Influenza Vaccines for the Future
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Waiting for a pandemic

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Abstract

After a quiet period of nearly 30 years, influenza strains with hemagglutinin types that have not been seen in humans previously started to jump from birds to man, suggesting the risk of a new influenza pandemic. However, in contrast to the situation with all the other influenza pandemics occurring in the 20th century and before, in the 21st century we have sophisticated technologies for diagnosis, therapy and prevention. Modeling of the possible spread of a pandemic suggests that vaccination is by far the only way to eliminate the risk of a new pandemic. In this chapter we review the development of new vaccines against H5N1 viruses, showing that effective vaccines adjuvanted with oil-in-water emulsion are about to be licensed and will soon be available. The race against an influenza pandemic has begun; it is a battle against time that mankind cannot afford to lose.

Introduction

Since 1580, at least ten influenza pandemics have occurred, with an average of one pandemic every 42 years. Analysis of the most recent and more accurate data predicts one pandemic every 30 years (see the chapter by Lattanzi). The last pandemic was in 1968, 40 years ago and therefore common sense and mathematical models predict that we should be prepared for a new pandemic. During the last 9 years, all the events that are expected to happen before a pandemic did happen. First, a new virus carrying the H5 antigen, that had never been in humans before jumped from chickens into man and killed six people in Hong Kong in 1997. This early outbreak was contained by culling chickens in the Hong Kong area. The virus momentarily disappeared and we forgot about it for a few years. However, the virus was not dead at all; it was successfully breading, multiplying and expanding in birds in South East Asia [1], until it suddenly blew up again with human cases in 2003 and 2004 in Vietnam, Thailand, Indonesia, and China. Clearly

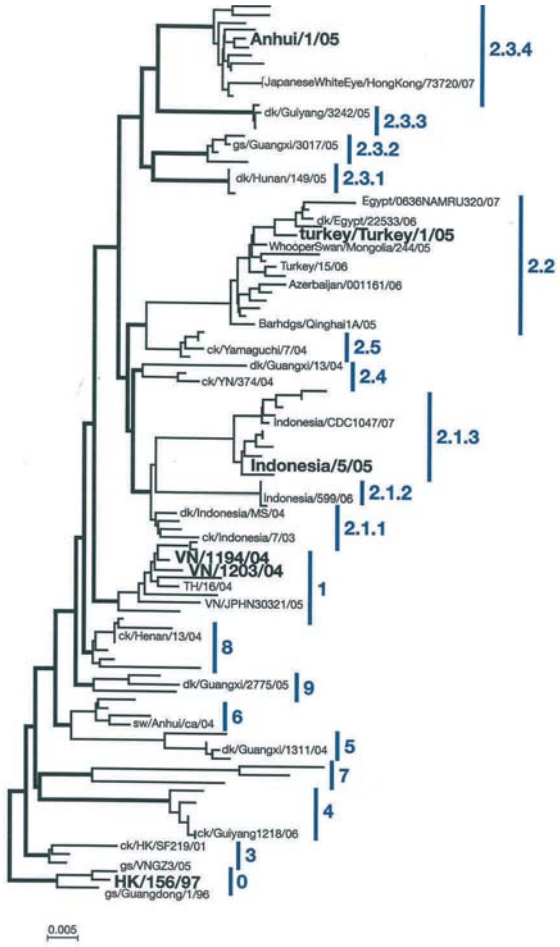

Figure 1. Evolution and nomenclature of the H5N1 virus isolates. Bold face indicates the most commonly studied viruses. Bold numbers on the right hand side indicate the clades and subclades.

the virus had escaped any control and was so widespread that since then culling hundreds of million of chickens in the areas of outbreak has provided only a temporary relief, but has never been able to control the spread of the virus. The virus, in fact, was spreading globally using migratory birds as vectors and soon appeared in the rest of Asia, Russia, Turkey, Egypt, and Nigeria. Today, the H5N1 virus is endemic in the bird population in Asia, Europe, and Africa. While spreading geographically, the virus has also been evolving and drifting antigenically, so that today we have many genetically distinct isolates of the H5N1 virus that can be classified into several clades and subclades (see Fig. 1). Up to 1 February 2008, the virus caused

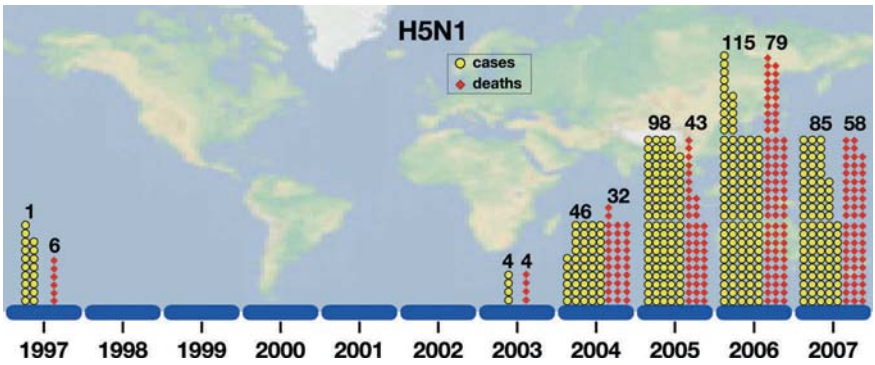

Figure 2. Yearly cases and deaths caused by the H5N1 virus from 1997 to 2007

Table 1. Cases and deaths caused by the H5N1 virus in different countries (WHO website, 1 February 2008)

Country	Total 2007	
	cases	deaths
Azerbaijan	8	5
Cambodia	7	7
China	27	17
Djibouti	1	0
Egypt	43	19
Indonesia	124	102
Iraq	3	2
Laos People's Democratic Republic	2	2
Myanmar	1	0
Nigeria	1	1
Pakistan	1	1
Thailand	25	17
Turkey	12	4
Viet Nam	102	48
Total	357	225

Total number of cases includes number of deaths. WHO reports only laboratory-confirmed cases.

357 reported cases and 225 deaths, with an overall mortality rate of 61% (see Fig. 2 and Tab. 1). All cases derived from close contacts with poultry, although in a few cases close contacts between people may have caused the infection. The virus continues to evolve, but is not yet able to be efficiently transmitted from human to human, and therefore the isolated viruses do not seem to be an immediate threat.

Table 2. Known influenza hemagglutinin types and species where they have been isolated

Hemagglutinin	Human	Swine	Equine	Avian
H1	+	+	–	+
H2	+	–	–	+
H3	+	+	+	+
H4	–	–	–	+
H5	–	–	–	+
H6	–	–	–	+
H7	–	–	+	+
H8–H16	–	–	–	+

In the meantime all predictions have been made. Some predict that the pandemic will come soon; others say that H5N1 will never cause a pandemic because the genetic background of the virus does not fit a pandemic strain. Others say that we may expect a pandemic from a virus different from H5N1. We should also consider the possibility that given the intense vaccination of the elderly population, the evolved healthcare system, the increased global surveillance, the 21st century may be the first period that will not see a pandemic influenza. Unfortunately, this scenario is unlikely to happen; in fact, the influenza virus is able to infect and evolve in several animal species (see Tab. 2), and considering that so far only 3 of the 16 known hemagglutinin (HA) types are present in human isolates, the virus seems to have enough fuel to continue to be a threat for mankind for the next several centuries.

In conclusion, although we have no certainty about when a pandemic is going to strike, these facts suggest that the risk of a pandemic should be taken into serious consideration, and that we should have a plan to be prepared for it. Considering that even a mild pandemic is predicted to cost to the USA economy alone more than 650 billion dollars and globally in excess of 3000 billion, a global investment of 30 billion (1% of the potential economic damage) seems a reasonable investment (insurance) against this risk.

Preclinical studies with vaccines against avian influenza

During the last few years, many academic laboratories, biotech companies and vaccine manufacturers have produced a plethora of vaccines potentially active against avian influenza. The most widely used are those manufactured in eggs using the same technology used to manufacture seasonal vaccines. These vaccines may be composed of whole inactivated virus, detergent-split virus or purified HA and neuraminidase (NA) subunits. These vaccines have already been tested in clinical trials and are described later on in this chapter. Here we describe the vaccine approaches for which results of clinical trials are not yet available or that have not yet been tested in humans.

The wild-type whole inactivated virus grown in cell culture (Baxter)

Baxter took the unusual approach of using fully virulent wild-type H5N1 viruses grown in Vero cells to make H5N1 vaccines. The rationale provided is that using a reverse genetic procedure, which delivers an attenuated non-dangerous virus that can be grown in biosafety level 2 laboratories, takes 3–4 weeks to generate the initial virus seed, while the wild-type virus can be used immediately for manufacturing. The caution with this approach is that thousands of liters of wild-type H5N1 virus even if grown under a biosafety 3 levels represent an extremely dangerous procedure, and in case of leakage may be enough to cause a pandemic in itself. A/Vietnam/1203/2004, A/Indonesia/05/2005 and other viruses were produced using this procedure [2]. The virus was first inactivated by a double procedure involving formaldehyde and UV treatment and then purified by sucrose gradient, ultracentrifugation, followed by diafiltration. The vaccine was immunogenic in preclinical studies, protective in mice, induced T cell responses and was cross protective. Preliminary clinical data have been reported at meetings but no published data are available yet.

Live attenuated vaccines (MedImmune/Astra)

Live attenuated H5N1 vaccines generated by reverse genetics and grown in eggs using the same procedure used for the live attenuated seasonal vaccine [3] have been reported. The preclinical studies have shown that the vaccines are immunogenic and induce protection and cross protection in mice even after one single dose [4]. However, these vaccines for the moment are not supposed to be used, i.e., not until a pandemic is declared, to avoid the possibility that the circulation of a live virus carrying the H5 gene may favor the evolution of a dangerous H5 strain and this may accelerate the pandemic.

Virus-like particles produced in baculovirus

Virus-like particles (VLPs) expressing the HA, NA, and M1 antigens of H5N1 virus have been produced in insect cell culture, using a baculovirus vector. The VLPs were immunogenic in mice. Recently, testing in a clinical trial in humans of an A/Indonesia/05/2005 H5N1 vaccine was reported [5]. Two doses of 15 and 45 μg were reported to induce a fourfold rise in neutralizing antibodies of 63% of subjects with the highest dose.

Vaccines based on M2

M2-based vaccines have been described in a number of studies. So far, nothing more than interesting preclinical reports are available [6–9]. Given the

absence of correlates of protection for vaccines based on M2, it is unlikely that it will be possible to see a fast development of these vaccines because in the absence of an efficacy trial it is virtually impossible to license these vaccines. It is possible, however, that in the long-term these components may find space first in improved seasonal vaccines and later in pandemic vaccines. The same may apply to vaccine constructs based on the internal conserved nucleoprotein (NP) [8, 10].

Clinical studies with vaccines against avian influenza

Many clinical studies have been performed during the last 10 years with vaccines against avian influenza. Below we describe mostly those studies that have been published, although in some cases we mention also data reported from meetings for which preliminary information is available. Unless otherwise stated in the text or in Table 3, immunogenicity was tested by hemagglutinin inhibition (HI) using horse red blood cells, by microneutralization (MN) using the RG A/Vietnam/1203/2004xAPR/8/34 influenza virus strain or by serum radial hemolysis (SRH) using turkey red blood cells coated with antigens derived from the tested virus (see the chapter by Montomoli). Seroconversion was considered positive when HI, or MN titers were <10 before immunization and >40 after immunization or when a fourfold increase in titer was observed in those subjects that had a pre-immunization titer >10. The overall data are reported in Table 3. SRH was considered positive when it was negative before immunization and had an area of hemolysis >25 mm^2 after immunization. Looking at the table, it is important to keep in mind that the data are not directly comparable since they have been produced at different times in different laboratories. HI studies in particular have been performed with horse erythrocytes using an assay that has never been validated across laboratories and for which no standards exist, so that large differences have been reported. Although MN also shows variability in different laboratories, it is probably a better assay for comparing results because it measures the ability of antibodies to neutralize viral infection, and this activity is biologically important.

The experience with MF59-adjuvanted H5N3 vaccine (Chiron/Novartis)

The first attempts to develop vaccines against H5N1 followed the 1997 outbreak in Hong Kong. At that time reverse genetics was not available, H5N1 could not be grown in eggs, reassortants could not be made and therefore only two attempts to make vaccines were made. One was a recombinant H5 subunit vaccine expressed in baculovirus produced by Protein Sciences (described below), the second one was a subunit vaccine prepared using a

non-virulent duck H5N3 virus that could grow in chicken eggs [11]. This H5N3 vaccine was a very interesting experience that provided most of the useful information we have today, and paved the way to further vaccine development. This vaccine was tested in human adult volunteers with and without the adjuvant MF59 (see the chapter by O'Hagan/Podda) at 7.5, 15 and 30 μg per dose (licensed vaccines contain 15 μg/dose). The data of the trial (reported in Fig. 4 in the chapter by Montomoli) were very surprising (Tab. 3). First, the conventional, non-adjuvanted vaccine did not elicit a significant protective antibody response (no HI response and only 10–45% MN). In retrospective we should not be too surprised by this result; while we use one dose of seasonal influenza vaccine in people that already have immunity against influenza, in the case of H5, people are naive to this antigen and therefore the vaccination needs to prime the immune system before it can induce a high antibody response. The really surprising finding instead was that the vaccine administered with the newly licensed MF59 adjuvant did induce a good immune response after one dose and a seroconversion by MN and SRH in nearly all subjects after two doses. Interestingly, the highest immune response was achieved with the 7.5 μg dose, which was the lowest used in the trial [11]. At 16 months after the primary immunization the test persons were given a boost with the same vaccine. Again the subjects vaccinated with the MF59-adjuvanted vaccine responded with a very strong and long-lasting immune response, while those vaccinated with the non-adjuvanted vaccine showed still a very low but detectable protective antibody response [12]. When the H5N1 virus started to circulate again in Thailand and Vietnam in 2003 and 2004, it was interesting to go back to the sera obtained from the trial and ask whether the people that had been immunized with the H5N3 virus had antibodies able to neutralize the H5N1 clade 1 viruses deriving from an antigenic drifting that had been taking place for 6–7 years. Figure 3 shows that, while the subjects vaccinated with the non-adjuvanted vaccines had no cross-clade neutralizing antibodies, the majority of the subjects vaccinated with MF59-adjuvanted vaccine had protective levels of antibodies against the heterologous clade 1 virus isolated in Vietnam and Thailand [13, 14]. In summary, the data had shown that MF59-adjuvanted vaccines could induce protective immunity against viruses not matching the vaccine strain and could cover the antigenic drift of the virus for 6–7 years. Finally, more recently, the people vaccinated with H5N3 in 1999 and boosted with H5N3 in 2001 were vaccinated in 2007 with a clade 1 vaccine. Preliminary data show that those who had been primed with the MF59-adjuvanted H5N3 vaccine produced very high level of antibodies against the clade 1 strain by 7 days after vaccination, while those primed without adjuvant also had a response but of much lower magnitude. People that had not been primed responded as expected only after two doses of vaccine and reached protective levels only 42 days after the first immunization. This latter study provided extremely important information showing that when people are primed with any H5N1 strain adjuvanted

Table 3. Clinical studies with vaccines against avian influenza.

						Seroconversion*					
Vaccine	Date	Dose	Adjuvant	Subjects	Pre-titers	HI	MN	SRH	Cross neutr.		Symptoms
									HI	MN	
Chiron/Novartis H5N3 subunit Nicholson et al. [11]	1999	7.5 15 30	MF59	65	3%	60[5] 45 20	**80**[2] **100** **100**	**(100)/90**[3] **(100)/82** **(100)/80**	n.d.	7–14[4] (2 doses)	No major findings
		7.5 15 30	None			0 0 9	10 18 36	(0) 0 (45) 0 (36) 9		43–71 (3 doses)	
Baculovirus recombinant H5 Treanor et al. [17]	1999	0 25 45 90	None	147	Not reported	n.d.	4[1] 17 28 52	n.d.	n.d.	n.d.	No major findings
Sanofi split H5N1Vietnam 2004 Treanor et al. [16]	April 2005	0 7.5 15 45 90	None	450	3%	0 13 24 41 57	0 7 20 41 53	n.d.	n.d.	n.d.	Pain, dose-dependent up to 50%
Sanofi split Vietnam 2004 Bresson et al. [18]	July 2005	7.5 15 30	Aluminium hydroxide	300	~1%	28 44 66	16 18 41	n.d.	n.d.	n.d.	Pain, dose-dependent up to 68%
		7.5 15 30	None			43 44 53	20 22 27				

GSK split H5N1 Vietnam 2004 Leroux-Roels et al. [19]	March 2006	3.8 7.5 4.5 30	ASO3	400	~1%	**82** **90** **96** **85**	**86** **86** **86** **98**	n.d.	20–32%	67–77%	Pain, dose-independent up to 100% Pain, dose-dependent up to 68%
		3.8 7.5 7.5 30	None			4 16 35 41	22 37 53 65				
Novartis subunit H5N1 Vietnam 2004 Banzhoff [20]	April 2005	7.5 15	MF59	312 adults	n.d.	n.d.	**85** **81**	**85** **80**	n.d.	n.d.	Pain in 50–60% of subjects Pain in 20–25% of subjects
		75 15	MF59	173 elderly			**79** **76**	**71** **68**			
Sinovac whole virus H5N1 Vietnam 2004 Lin et al. [21]	July 2006	1.25 2.5 5 10	Aluminium hydroxide	120	3%	13[5] 21 33 78	9 21 33 65	n.d.	n.d.	n.d.	No major findings
Hungarian whole virus Vietnam 2004			Alum phosphate	146	0	645	n.d	n.d.	n.d.	n.d.	No major findings
Chiron/Novartis subunit H9N2 [23]	March 2005	3.75 7.5 15 30	MF59	96	n.d.	**100** **92** **100** **100**	**100** **92** **100** **100**	n.d.	n.d.		No major findings
		3.75 7.5 15 30	None			67 58 50 75	67 58 50 83				

*Seroconversion is regarded as positive when HI or MN were <10 before immunization and ≥40 after second immunization. When pre-titers were >10, fourfold rise in titer may be needed to score positive in seroconversion. SRH was considered positive when pre-immunization tests were negatives and post-immunization had a an area of hemolysis >25 mm^2.

[1]% with MN > 1/80; [2]% with NeuT > 1/32; [3]Seroconversion against H5N3 and H5N1, respectively; [4]Against Vietnam 2004 and Thailand 2004, respectively; [5]HI was done with chicken or turkey red blood cells.

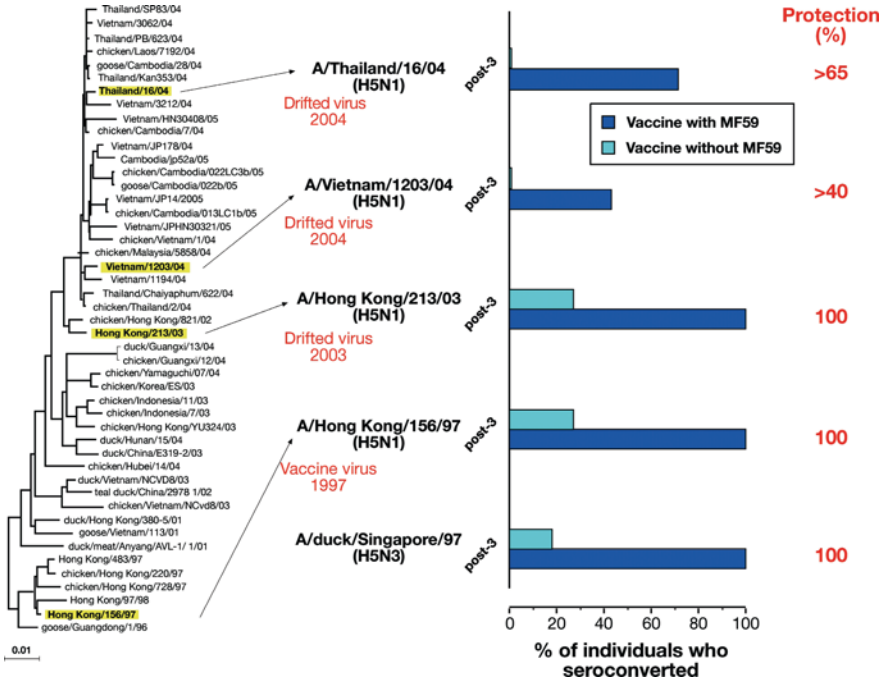

Figure 3. Phylogenetic tree of the H5N1 viruses circulating from 1997 to 2004 (left). On the right are reported the % of people with neutralizing titers ≥40 against the virus strains. The graph shows that vaccination with MF59-adjuvanted clade 0 vaccine induces protective antibodies against clade 1 strains, while the non-adjuvanted vaccine is unable to do so.

with MF59, not only do they develop a cross-protective immune response that may cover the strains that can come during the 6–7 years following vaccination, but also that at any time it is sufficient to give a boost with one dose of adjuvanted vaccine to get an almost immediate protection against the new strain. These data are of paramount importance in designing pre-pandemic vaccination strategies that are discuss later on the policy section of this chapter.

Recombinant HA (Protein Sciences)

The recombinant HA cloned from the H5N1 strain isolated in Hong Kong in 1997 was produced in baculovirus-infected cells and purified. Placebo, 25, 45 or 90 μg protein were used to immunize 147 subjects. Two doses were given, at day 0 and 21 or 42. An additional blood sample was taken at day 56. Respectively, 4, 17, 28 and 52% of the subjects had a MN > 1/80 after the second immunization (Tab. 3). The trial showed that, although some

immunity to H5N1 could be induced, large amounts of antigen were necessary. More recently these subjects were boosted with an H5N1 vaccine from Sanofi Pasteur without adjuvant. Preliminary available data suggest the 90 μg of adjuvant-free vaccine can boost the antibody response, but with a strength lower and a kinetic slower than that provided by MF59-adjuvanted vaccine. No data on cross-neutralizing antibodies have been reported [15].

Non-adjuvanted H5N1split vaccine (Sanofi Pasteur)

The real race to develop H5N1 vaccines started in 2004 and 2005 when, after the quiet period between 1998 and 2002, H5N1 started to cause new human cases in Vietnam and Thailand (see Fig. 2). This time it became clear that H5N1 not only had not gone away, but it was there to stay, and the global community started to panic fearing that the pandemic could come before the world had the time to be prepared. The first egg-based H5N1 vaccine was produced by Sanofi Pasteur. This was a split vaccine. The virus strain was generated by reverse genetics containing the HA and NA gene segments from the influenza A/Vietnam 1203/2004 and all the other genes from the strain A/PR/8/34 that grows well in embryonated eggs and is normally used to generate vaccine strains. The HA gene was further modified to replace the six basic amino acids at the cleavage site between domain 1 and 2 of the HA, which are known to be associated with increased virulence. Thanks to these modifications the new virus carried the HA and NA genes of the pathogenic H5N1 virus but was non-virulent and could be grown in a biosafety level 2 laboratory. After growth in eggs, the virus was purified by ultracentrifugation, inactivated with formaldehyde and split by addition of Triton X-100. The vaccine was finally ready after sterile filtration and additional steps of purification. Clinical trials started in April 2005 [16]; 450 subjects, with a median age of 39 years, were enrolled in a randomized, placebo-controlled, double-blind, multicenter trial. Groups of approximately 100 people received 7.5, 15, 45 or 90 μg vaccine antigen, 36 people received a placebo intramuscularly (i.m.) at days 0 and 28. A blood sample was also taken 28 days after the second dose. Safety of the vaccine was fine overall, with local pain and tenderness being the most frequently reported symptoms. These symptoms occurred after both doses, were dose-dependent and were reported by 40–50% of the subjects that received the highest vaccine doses. The immunogenicity results were clearly disappointing and completely aligned with the non-adjuvanted group of the H5N3 vaccine study [11] and with those from the Protein Sciences study with recombinant H5N1 [17]. Seroconversion using HI and MN were observed only after the second dose in approximately 50% of the subjects that received the high dose of 45 and 90 μg (see Tab. 3). Clearly these results indicated that, while a vaccine against H5N1 was possible, without an adjuvant up to 180 mg per subject were required. With this vaccine, the global

influenza vaccine manufacturing capacity (approximately 400 M doses of trivalent vaccine/year) was able to provide a vaccine for not more than 200 million people/year. This vaccine has been licensed in the USA by FDA.

Split vaccine adjuvanted with alum (Sanofi Pasteur)

In July 2005 Sanofi Pasteur performed a trial in Europe using a similar split vaccine in a randomized, multicenter, open label trial in 300 subjects aged 18–40 years [18]. The strain used was influenza A/H5N1/Vietnam/2004 prepared by reverse genetics by the National Institute for Biological Standards and Control (NIBSC). The vaccine was adjuvanted by aluminum hydroxide. Three doses 7.5, 15 and 30 μg were injected i.m. at days 0 and 21, a further blood sample was obtained at day 42. HI and MN were measured as in the previous trial. The results were disappointing. Neither the vaccine without the adjuvant nor the one adjuvanted with alum were able to induce substantial HI or MN seroconversions (Tab. 3). Some dose-dependent immunogenicity was observed; however, seroconversion was usually achieved in less than 50% of the cases and the only group that had an HI seroconversion above 50% was the one with 30 μg and alum. However, this group had very low seroconversion in MN, raising questions on the how the HI titers should be interpreted. In conclusion, this study confirmed what had already been well described in the 1960s and 1970s, that is, that the aluminum is not a good adjuvant for the influenza vaccine (see the chapter by Lattanzi).

AS03 adjuvanted H5N1 split vaccine (Glaxo SmithKline)

The split vaccine was made using the strain A/Vietnam/1194/2004, NIBRG-14 grown in eggs. The strain was generated by reverse genetics and contained the HA and NA gene segments from the H5N1 Vietnam 2004 and the other gene segments from the A/PR/8/34 strain. In this case as well, the HA gene was engineered to remove the six basic amino acids, so that the strain could be grown under biosafety level 2 conditions. The trial started in March 2006 and 400 subjects 18–60 years old were enrolled in an observer-blind, randomized trial [19]. Groups of 50 received 3.8, 7.5, 15 or 30 μg of vaccine alone or adjuvanted with the AS03 oil in water emulsion (5% DL-α-tacopherol and 95% squalene oil in water containing 2% Tween 80), a composition very similar to that of MF59 (see the chapter by O'Hagan/Podda). Two i.m. doses, at days 0 and 21 were given. Blood was also taken at day 42. Overall, safety was fine, with local pain being the most frequently reported symptom, and was dose dependent in up to 68% of the non-adjuvanted groups and dose independent in virtually all subjects (90–96%) in the adjuvanted groups. HI and MN were measured against the homologous vaccine strain and also against the heterologous, clade 2

A/Indonesia/5/2005 strain. As shown in Table 3, data obtained with the AS03-adjuvanted vaccine were very similar to those previously described for the MF59-adjuvanted H5N3 vaccine: seroconversion measured by MN and HI was achieved in 86–98% and 85–96% of the subjects, respectively, independently of the dose used. The data obtained with the vaccine without adjuvant were as disappointing as those previously obtained with the non-adjuvanted H5N3 and H5N1 vaccines: seroconversion was dose dependent and reached a maximum of 65% and 41% in MN and HI, respectively, at the maximum dose used. Interestingly, in this case, CHMP criteria of seroconversion rates in at least 40% of the subjects were achieved also after the first dose in those subjects that received 7.5 μg or more of adjuvanted vaccine. The vaccine was also shown to induce seroconversion against the heterologous clade 2 Indonesia strain, which was observed in 67–77% of the subjects when using the MN, but could be detected in only 20–32% of the subjects when using the HI.

Subunit vaccine adjuvanted with MF59 (Novartis)

The subunit vaccine was produced in eggs using the strain A/Vietnam/1194/2004, NIBRG-14 and according to the procedures used for the seasonal influenza vaccine [20]; 7.5 and 15 μg were used to immunize 312 adults and 173 elderly subjects i.m., at days 0 and 21. A blood sample was taken also at day 42. Table 3 shows that both doses induced seroconversion in the majority of the subjects (80–85% of the adults and 68–79% of the elderly seroconverted by MN or SRH). These data confirmed that the data obtained with H5N3 also apply to H5N1. These are also the first data available of the immunogenicity of the vaccine in the elderly population, and they indicate that the adjuvanted vaccine is suitable for the immunization of 65 years and older subjects [20].

Whole inactivated virus vaccine adjuvanted with aluminum hydroxide (Sinovac)

The strain A/Vietnam/1194/2004, NIBRG-14 grown in eggs, inactivated by formalin, concentrated and purified by chromatography, and formulated with aluminum hydroxide was used to immunize 120 subjects [21]. The randomized, placebo-controlled, double-blind study, started in July 2006 in 18–60 years old subjects in Beijing, China; 1.25, 2.5, 5 and 10 μg antigen per dose were used for immunization at day 0 and 28. A blood sample was also obtained at day 42. The vaccine was reported to be safe; however, the number of adverse events reported is so low that it is likely that the standards for reporting in this trial were different from the other trials. The HI titers were measured using turkey erythrocytes, and therefore cannot be compared to

any of the other data. In any case a dose-dependent seroconversion was observed in 13–78% of the subjects (Tab. 3). MN seroconversion occurred in only 9–65% of the subjects. So far, this is the only published trial using whole inactivated virus. The data (as judged from the MN seroconversion) are not very exciting. A second whole virus vaccine produced by the Hungarian Center for Allergy and Immunology reported 64% seroconversion by HI. However, even in this case HI was measured by chicken erythrocytes and therefore data cannot be compared with the others. [22]. Many other claims were made with whole inactivated viral vaccines by several independent groups. However, so far, none of them has provided robust data able to support the claims made often in press releases.

H9N2 vaccine adjuvanted with MF59 (Novartis)

The vaccine strain was a reassortant containing the HA and NA gene segments from the influenza A/chicken/Hong Kong/G9/97 and the remaining gene segments from the A/PR/8/34 strain. The virus was grown in eggs and the vaccine produced according to the procedures used for the seasonal subunit vaccine. In April 2005, 96 subjects (12 per group) received 3.75, 7.5, 15, or 30 μg vaccine with or without MF59 adjuvant. Immunization was i.m., at days 0 and 28. A further blood sample was taken at day 56.

The results, reported in Table 3, confirmed the data obtained with all the other trials performed with H5N1 and H5N3 [11–13]. Basically, nearly all subjects immunized with the adjuvanted vaccine achieved seroconversion both in HI and MN, independently of the dose used, showing that 3.75 μg are still able to induce a full immune response. In marked contrast, the non-adjuvanted vaccine showed a much lower, dose-dependent response [23].

Policy

During the 1997–2007 period we have seen the most diverse reactions of policymakers towards the potential risk of a pandemic influenza. The approaches have been from totally ignoring the problem (1998–2003), to panic (2005–2006), to a more recent balanced rational approach. Below are the possible options that policymakers have. A summary of the different options is reported in Figure 4.

Options 1 and 2: Vaccinating pandemic survivors

Options 1 is based on the strategy to wait for the WHO to declare the beginning of the pandemic, identify the strain causing the pandemic, rush to manufacture the vaccine and then vaccinate people with two doses of vaccine.

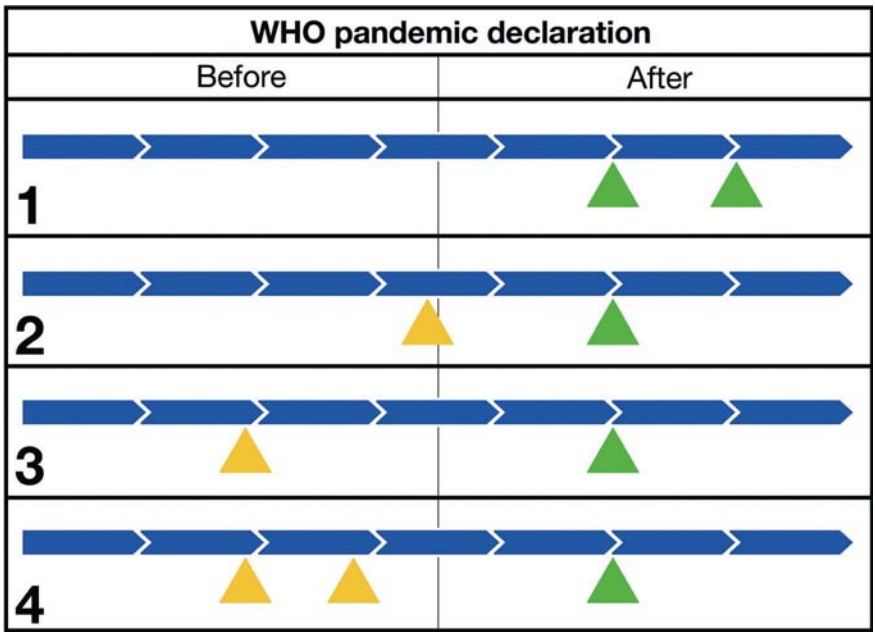

Figure 4. Options available to policymakers to vaccinate against a pandemic influenza virus. Options 1–4 are described in the text. Yellow triangles show immunizations before the beginning of the pandemic; green triangles indicate vaccination after the beginning of the pandemic.

Option 2 is to stockpile enough vaccine to administer one dose when WHO declares the pandemic and then manufacture the vaccine using the pandemic strain and give the second dose. Clearly these options have the insurmountable limit that the vaccination campaign becomes effective when it is too late, and therefore vaccination can only help those people that have already survived the pandemic. In fact, while all modeling studies [24–26] predict that in only 3 months a pandemic will sweep a country, preparing and delivering two doses of vaccines will take at least 8–10 months, independently of the capacity and technology available (3–4 weeks to prepare the seed strain, 3–6 months to manufacture any significant amount of vaccine, 1 month to build immunity). These two options have been very popular between 2004 and 2007 when vaccines had not yet been tested in clinical trials, safety data were not yet available, and cross protection had not been convincingly demonstrated. Both options are based on the assumption that the vaccines need to be made using the same strain causing the pandemic and that cross protection is not effective. Option 2 has the additional disadvantage that if the strain causing the pandemic is of a different clade of the one stockpiled it would be not useful. Today we know that, while non-adjuvanted or alum-adjuvanted vaccines have not been shown to induce cross protection among

different clades of H5N1, the vaccines adjuvanted with oil in water emulsions have been shown to induce cross protection- and therefore it is possible to vaccinate without waiting for the strain that causes the pandemic.

Options 3 and 4: Pre-pandemic vaccination

Option 3 is to vaccinate people with one dose of adjuvanted vaccine before the pandemic starts and then to give a boost with the pandemic strain once the pandemic starts. This option is based on the observation that, while priming with one dose is not able to produce a fully protective and long lasting immunity, it will be able to prime the immune system and induce a memory that can be boosted later with the pandemic strain. This option, although not ideal would have the great merit to prime the immune system against an H5 virus so that the basic assumptions for pandemic to happen (which is the circulation of the virus in an unprimed population) would be eliminated. If option 3 was adopted, we should no longer have to fear a pandemic caused by an H5 virus, because this virus would circulate in a primed population and in the worse case scenario the virus would be able to cause only a bad influenza season.

Option 4 is to induce fully protective and cross protective titers of antibodies and memory B and T cells by vaccinating the population with two doses of adjuvanted vaccine using any H5 virus and still have the option to boost the immunity with a pandemic vaccine when the pandemic is declared. Today, this is possible using the vaccines adjuvanted with MF59 or AS03. In fact, these vaccines have been shown to induce highly protective level of antibodies against the vaccine strain, which are cross reactive with strains from different clades, and to induce a memory that when boosted by any distantly related H5 virus it provides, in just 7 days, a fully protective response against the new virus. This implies that regardless of the virus strain used for the pre-pandemic vaccination, the appropriate immunity will be there when needed. In fact, the boost can be provided by a new immunization as shown in Figure 4, or by the infection with the pandemic strain that will be able to induce a protective immunity before it induces the disease.

In conclusion, pre-pandemic vaccination is the only option that we have to make sure that we are in the position to prevent and control the risk of a pandemic. In this way, the vaccine would block the disease or would significantly affect its morbidity/mortality.

Barriers to pre-pandemic vaccination

If pre-pandemic vaccination has the potential to eliminate the risk of a pandemic, why are policymakers not rushing to implement it? There are several barriers between the policymakers and the implementation. The first one is

that pre-pandemic vaccines have not yet been licensed and therefore they are not yet available. This barrier is about to disappear because the Novartis and GSK vaccines are likely to be licensed in Europe in the near future and will be submitted for registration in the USA shortly. The second barrier has been that, while cross protection had been amply demonstrated with H5N3, and in pre-clinical studies, human data on H5N1 were not yet available. Today, these data are available and strong. Perhaps the strongest data derive from the boost in 2007 using a clade 1 virus of the people immunized in 1999 and 2001 with H5N3. These data show that when the appropriate memory is present, a few days after boost are sufficient to induce a fully protective response against a drifted virus. This can be achieved by vaccinating with an adjuvanted vaccine or by infection.

In conclusion, the technically sound barriers to pre-pandemic vaccination are disappearing and we see no reason why this should not be implemented. However, there are probably even more important barriers that are likely to be in the way of making the right decisions. The first and most important is that a decision to actively vaccinate people implies taking a risk, while waiting is not risky for the policymakers. (However, it is a risk for the population.) The major fear of the policymakers is safety. What happens if they recommend a vaccination and then we have a safety problem with the vaccine? This is reasonable concern that should be addressed. Policymakers look at the swine flu experience of 1977 (see the chapter by Lattanzi) and they do not want to find themselves in a similar situation. They are absolutely right in this; no compromise is acceptable for safety in a pre-pandemic situation. On the other hand, there are a number of reassuring data that should make policymakers confident. First, the swine flu vaccination was mostly done with a whole inactivated virus, which at that time was known to be not pure and to induce a lot of side effects. (That is the reason why split and subunit vaccines were developed and replaced the whole virus in seasonal vaccines.) Today, we have the option to use vaccines that are not based on whole, inactivated virus. Second, the safety of some of the adjuvants has been amply demonstrated. MF59 has been safely given to >30 million people, and passive surveillance showed that there is no risk of Guillain Barré (reported cases were similar to those of non-adjuvanted vaccines). In conclusion, all the barriers to pre-pandemic vaccination are disappearing and we are confident that our generation will be the first one in centuries that will be able to prevent an influenza pandemic.

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