INTRODUCTION

History and evolution of influenza vaccines

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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 tech-
nique did not allow large amounts of vaccine to be pro-
duced, while the other methods did not yield satisfactory
results in terms of tolerability.

The introduction of the Sharpie centrifuge for the clarifi-
cation of the allantoid fluid, saccharose-gradient centrifugation and the availability of continuous-flow zonal cen-
trifuges 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 vac-
cine producers in various countries [16-18].
Numerous controlled clinical studies demonstrated that
fragmentation of the virions did not impair the immuno-
geticity of the vaccine, while it did reduce reactivity,
especially in young subjects. These studies, however,
also revealed that the split vaccines were not as immuno-
getic 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 develop-
ment 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 iso-
lated 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 establish-
ment by the WHO of an International Standard, which
enabled the potency of a vaccine to be expressed in In-
ternational 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

Adjuvants

The idea of potentiating the immunogenicity of influ-
enza vaccines through the addition of adjuvants can be
traced back throughout the entire history of inactivated
influenza vaccines. This need became particularly evi-
dent 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 expec-
tation 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 char-
acteristics. 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 vac-
cines were in Europe; only recently they were licensed
in the U.S. In Italy, the first adjuvants to be incorpo-
rated into the vaccines prepared for seasonal influenza
prevention campaigns were MF59 and virosomes. The
first controlled clinical trial involving MF59 was under-
taken in 1992-93 by Chiron Vaccine (today Novartis) in
collaboration with our institute, and was prolonged for
three consecutive seasons [26]. Since then, several mil-
lion elderly subjects have received doses of the vaccine,
especially during the seasonal prevention campaigns;
the results have been very satisfactory in terms of im-
munogenicity, 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 in-
duce cross-protection against moderately drifted strains
of the virus A/H5N1. Finally, MF59 was used as an ad-
juvant in the vaccine (Focetria®) against the 2009-2010
pandemic caused by a virus of swine origin, the proto-
type 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-arrangement, 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


[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.


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