantadanes are rarely used today. Resistant H3N2 viruses emerged in China during the 2003 season and quickly spread worldwide, reaching resistance rates >90% in Asia and the US, and nearly 50% in Europe for H3N2 subtypes; rates for H1N1 are slightly lower (42). Notably, most Asian H5N1 isolates are also resistant to this class of drugs, and the pandemic H1N1 2009 virus carries the resistance-conferring mutation (42, 43), rendering these drugs mostly obsolete for the treatment of circulating influenza strains.

**Neuraminidase Inhibitors**

The viral neuraminidase (NA) protein is involved in the early and late steps of infection. During virus entry, NA is thought to cleave sialic acid residues from mucin, a class of glycoproteins abundantly present in the airways, which may impede access to the target cell membrane. NA also plays a role in virus egress by removing sialic acids from the viral particle and surrounding cell surfaces to avoid aggregation of newly formed virions (44, 45). The class of approved NA inhibitors currently includes oseltamivir and zanamivir, which are small molecules that bind with high affinity to the active site of the NA enzyme (Fig. 1.). This region is highly conserved among influenza A and B viruses and, consequently, NA inhibitors are active against viruses from both genera (46), but oseltamivir is less effective than zanamivir against influenza B (47). Both drugs have been approved by the US Food and Drug Administration for the treatment and prevention of influenza since 1999.

In ferrets, the oseltamivir regimen effective against seasonal influenza does not control highly pathogenic H5N1 infections (48). Subsequent studies using H5N1 viruses revealed that the effective dose depends on the virulence and the time after infection. A low dose of oseltamivir is sufficient to protect ferrets against a lethal challenge when treatment is started within 4 hrs after infection, whereas the dose has to be almost tripled when the treatment starts after 24 hrs (48). In a H5N1 macaque model, prophylactic treatment with 10 and 20 mg/kg zanamivir intravenously resulted in an important reduction of gross pathology, pneumonia, and viral load, whereas treatment with the higher dose 4 hrs after infection was associated with a similar effect on the viral load without improving lung pathology and pneumonia (49), illustrating the importance of rapid diagnosis and treatment. One concern unique to highly virulent influenza viruses, especially of the H5N1 subtype, is their possible dissemination to the brain. Drug distribution studies in rats suggest that oseltamivir is limited in its ability to cross the brain–blood barrier, and thus it may not be the drug of choice if neuro-invasion is suspected (50).

During the past 2 yrs, oseltamivir-resistant H1N1 viruses have emerged in Europe and Asia, and they are spreading rapidly across the world (51–53). So far, there is little resistance in the H3N2 subtypes, and most H5N1 subtypes remain NA inhibitor-sensitive (4, 54, 55). However, the first oseltamivir-resistant pandemic H1N1 2009 isolates have been reported in Asia and North America (56, 57), and these viruses are likely to spread because of the ongoing use of oseltamivir. In contrast to oseltamivir, resistance to zanamivir is less frequent, even though a recently identified mutation is associated with decreased susceptibility to zanamivir without affecting oseltamivir susceptibility. Viruses carrying this mutation accounted for 2.3% of isolates between 2006 and early 2008, and they were found to retain wild-type fitness in ferrets (58), suggesting that increased zanamivir use will also lead to the rapid emergence of resistance.

**Ribavirin and Viramidine**

Ribavirin is a guanosine analogue licensed for the treatment of hepatitis C in combination with IFN-α. It inhibits viral replication either indirectly by decreasing intracellular guanosine triphosphate levels through the inhibition of inosine 5'-monophosphate dehydrogenase or directly by interfering with transcription and genome replication (59) (Fig. 1.). Because of its multiple mechanisms of action, ribavirin inhibits a broad range of DNA and RNA viruses and resistance rarely develops (60).

In mice, prophylactic and postexposure oral or intraperitoneal ribavirin treatment results in decreased viral titers in the lungs and faster virus clearance but does not reduce mortality, whereas administration by aerosol greatly reduces viral load and increases survival rates (61–64). On aerosol administration, ribavirin is found in high concentrations in the lungs and lower concentrations in the blood, other organs, and the brain (65); this may be an advantage for the treatment of avian influenza viruses, which...
considerably reduced lung titers and pre-treatment with a single dose of IFN-
models. In mice and guinea pigs, pre-
been assessed systematically in animal
recently, the anti-influenza efficacy has
shown that intranasal IFN treatment re-
duced clinical signs of influenza, but it
had no beneficial effect against H5N1 (79). In
addition to IFN, the therapeutic effect of
IFN inducers such as poly(I:C) against
influenza is being investigated. Prophy-
lactic treatment with liposome-encapsu-
lated poly(I:C) protected mice against
mouse-adapted influenza strains (80),
and clinical trials are underway to evalu-
ate the toxicity of the compound.

Corticosteroids

The use of immunomodulators, par-
ticularly steroids and their derivatives,
has been examined mostly in the context
of H5N1 infections, which cause severe-
to-fatal disease that is characterized by
fulminant pneumonia and multi-organ
failure associated with an excessive in-
flammatory response (2, 81). Treatment
with corticosteroids was not associated
with increased patient survival during
H5N1 outbreaks in Hong Kong, Vietnam,
and Thailand (2, 81, 82). The World
Health Organization does not recom-
 mend the use of corticosteroids except in
cases of septic shock with suspected ad-
renal insufficiency.

Only triamcinolone doses >4 mg/kg
resulted in notable reduction of pulmo-
ry lesions and suppression of immune
activation in cotton rats infected with
seasonal influenza viruses (83), demon-
strating that a high-threshold steroid
concentration is necessary for efficient
treatment. However, no improvement as-
associated with corticosteroid treatment
was seen in mice infected with a highly
virulent H5N1 strain (84).

Chloroquine

As mentioned, influenza viruses enter
target cells by endocytosis, and a low pH
is required to trigger fusion of viral and
endosomal membranes. Chloroquine is a
weak base that is commonly used to treat
malaria. In the cell, the drug accumulates
in acidic organelles, such as endosomes
and lysosomes, where it increases the pH
(85, 86) (Fig. 1.). Chloroquine has been
shown to inhibit many viruses that re-
quire low pH for entry and is considered
a possible treatment for severe acute re-
spiratory syndrome and human immuno-
deficiency virus (87). Even though in vitro
studies show that chloroquine inter-
feres with H1N1 and H3N2 replication
(88), it does not prevent weight loss as-
sociated with infection in mice or result
in decreased viral replication in the nose
of the ferrets (89).

Antiviral Drugs in Development

All available influenza-specific drugs
are limited by the rapid emergence of
resistance once they are broadly used.
Therefore, a global effort is ongoing to
develop new antiviral drugs that either
target different steps in the viral life cycle
or modulate the immune system. Several
of the more promising candidates are al-
ready undergoing preclinical evaluation
or are in clinical trials.

Attachment Inhibitors

To prevent virus attachment to the
target cell, molecules can target the re-
ceptor-binding or fusion domains in the
viral attachment protein HA (or sialic ac-
ids), the cellular receptor. Different mole-
cules, mainly multivalent substrate ana-
logues and blocking peptides, have been
designed that interfere with these initial
virus–host cell interactions (90 –92) (Fig.
1.).

The recombinant fusion protein
DAS181 (Fludase; NexBio, San Diego,
CA), which is composed of the catalytic
domain of Actinomyces viscosus and an
epithelium-anchoring domain, prevents
infection by cleaving-off sialic acids
present at the cell surface of the airway
epithelium. In mice, DAS181 pretreat-
ment resulted in improved lung function,
less pathology, significantly reduced lung
titers, and inhibition of systemic dissem-
ination after H5N1 infection (93, 94).
Postexposure treatment was beneficial if
initiated 48 hrs after infection in the case
of H1N1 infection (94), whereas the con-
rol of H5N1 infection required treatment
within 24 hrs (93). In ferrets, treatment
with 1000 U/day (>1 mg/kg/day) for 7
days starting 2 days before infection
greatly reduced virus shedding and pre-
vented disease after challenge with a hu-
man seasonal virus without signs of tox-
icity (94). Recently completed phase Ia
studies with this compound demonstrate
that repeat doses of DAS181 are well-
tolerated and have no toxic effects (95).

Because activation of the HA protein
requires extracellular proteases, the anti-
viral activity of different protease inhibitors has been evaluated. Given intraperitoneally or intranasally, the trypsin-like protease inhibitor aprotinin reduced lung titers and resulted in a 35% mortality reduction on challenge with a mouse-adapted virus (96). Aprotinin inhalation also reduced clinical signs of influenza and parainfluenza infections in clinical trials (97). The development of inhibitors specifically targeting the HA receptor-binding domain is less advanced. Given prophylactically, a synthetic polymeric attachment inhibitor prevented H5N1 morbidity and mortality in mice. However, when treatment was initiated 6 hrs after infection, only a modest decrease in lung titers was observed (92).

**Neuraminidase Inhibitors**

Several new NA inhibitors are in advanced stages of development, such as peramivir (RWJ-270201, BCX-1182) and other cyclopentane and cyclopentane amide derivatives, pyrrolidines (A-192358, A-315675) and other pyrrolidine derivatives, and 2,3-disubstituted tetrahydrofururan-5-carboxylic acid derivatives. Peramivir has been most extensively studied. A prophylactic dose of 10 to 20 mg/kg injected intramuscularly prevented or greatly reduced mortality in mice after H1N1 or H3N2 challenge, respectively (98). A single intramuscular dose followed-up by 7 days of oral treatment was as effective as oseltamivir in protecting against infection with a highly virulent H5N1 strain. When treatment was started 24 or 48 hrs after a lethal challenge, 78% and 56% of the mice survived, respectively (99). In ferrets, peramivir treatment initiated 1 hr after infection and daily for 4 days thereafter resulted in improved survival after infection (100). However, in humans, oral peramivir did not provide good protection, which was attributed to the low oral bioavailability (<5%) of the drug (101). The efficacy of intravenous or intramuscular routes of inoculation is being evaluated in phase III clinical trials.

**RNA Polymerase Inhibitors**

In addition to viral transcription and replication, the influenza polymerase complex also possesses an endonuclease activity that allows the virus to synthesize the viral messenger RNA using the capped primers of the host (102). Several compounds targeting the replication or endonuclease activities are in development (Fig. 1).

To date, two nucleoside analogues have been tested for anti-influenza activity: 2'-deoxy-2'-fluoroguanosine and T-705; 2'-deoxy-2'-fluoroguanosine has only been evaluated in vitro (103, 104), but T-705 (Toyama Chemical, Tokyo, Japan), which acts as a purine nucleoside analogue, is better-characterized (105). Unlike ribavirin, T-705 does not affect the host DNA or RNA synthesis and has a better therapeutic index in preclinical tests. Treatment of mice once, twice, or four times daily for 5 days starting 1 hr after H5N1 infection with 30 to 300 mg/kg daily prevented lung pathology and mortality. In a direct comparison, T-705 was less efficient than zanamivir and oseltamivir but better than ribavirin (106). Phase I/II trials with T-705 are ongoing in the US and Japan, and clinical data are accumulating (107).

**Small Interfering RNA**

Small interfering RNA are RNA duplexes of 21 t o 25 nucleotides that recognize specific RNA and trigger their degradation through a process called RNA interference (108). Directed against conserved regions of different viral proteins, small interfering RNA are very efficient in vitro (109) (Fig. 1.). In mice, pretreatment with influenza-specific small interfering RNA reduced the viral load in the lungs and protected against lethal challenge with H5N1, H1N1, and H7N7 viruses (110, 111). In a rhesus macaque severe acute respiratory syndrome model, small interfering RNA were efficient when used 5 hrs or 24 hrs after infection, highlighting their therapeutic potential against respiratory infections (112).

**Passive Immunotherapy and Vaccine Development**

To date, passive immunotherapy has been used for patients at high risk for several viruses, including rabies, hepatitis A, and respiratory syncytial virus (113). During the 1918 pandemic, severely ill patients treated with convalescent sera had a case fatality rate of 16%, whereas 37% of untreated patients died (114). More recently, treatment of two H5N1-infected patients with plasma from a convalescent patient resulted in fast recovery (115, 116).

Monoclonal antibodies against the HA of H1 and H2 viruses administered 2 days after infection increased survival in mice (117), and whole antibodies or Fab fragments protected SCID mice from lethal H1N1 infection (118, 119). Furthermore, humanized antibodies and human monoclonal antibodies developed from a patient with Vietnamese H5N1 protected mice against lethal challenge, even when administered up to 3 days after infection (120, 121). These animal studies demonstrate the potential of passive antibody transfer as influenza treatment, and much work is focusing on this therapeutic approach, especially with the emergence of biologically robust NA-resistant strains.

**Approved Vaccines**

Because of the acute nature of influenza infections, prophylactic vaccines will remain the most efficient and cost-effective control measure. With the exception one cold-adapted live-attenuated vaccine, all North American influenza vaccines are inactivated and contain either detergent-split virions or further purified viral glycoproteins. All these vaccines are grown in embryonated chicken eggs and include one previously chosen H1N1 and H3N2 strain together with an influenza B virus, which are changed annually in response to the ongoing antigenic drift (122). In Europe, a subunit vaccine adjuvanted with MF59, an oil-in-water emulsion, has been in use since 1997 (123), and a cell culture-produced inactivated vaccine was approved in 2007 (124). It is thus likely that these or similar formulations will be approved in North America in the foreseeable future.

**CONCLUSION**

**Candidate Vaccines**

The worldwide effort to prepare for a possible influenza pandemic has resulted in the development of a broad range of candidate vaccines. Even though a universal vaccine that protects against all influenza subtypes remains elusive, the inclusion of more conserved internal viral proteins has resulted in protection against different strains from the same subtype and even a certain level of heterosubtypic immunity in different animal models (125, 126). To increase the level and duration of the immune response, various adjuvants have been developed, including specific stimulators of immune-signaling pathways and immuno-
genic proteins, in addition to the latest generation of oil-in-water emulsions (127). Several of these compounds have shown efficacy in different animal models and are being evaluated in clinical trials.

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