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Report on the first WHO integrated meeting on development and clinical trials of influenza vaccines that induce broadly protective and long-lasting immune responses Hong Kong SAR, China, 24–26 January 2013☆

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Abstract

On January 24–26, 2013, the World Health Organization convened the first integrated meeting on "The development and clinical trials of vaccines that induce broadly protective and long-lasting immune responses" to review the current status of development and clinical evaluation of novel influenza vaccines as well as strategies to produce and deliver vaccines in novel ways. Special attention was given to the development of possible universal influenza vaccines. Other topics that were addressed included an update on clinical trials of pandemic and seasonal influenza vaccines in high-risk groups and vaccine safety, as well as regulatory issues.

Keywords

Universal influenza vaccines; Seasonal influenza vaccines; Pandemic influenza vaccines; Neutralizing antibodies; Live attenuated influenza vaccines; Inactivated influenza vaccines; Adjuvants

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1. Introduction

Influenza viruses remain a serious threat to public health, due to their ability to escape the human immune system through frequent antigenic drift and occasional antigenic shift. The unabated circulation of highly pathogenic avian influenza (HPAI) A H5N1 influenza virus and the recent demonstration that relatively few mutations could confer mammalian transmissibility to the virus underscore the pandemic potential of HPAI viruses. The emergence of the 2009 pandemic H1N1 influenza virus (A(H1N1)pdm09) illustrates the risk of emergence of a new pandemic from an animal influenza virus and the lengthy production timeline of a strain-specific pandemic vaccine. It is thus highly desirable to develop influenza vaccines that offer broad cross-subtype protection to combat any possible new influenza A virus pandemic.

As reviewed by Robert Huebner (US Biomedical Advanced Research and Development Authority, Department of Health and Human Services (HHS), Washington, DC, USA) in his keynote address, current seasonal influenza vaccines confer protection only against homologous virus strains, which necessitates frequent reformulation to include newly emerging strains. Trivalent inactivated vaccines (TIV) show only 50–70% protective effectiveness in adults, with lower effectiveness in the elderly and young infants. It is recognized that mammalian cell-based influenza vaccines, recombinant vaccines, and adjuvanted vaccines, should be encouraged to provide greater pandemic vaccine production capacity. Currently, at least one cell-based vaccine and one recombinant vaccine produced in insect cells have been licensed in the USA. Quadrivalent inactivated vaccines (QIV) with two influenza A and two influenza B strains, and one quadrivalent live attenuated vaccine have also been approved. However, an effective universal influenza vaccine is still far from reality, requiring a long development process, with large-scale efficacy trials. It will probably also necessitate the development of new potency assays for its evaluation.

John Tam (World Health Organization (WHO), Geneva, Switzerland) summarized the 2012 recommendations of the WHO Strategic Advisory Group of Experts (SAGE) on Influenza vaccination [1]. Seasonal influenza vaccination is recommended for populations at risk of severe influenza infection including pregnant women, children less than 5 years and in particular less than 2 years of age, the elderly, and individuals with underlying health conditions, as well as for health-care workers, who are at increased risk of exposure and may also spread the infection to vulnerable patients.

2. Broadly protective and universal influenza vaccine strategies

The development of a universal influenza vaccine remains challenging, requiring in-depth knowledge of conserved epitopes on viral proteins that can elicit cross-protective antibody (Ab) responses. Identified epitopes are located in the virus matrix protein 2 (M2) and especially its 23 N-terminal amino acid ectodomain (M2e), in the highly conserved HA2 region of the hemagglutinin (HA) and in the neuraminidase (NA). Potentially cross-protective T cell epitopes were also identified within internal virion proteins, primarily the matrix protein 1 (M1) and the nucleoprotein (NP) [2–4].

As reviewed by Florian Krammer (Mount Sinai Hospital, New-York, NY, USA) and by Guus Rimmelzwaan (Erasmus University, Rotterdam, The Netherlands), the M2e domain can induce a broadly protective Ab response in animals. Immunization with an M2e-HBc fusion antigen provided 90–100% protection against lethal virus challenge in mice and ferrets [5]. Abs to M2e act through antibody-dependent cellular cytotoxicity (ADCC) [6]. Influenza-specific, cross-reactive ADCC Abs that can trigger *in vitro* elimination of influenza-infected human blood and respiratory epithelial cells in the presence of NK cells have been detected in human sera devoid of neutralization activity [7].

Broadly neutralizing Abs that bind to a highly conserved conformational epitope on the globular head of the HA molecule were recovered from H5N1-infected individuals [8,9] and from mice immunized with H5 vaccine [10]. Theodore Ross (University of Pittsburgh, PA, USA) reported that insect cell-produced virus-like particles (VLPs) made of the HA and NA proteins of avian H5N1 with computationally optimized sequences (COBRA) elicited hemagglutination-inhibiting (HAI) Abs and protected mice and macaques against challenges with pathogenic H5N1 virus strains from different genetic clades [11]. A H5 HA DNA prime followed by a H5 HA VLP boost produced similar results with cross-clade protection in mice, as reported by Paul Zhou (Pasteur Institute, Shanghai). The neutralizing Abs elicited by these vaccination regimens were shown to bind to the globular head of HA.

However, as summarized by Krammer, the most potent broadly reactive influenza virus neutralizing Abs identified to date are those that bind to a highly conserved region in the stem of HA [12–15]. Such Abs, which were effective against all group 1 influenza A viruses tested, were shown to target the membrane-proximal region of the HA molecule and prevent membrane fusion [16,17]. Screening of libraries of human neutralizing monoclonal Abs (mAbs) identified Abs that bind to a conserved epitope in the fusion domain of the influenza virus HA subunit 2 (HA2) protein located on the HA stem. Such mAbs protect mice against lethal challenges with influenza A and B viruses [16,18]. A synthetic peptide vaccine based on this conserved neutralization epitope demonstrated protective activity in mice against influenza viruses of subtypes A(H3N2), A(H1N1) and A(H5N1) [19], thus providing proof of concept for a broadly protective HA2-based influenza vaccine. These mAbs are derived from a specific Ab gene heavy-chain variable region IGHV1–69, and only require limited affinity maturation from the germline ancestor [20]. The development of HA2-based influenza immunogens that afford good protection in a mouse challenge model is in progress [21,22], as well as a vaccine strategy based on the use of chimeric HA molecules that express the same stalk but different HA heads and provide heterologous and heterosubtypic protection in mice [23].

The virus neuraminidase (NA) antigen can also provide cross-reactive immunity and partial protection against heterotypic virus challenge [24,25] that seems to correlate with sialic acid cleavage-inhibiting Ab titers [26].

In addition to Abs, T cells also can confer broad protection against multiple influenza virus subtypes [27]. Cross-reactive T cell responses involving both $CD4^+$ and $CD8^+$ T cells were found to mediate early clearance of an antigenically novel influenza virus in nonhuman primates [28]. Cross-reactive and protective cellular immune responses were found in

humans after infection with a novel influenza virus [29,30]. T cell responses are mainly directed against the relatively conserved internal matrix (M1) protein and nucleoprotein (NP) of the virus [31], which ought therefore to be considered for inclusion in a universal vaccine.

As reviewed by Sarah Gilbert (The Jenner Institute, Oxford, UK), vaccination of human volunteers with a modified vaccine Ankara (MVA) recombinant virus expressing a NP-M1 fusion protein successfully boosted pre-existing cellular immune responses to seasonal influenza vaccine and elicited increased T cell IFN-γ responses to NP and M1 antigens [31,32], as well as significant reduction in duration of virus shedding following challenge [33]. Clinical evaluation of a chimpanzee adenovirus NP-M1 prime followed by MVA NP-M1 boost is currently underway. In another study, mice vaccinated with an equimolar mixture of synthetic peptides corresponding to conserved T cell epitopes in M1, M2, NP and PB1 mixed with the adjuvant montanide ISA-51 (the Flu-v vaccine) were protected against lethal influenza virus challenge. The same vaccine elicited significant T cell IFN-γ responses in human volunteers [34].

Tania Gottlieb (Biond Vax Pharmaceuticals, Tel-Aviv, Israel) described the M-001 fusion protein, made by fusing peptides corresponding to conserved linear epitopes from the HA, NP, and M proteins. M-001 was tested for safety in clinical trials and found to elicit a $CD4^+$ $IFN\gamma^+$ T cell response that primed efficiently for HAI Ab responses to TIV. It was suggested that M-001 could be used yearly as a primer to TIV vaccination in the elderly, or as a prepandemic primer to new pandemic vaccines. Baoying Huang (Chinese Center for Disease Control and Prevention, Beijing, China) reported that a NP-M2e fusion protein produced in *E coli* and administered with alum at a dose of a few μg was able to provide broad protection against lethal challenge in mice.

Emphasis was made on the need to develop consensus standard assays and reagents that would allow relevant comparisons between the different vaccine approaches. The comparison of candidate vaccines for effectiveness in human volunteers was considered highly desirable. Rather than costly and lengthy clinical efficacy trials, it was suggested that human challenge studies could provide a faster and more efficient approach. The duration of the Ab response elicited by various vaccines is also important, and immune memory is essential, yet more difficult to measure.

3. Live attenuated influenza vaccines and new approaches in vaccination

As reviewed by Alain Townsend (Oxford University, UK), live attenuated influenza vaccines (LAIV) are based on cold-adapted (*ca*) mutants. Intranasal LAIV provides cross-protective immunity with a moderate strain-specific Ab response but a strong T cell response, particularly in the lungs. Their safety and efficacy have been demonstrated even in young children with asthma [35]. The possibility of vaccine delivery by self-administration would enhance efficiency in mass vaccination [36].

The development of new LAIVs for viruses with pandemic potential was reported by Kanta Subbarao (NIAID, NIH, Bethseda MD, USA), Larisa Rudenko (Institute of Experimental

Medicine, St Petersburg, Russia) and Punnee Pitisuttithum (Mahidol University, Bangkok, Thailand), who described the results of clinical trials with A(H5N1), A(H7N3) and A(H5N2) LAIVs. The vaccines were well tolerated but moderately immunogenic, requiring two doses to elicit adequate Ab responses in the majority of subjects [37,38]. Administration of H5N1 LAIV primed for a rapid and robust neutralizing antibody response to an inactivated subunit H5N1 vaccine boost, resulting in a broader cross-reactive response against the different clades of A(H5N1). As discussed by Irina Isakova-Sivak (Institute of Experimental Medicine, St Petersburg, Russia) it should be possible to modify the LAIV master donor virus A/Leningrad/134/17/57 to improve further vaccine immunogenicity. Christopher Ambrose (MedImmune, Gaithersburg, MD, USA) reported that serum and nasal IgAs were the most sensitive measure of LAIV immunogenicity [39], but that heterogeneity in sampling nasal secretions, especially in young children, often hindered precise Ab level determination. Huan H Nguyen (International Vaccine Institute, Seoul, Korea) reported that administration of a A(H5N1) or A(H1N1) LAIV by the sub-lingual route also elicited mucosal and systemic antibody responses in mice and humans similar to those observed after intranasal vaccination.

Pamuk Bilsel (FluGen Inc, Madison, WI, USA) described a different LAIV, a non-replicating influenza virus (ΔM2) with deletion of the M2 gene. The virus could only replicate in Madin-Darby canine kidney (MDCK) cells engineered to constitutively express the M2 protein. Injection of ΔM2 A(H1N1) virus to mice elicited systemic, cellular and mucosal immunity and resulted in broad cross-protection against challenges with A(H3N2) or A(H5N1) viruses. Cross-protection experiments in ferrets are in progress.

A panel discussion on LAIVs concluded that the identification of immune correlates of protection for LAIVs and the development and standardization of corresponding assays are of high priority. The issue of LAIV prime followed by boost with another vaccine was also discussed, based on the hypothesis that using two divergent strains for priming and boosting might increase the breadth of the immune response.

A number of reports were made on the development of new influenza vaccines based on virus-like particles (VLPs) consisting of recombinant HA produced in plants or insect cells [40]. Nathalie Landry (Medicago, Quebec, Canada) described the expression of HA VLPs in tobacco plants using a recombinant *Agrobacterium* strain. A single dose of VLPs mixed with alum (5 μg HA for H1 VLPs or 20 μg for H5 VLPs) was shown to elicit seroconversion together with as cross-reactive cell-mediated immune responses including $CD8^+$ IFN-γ^+ T cells in approximately 60% of subjects. A 600 kg of plant material could yield 10 million doses of purified influenza HA VLP vaccine. Dr. Dominic Lam (Hong Kong Baptist University, Hong-Kong SAR, China) described the development of edible influenza vaccines derived from recombinant plants or *Lactobacilli*. Live recombinant *Lactococcus lactis* expressing H5 HA was formulated into mini-capsules for oral administration. Four doses of the vaccine conferred full protection to mice against a lethal A(H5N1) challenge [41]. A similar vaccine was developed against A(H9N2) influenza virus. Manon Cox (Protein Sciences Corporation, Meriden, CT, USA) described the production of the first recombinant trivalent influenza vaccine (Flublock™), which was recently licensed. The vaccine consists of purified HA produced in SF9 insect cells using a recombinant baculovirus vector.

The issue was discussed of possible allergenic side effects due to glycan molecules from either plant or insect cells in the recombinant vaccines, but no such side effect has been reported to date. Additional assays other than HAI test will be needed to fully validate the recombinant HA vaccines and large scale efficacy trials will likely be required.

Attempts were made at improving the immunogenicity of classical TIV in the elderly by increasing the dose of antigens in the vaccine. As reviewed by Robert Atmar (Baylor College of Medicine, Houston, TX, USA), a high-dose TIV containing 60 μg HA per influenza virus strain was successfully tested in adults 65 years of age or older and shown to elicit a significantly increased HAI titer, higher rates of seroconversion and achievement of HAI titers >40 [42,43]. A similar observation was made when injecting TIV by the intra-dermal (ID) route [44], as reported by Filipo Ansaldi [University of Genoa, Italy), who showed that HAI titer, rates of seroconversion and seroprotection were higher after ID than after IM vaccination in subjects aged 60 years or older [45]. Akira Ainai (National Institute of Infectious Diseases, Japan) reported that an inactivated, wholevirion vaccine without adjuvant administered to healthy adults at a dose of 45 μg HA by the intranasal route at 0 and 3 weeks induced a 44% HAI seroconversion rate and detectable HAI and neutralizing Abs in nasal washes. Another approach for improving TIV was reviewed by Timo Vesikari (University of Tampere School of Medicine, Tampere, Finland), who reported the development of quadrivalent inactivated vaccines with two influenza A and two influenza B viruses. This is deemed necessary in view of the co-circulation of the two influenza B lineages, B/Yamagata and B/Victoria, in different parts of the world, and the fact that there is little or no cross-protection between the two [46]. Several quadrivalent inactivated vaccines are being developed [47,48] and a live attenuated quadrivalent vaccine (Q/LAIV) has recently been licensed in the USA.

Finally, the advantage of growing influenza virus in cells other than embryonated eggs was reviewed by both Otfried Kistner (Baxter BioScience, Orth/Donau, Austria) for Vero cell–derived vaccines, and Theodore Tsai (Novartis Vaccines, Cambridge, MA, USA) for MDCK cell-derived vaccines. Numerous comparative clinical studies have demonstrated the safety, immunogenicity and efficacy of both the whole-virus vaccine prepared from Vero cells [49,50] and the TIV prepared from virus grown in MDCK cells [51]. A two-dose Vero cell-derived whole-virus A(H5N1) vaccine with 7.5 μg HA without adjuvant was previously shown to elicit a significant cross-clade neutralizing antibody response in humans [52,53].

4. The use of adjuvants and the safety of influenza vaccines

The immunological bases for using adjuvants in influenza vaccines was reviewed by Giuseppe Del Giudice (Novartis, Sienna, Italy), who outlined their beneficial impact on the immune response leading to antigen dose-sparing, better priming of immune memory including Th1 $CD4^+$ T cell responses, increased breadth of the Ab response, increased avidity of the Abs and enhanced effectiveness of the vaccine in young children and older adults, as demonstrated with the oil-in-water adjuvant MF59 [54–56]. Dr. Rebecca Cox (University of Bergen, Norway) described similar properties of the new Matrix-M adjuvant, which was successfully tested with a virosomal A(H5N1) vaccine [57–59].

Preclinical evaluation of two other new adjuvants were presented: cationic liposomes combined with a plasmid DNA (JVRS-100), which was used with an inactivated split A(H5N1) vaccine by Xiuhua Lu (CDC, Atlanta, GA, USA); and a synthetic TKPR tetrapeptide, tuftsin, which was fused to a branched M2e multiple peptide system [(M2E)4-Tuftsin], as described by Xiaoyu Liu (Institute for Viral Disease Control and Prevention, Beijing, China). JVRS-100 provided antigen-sparing, cross-clade Ab responses and cross-clade protection with enhanced Th1/IgG2a responses in mice. The (M2e)4-tuftsin vaccine showed promising results against PR8 challenge in mice.

The risk of adverse events following vaccination with adjuvanted influenza vaccines was reviewed by Janet Englund (Washington University, Seattle, WA, USA), Hanna Nohynek (Natl Institute of Health and Welfare, Helsinki, Finland), Susanna Esposito (University of Milan, Italy) and Katherine Donegan (Medicines and Healthcare Products, Regulatory Agency, London, UK). The risk of adverse events following vaccination for pregnant women and their fetus appears to be very low, as demonstrated in a number of studies [60–63]. Likewise, the safety of influenza vaccination has been demonstrated for children with chronic diseases, including asthma and respiratory disabilities [64].

Northern European countries, particularly Sweden and Finland, reported the occurrence of narcolepsy in children and adolescents 4–19 years of age after vaccination with Pandemrix™, an AS03-adjuvanted, A(H1N1)pdm 2009 vaccine. Narcolepsy is characterized by excessive diurnal sleepiness together with episodes of sudden loss of muscle control (cataplexy). The event was found to occur in 7 per 100,000 vaccinations. A specific HLA allele (HLA DQ B1 0602) in the vaccinees has been implicated but no formal explanation can be offered at this time. A recent study from the United Kingdom also reported the occurrence of narcolepsy after administration of Pandemrix™ [65].

Arnold Monto (University of Michigan, Ann Harbor, MI, USA), Janet Englund, and Ralf Wagner (Paul-Ehrlich Institut, Langen, Germany) discussed the vaccine effectiveness (VE) of current influenza vaccines. In general, VE among children was higher for LAIV (~80%) than for TIV (50–60%). The elderly show the highest rate of influenza-associated mortality (75–135 per 100,000) and the lowest VE (~30%). A household-based study showed that VE during the 2010–11 season was lower in those who had received influenza vaccine the previous year as compared with those who had not. Results from the US CDC influenza VE study network for the 2011–12 season showed a similar effect. Crude VE estimates for all influenza and among all ages was 32% in those who had received influenza vaccine the previous year compared with 62% among those who had not. Furthermore, adjusted VE was considerably lower (37%) against influenza A(H3N2) than against influenza H1 (60%) or B (64%) viruses.

Influenza VE in HIV seropositive individual was reviewed by Marta Nunes (Witwatersand, SA), who reported that influenza-associated mortality was much higher in HIV-infected than uninfected individuals [66,67]. Lower $CD4^+$ T cell counts appear to correlate with lower response rates to TIV. Doubling the dose of HA in the vaccine [68] and/or using two doses of TIV one month apart [69] increased the seroconversion rate, especially with the AS03- or MF59-adjuvanted vaccines. However, a lack of efficacy of a two-dose TIV regimen in

HIV positive young children was reported from South Africa, in part due to a drift of the circulating A(H3N2) virus [70]. A randomized, controlled Phase II trial was recently initiated to evaluate the safety and immunogenicity of TIV in HIV-infected pregnant women and their offspring.

Finally, Arnold Monto, on behalf of Joseph Bresee (CDC, Atlanta, GA, USA) provided highlights from the International Meeting on Influenza Vaccine Effectiveness hosted by WHO in December 2012. The meeting addressed the need for standardization of VE studies and for more observational VE studies in low- to mid-income countries. Factors such as standard of living, general hygiene and circulation of other viruses may all affect VE of influenza vaccines.

5. Concluding remarks

Vaccine strategies involving the M2 protein, the stalk domain of the hemagglutinin, or internal viral proteins have shown promise in animal models for the development of influenza vaccines that elicit broad, heterosubtypic protective immune responses. However, there remains limited information as to their potential in humans. The conduct of vaccine efficacy trials with these new vaccines remains a challenge that could be addressed, in part, by the use of human challenge studies, which could also provide insight on the identification of immune correlates of protection. Non-HA-based vaccine candidates will require novel standardized assays to measure vaccine immunogenicity and potency, such as quantitative assays to measure anti-HA2 broadly neutralizing Ab levels, M2e-dependent ADCC, mucosal immunity and/or T cell responses. Finally, it will be most important to measure the duration of immunity elicited by these new vaccines.

Regarding new approaches in influenza vaccination, the development of QIV represents the latest improvement but such vaccines still need to be formally tested for clinical efficacy. The use of high-dose inactivated vaccines for the elderly may also represent an important step forward, but data on their effectiveness is missing. An important domain which remains to be explored is that of the burden of disease in low-income, resource-poor countries and the study of VE in these countries. For example, poor TIV immunogenicity in HIV-infected children is a major challenge to be addressed [70].

The recent licensure of the first recombinant influenza vaccine may herald in a new generation of vaccines. Although much progress has been made toward the development of vaccines that provide broad-based protection against influenza virus infection, more work is still needed to determine whether an effective universal influenza vaccine is achievable.

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