INTRODUCTION

History and evolution of influenza vaccines

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Key words

Influenza • Vaccine • Adjuvants • Split vaccines

Summary

Since the isolation of influenza virus in 1933, a great deal of work was carried out in order to develop influenza vaccines and improve these fundamental tools of prevention in terms of production, quality control, safety and tolerability, and immunogenicity. The paper summarizes the cornerstones of the continuous evolution of influenza vaccines and the most recent and promising developments in this field.

Introduction

Research into the possibility of developing a vaccine against influenza began soon after the virus was isolated in 1933 [1]. In the following years, a great deal of work was carried out in order to achieve this objective, and in 1945 the first licence to produce a vaccine for civilian use was granted in the United States (U.S.), as described in the reviews by Francis and by Wood and Williams [2, 3]. This vaccine, which was prepared in anticipation of the 1945-46 influenza season, contained two viral strains: one type A (A/PR8/34) and one type B (B/Lee/40). These strains were cultivated on embryonated chicken eggs in accordance with the technique of Burnett [4], inactivated with formol, and purified and concentrated by means of erythrocyte adsorption/elution [5]. Erythrocyte agglutination testing was utilised in order to measure the quantity of antigen present in a dose, while immunogenicity was evaluated by means of the agglutination inhibition test [6]. The vaccine was authorised chiefly on the basis of a series of controlled clinical studies conducted by the U.S. Armed Forces, which documented its safety and effectiveness.
The interest of the U.S. military in developing an influenza vaccine stemmed from the experience of the 1918 influenza pandemic. Having broken out in North Carolina in March 1918, the pandemic swept through the troops of the American Expeditionary Force, who had been sent to support the Western allies in April of that year. The effect was devastating both for the soldiers and for the civilian populations to whom the virus was transmitted. For this reason, since 1946 the entire U.S. Army has been vaccinated against influenza.
The virus B epidemic of the winter of 1945-46 (the first year that the vaccine was used) provided further evidence of the efficacy of the vaccine [7]. However, in the winter of 1946-47, the new vaccine ran up against an obstacle: antigenic drift. Indeed, an antigenic variant of the strain A, named A/FM1/47, appeared in Australia and rapidly spread worldwide, reducing the protective efficacy of the vaccine to very low values [8-10]. This event not only prompted the U.S. Commission on Influenza to incorporate the new strain into the vaccine for the 1947-48 winter season, instead of the previous strain A/PR8/34, but also brought to light the problem of appropriate selection of the viral strains to be used in vaccines.

Selection of vaccine strains

Convincing evidence of the need to carefully select the strains for insertion into the influenza vaccine prompted the World Health Organization (WHO) in April 1957 to constitute a small committee to study the vaccine. It was established that the World Influenza Centre should coordinate the work of laboratories and spread appropriate information. The first centre to be set up was in London, followed, a few years later, by that of Bethesda. The centres worked together to establish a worldwide viral surveillance network, and by 1953, 54 centres in 42 countries were able to provide information on circulating viruses.
In the same year, the WHO also began informing governments as to the correct choice of vaccine strains. Recommendations regarding the northern hemisphere are still issued in the middle of February, while for more than 10 years, recommendations for the southern hemisphere have been issued in the middle of September. The strains recommended by the WHO are selected on the basis of their antigenic and genetic features.

Improvement in vaccine production

Antigenic drift was not the only obstacle to the diffusion of influenza vaccination. A very delicate step was that of the concentration/purification of the viral suspensions in

the first place. The erythrocyte adsorption/elusion technique did not allow large amounts of vaccine to be produced, while the other methods did not yield satisfactory results in terms of tolerability.
The introduction of the Sharple centrifuge for the clarification of the allantoid fluid, saccharose-gradient centrifugation and the availability of continuous-flow zonal centrifuges at the industrial level enabled highly purified and concentrated viral suspensions to be prepared [11-14].
Whole-virus vaccines proved to be well tolerated by adults and the elderly, but less so by children and young people. For this reason, attention was again turned to the research by Davenport et al. [15], which had shown that vaccines prepared with influenza virions split by means of ether and Tween80 caused fewer febrile reactions than whole-virus vaccines, while maintaining good immunising properties. This gave rise to the production of "split vaccines", which were authorised in the United States in 1968 and subsequently throughout the world. New splitting techniques were also developed and utilised by vaccine producers in various countries [16-18].
Numerous controlled clinical studies demonstrated that fragmentation of the virions did not impair the immunogenicity of the vaccine, while it did reduce reactogenicity, especially in young subjects. These studies, however, also revealed that the split vaccines were not as immunogenic as whole-virus vaccines in unprimed subjects.
A further step forward was the production of vaccines containing only viral haemagglutinin and neuraminidase and minimal traces of internal proteins [19-23]. These vaccines are well tolerated both by young children and by subjects who are sensitive to exogenous antigens, such as asthmatics. Nevertheless, like split vaccines, they are less immunogenic than whole-virus vaccines.
Another important practical advance was the development of a method of obtaining re-assortant viral strains with a high capacity to grow in embryonated chicken eggs [24]. This enabled larger amounts of vaccine to be produced in a shorter time, even though recently isolated viral strains have displayed scant ability to grow in eggs.

Improvement of vaccine standardization and quality control

The evolution of the methods of vaccine preparation also necessitated the development of new techniques for evaluating the potency of vaccines. Initially, potency was measured in Chicken Cell Agglutination (CCA) units, according to the erythrocyte agglutinating titre. This technique was further refined through the establishment by the WHO of an International Standard, which enabled the potency of a vaccine to be expressed in International Units.
The development of split vaccines and subunit vaccines, however, raised the need to work out a new method of comparative evaluation of the antigenic content of the various influenza vaccines. To this end, the radial single immunodiffusion test [25] was adopted, which enables the antigenic content of a vaccine to be expressed in micrograms of haemagglutinin. In Europe, the potency of inactivated vaccines for use in annual influenza prevention campaigns is currently measured also by determining the appearance or increase of agglutination-inhibiting antibodies following administration of the vaccine to volunteers in clinical trials.
Methods of assessing vaccine purity have also been steadily updated, and limits have been placed on the levels of ovalbumin and endotoxins.

Adjuvants

The idea of potentiating the immunogenicity of influenza vaccines through the addition of adjuvants can be traced back throughout the entire history of inactivated influenza vaccines. This need became particularly evident when controlled clinical studies clearly showed that the classical split vaccines were less efficacious not only in young children, but also, owing to a physiological mechanism of "immunosenescence", in subjects aged over 65 years, who account for a large portion of the population requiring priority protection.
In addition, since the early 2000s, the increasing expectation of a severe pandemic caused by a virus of avian origin has intensified research into adjuvants. The main advantages yielded by an appropriate adjuvant are:
antigen saving (i); enhanced immunogenicity of the vaccine in hyporesponsive subjects (ii); "broadened" immune responses, with protection also against drifted viral strains that are not present in the vaccine (probable cross-protection) (iii).
The key issue, which needs to be examined with great care and attention, is that of safety, in both the short and long term, in groups of individuals with different characteristics. The substances used and the experimental procedures adopted in order to achieve these objectives are too numerous to be listed here.
The first countries to authorise the use of adjuvated vaccines were in Europe; only recently they were licensed in the U.S. In Italy, the first adjuvants to be incorporated into the vaccines prepared for seasonal influenza prevention campaigns were MF59 and virosomes. The first controlled clinical trial involving MF59 was undertaken in 1992-93 by Chiron Vaccine (today Novartis) in collaboration with our institute, and was prolonged for three consecutive seasons [26]. Since then, several million elderly subjects have received doses of the vaccine, especially during the seasonal prevention campaigns; the results have been very satisfactory in terms of immunogenicity, tolerability and safety [27].
MF59 has been thoroughly investigated as an adjuvant to vaccines prepared against avian influenza, and has proved able both to potentiate immunogenicity and to induce cross-protection against moderately drifted strains of the virus A/H5N1. Finally, MF59 was used as an adjuvant in the vaccine (Focetria®) against the 2009-2010 pandemic caused by a virus of swine origin, the prototype of which is the strain A/California09/H1N1 [28].

The virosomal vaccine is a particular form of liposomal vaccine in which the surface glycoproteins of the virus are attached to both surfaces of the liposome [29]. A recent review by Gasparini et al. documents the very good tolerability and good immunogenicity of virosomal vaccines in various age-groups [30].
Other recent developments include:

- the availability of a vaccine made up of live attenuated viruses for intranasal administration (LAIV); this vaccine was authorised in the U.S. in 2003, and in Italy and Europe for the 2011-2012 season [31, 32];
- the availability, since the 2010-2011 season, of an inactivated vaccine for intradermal administration. The immunising dose, which is concentrated in a volume of 0.1ml, comes in a syringe-container which ensures release of the vaccine at the level of the dermis [33];
- the "reverse transcriptase" method of handling viral strains to be used as seeds for vaccine production. This is more complex than re-assortment, but requires less time to produce the seed virus [34, 35];
- the production of vaccine viruses in mammalian cell cultures [36-39].

Conclusions

No other vaccine has undergone the almost continuous evolution that influenza vaccines have seen. It therefore follows that studies which compare or elaborate the results of vaccination campaigns or trials carried out in different years must take into account the fact that the characteristics of vaccines differ, even considerably, from one year to another. This evolution has surely not come to an end, as can be deduced from the capacity for transformation displayed by influenza viruses, the availability of numerous reservoirs and the continuous development of technologies for the preparation of vaccines. Proof of this can be seen in the hundreds of publications on the subject which appear every year in the ongoing effort to develop vaccines that increasingly meet the needs of recipients.

References

[1] Smith W, Andrewes CH, Laidlaw PP. *A virus obtained from influenza patient.* Lancet 1933;ii:66-8.

[2] Francis T. *Vaccination against influenza.* Bull Who 1953;8:725-41.

[3] Wood JM, Williams MS. *Hystory of inactivated Influenza vaccines.* In: *Textbook of Influenza.* Oxford: Blackwell Scienze 1998, pp. 317-323.

[4] Burnett FM. *Influenza virus infection of the chick embryo lung.* Br J Exp Pathol 1940;21:147-53.

[5] McClelland L, Hare R. *The adsorption of influenza virus by red cells and a new in vitro method of measuring antibodies for influenza virus.* Can J Public Health 1941;32:530-8.

[6] Hirst GK. *Agglutination of red cells by allantoic fluid of chick embryos infected with influenza virus.* Science 1941;94:22-3.

[7] Francis T, Salk JE, Bruce WM. *The protective effect of vaccination against epidemic influenza.* B. J Am Med Assoc 1946:131:275-8.

[8] Francis T, Salk JE, Quilligan JJ. *Experience with vaccination against influenza in the spring of 1947.* Am J Public Health 1947;37:1013-6.

[9] Sartwell PE, Long AP. *Army experience with influenza 1946-47; epidemiological aspects.* Am J Hygiene 1948;47:135-41.

[10] Mellanby H, Dudgeon JA, Andrewes CH, et al. *Vaccination against influenza A.* Lancet 1948;I:978-82.

[11] Stanley WM. *The preparation and properties of influenza virus vaccines concentrated and purified by differential centrifugation.* J Exp Med 1945;81:193-218.

[12] Reimer CB, Baker RS, Newlin JE, et al. *Influenza virus purification with the zonal ultracentrifuge.* Science 1966;152:1379-81.

[13] Reimer CB, van Baker RS, Frank RM, et al. *Purification of large quantities of influenza virus by density gradient centrifugation.* J Virol 1967;1:1207-16.

[14] Mistretta AP, Crovari-Cuneo P, Giacometti G, et al. *Purification and concentration of influenza inactivated viruses by continuous-flow zonal centrifugation.* Boll Ist Sleroter Mllan 1975;54:45-56.

[15] Davenport FM, Hennessy AV, Brandon FM, et al. *Comparisons of serologic and febrile responses in humans to vaccination with influenza A viruses or their haemagglutinins.* J Lab Clin Med 1964;63:5-13.

[16] Webster RG, Laver WG. *Influenza virus sub-unit vaccines: immunogenicity and lack of toxicity for rabbits of ether and detergen-disrupted virus.* J Immunol 1966;96:596-605.

[17] Brandon FB, Cox F, Lease GO, et al. *Respiratory virus vaccines III. Some biological properties of sephadex-purified ether.extracted influenza virus antiges.* J Immunol 1967;98:800-5.

[18] Neurath AR, Rubin BA, Sillaman J, et al. *The effect of non-aqueous solvents on the quarternary structure of viruses: a procedure for the simultaneous concentration, purification and disruption of influenza viruses.* Microbios 1971;4:145-50.

[19] Lever WG, Valentine RC. *Morphology of the isolated haemagglutinin and neuraminidase subunit vaccine.* Virology 1969;96:511-22.

[20] Bachmayer H. *Selective solubilization of haemagglutinin and neuraminidase: from influenza virus.* Intervirology 1975;5:260-72.

[21] Lever WG, Webster RG. *Preparation and immunogenicity of an influenza virus haemagglutinin and neauraminidase subunit vaccine.* Virology 1976;69:511-22.

[22] Brady MI, Furminger IGS. *A surface antigen influenza vaccine. 1. Purification of haemagglutinin and neuraminidase.* J Hygiene 1976;77:161-72.

[23] Brady MI, Furminger IGS. *A surface antigen influenza vaccine. 2. Pyrogenicity and antigenicity.* J Hygiene 1976;77:173-80.

[24] Kilbourne ED. *Future influenza vaccines and use of genetic recombinants.* Bull WHO 1969;41:643-5.

[25] Schild GC, Wood JM, Newman RW. *A single radial immunodiffusion technique for the assay of haemagglutinin antigen.* Bull WHO 1975;52:223-30.

[26] Minutello M, Senatore F, Cecchinelli G, et al. *Safety and immunogenicity of an inactivated subunit influenza virus vaccine combined with MF59 adjuvant emulsion in elderly subjects, immunized for three consecutive influenza Seasons.* Vaccine 1999;17:99-104.

[27] Schultze V, D'Agosto V, Wack A, et al. *Safety of MF59 adjiuvant.* Vaccine 2008;26:3209-3222.

[28] Clark TW, Pareek M, Hoschler K, et al. *Trial of Influenza A (H1N1) 2009 Monovalent MF59-Adjuvanted Vaccine.* N Eng J Med 2009;361:2424-35.

[29] Glück R, Mischler R, Finkel B, et al. *Immunogenicity of new virosome influenza vaccine in elderly people.* Lancet 1994;344:160-3.

[30] Gasparini R, Lai P. *Utility of virosomal adjuvated influenza vaccines: a review of the literature.* J Prev Med Hyg 2010;51:1-6.

[31] Food and Drug Administration (FDA). *Approval Letter - Influenza Virus Vaccine Live, Intranasal. June 17, 2003.* Available at http://www.fda.gov/BiologicsBloodVaccines/Vaccines/ApprovedProducts/ucm123753.htm (Last accessed 31st August 2011).

[32] European Medicines Agency (EMA). *European public assessment reports – Fluenz. Authorisations details.* Available at http://www.ema.europa.eu/ema/index.jsp?curl=pages/medicines/human/medicines/001101/human_med_001405.jsp&mid=WC0b01ac058001d125&murl=menus/medicines/medicines.jsp (Last accessed 31st August 2011).

[33] European Medicines Agency (EMA). *European public assessment reports – Intanza. Authorisations details.* Available at http://www.ema.europa.eu/ema/index.jsp?curl=pages/medicines/human/medicines/000957/human_med_000842.jsp&mid=WC0b01ac058001d125&murl=menus/medicines/medicines.jsp (Last accessed 31st August 2011).

[34] Lee CW, Senne DA, Suarez DL. *Generation of reassortant influenza vaccines by reverse genetics that allows utilization of a DIVA (Differentiating infecter from Vaccinated Animals) Strate the control of avian Influenza.* Vaccine 2004;22:3175-81.

[35] Robertson JS, Engelhardt OG. *Developing vaccines to combat pandemic influenza.* Viruses 2010;2:532-46.

[36] Tree JA, Richardeon C, Fooks AR, et al. *Comparison of large-scale mammalian cell culture systems with egg culture for the production of Influenza virus A vaccine astrains.* Vaccine 2001;19:3444-50.

[37] Halperin SA, Smith B, Mabrouk T, et al. *Safety and Immunogenicity of a trivalent, Inactivated, mammalian cell culture-derived influenza vaccine in healthy adults, seniors and children.* Vaccine 2002;20:1240-7.

[38] CPMP/BWP/2490/00: Cell culture inactivated influenza vaccines. Annex to Note for Guidance on Harmonisation of Requirements for Influenza Vaccines (CPMP/BWP/214/96). Issued January 2002. Available at http://www.ema.europa.eu/docs/en_GB/document_library/Scientific_guideline/2009/09/WC500003877.pdf (Last accessed 31st August 2011).

[39] Perdue ML, Arnold F, Li S, et al. *The future of cell culture-based influenza vaccine production.* Expert Rev Vaccines 2011;10:1183-94.

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