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SYMPOSIUM

Pandemic Influenza: Overview of Vaccines and Antiviral Drugs

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Pandemic influenza has become a high priority item for all public health authorities. An influenza pandemic is believed to be imminent, and scientists agree that it will be a matter of when, where, and what will be the causative agent. Recently, most attention has been directed to human cases of avian influenza caused by a H5N1 avian influenza virus. An effective vaccine will be needed to substantially reduce the impact of an influenza pandemic. Current influenza vaccine manufacturing technology is not adequate to support vaccine production in the event of an avian influenza outbreak, and it has now become clear that new innovative production technology is required. Antiviral drugs, on the other hand, can play a very important role in slowing the disease spread but are in short supply and resistance has been a major issue. Here, we provide an update on the status of pandemic vaccine development and antiviral drugs. Finally, we conclude with some proposed areas of focus in pandemic vaccine preparedness.

INTRODUCTION

On November 25, 2005, the World Health Organization updated the cumulative number of confirmed human cases of avian influenza caused by A/H5N1 [1]. The result: a case total of 132 with a case fatality of 68, up from 117 with a case fatality of 60 from just four weeks earlier. Avian influenza in poultry is widespread in Asian countries, including Cambodia, Indonesia, Thailand, Vietnam, and China, where human cases have been reported as well.

Avian influenza has been a problem in the poultry industry for many years. Examples include the North American highly pathogenic outbreak in Pennsylvania in 1983 [2] and in Central Mexico during 1994 to 1995 [3]. Human cases of avian influenza have only been reported since 1997. Table 1 summarizes the occurrence of human cases and the disease outcome associated with concurrent poultry outbreaks in various countries. The fact that human cases were first identified in Hong Kong and, subsequently, the United States, the Netherlands, and Canada suggests that the availability of improved diagnostic methods in these countries enabled the identification of these avian influenza viruses in humans.

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†Abbreviations: CDC-ILI, influenza-like illness; DHHS, Department of Health and Human Services; HA, hemagglutinin; NIAID, National Institute of Allergy and Infectious Diseases; NA, neuraminidase; rHA, recombinant HA; RNA, ribonucleic acid.

Table 1. Human impact of avian influenza outbreaks in poultry since 1997.

Year	Strain	Impact	Country
1997	H5N1	18 (6)	Hong Kong
1999	H9N2	2	Hong Kong
2002	H7N2	1	US Virginia
2003	H5N1 H7N7	3 (2) 89 (1)	Hong Kong Netherlands
2004-2005	H5N1 H7N3	132 (68) 2	Asia Canada

In other words, human infection with avian influenza viruses may have previously gone undiagnosed and may have been more commonly associated with outbreaks in poultry. The other important finding presented in Table 1 is that, besides H5N1, a wide variety of avian influenza viruses, including the H7 and H9 subtypes, are capable of infecting and causing disease in humans [4].

When Hong Kong in 1997 suffered from a severe H5N1 outbreak in poultry, the authorities undertook the following actions: 1.5 million chickens were culled, ducks and geese were removed, two clean days per month were introduced in the live bird markets, and, finally, poultry flocks were vaccinated with an inactivated H5 vaccine [5]. Unfortunately, the above measures are not followed throughout Asia because they are too expensive. Bird culling is the most common and widespread approach to eradicate avian influenza in developed countries. Despite the availability of poultry vaccines, countries often elect not to vaccinate their birds because of a potential negative impact on the ability to export the birds.

INFLUENZA VIRUSES

Influenza viruses are single-stranded ribonucleic acid (RNA)† viruses with a segmented genome encoding 10 proteins. The viruses are surrounded by a lipid containing envelope through which two major glycoproteins spike: hemagglutinin (HA) and neuraminidase (NA). Both proteins have been recognized as key antigens in the host response to influenza virus in both natural infection and vaccination. Antibodies against HA have the ability to neutralize the virus, and, for this reason, the HA is generally considered to be the active ingredient in an influenza vaccine. The HA protein consists of two subunits: HA1 and HA2. The HA1 domain contains all the structural epitopes and is connected with the HA2 domain by several amino acids. Highly pathogenic viruses contain a connector that consists of negatively charged amino acids [6, 7] causing the HA0 to break apart into HA1 and HA2, resulting in activation of HA without the presence of a protease. The exact mechanism by which this activation occurs is presently unknown. By using a technique referred to as "reverse genetics," scientists are now able to replace this stretch of basic amino acids with other amino acids and thus convert the highly pathogenic virus into a mild or non-pathogenic H5N1 virus [8].

INFLUENZA VACCINES

Currently, there are three inactivated viral vaccines and one live attenuated viral vaccine approved for use to prevent influenza in the United States. The manufacturing of all these vaccines involves the adaptation of the selected variants for high yield in eggs by serial passage or reassortment with other high-yield strains. Selected influenza viruses are grown in embryonated chicken eggs, and the influenza virions are purified from allantoic fluid. For the inactivated virus vaccines, the influenza virus preparations are then killed by treatment with an inactivating agent, such as formaldehyde [9]. Split virion vaccines such as FluZone (Sanofi Pasteur) are produced by splitting the virus particles by use of detergents or solvents. The subunit vaccines, such as Fluvirin (Chiron) are further purified to remove the internal proteins, leaving only hemagglutinin and neuraminidase. It is obvious that chickens will be affected first in an avian influenza outbreak, and, therefore, the availability of embryonated eggs to support vaccine manufacturing is highly unlikely. Prior to the development of "reverse genetics," it was impossible to grow highly pathogenic avian viruses in chicken eggs because the virus killed the embryos.

CELL-BASED INFLUENZA VACCINE PRODUCTION

Alternative methods for the production of influenza vaccines are needed. Influenza vaccines have historically been cheap, and, as a result, vaccine manufacturers were not motivated to invest in developing new or innovative technology or products to replace the out-dated egg-based manufacturing process.

The influenza vaccine composition is adjusted annually based on influenza surveillance data generated by WHO/CDC. Therefore, the new manufacturing technology has to be able to respond at least as quickly to changes in the influenza vaccine composition as the current egg-based technology.

Most pharmaceutical vaccine development efforts are aimed at producing live influenza viruses in cell culture. Solvay [10], Chiron and ID Biomedical (former Shire or Biochem Pharma) [11] are all in various stages of obtaining licensure for the production of influenza viruses using MDCK (Madin Darby Canine Kidney) cells. Baxter has elected the VERO (African Green Monkey Kidney) cell line for the production of their influenza vaccine, and Sanofi Aventis is working on various different cell-based approaches including a stem cell line in collaboration with Vivalis, a collaboration with Nautilus, and a human retina cell line (Per.C6) in collaboration with Crucell [12].

Most manufacturers plan to use the current (egg-based) virus inactivation and purification process for the downstream processing of their cell-based vaccine. Baxter, however, plans to proceed with licensure of a "whole-inactivated" viral vaccine (partly purified), most likely out of economic considerations.

The yields per liter of influenza virus are highest in the MDCK cell line and lowest in the VERO cell line. The VERO cell line offers the advantage that there is already one example of a licensed vaccine on the market that uses this cell line. The main hurdle for the MDCK cell line is that it is considered to be tumorigenic [13] and that regulatory authorities are concerned with the potential risk of carry-over. It has been speculated that regulatory authorities will require the generation of large safety databases in human subjects (exceeding 50,000 subjects) to address potential safety concerns [unpublished data].

A general limitation of using cell culture to produce human influenza viruses is that the process still requires the production of a high-yielding re-assortant virus, this time not egg-adapted but a mammalian cell line adjusted re-assortant. This process may introduce cell line-specific mutations in the genes that can lead to the selection of variants characterized by antigenic and structural changes in the hemagglutinin protein [14-16], potentially resulting in less-efficacious vaccines. In order to

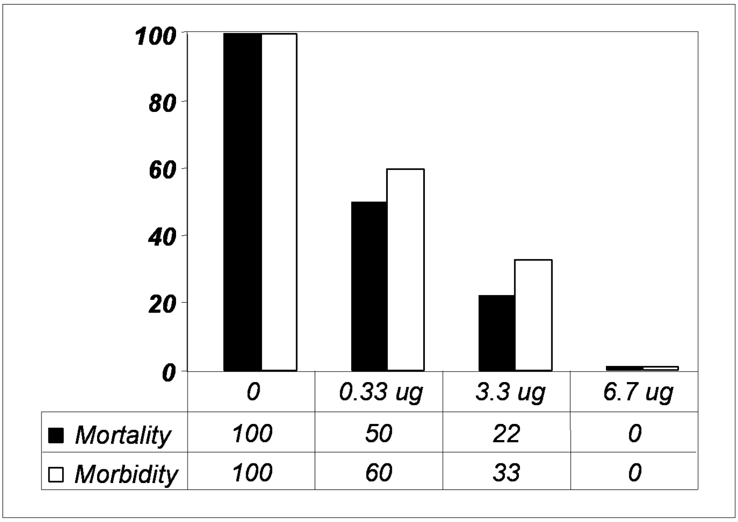

Figure 1. Chicken challenge study. Birds were vaccinated with increasing concentrations of non-adjuvanted recombinant HA derived from A/Hong Kong/156/95. A vaccine dose of 6.7 μg prevented the birds from illness, shedding the virus, and death following a lethal challenging with a highly pathogenic avian influenza virus.

increase the yields obtained in VERO cells, methods that are used include reverse genetics techniques. While this technique enables one to introduce specific changes to the virus, it is yet unclear what the impact of such changes on vaccine efficacy will be.

The cell-based influenza vaccine closest to market introduction is the product from Solvay, which already has obtained regulatory approval in the Netherlands. Solvay is in the process of completing a large-scale manufacturing facility and plans product introduction in 2006.

RECOMBINANT HA PROTEIN VACCINE FOR INFLUENZA

Resistance to influenza infection correlates with serum anti-HA antibody levels [17, 18] and resistance to disease can be correlated with local neutralizing antibody and secretary IgA antibody to HA as well as serum anti-HA antibody [19].

Progress in recombinant DNA technology has allowed for the rapid cloning of influenza virus HA genes, expression of correctly folded and biologically active hemagglutinin in a eukaryotic system, and high levels of production of recombinant HA (rHA).

rHA had been tested in several Phase I/II human clinical trials conducted by the National Institute of Allergy and Infectious Diseases (NIAID) and academic institutions involving over 600 subjects that demonstrated safety, immunogenicity, and efficacy as reported in four published studies [20-23], when Hong Kong bird flu first emerged in 1997. Recently, Protein Sciences Corporation also completed a field efficacy study with a trivalent recombinant HA vaccine. The trivalent recombinant hemagglutinin vaccine was safe, immunogenic, and effective in the prevention of influenza disease, with the higher dose showing a 100 percent protective efficacy against cell culture confirmed influenza in subjects presenting with influenza-like illness (CDC-ILI). In addition, the number of subjects presenting with CDC-ILI was reduced by 54.4 percent compared to placebo in this dose group.

Table 2. Antiviral Drugs currently in use or approved in the US.

Generic name	Trade name	Year of approval	Mechanism of action
Amantidine	Symmetrel	1966	Interferes with function of M2
Rimantidine	Flumadine	1993	Interferes with function of M2
Oseltamivir Active against A & B	Tamiflu	1999	Neuraminidase inhibitor
Zanamivir* Active against A & B	Relenza	1999	Neuraminidase inhibitor

In collaboration with NIAID, Protein Sciences produced a vaccine candidate within eight weeks in response to the threat posed by the Hong Kong bird flu. The vaccine candidate proved to be efficacious in chickens in a challenge study (100 percent prevention of illness, shedding of the virus, and death) conducted by the United States Department of Agriculture in a high-containment facility in Georgia. The results are shown in Figure 1. It subsequently was administered to over 200 healthcare workers and researchers and produced antibody responses that were believed to be protective in 50 percent of the recipients who received two doses of the vaccine [24]. In August 2005 (more than six years later), Anthony Fauci reported similar results with a "reverse genetic" vaccine candidate produced in embryonated chicken embryos by Sanofi Pasteur [25].

ANTIVIRAL DRUGS

Four antiviral drugs, listed in Table 2, are approved in the United States for use in influenza disease. Their use is recommended when vaccines are contra-indicated and/or in high-risk populations. The disadvantage of these antiviral drugs is that they must be used within 24 to 48 hours after onset of the disease, and the use, in general, is cautioned because of the potential side effects.

ANTIVIRAL DRUG RESISTANCE

There are reports of broad resistance of current circulating H5N1 against amantidine [26]. Similar reports also suggest that this resistance is observed against rimantidine [27]. The widespread use of antiviral drugs such as amantidine in chickens reported in the press [28] may in part be responsible for this resistance. Resistance has also been reported for the newer drugs oseltamivir and zanamivir [29]. The product label information for zanamivir states that a single point mutation in the NA gene can render the drug 1,000-fold less efficacious. Also, a case study report by Le et al. [30] suggests that drug resistance against oseltamivir can evolve within a two-week treatment period.

UNITED STATES GOVERNMENT ACTIONS

The United States government is, like many governments, stockpiling antiviral drugs. To date, 4.3 million doses of oseltamivir and 5 million doses of rimantidine have been stockpiled [27] . However, the U.S. has at least 10 million first responders, and the rimantidine is not effective against the currently circulating H5N1 virus.

In addition, the Department of Human Health Services (DHHS) has awarded over $300 million to Sanofi-Pasteur to secure year-round egg supply, to stockpile H5N1 vaccine produced in embryonated chicken eggs, and to develop an influenza vaccine produced in PER.C6 cell culture. These awards are curious, however, because, since there will likely be no chickens to secure the egg supply in the true event of an avian influenza outbreak, the efficacy of the egg-grown H5N1 vaccine is unknown and the expected shelf life of this vaccine is less than one year. Further, it is unclear whether the next pandemic will be caused by an H5 virus, and, finally, the cell-based vaccine using the PER.C6 cell line is the least advanced of the earlier described alternatives. Very recently, Chiron also received an order to produce H5N1 vaccine in embryonated chicken eggs. DHHS put out a new Request for Proposals in June 2005 to the vaccine industry for alternative manufacturing methods, but to date no rewards have been made. DHHS is not exactly stimulating innovation with their actions to date by ignoring innovative approaches being pursued by smaller companies and awarding contracts to companies that have otherwise no interest in developing alternative production technologies.

CONCLUSIONS

It is probably not possible to prevent the next pandemic. At this moment, our level of preparedness is low. While antiviral drugs may be efficient in slowing the spread of disease when used in the center of an outbreak, it is probably not so useful to stockpile these drugs in countries where an outbreak is unlikely to start. There is clearly insufficient support for and, therefore, progress in the development of an innovative vaccine that can effectively respond in case of an emergency.

Proposed areas of focus in pandemic preparedness should include avoiding disease spread in animals by using vaccines, providing resources to Asia to ensure implementation of control measures, developing vaccines that can be used in a prophylactic manner and continuing to monitor disease spread.

The use of influenza vaccines has long been hindered by the impact vaccine use may have on export of poultry meat. By establishing international criteria for vaccine use, such as inclusion of sentinel birds in vaccinated poultry flocks or by using markers or subunit vaccines, there should be a way to safely and effectively use influenza vaccine in animals.

By providing resources to Asia, we cannot only control disease in the center of an outbreak of which epidemiological studies have shown that this would be the most effective way to control an outbreak [31], but we could also think of providing compensation to people who have infected birds. In this way, we may be able to foster a situation in which avian influenza will not go unnoticed and likely prevent the development of a pandemic virus that can effectively spread from human to human. Finally, we should also make sure that antiviral drugs are not used in ways that may render the drugs ineffective when we really need them by imposing strict guidelines on use for veterinary purposes.

Developing a safe, prophylactic vaccine containing, for example, H5, H2, H7, and/or H9 hemagglutinin proteins that could stimulate a low-level immune response against these viruses to which many people do not have pre-existing antibodies, since the viruses, H5, H7, and H9, are not sufficient to infect humans or like H2 and have not circulated for the past 40 years. Such a vaccine may be the most effective proactive response to the threat of potential pandemic.

When Dr. John La Montagne speculated that our strengthened surveillance systems to monitor disease spread and modern diagnostics tools would allow us to slowly see a disease unfold [32], he was probably right. Currently, the avian influenza viruses have not acquired the ability to transmit easily from human to human, but as we continue to monitor the disease and the genetic composition of the viruses as suggested by the work of Taubenberger et al., [33] we may be able to make useful predictions as to when, where, and what the next pandemic will be.

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