Recent Advances in the Management of Viral Flu Infections

Department of Pharmaceutics, Maharishi Arvind College of Pharmacy, Ambabari, Jaipur, Rajasthan, India.
Received February 25, 2013; accepted May 29, 2013

ABSTRACT
Flu is an infectious respiratory disease which is caused by influenza viruses. The most common symptoms of flu are nausea, vomiting and diarrhoea. Different subtypes of influenza viruses are identified based on the combinations of these antigenic structures. Influenza viruses are negative-sense, single-strand RNA viruses that belong to the family Orthomyxoviridae. Antiviral medications are the current pharmacotherapy of flu because they are approximately 70% to 90% effective in preventing influenza. Influenza virus type A is readily prone to mutations, which can lead to minor antigenic changes (antigenic drift) or major antigenic changes (antigenic shift). These changes can result in viral strains capable of causing either an epidemic or a pandemic, because overall immunity in the population is lower. Flublok is the first trivalent influenza vaccine made using an insect virus (baculovirus) expression system and recombinant DNA technology (i.e., without the use of actual influenza virus), so there is little risk of getting the flu from the vaccine. Flublok is highly purified, has three times the amount of active ingredient in traditional influenza vaccines, and contains no preservatives, antibiotics or adjuvants. It provides protection against the latest strains of influenza by containing the corresponding hemagglutinin antigens for those strains. This review article provides brief information of influenza and its treatment by biotechnology-based vaccines introduced within the past few years.

KEYWORDS: Influenza; Hemagglutinin; Flu; Flublok; Baculovirus; Antigenic shift.

Introduction
The word influenza may have been derived from the Latin word influo, which means “to flow in,” indicating airborne transmission, or from the Italian word influence, which indicates influence of weather or an astrological influence. In addition to humans, influenza also infects a variety of animal species. Some of these influenza strains are species specific, but new strains of influenza may spread from other animal species to humans (Mohan et al., 2012).

The term avian influenza used in this context refers to zoonotic human infection with an influenza strain that primarily affects birds. Influenza is a leading cause of illness and death around the world, resulting on average in more than 200,000 hospitalizations and ranging from 3,000 to 49,000 deaths annually in the U.S. alone. More than 90% of influenza-related deaths occur in people over the age of 65, and individuals of all ages with weakened immune systems are at particularly high risk for developing complications from the illness. Influenza, also known as the flu, is a contagious respiratory disease that is caused by influenza viruses. Influenza viruses infect the respiratory tract (nose, throat, and lungs) in humans. Flu season typically peaks in January or February but can extend as late as May. The flu is different from a cold, mainly because the symptoms and complications are more severe. (Abramson et al., 2012; Thompson et al., 2003; 2004).

Biology of Influenza Virus
The structure of the influenza virus is somewhat variable, but the virion particles are usually spherical or ovoid in shape and 80 to 120 nanometers in diameter (Figure 1). Sometimes filamentous forms of the virus occur as well, and are more common among some influenza strains than others. The influenza virion is an enveloped virus that derives its lipid bilayer from the plasma membrane of a host cell. Two different varieties of glycoprotein spike are embedded in the envelope. Approximately 80 percent of the spikes are hemagglutinin, a trimeric protein that functions in the attachment of the virus to a host cell. The remaining 20 percent or so of the glycoprotein spikes consist of neuraminidase, which is thought to be predominantly involved in facilitating the release of newly produced virus particles from the host cell.

Fig. 1. Core structural features of influenza virus.
On the inner side of the envelope that surrounds an influenza virion is an antigenic matrix protein lining. Within the envelope is the influenza genome, which is organized into eight pieces of single-stranded RNA (A and B forms only; influenza C has 7 RNA segments). The RNA is packaged with nucleoprotein into a helical ribonucleoprotein form, with three polymerase peptides for each RNA segment (Louie, 2012; Petsch et al., 2012). Mutations in the antigenic structure of the influenza virus have resulted in a number of different influenza subtypes and strains. Specific varieties of the virus are generally named according to the particular antigenic determinants of hemagglutinin (13 major types) and neuraminidase (9 major types) surface proteins they possess, as in influenza A (H2N1) and A/H3N2). New strains of the influenza virus emerge due to a gradual process known as antigenic drift, in which mutations within the virus antibody-binding sites accumulate over time. Through this mechanism, the virus is able to largely circumvent the body’s immune system, which may not be able to recognize and confer immunity to a new influenza strain even if an individual has already built up immunity to a different strain of the virus. Both A and B influenza viruses continually undergo antigenic drift, but the reformulation of influenza vaccines each year often enables scientists to take into account any new strains that have emerged (Hunt, 2012).

Influenza A also experiences another type of mutation called antigenic shift that results in a new subtype of the virus. Antigenic shift is a sudden change in antigenicity caused by the recombination of the influenza genome, which can occur when a cell becomes simultaneously infected by two different strains of type A influenza. The unusually broad range of hosts susceptible to influenza A appears to increase the likelihood that this event will occur. In particular, the mixing of strains that can infect birds, pigs, and humans is thought to be responsible for most antigenic shifts. Notably, in some parts of the world, humans live in close proximity to both swine and fowl, so that human strains and bird strains may readily infect a pig at the same time, resulting in a unique virus. New subtypes of influenza A develop abruptly and unpredictably so that scientists are unable to prepare vaccines in advance that are effective against them. Consequently, the emergence of a new subtype of the virus can cause a global pandemic in a very short amount of time (Abramson et al., 2012; Glezen, 1982).

**Pathophysiology of Influenza Infections**

Influenza viruses are negative-sense, single-strand RNA viruses that belong to the family Orthomyxoviridae (MMWR et al., 2012). Human influenza viruses are divided into 3 major types: A, B, and C. Influenza type A viruses cause disease in humans and many animal species. Waterfowl (e.g., ducks, geese) are the natural reservoir for type A. In addition, in freshwater lakes, influenza A virus can stay alive for 4 days at 22°C and for more than a month at 0°C. Influenza type B viruses primarily cause disease in humans, particularly children. Infections with influenza type C viruses are rare.

Influenza viral RNA has 8 genetic elements. The RNA has a lipid envelope with 2 major antigenic components on its surface, hemagglutinin (H) and neuraminidase (N). These components enable the replication and subsequent release of the virus, leading to its spread. Influenza type A viruses also have ionic channel proteins, termed M2 proteins (Morb et al., 2012).

The H antigen is the major virulence determinant because these antigens help viral attachment to the cell. H proteins are divided into 16 types, whereas N proteins are divided into 9 types. The N act on the sialic acid component of the cell which enables viral detachment.

Different subtypes of influenza viruses are identified based on the combinations of these antigenic structures, with 144 combinations possible. For example, influenza A subtype H3N2 expresses hemagglutinin 3 and neuraminidase 2. Influenza A subtype H5N1, or avian influenza, has been found in chickens, ducks, and migratory fowl throughout Asia and is now spreading west through Europe and North Africa. It is highly virulent in humans but is poorly transmissible between humans. Currently, influenza A/H1N1, A/H3N2, and influenza type B viruses are the circulating influenza strains that cause seasonal human infections. The influenza vaccine components for the current year are intended to provide protection from these strains (Abde et al., 2008; Louie et al., 2012).

In contrast with the typical course of disease caused by seasonal influenza, a higher viral load and prolonged viral replication are observed in infections caused by avian influenza virus A/H5N1. Current research is focusing on broadly neutralizing antibodies against influenza viruses for potential treatments and vaccines. One group recently reported isolating and characterizing human monoclonal antibody CR8020 with broad neutralizing activity against most group 2 viruses, including H3N2 and H7N7, and another group isolated a neutralizing monoclonal antibody that recognized the hemagglutinin glycoprotein of all 16 subtypes and neutralized both group 1 and group 2 influenza A viruses.

**Antigenic drift and shift**

Influenza virus type A is readily prone to mutations, which can lead to minor antigenic changes (antigenic drift) or major antigenic changes (antigenic shift). These changes can result in viral strains capable of causing either an epidemic or a pandemic, because overall immunity in the population is lower or nonexistent (De Jong et al., 2006). With antigenic drift, the surface hemagglutinin or neuraminidase proteins are slightly altered by accumulated point mutations and nucleotide substitutions, insertions, or deletions. Because the antigens are only slightly altered, an infected person’s immune system still recognizes the virus to some extent, and the infection is usually less severe. Antigenic shift
refers to a major change in the viral RNA caused by gene reassortment.

This results in replacement of the surface hemagglutinin or neuraminidase proteins and these new proteins may be unrecognizable by an infected person’s immune system. The newly reassorted viral strain may cause a pandemic in an immunologically naïve population. Because avian influenza viruses attach to receptors specific to birds, whereas human influenza viruses attach to receptors specific to humans, transmission of influenza from birds to humans is rare. However, an intermediate host that has receptors used by both viruses (e.g., pigs) can become co-infected with both avian and human influenza viruses; this permits reassortment that may produce major changes in the genetic component of surface proteins. The resulting viral strain may be more capable of transmission to humans (de Jong et al., 2006; Yu et al., 2011).

Modes of Transmission of Flu
(a) **Person to Person:** People with flu can spread it to others up to about 6 feet away. Most experts think that flu viruses are spread mainly by droplets made when people with flu cough, sneeze or talk. These droplets can land in the mouths or noses of people who are nearby or possibly be inhaled into the lungs. Less often, a person might also get flu by touching a surface or object that has flu virus on it and then touching their own mouth or nose.

(b) **Flu is infectious:** Most healthy adults may be able to infect others beginning 1 day before symptoms develop and up to 5 to 7 days after becoming sick. Children may pass the virus for longer than 7 days. Symptoms start 1 to 4 days after the virus enters the body. Some persons can be infected with the flu virus but have no symptoms. During this time, those persons may still spread the virus to others (Booy et al., 2012).

Flu is unpredictable and how severe it is can vary widely from one season to the next depending on many things, including what flu viruses are spreading, how much flu vaccine is available, when vaccine is available, how many people get vaccinated, and how well the flu vaccine is matched to flu viruses that are causing illness.

Types of Flu
1. **Seasonal Flu:** Seasonal flu is a contagious respiratory illness caused by flu viruses. Keep in mind that even seasonal influenza can be a serious disease. Sometimes seasonal influenza can lead to complications (like pneumonia). It also can lead to hospitalization and even death.

2. **H3N2v:** Influenza A H3N2 variant viruses (also known as “H3N2v” & non-human influenza viruses) that normally circulate in pigs are called “variant” viruses when they are found in people. H3N2v is a virus that normally circulates in pigs and that has infected humans. When these viruses infect humans, they are termed “variant” viruses.

3. **H1N1 (Swine Flu):** Swine influenza (swine flu) is a respiratory disease of pigs caused by type A influenza viruses that regularly cause outbreaks of influenza in pigs. Influenza viruses that commonly circulate in swine are called “swine influenza viruses” or “swine flu viruses.” Like human influenza viruses, there are different subtypes and strains of swine influenza viruses. The main swine influenza viruses circulating in U.S. pigs in recent years are:
   (i) swine triple reasserting (tr) H1N1 influenza virus
   (ii) trH3N2 virus
   (iii) trH1N2 virus (Viasus et al., 2011; MMWR, 2009)

4. **H5N1 (Avian/Bird Flu):** Avian influenza refers to the disease caused by infection with avian (bird) influenza (flu) Type A viruses. These viruses occur naturally among wild aquatic birds worldwide and can infect domestic poultry and other bird and animal species. Avian flu viruses do not normally infect humans (Uyeki, 2009).

Avian influenza A viruses are classified into the following two categories: low pathogenic avian influenza (LPAI) A viruses, and highly pathogenic avian influenza (HPAI) A viruses.

Infection of poultry with LPAI viruses may cause no disease or mild illness (such as ruffled feathers and a drop in egg production) and may not be detected. Infection of poultry with HPAI viruses can cause severe disease with high mortality. Both HPAI and LPAI viruses can spread rapidly through poultry flocks (Yu et al., 2011).

5. **Current Flu Season:** Flu is still at epidemic levels as reported by the U.S. Centers for Disease Control and Prevention (CDC).

Prevalence of Flu
According to the current WHO Global Influenza Update [see ProMED-mail Influenza (14): WHO global update 20130202.1526486, dated 31 Jan 2013, “Influenza activity in India remained at inter-seasonal levels, with low detections of mainly influenza A(H1N1) pdm09 and some influenza B “(Mohan, 2010). Every year, 10 percent to 20 percent of Americans get sick with the flu. In the United States, approximately 25 percent of the population has flu-associated illness annually, leading to an average of 20,000 to 40,000 deaths per year. The CDC estimates that 35 to 50 million Americans come down with the flu during each flu season, which typically lasts from November to March (Mukherjee et al., 2010).

Pandemic Flu
Pandemic years are associated with many more cases of influenza and a higher case fatality rate than that seen in seasonal flu outbreaks. It is common to encounter clinical attack rate ranges for seasonal flu of 5% to 15% in the literature. For pandemic flu, clinical attack rates are reported in the range of 25% to 50% (Yu et al., 2011). During a typical year in the United States,
30,000 to 50,000 persons die as a result of influenza viral infection. Frequently cited numbers are 20,000 deaths each year, and 37,000 annual deaths. About 5-10% of hospitalizations for influenza lead to fatal outcome in adults.

In normal years, although most influenza infection is in children, the serious morbidity and mortality is almost entirely among elderly people with underlying chronic disease. During influenza epidemics from 1979-80 through 2000-01, the estimated overall number of influenza-associated hospitalizations in the United States ranged from approximately 54,000 to 430,000/epidemic. An average of approximately 226,000 influenza-related excess hospitalizations occurred per year, with 63% of all hospitalizations occurring among persons aged > 65 years (Glezen 1982).

Influenza-related deaths can result from pneumonia and from exacerbations of cardiopulmonary conditions and other chronic diseases. Deaths of older adults account for > 90% of deaths attributed to pneumonia and influenza. In one study of influenza epidemics, approximately 19,000 influenza-associated pulmonary and circulatory deaths per influenza season occurred during 1976-1990, compared with approximately 36,000 deaths during 1990-1999. Estimated rates of influenza-associated pulmonary and circulatory deaths/100,000 persons were 0.4-0.6 among persons aged 0-49 years, 7.5 among persons aged 50-64 years, and 98.3 among persons aged > 65 years (Yu et al., 2011).

The number of hospitalizations and deaths will depend on the virulence of the pandemic virus. Estimates differ about 10-fold between more and less severe scenarios. Published estimates based on extrapolation of the 1957 and 1968 pandemics suggest that there could be 839,000 to 9,625,000 hospitalizations, 18-42 million outpatient visits, and 20-47 million additional illnesses, depending on the attack rate of infection during the pandemic. Estimates based on extrapolation from the more severe 1918 pandemic suggest that substantially more hospitalizations and deaths could occur. The demand for inpatient and intensive-care unit beds and assisted ventilation services could increase by more than 25% under the less severe scenario.

Because the virulence of the influenza virus that causes the next pandemic cannot be predicted, two scenarios were presented by CDC, HHS and DHS based on extrapolation of past pandemic experience. The DHS estimates are suspect, since they appear to derive from a 1999 analysis that was based on the 1997 US population of 265 million. By 2005 the US population was about 295 million, so the DHS estimates are about 10% low simply due to the growth in population.

According to the Centers for Disease Control and Prevention (CDC), it has been estimated that in the absence of any control measures such as vaccination and drugs, a "medium-level" influenza pandemic in the United States could kill 89,000 to 207,000 people, affect from 15 to 35 percent of the U.S. population, and generate associated costs ranging from $71 billion to $167 billion. Another CDC estimate suggested that, in the United States alone, up to 200 million people will be infected, 50 million people will require outpatient care, and two million people will be hospitalized, and between 100,000 and 500,000 persons will die. These numbers are significantly higher than the estimates used by the Department of Homeland Security. The HHS notes that the death rate associated with the 1918 influenza applied to the current population would produce 1.9 million deaths in the United States and 180 million to 360 million deaths globally. It is most noteworthy that the "Low" scenario presented by HHS corresponds to the "High" scenario presented by DHS.

Etiology of Flu

Influenza is an acute infection caused by any of 3 types of influenza viruses (A, B, C). Types A and B cause epidemic disease, and type C causes sporadic disease. Type A is the most common. Influenza is highly contagious. The virus is spread when an individual inhales contaminated airborne droplets (following coughing or sneezing by an infected person) or comes in direct contact with an infected person's secretions (kissing, sharing of handkerchiefs and other items, sharing of objects such as spoons and forks). Viruses may also be transmitted via touching of smooth surfaces, such as doorknobs, handles, and telephones (Mukherjee et al., 2010).

Certain populations are at higher risk for influenza. Risk factors include the following:

- Children younger than 5, but especially children younger than 2 years old.
- Adults 65 years of age and older.
- Pregnant women.
- People who have medical conditions including:
  - Asthma; Neurological and neurodevelopmental conditions (including disorders of the brain, spinal cord, peripheral nerve, and muscle such as cerebral palsy, epilepsy (seizure disorders), stroke, intellectual disability (mental retardation), moderate to severe developmental delay, muscular dystrophy, or spinal cord injury); Chronic lung disease (such as chronic obstructive pulmonary disease [COPD] and cystic fibrosis).
  - Heart disease (such as congenital heart disease, congestive heart failure and coronary artery disease);
  - Blood disorders (such as sickle cell disease); Endocrine disorders (such as diabetes mellitus); Kidney disorders; Liver disorders; Metabolic disorders (such as inherited metabolic disorders and mitochondrial disorders); Weakened immune system due to disease or medication (such as people with HIV or AIDS, or cancer, or those on chronic steroids); People younger than 19 years of age who are receiving long-term aspirin therapy; People who are morbidly obese (Yu et al., 2011).

Symptoms and Diagnosis of Flu

Flu symptoms include abdominal pain, arm pain, back pain, breathing difficulty, chest pain, congestion,
The diagnosis and evaluation of influenza A or B can involve the following:

- Viral culture of nasopharyngeal and/or throat samples
- Hemagglutination inhibition techniques
- Immunofluorescence assay
- Enzyme-linked immunosorbent assay (ELISA)
- Rapid influenza diagnostic tests (RDTs)

Prevention and Treatment of Flu

Prevention from flu is achieved by flu vaccination or antiviral agents.

Vaccination: The single best way to prevent the flu is to get a flu vaccine each season (LaMontagne et al., 1983; Quinnan et al., 1983; Barker and Mullooly, 1980; Patriarca et al., 1985; Nichol et al., 1994; Nichol et al., 2003). There are two types of flu vaccines:

- "Flu shots" inactivated vaccines (containing killed virus) that are given with a needle. There are three flu shots being produced for the United States market now. The regular seasonal flu shot is "intramuscular" which means it is injected into muscle (usually in the upper arm). It has been used for decades and is approved for use in people 6 months of age and older, including healthy people, people with chronic medical conditions and pregnant women. Regular flu shots make up the bulk of the vaccine supply produced for the United States.
- A hi-dose vaccine for people 65 and older which also is intramuscular.
- An intradermal vaccine for people 18 to 64 years of age which is injected with a needle into the "dermis" or skin. (Fiore AE guidelines et al., 2010)

The following groups should not receive the flu shot:

- People who have ever had a severe allergic reaction to eggs. People who have had a mild reaction to egg—that is, one which only involved hives—may receive Trivalent Inactivated Vaccine (TIV) (not LAIV) with additional precautions (Trenor et al., 1996; Lakey et al., 1996). Make sure your healthcare provider knows about any allergic reactions.
- People who have had a severe reaction to the vaccine in the past.
- People with asthma and children younger than 5 years with one or more episodes of wheezing within the past year.
- Pregnant women (Siston et al., 2010).
- Anyone with certain muscle or nerve disorders (such as seizure disorders or cerebral palsy) that can lead to breathing or swallowing problems.
- Anyone with a weakened immune system.
- Anyone in close contact with someone whose immune system is so weak they require care in a protected environment (such as a bone marrow transplant unit). Close contacts of other people with a weakened immune system (such as those with HIV) may receive LAIV. Healthcare personnel in neonatal intensive care units or oncology clinics may receive LAIV.
- Children or adolescents on long-term aspirin treatment.
- People with a history of Guillain–Barré Syndrome (a severe paralytic illness, also called GBS) that occurred after receiving influenza vaccine and who are not at risk for severe illness from influenza should generally not receive vaccine. Tell your doctor if you ever had Guillain–Barré Syndrome. Your doctor will help you decide whether the vaccine is recommended for you.
- People under 65 years of age should not receive the high-dose flu shot.
- People who are under 18 years old or over 64 years old should not receive the intradermal flu shot.
- If you are sick with a fever when you go to get your flu shot, you should talk to your doctor or nurse about getting your shot at a later date. However, you can get a flu shot at the same time you have a respiratory illness without fever or if you have another mild illness.

The nasal-spray flu vaccine: A vaccine made with live, weakened flu viruses that are given as a nasal spray (sometimes called LAIV for “Live Attenuated Influenza Vaccine”). The viruses in the nasal spray vaccine do not cause the flu. LAIV is approved for use in healthy people 2 to 49 years of age who are not pregnant. About two weeks after vaccination, antibodies develop that protect against influenza virus infection. Flu vaccines will not protect against flu-like illnesses caused by non-influenza viruses.

The following groups should not receive the nasal spray vaccine (LAIV):

- Adults 50 years of age and older or children from 6 through 23 months of age. (Children younger than 6 months should not get either influenza vaccine.)
- People who have ever had a severe allergic reaction to eggs. People who have had a mild reaction to egg—that is, one which only involved hives—may receive Trivalent Inactivated Vaccine (TIV) (not LAIV) with additional precautions (Trenor et al., 1996; Lakey et al., 1996). Make sure your healthcare provider knows about any allergic reactions.
- People who have had a severe reaction to the vaccine in the past.
- People with asthma and children younger than 5 years with one or more episodes of wheezing within the past year.
- Pregnant women (Siston et al., 2010).
- Anyone with certain muscle or nerve disorders (such as seizure disorders or cerebral palsy) that can lead to breathing or swallowing problems.
- Anyone with a weakened immune system.
- Anyone in close contact with someone whose immune system is so weak they require care in a protected environment (such as a bone marrow transplant unit). Close contacts of other people with a weakened immune system (such as those with HIV) may receive LAIV. Healthcare personnel in neonatal intensive care units or oncology clinics may receive LAIV.
- Children or adolescents on long-term aspirin treatment.
- People with a history of Guillain–Barré Syndrome (a severe paralytic illness, also called GBS) that occurred after receiving influenza vaccine and who are not at risk for severe illness from influenza should generally not receive vaccine. Tell your doctor if you ever had Guillain–Barré Syndrome (Abramson et al., 2012; LaMontagne et al., 1983; Quinnan et al., 1983).
TABLE 1
Influenza vaccine information, by age group

<table>
<thead>
<tr>
<th>Vaccine</th>
<th>Trade name</th>
<th>Age group</th>
<th>No. of doses</th>
<th>Route*</th>
</tr>
</thead>
<tbody>
<tr>
<td>TIV</td>
<td>Fluzone</td>
<td>6 – 35 months</td>
<td>1 or 2</td>
<td>IM</td>
</tr>
<tr>
<td>TIV</td>
<td>Agriflu</td>
<td>≥ 18 years</td>
<td>1</td>
<td>IM</td>
</tr>
<tr>
<td>TIV</td>
<td>Fluvirin</td>
<td>≥ 4 years</td>
<td>1 or 2</td>
<td>IM</td>
</tr>
<tr>
<td>TIV</td>
<td>Fluarix</td>
<td>≥ 3 years</td>
<td>1 or 2</td>
<td>IM</td>
</tr>
<tr>
<td>TIV</td>
<td>FluLaval</td>
<td>≥ 18 years</td>
<td>1</td>
<td>IM</td>
</tr>
<tr>
<td>TIV</td>
<td>Afluria</td>
<td>≥ 9 years</td>
<td>1</td>
<td>IM</td>
</tr>
<tr>
<td>TIV high dose</td>
<td>Fluzone</td>
<td>≥ 65 years</td>
<td>1</td>
<td>IM</td>
</tr>
<tr>
<td>TIV intradermal</td>
<td>Fluzone</td>
<td>18-64 years</td>
<td>1</td>
<td>ID</td>
</tr>
<tr>
<td>LAIV</td>
<td>FluMist</td>
<td>2 – 49 years</td>
<td>1 or 2</td>
<td>IN</td>
</tr>
</tbody>
</table>

*ID intra dermal, IN intra nasal (Treanor, 2011).

Anti-viral drugs

Antiviral medications are prescription pills, liquids, or inhalers used to prevent or treat flu viruses. They are approved for adults and children one year and older. (Hsu J, et al., 2012)

(i) **Anti-influenza virus drugs**: Amantadine, rimantadine, oseltamivir (Tamiflu), and zanamivir (Relenza)

(ii) **Non selective anti-viral drugs**: Ribavirin (Jefferson et al., 2012; Tripathi, 2010).

Oseltamivir (Tamiflu): This is recently developed anti-influenza virus prodrugs with a broader spectrum activity covering influenza A (amantadine sensitive as well as resistant), influenza B and avian influenza (bird flu) H5N1 and other strains. It acts by inhibiting influenza virus neuraminidase enzyme which is needed for release of progeny virion from the infected cell (Louie, 2012). It is an ester prodrug that is rapidly and nearly completely hydrolyzed during absorption in intestine and by liver to the active form oseltamavir carboxylate. The active metabolite is not further metabolized and is excreted by the kidney with a t½ of 6-10 hours. It is used both for prophylaxis as well as treatment of influenza A,B and bird flu (Tripathi, 2010; Viasus, 2011).

TABLE 2
Antiviral Medications Recommended for Treatment and Chemoprophylaxis of Influenza.

<table>
<thead>
<tr>
<th>Antiviral Agent</th>
<th>Activity Against</th>
<th>Use</th>
<th>FDA Approved For</th>
<th>Not Recommended for Use in</th>
<th>Adverse Events</th>
</tr>
</thead>
<tbody>
<tr>
<td>Oseltamivir (Tamiflu®)</td>
<td>Influenza A and B</td>
<td>Treatment</td>
<td>2 wks and older</td>
<td>N/A</td>
<td>Adverse events: nausea, vomiting. Sporadic, transient neuropsychiatric events (self injury or delirium) mainly reported among Japanese adolescents and adults.</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Chemo- prophylaxis</td>
<td>1 yr and older</td>
<td>N/A</td>
<td></td>
</tr>
<tr>
<td>Zanamivir (Relenza®)</td>
<td>Influenza A and B</td>
<td>Treatment</td>
<td>7 yr and older</td>
<td>people with underlying respiratory disease (e.g., asthma, COPD)</td>
<td>Allergic reactions: oropharyngeal or facial edema. Adverse events: diarrhea, nausea, sinusitis, nasal signs and symptoms, bronchitis, cough, headache, dizziness, and ear, nose and throat infections.</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Chemo- prophylaxis</td>
<td>5 yr and older</td>
<td>people with underlying respiratory disease (e.g., asthma, COPD)</td>
<td></td>
</tr>
</tbody>
</table>

(Tripathi 2010; Yang et al., 2012)

TABLE 3
Recommended Dosage and Duration of Treatment or Chemoprophylaxis for Influenza Anti-viral Medications.

<table>
<thead>
<tr>
<th>Antiviral Agent</th>
<th>Use</th>
<th>Children</th>
<th>Adults</th>
</tr>
</thead>
<tbody>
<tr>
<td>Oseltamivir (Tamiflu®)</td>
<td>Treatment</td>
<td>If younger than 1 yr old, the dose is 3 mg/kg/dose twice daily</td>
<td>75 mg twice daily</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(Dose varies by child’s weight) If 1 yr or older and weigh 15 kg or less, the dose is 30 mg twice a day.</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>If 1 yr or older and weigh more than 15 to 23 kg, the dose is 45 mg twice a day.</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>If 1 yr or older and weigh more than 23 to 40 kg, the dose is 60 mg twice a day.</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>If 1 yr or older and weigh more than 40 kg, the dose is 75 mg twice a day.</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Chemo- prophylaxis</td>
<td>(Not FDA approved for use in children younger than 1 yr old) If child is younger than 3 months old, chemoprophylactic use is not recommended unless situation is judged critical due to limited data on use in this age group.</td>
<td>75 mg once daily</td>
</tr>
</tbody>
</table>

Table 3 contd...
Chemoprophylaxis of Flu

Annual influenza vaccination is the best way to prevent influenza because vaccination can be given well before influenza virus exposures occur, and can provide safe and effective immunity throughout the influenza season. Antiviral medications are approximately 70% to 90% effective in preventing influenza and are useful adjuncts to influenza vaccination (Hsu et al., 2012).

**TABLE 4**
Duration of treatment of chemoprophylaxis.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Recommended duration</th>
<th>Recommended duration of antiviral treatment is 5 days. Longer treatment courses for patients who remain severely ill after 5 days of treatment can be considered.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chemoprophylaxis</td>
<td></td>
<td>For control of outbreaks in long-term care facilities (e.g. elderly nursing homes) and hospitals, CDC recommends antiviral chemoprophylaxis for a minimum of 2 weeks, and continuing up to 1 week after the last known case was identified. Antiviral chemoprophylaxis should be considered, especially for elderly long-term care facilities, for all exposed residents, including those who have received influenza vaccination.</td>
</tr>
</tbody>
</table>

Flublok

Flublok is the first trivalent influenza vaccine made using an insect virus (baculovirus) expression system and recombinant DNA technology (Trenar et al., 2011; Oers, van 2006; Treanoret et al., 2006). Flublok is designed to protect against the H1N1, H3N2, both A strains and one B strain of the influenza virus, and is approved for people between ages 18 and 49. Unlike current flu vaccines, Flublok does not use the influenza virus or eggs in its production. Flublok’s novel manufacturing technology allows for production of large quantities of the influenza virus protein, hemagglutinin (HA) – the active ingredient in all inactivated influenza vaccines that is essential for entry of the virus into cells in the body (Frey et al., 2012). The majority of antibodies that prevent influenza virus infection are directed against HA. The closer the match between the circulating strains causing disease and the strains in the vaccine, the better the protection against influenza.

Flublok is a novel protein vaccine for the prevention of seasonal influenza disease and is the first to be made in a 100% egg-free system without growing influenza viruses – so the vaccine can be made quickly and without any of the infectious risk traditionally associated with vaccine manufacture. The Flublok technology avoids those time-consuming steps in a process that will be faster, easier to control and easier to scale up in an emergency. Flublok is highly purified, has three times the amount of active ingredient in
traditional influenza vaccines, and contains no preservatives (thimerosal), antibiotics or adjuvants (Treanor et al., 2011; Cox and Anderson, 2007, Holtz et al., 2003). It has a shelf life of 16 weeks from the date of manufacture.

Flublok is tailored annually to provide protection against the latest strains of influenza by containing the corresponding hemagglutinin antigens for those strains. Flublok is made using recombinant technology (i.e., without the use of actual influenza virus), so there is no risk of getting the flu from the vaccine.

Flublok contains three, full-length, recombinant HA proteins to help protect against two influenza virus A strains, H1N1 and H3N2, and one influenza virus B strain.

According to Protein Sciences, the vaccine’s manufacturer, Flublok production involves programming insect cell grown in steel tanks to produce large amounts of a particular flu virus protein, known as hemagglutinin. HA is a protein expressed on the surface of influenza viruses that is strain-specific (for example, the H1 in H1N1). Flublok advantages include higher antigen content, potentially superior immunogenicity for the elderly and immune-compromised, manufacturing platform requires less lead time, allowing regulators more time to make accurate flu response decisions, no influenza viruses used in production, no antibiotics, adjuvants or preservatives (e.g., thimerosal), no egg-related by products (Holtz et al., 2003; Cox and Anderson, 2007).

**Indications and Dosage**

Flublok is a sterile, clear, colorless solution of recombinant hemagglutinin (HA) proteins from three influenza viruses for intramuscular injection. It contains purified HA proteins produced in a continuous insect cell line that is derived from Sf9 cells of the fall armyworm, Spodoptera frugiperda, and grown in serum-free medium composed of chemically-defined lipids, vitamins, amino acids, and mineral salts. Each of the three HAs is expressed in this cell line using a baculovirus vector Autographa californica nuclear polyhedrosis virus), extracted from the cells with Triton X-100 and further purified by column chromatography. The purified HAs are then blended and filled into single-dose vials. The stoppers used for the single-dose vials do not contain latex.

Flublok is a vaccine indicated for active immunization against disease caused by influenza virus subtypes A and type B contained in the vaccine. It is for intramuscular injection only. Administer Flublok as a single 0.5-mL dose. Shake the single-dose vial gently before withdrawing the vaccine dose. Parenteral drug products should be inspected visually for particulate matter and discoloration prior to administration whenever solution and container permits. If either of these conditions exists, the vaccine should not be administered. The preferred site for injection is the deltoid muscle. Administration is by sterile needle and syringe. Flublok should not be mixed with any other vaccine in the same syringe or vial. Flublok is contraindicated in individuals with known severe allergic reactions (e.g., anaphylaxis), to any component of the vaccine. The most common (≥10%) injection-site reaction was pain (>37%); the most common (≥10%) solicited systemic adverse reactions were headache (>15%), fatigue (>15%) and myalgia (>11%).

**Pharmacology**

Flublok contains recombinant HA proteins of the three strains of influenza virus specified by health authorities for inclusion in the annual seasonal vaccine. These proteins function as antigens which induce a humoral immune response, measured by hemagglutinin inhibition antibody (HAI). Antibodies against one influenza virus type or subtype confer limited or no protection against another.

Furthermore, antibodies to one antigenic variant of influenza virus might not protect against a new antigenic variant of the same type or subtype. Frequent development of antigenic variants through antigenic drift is the virologic basis for seasonal epidemics and the reason for the usual replacement of one or more influenza virus strains in each year’s influenza vaccine. Therefore, influenza vaccines are standardized to contain the hemagglutinins of influenza virus strains (i.e., typically two type A and one type B), representing the influenza viruses likely to be circulating in the U.S. in the upcoming winter (Treanor et al., 2006; Cox and Anderson, 2007; Treanor et al., 2007). Flublok has not been evaluated for carcinogenic or mutagenic potential, or for impairment of male fertility in animals. Reproduction studies performed in female rats revealed no evidence of impaired fertility due to Flublok. Clinical trials with Flublok suggest that it is more effective at generating protective antibody titers against influenza in elderly and immune compromised individuals compared to a licensed vaccine. (Keitel et al., 1994 and Keitel et al., 1996). The safety of FluBlok was tested in a study of 2,300 people. It was found to be 44.6 percent effective against all strains of the flu (Treanor et al., 2011).

**Conclusions**

Evidence exists to support the transmission of influenza viruses by direct and indirect contact and by droplet and droplet nuclei (i.e., airborne) transmission. The use of current droplet and contact precautions assumes placement of patients in rooms with standard air exchange rates. Whether the use of negative-pressure rooms would result in a measurable decrease in the rate of transmission, compared with the use of droplet precautions in a private positive-pressure room with appropriate air exchange and ventilation, is unknown and may only be determined through carefully planned studies. Immunity in the general population to such a virus would be poor, and the viral inoculum necessary for infection may be low. Improved rates of vaccination of all persons at increased risk of influenza-
related complications, their household contacts, and health care personnel, would substantially limit the introduction of influenza into health care facilities and impede person-to-person transmission when influenza is introduced. High priority should be given to improvements in influenza-vaccine coverage and improvements in the diagnosis and treatment of influenza to reduce childhood mortality from influenza.

References


Kimberlin DW et al., Oseltamivir pharmacokinetics, dosing, and resistance in children from birth to two years of age with influenza. Journal of Infectious Diseases 2012;published ahead of print.


Kimberlin DW et al., Oseltamivir pharmacokinetics, dosing, and resistance in children from birth to two years of age with influenza. Journal of Infectious Diseases 2012;published ahead of print.


Rocio Coloma, José M. Valpuesta, Rocío Arranz, José L. Carrascosa, Juan Ortín mail, Jaime Martín-Benito (2009). The Structure of a Biologically Active Influenza Virus Ribonucleoprotein Complex. *PLOS* **10**:1371


