Development of Vaccines against Influenza A Virus (H5N1)

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Three influenza pandemics took place during the 20th century, including the 1918 pandemic, which killed an estimated 50 million people. We are facing the threat of another pandemic, which may be caused by an A/H5N1 influenza virus. These viruses have expanded their territory from Asia to the Middle East, Africa and Europe and have caused more than 190 human deaths up to the present. Vaccines in response to this pandemic threat are currently under development. Reverse-genetics-based inactivated whole-virion vaccines and adjuvanted split-virion vaccines are undergoing clinical trials and are among possible candidates to be approved as H5N1 vaccines for human beings. Problems, including low immunogenicity in the generally naive human population, a lack of data on these vaccines in relation to immunocompromised patients, young children and the elderly and the currently limited global capacity to manufacture influenza vaccines, all need to be resolved. Several innovative approaches, such as the use of novel adjuvants, an antigen-sparing policy and the use of adenoviral-vector-based or DNA vaccines, are being used to develop more efficient vaccines. Every effort should be made to shorten the gap that remains and improve greatly influenza pandemic vaccine access. *(Chang Gung Med J 2007;30:294-304)*

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Influenza pandemics

Influenza virus is an enveloped single strand RNA virus of negative polarity and belongs to the family *Orthomyxoviridae*. There are three types of influenza virus, A, B and C. The genome of influenza virus is composed of eight (influenza A and B) or seven (influenza C) segments. The virus capsid contains two major antigenic proteins, hemagglutinin (HA) and neuraminidase (NA). There are 16 known different HA subtypes (H1-H16) and nine known different NA subtypes (N1-N9), all of which have been found among influenza A viruses.

An influenza pandemic may occur when a new influenza strain carrying a novel HA (sometimes with a novel NA) antigen emerges and spreads in the human community among which the general population are immunogenically naive to this antigen. Three pandemics occurred during the 20th century, in 1918, 1957 and 1968, and all three were associated with substantial numbers of illnesses and deaths. The complete sequence of the 1918 pandemic strain, which resulted in an estimated 50 million deaths, revealed that the virus had evolved directly via mutation from an avian H1N1 virus and adapted to

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humans. The next two pandemics were caused by reassortants of an avian influenza virus and a human influenza virus. The reassortant of H2N2 avian influenza virus (HA, NA and PB1 segments) and H1N1 human virus (the remaining 5 segments) gave rise to the 1957 pandemic and the reassortant of H3 avian (HA and PB1 segments) and H2N2 human virus produced the 1968 pandemic. We are now facing the threatening of the first pandemic of the 21st century, which will probably be caused by one of the A/H5N1 influenza viruses. By 2006, the H5N1 viruses have expanded their geographical distribution across Asia to the Middle East, Africa and Europe and in the process caused large scale poultry and migratory bird death; up to July 11, 2007 there had also been 318 cumulative confirmed human cases reported to World Health Organization (WHO), resulting in 192 deaths (Table 1).[1] Most of these cases could be traced to contact between the human and poultry. If H5N1 evolves to be able to easily cross the species barrier, a pandemic influenza may occur, and this will have catastrophic consequences for world human health and the global economy.

In order to contain pandemic influenza at the source, a simulation model for rural Southeast Asia suggests that a combination of local pre-pandemic vaccination, quarantine and targeted antiviral prophylaxis may be able to contain the pandemic virus most effectively.[2] Immunization against influenza is considered to be an essential public-health intervention to control seasonal influenza epidemics as well as pandemic influenza. Influenza (H5N1) vaccine development and deployment are thus critical elements in pandemic influenza preparedness.

The important issues associated with the development of a H5N1 vaccine include the highly-pathogenic character of the virus and the present immunogenic naivity of the world's human population. These are harsh challenges to a traditional vaccine producer. In this analysis, we describe current vaccine preparation, enumerate possible H5N1 vaccine strategies, discuss the results from published clinical trials and elucidate innovative vaccine technologies.

Current vaccine preparations for seasonal human influenza

Current influenza vaccine types include inactivated virus vaccines (including whole-virion, split and subunit vaccines) and live-attenuated virus vaccines. Inactivated influenza vaccines are produced by seeding a candidate virus in chicken embryonated eggs, purifying the propagated virus from the allantoic fluids of the inoculated eggs and then inactivating the virus with chemicals. For the preparation of a whole-virus vaccine, formaldehyde or β-propiolactone is utilized. Ether or detergent is utilized for split or subunit vaccine formulation. Vaccine reference strains are reassortants and produced by double infection of embryonated hens' eggs using the recommended strain and the laboratory strain PR8, which grows to high titer in eggs. High growth reassortants (HGRs) are provided by WHO reference

Table 1. Cumulative Numbers of Confirmed Human Cases of Avian Influenza A/H5N1 Reported to WHO.[1] (dated the 11, July, 2007)

Country	2003		2004		2005		2006		2007		Total	
	cases	deaths	cases	deaths	cases	deaths	cases	deaths	cases	deaths	cases	deaths
Azerbaijan	0	0	0	0	0	0	8	5	0	0	8	5
Cambodia	0	0	0	0	4	4	2	2	1	1	7	7
China	1	1	0	0	8	5	13	8	3	2	25	16
Djibouti	0	0	0	0	0	0	1	0	0	0	1	0
Egypt	0	0	0	0	0	0	18	10	19	5	37	15
Indonesia	0	0	0	0	20	13	55	45	27	23	102	81
Iraq	0	0	0	0	0	0	3	2	0	0	3	2
Lao	0	0	0	0	0	0	0	0	2	2	2	2
Nigeria	0	0	0	0	0	0	0	0	1	1	1	1
Thailand	0	0	17	12	5	2	3	3	0	0	25	17
Turkey	0	0	0	0	0	0	12	4	0	0	12	4
Viet Nam	3	3	29	20	61	19	0	0	2	0	95	42
Total	4	4	46	32	98	43	115	79	55	34	318	192

laboratories to the vaccine manufacturing industry and these companies use these seed viruses to manufacture the vaccine in bulk.[9] The current seasonal influenza vaccine is a trivalent vaccine and each influenza vaccine contains an A/H3N2, an A/H1N1 and a B virus strain.

Challenges encountered in H5N1 vaccine development

Using the same strategy of vaccine production as is used for annual influenza, several challenges are encountered when developing an effective vaccine against H5N1 viruses. These include the high pathogenicity of H5N1 viruses, the genetic diversity of the circulating H5N1 viruses and the immunogenetic naivety of the human population.

The high pathogenicity of H5N1 viruses not only threatens the workers handling vaccine production, but also reduces the efficiency of viral propagation in eggs. This will result in a low yield of virus from the embryonated eggs. In addition, during a pandemic caused by H5N1 viruses, the supply of eggs might be reduced due to a large-scale slaughter of and high fatalities among infected chicken.

The majority of H5N1 viruses circulating between 2003 and 2006 can be separated into two main phylogenetic clades using their HA sequences (Fig. 1).[4] Clade 1 viruses, circulating in Cambodia, Vietnam and Thailand, were responsible for human infections between 2004 and 2005. Clade 2 viruses circulated in birds in China and Indonesia between 2003 and 2004 and then spread westward into the Middle East, Europe and Africa during 2005 and 2006. The viruses in clade 2 can be further separated into six subclades. Subclades 1, 2 and 3 were responsible for human infections in Indonesia, in Turkey, Iraq, Egypt and Azerbaijan and in China, respectively. Results from the Center for Disease Control and Prevention of the United States indicated that the antibodies generated by clade 1 viruses poorly cross-react with clade 2 viruses.[10] Since we can not predict which strain will evolve into the pandemic strain, it is difficult to choose one strain for the development of a pre-pandemic vaccine and for stockpiling. Therefore, more than one candidate strain needs to be included in any developed vaccine.

Published clinical trials (Table 2) have revealed the fact that only high antigen doses of inactivated whole-virion or adjuvanted split-virion H5 vaccines can induce satisfactory immune protection in healthy adult volunteers. This further decreases the number of vaccine doses that will be available at any one time because of the need to increase the unit dosage. In addition, there is no available data on these vaccines relative to vulnerable populations, including infants, children, the elderly and immunocompromised persons.

Another crucial problem is that there is no validated method to detect heterotypic and heterosubtypic immunity. The hemagglutination-inhibition (HAI) assay, which is used to detect anti-influenza antibodies, is well-known to correlate with immune-protection. However, the HAI assay is thought to be less sensitive when detecting antibodies induced by avian influenza viruses.[11] The micro-neutralization (NT) assay may provide a sensitive and possible alternative.[12,13] However, there is significant inter-laboratory endpoint variability with this assay and there is also a lack of evidence correlating immunity with the assay; these are both obstacles when trying to license a vaccine. To develop an international standard for assessing serological responses to the various H5N1 vaccines is thus crucial for the comparative analysis of such vaccines.

Approaches to the development of H5N1 vaccines

To overcome these obstacles, several strategies have been explored. These can be generally categorized into two groups: the use of a low-pathogenicity H5 avian influenza (LPAI) virus that is antigenically related to circulating strains and the use of viral H5 HA as an antigen.

Following the first H5N1 outbreak among human beings in Hong Kong in 1997, some LAPI viruses were selected for the development of a H5 vaccine. A/duck/Singapore/F119-3/97 (H5N3) was used as a surface antigen vaccine and was tested on healthy volunteers.[12-14] The vaccine was safe and well tolerated but poorly immunogenic. Due to the lack of available LPAI candidate viruses, viral H5 HA has been used for the development of later H5N1 vaccines and several strategies, such as reverse genetics, virus vectors and live attenuated virus, have been tried in order to express the viral H5 HA (Table 3). Since the original development of the reverse genetic technology by Palese and colleagues,[16] plasmid-based systems have replaced the previously

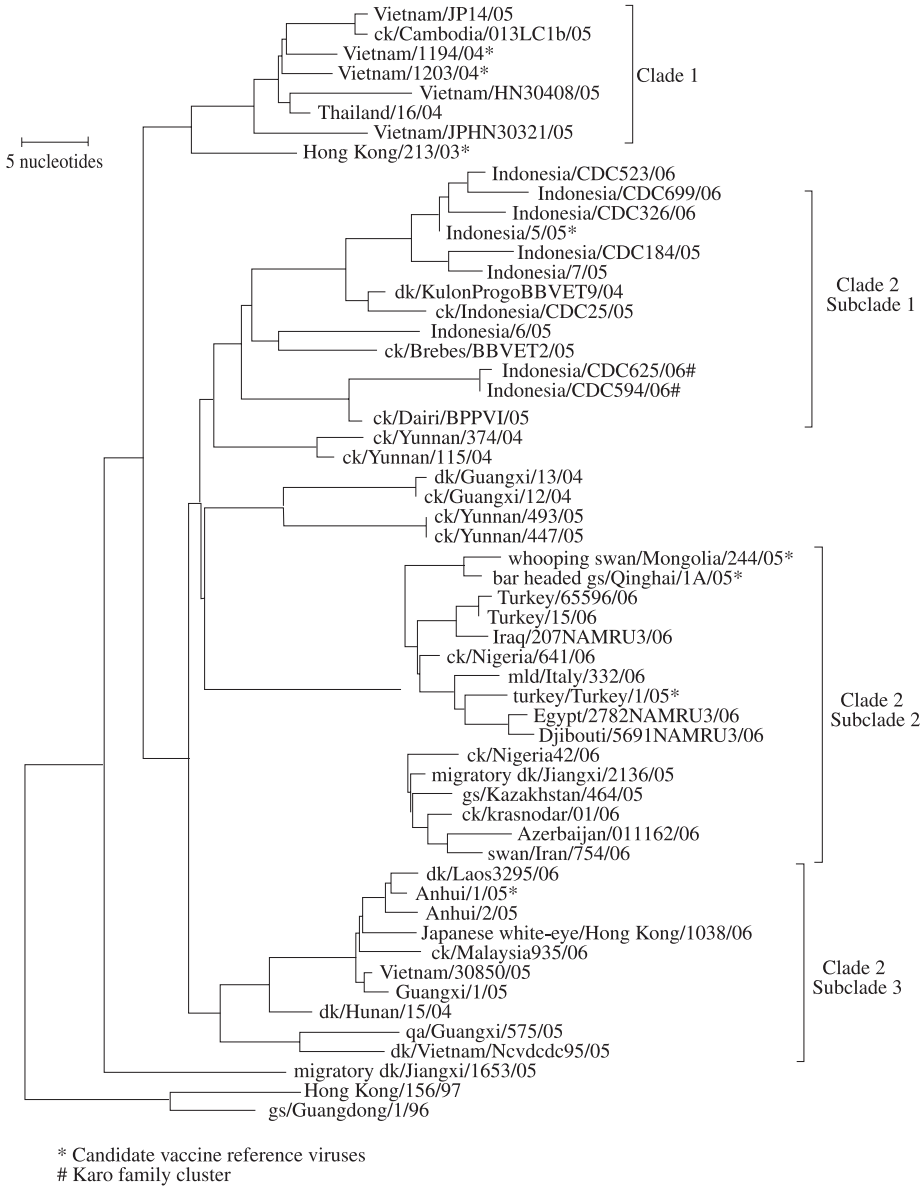

Fig. 1 Evolution of the H5N1 Hemagglutinin Gene. From 2003 to 2006, the hemagglutinin gene of the H5N1 viruses has evolved into clade 1 and clade 2. More recently, the clade 2 viruses were further grouped into three subclades. Clade 1 viruses were principally responsible for human cases in Vietnam, Thailand and Cambodia during 2004-5. Subclade 1 of clade 2 viruses were associated with human cases in Indonesia during 2005-6. Subclade 2 viruses were found in infected birds in Mongolian, Middle East, China, Europe and Africa and have also been isolated in some human cases in the same geographic areas. Subclade 3 viruses have been responsible for human infections in China and in birds from neighboring countries.[20]

demanding methods used to produce mutant or reassortant viruses.[17-19] The current H5N1 vaccine strategy is to use either an inactivated whole-virion vaccine or an adjuvanted-split virus vaccine in order to enhanced immune protection. A lowest antigen dose strategy is used to ensure the recipient safety and to improve mass vaccine production efficiency. Currently approved adjuvants are MF-59 and aluminum compounds and these have been utilized in clinical trials.

Table 2. Current Published Clinical Trials of Human Influenza Virus H5 Vaccines

Vaccine strain and type	Subjects	Regimen	Study design	Major Results	Country	Published year	Reference
A/duck/Singapore/F119-3/97 (H5N3) surface antigen (monovalent) vaccine	65 healthy adults	Two doses at 0, 21 days	Single center, observer-blind, randomized, phase I	Two doses of MF59 adjuvanted vaccines gave highest seroconversion rates.	UK	2001	Nicholson KG et al.[12]
Recombinant baculovirus-expressed H5 HA	147 healthy adults	Two doses at 0, 21 (or 28, 42 days)	Single center, double-blind, randomized, phase I	52% with neutralizing antibody titers ≧1:80 only in 90 μg after two doses	USA	2001	Treanor JJ et al.[15]
rg A/Vietnam/1203/04 (H5N1) X A/PR/8/34 (H1N1) inactivated, split-virion	451 healthy adults	Two doses at 0, 28 days	Multicenter, double-blind, placebo-controlled, phase I	54% with neutralizing antibody titers ≧1:40 only in 90 μg after two doses	USA	2006	Treanor JJ et al. [25]
rg A/Vietnam/1194/04 (H5N1) X A/PR/8/34 (H1N1) inactivated, split-virion	300 healthy adults	Two doses at 0, 21 days	Multicenter, randomized, open-label, non-controlled, phase I	67% with hemmagglutin-inhibition seroconversion rate in $Al(OH)_3$-adjuvanted 30 μg after two doses	France	2006	Breason JL et al.[26]
Not-published inactivated whole-virion	About 100 volunteers	Unknown	Unknown	Significant antibody responses with 15-30 μg adjuvanted vaccine	Hungary	2005	Only published in Hungary
rg A/Vietnam/1194/04 (H5N1) X A/PR/8/34 (H1N1) inactivated, whole-virion	120 healthy adults	Two doses at 0, 28 days	Single center, stratified randomized, double-blind, placebo-controlled, phase I	78% with seropositivity in $Al(OH)_3$-adjuvanted 10 μg after two doses	China	2006	Lin JT et al.[28]

Table 3. Vaccine Strategies for Pandemic Influenza[43]

Characteristics	Protein	DNA‡	Viral vector‡	Cold-adapted‡
Source	Cell derived; virus-like particles; inactivated; subunit	Plasmid containing DNA of interest	Adenoviral; Fowlpox; RNA replicon	Attenuated influenza virus
Route	Intradermal; intramuscular;	intranasal; subcutaneous;	transdermal patch	Intranasal
Adjuvant	MF-59; alum;CpGs*; immunostimulatory patch			
Formulation	ISCOM†; liposomes‡			

CpG* is synthetic DNA adjuvant.
ISCOM † is an immunostimulating complex and a combined form of adjuvant and antigen.
Liposomes ‡ involve enclosure of the delivered molecule in a membrane vesicle.
DNA vaccines, viral vector vaccines and live attenuated cold-adapted vaccines are possibly associated with cell-mediated immunity. Other vaccines give rise to humoral immunity.

Candidate vaccine strains for H5N1 vaccines

Due to the lack of an available suitable LPAI candidate virus for vaccine production, reassortant technology using the HA of a high-pathogenicity avian influenza (HPAI) virus modified by removal of multiple basic amino acids within the HA connecting peptide is being developed. For the development of a candidate vaccine strain for H5N1, both the HA and NA are derived from a H5N1 strain and the other 6 viral gene segments are from a so called "backbone" virus that grows well in the eggs and has been proved to be attenuated in humans. WHO has approved A/Puerto Rigo/8/34/H1N1 as a backbone virus to offer internal genes. Several high-growth

PR8-H5N1 6:2 reassortant viruses, which have been prepared using reverse genetics, are undergoing pre-clinical and clinical testing as potential vaccine seed viruses.[9] The PR8-H5N1, 6:2 reassortant viruses, include NIBRG-14 (produced by the National Institute for the Biological Standards and Control, UK), VN/04xPR8-rg (produced by St. Jude Children's Research Hospital, USA) and VNH5N1-PR8/CDC-rg (produced by Centers for Disease Control and Prevention, USA).[9] The prototype virus strains, belonging to clade 1, were selected under the recommendation of WHO in February 2005, and are A/Vietnam/1194/04 (H5N1) and A/Vietnam/1203/04 (H5N1).[21] New prototype virus strains from clade 2 have been released by WHO based on phylogenetic analysis of the circulating H5N1 viruses during 2005-6. Viruses representative of subclade 1 (A/Indonesia/5/2005), of subclade 2 (A/Bar headed goose/Quinhai/1A/2005, A/Whooper swan/Mongolia/244/2005 and A/turkey/Turkey/1/2005) and of subclade 3 (A/Anhui/1/2005) were selected for the preparation of reverse genetics modified reassortant vaccine strains.[22]

A new reverse genetics system has been developed to improve transfection efficacy.[23] Four plasmids have been constructed for the generation of vaccine seed viruses. A Pol I plasmid synthesizes the modified HA and NA segments derived from the wild-type circulating strains. Another Pol I plasmid synthesizes the other six internal gene segments from a backbone virus that grows well in eggs or cell culture. Two viral-protein expressing plasmids also used to express NP and PB1, PB2 and PA respectively. After transfection into Vero cells, this system is more efficient than the 12-plasmid system previously used for virus generation. Furthermore, it, might be suitable for the production of pandemic vaccine seed viruses.

Pre-clinical and clinical trials of human H5N1 vaccines

A vaccine prepared from a LPAI virus, A/duck/Singapore/F119-3/97 (H5N3), was assessed in an observer-blind randomized phase I trial in healthy volunteers from May to July 1999 in the UK.[2-14] The results showed that only MF-59 adjuvanted vaccine gave satisfactory immune protection. The H5 surface-antigen vaccine has been shown to be poor antigenic in a poultry study where no protection against lethal challenge was found.[24] A follow-up study after 16 months showed that there was no protective antibody titer after two-dose priming.[14] After boosting with one more dose of the MF-59 adjuvanted vaccine, cross-reactive neutralizing antibody responses to heterologous H5N1 viruses from 1997-2004 was induced.[15] Despite high seroconversion rates for A/HongKong/156/97 (100%), A/HongKong/213/03 (100%) and A/Thailand/16/04 (71%), the seed virus is antigenically distinct from A/Vietnam/1203/04 (43%). As a result, this H5N3 based inactivated vaccine induced low protective immunity against the current circulating H5N1 strains.[13] Another clinical trial with a recombinant baculovirus-expressed H5 HA from A/HongKong/56/97 showed only modest immunogenicity at high dose.[14]

In a meeting hosted by WHO on 27th April, 2006, thirteen companies were stated to be proceeding with twenty eight different clinical trials.[25] Up to the present, four clinical trials with PR8-H5N1 6:2 reassortant viruses from genetically modified HA and NA from A/Vietnam/1203/04 or A/Vietnam/1194/04 have been published.[25-28] A phase I clinical trial with a split H5 vaccine achieved a presumed protective antibody titer in 54% of 451 adult volunteers after two-dose of 90 μg HA antigen separated by 28 days.[26] Although this vaccine might prevent human H5N1 infection, the HA antigen dose is 12 times that of the seasonal human influenza vaccine. Lower antigenic doses with similar immunogenicity have been demonstrated by adding an adjuvant. However, this vaccine, which is manufactured by Sanofi Pasteur Inc., became the first approved US vaccine for humans against the avian influenza virus H5N1 in April 2007. In France, a two-dose regimen separated 21 days with an aluminum hydroxide-adjuvanted inactivated split vaccine was used on 300 adults and showed a 67% seroconversion rate; the vaccine was safe at an antigen dose of 30 μg.[27] A Hungarian report describes a clinical trial made up of about 100 volunteers using an aluminum phosphate-adjuvanted inactivated whole virus vaccine. In this study, doses of 15-30 μg of antigen only were needed to provoke a significant antibody response.[28]

Another double-blind, placebo-control, phase I clinical trial conducted in China, which included 120 healthy adults, utilized an inactivated whole-virion H5N1 vaccine with aluminum hydroxide adjuvant

(the vaccine strain was NIBRG14). This well-tolerated vaccine achieved 78% seropositivity after 2 doses of 10 μg of antigen.[29] The known current clinical trials of human H5 influenza vaccines are summarized in Table 2.

Cross-reactivity of the different clades of H5N1 remains a great concern. Previously published human trial have shown some intra-subtypic cross protection with a MF59-adjuvanted H5 vaccine.[13] One inactivated whole-virion H5N1 vaccine did protect mice from lethal challenge with antigenically different viruses.[30] In a more recent study, cross protection by a rgHK/213/03(H5N1)xPR8 inactivated whole virus vaccine was demonstrated in vaccinated ferrets against challenge with antigenically distinct H5N1 influenza viruses (A/HK/156/97, A/Vietnam/1203/04).[32] Data from the Center for Disease Control and Prevention indicate antibodies generated by clade 1 viruses poorly cross-react with clade 2.[10] Thus, it would seem that any pandemic vaccine should include one clade 1 and three clade 2 viruses, specifically one each from within subclade 1, 2 and 3.

Further research on innovative approaches to H5N1 vaccine development

In order to fulfill the urgent need for pre-pandemic vaccines, scientists are developing innovative approaches to the production of influenza vaccines and these include the following:

Egg-free cell culture approaches

During a pandemic, rapid and mass vaccine productions may be impossible using chicken embryonated eggs. Cell-culture-based H5 vaccines are under development. Validated cell culture systems for this purpose have been reported for Madin-Darby canine kidney cells[32] and Vero cells.[9]

Live attenuated cold-adapted H5N1 vaccines

Live-attenuated H5N1 vaccines have been developed to overcome the low immunogenicity of inactivated vaccines for humans. One study showed a recombinant H5N1 vaccine with modified HA and NA from H5N1/97 viruses and internal genes from a cold-adapted attenuated virus strain was safe and immunogenic in ferrets and also protected chicken from a wild-type H5N1 challenge.[33] In another preclinical trial, a live-attenuated cold-adapted H5N1 vaccine was effective for ferrets and mice against homologous and heterologous wide-type H5N1 infections.[34] For rapid immunization of an immunologically naive population, live-attenuated vaccines potentially offer a number of advantages over inactivated vaccines. These include the production of a better humoral response (including mucosal IgA antibodies) and cellular immunity, the induction of protective immunity more quickly (7-10 days following initial immunization), the fact that they require fewer doses and that they offer broader range of protection against antigenically drifted strains.[35] However, a major concern is the possible introduction of a novel HA or NA gene into the human populations before a wide-spread pandemic occurs.[36]

Replication-incompetent adenoviral-vector-based vaccines

An adenoviral-vector-based hemagglutinin subtype 5 influenza vaccine was shown to provide protection against antigenically distinct H5N1 strains and induced HA epitope-specific cytotoxic CD8 T cell responses in mice.[37] Another similar study showed effectiveness in chickens.[38] Clinical trials with adenoviral-vector-based vaccines against H1N1 have shown them to be safe and effective despite the presence of pre-existing anti-adenoviral antibodies.[36] This class of vaccines can be produced rapidly and safely due to their replication-incompetence and have the potential to protect both poultry and humans.[39] This type of vaccine has the advantage of inducing both humoral and cellular immunity and conferring cross-protection against continuously evolving H5N1 viruses without the need of an adjuvant. This approach is a feasible vaccine strategy against a potential pandemic and provides a viable option for potential vaccine stockpiling in the face of a potential influenza pandemic.

M2 protein-based vaccines

In humans, antibodies against the M2 ectodomain, though not consistently induced by infection or inactivated vaccine, exhibit significant protective activity against influenza strains in animal models.[40,41] A number of approaches have been tried to enhance such antibody responses, including fusion with various proteins, coupling with carrier proteins and delivery using a viral vector or in virus-like particles. Despite evidence from animal trials showing

some degree of heterotypic protection against human influenza A viruses, there remain some problems. M2 protein is not present in influenza B viruses, the mechanism whereby anti-M2 antibody provides protection is unknown and the safety of M2 based vaccines requires further evaluation.[40]

Other approaches that have been demonstrated to be promising in animal models include the conjugation of a conserved viral antigen (nucleoprotein) to an immunostimulatory DNA sequence (ISS) and the conjugation of a conserved epitope of hemagglutinin to a bacterial flagella protein.

DNA vaccines

Vaccines based on plasmid DNA may save time by reducing the length of the production stage of conventional vaccines from 4 to 9 months to 1 month or so. This would have the effect of alleviating any vaccine crisis. Individuals receiving a DNA vaccine are injected with plasmid DNA containing an appropriately encoded antigen, rather than the antigen protein itself. The plasmid is taken up by dendritic cells in the skin or muscle. Replication then takes place in these cell independent of the chromosomal DNA and there is transcription and translation of the antigen of interest. Degraded peptide fragments of the antigen are presented by major histocompatibility complex (MHC) class I or II, depending on the cell type that has taken up the DNA or the method of administration (gene gun or injection).[42,43] Both humoral and cellular responses can be induced by a very small amount of plasmid that encodes the antigen.

Some specific features of DNA vaccines make them more attractive than conventional vaccines. DNA is easy to manipulate and the approach allows expression in the recipient's own cells. This is apparently a favorable platform. The plasmid is capable of carrying multiple antigens, is designed to be non-infectious and the addition of adjuvant also enhances the immune response. DNA vaccines also have excellent safety profiles and have shown no toxicity or immunogenicity unlike other vaccine types. However, some problems do remain. In some cases, DNA vaccines do not elicit equally strong immune responses in primate models as they do in mouse models. There are also some concerns about the possibility of inducing anti-double-stranded DNA antibodies, of activating the Toll-like receptors and because there is a low efficiency of transformation. Currently, there are several DNA vaccines that have been developed for veterinary and human purposes worldwide, but none have as yet proceeded beyond phase 2 trials. Much effort is needed to improve their performance in the near future to fulfill their promise.

Conclusions

H5N1 influenza virus is considered to be the most likely candidate for next influenza pandemic in humans. When an influenza pandemic occurs, vaccination will be one of the key interventions. The U.S. Food and Drug Administration approved first U.S. vaccine for humans against H5N1 influenza virus in April 2007. A summary of the currently available data on the strategies being used for the development of H5N1 influenza vaccines for human use are:

1. Genetic manipulation is being carried out to modify surface genes from high-pathogenic wild-type viruses. These are rescued with well-adapted backbone viruses to allow the rapid mass production of safety vaccines.

2. Inactivated whole-virion or adjuvanted sub-virion virus vaccines are being considered in pandemic vaccine planning. More information is required on these vaccines with respect to infants, children, the elderly and the immunocompromised persons.

3. Multiple reference strains may need to be included in a single vaccine since current studies show a lack of cross reactivity between the different H5N1 virus clades.

4. In order to produce sufficient doses of any antigen during a pandemic, an egg-free system or new strains that grow well in eggs are crucial. To help solve this problem, cell culture-based systems and DNA vaccines are under development.

More efforts are still needed to fight against the pandemic threat posed by influenza virus (H5N1). The strategies used for H5N1may then be applied to the H2N2, H9N2 and H7N7 viruses, which are also possible virus candidates for an influenza pandemic.

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禽流感 (H5N1) 疫苗的研發

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20 世紀人類曾歷經三次流行性感冒病毒大流行，其中最著名的就是1918 年左右的疫情，估計將近五千萬人因此死亡。本世紀人類面臨另一個禽流感病毒 H5N1 的威脅；2006 年它擴展領土，從亞洲、中東、非洲，襲捲到歐洲，已造成超過 150 名人類死亡。科學家以數學模式模擬流感流行，大流行期前，七成以上的預防注射能結合抗病毒藥物，發揮最有效的控制疫情功效。目前爲了因應全球大流行的威脅，在世界衛生組織的主導之下，H5N1 疫苗正在全世界頂尖研究機構進行研發與臨床試驗。運用反轉基因技術製成之去活性全病毒疫苗或是添加輔劑之單位病毒疫苗，將是最有希望成爲最早通過認證的人類 H5N1 疫苗。然而，許多問題仍考驗著我們：H5 抗原普遍無法在未曾接觸過的人類產生有效抗體，需仰賴高劑量抗原或多次注射達成免疫；目前臨床試驗的對象均爲健康的成年人，對於易受流感之害的免疫不全者、嬰幼兒及老年人是否有效，仍然未知；疫苗製造商是否願意投資大量金錢及風險，及能否在短時間內製造大量疫苗供應全球，都是迫在眉睫急需解決的問題。未來，積極研發新疫苗的技術，例如加入新的輔劑，減少抗原劑量或輔劑帶來的副作用，腺病毒載體及 DNA 疫苗等，將是預防下一個世紀流感疫病的重要努力方向。(長庚醫誌2007;30:294-304)

關鍵詞：全球性大流行，H5N1 流感病毒，疫苗，反轉錄基因學

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