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Influenza Vaccination

Grippeschutzimpfung

Influenza places a heavy burden on society. Distress of the community resulting from the disease translates into difficulties in family management as well as absence from work, school and social work. Moreover, there is still uncertainty in the current knowledge of anti-influenza immunity, even though, thanks to advances in molecular biology, the structure, chemistry and genetics of the virus are by now almost completely known. The greatest difficulty of the vaccine lies in the great variability of the influenza virus. The A and B influenza viruses are the most important ones. The A viruses include several subtypes, H3N2 and H1N1 being presently the most important ones. The present vaccine, therefore, must be updated every year with strains that have the greatest probability of spreading in the human population during the influenza season. New influenza vaccines based on molecular biotechnology, such as DNA-recombinant or naked DNA vaccines, are currently widely studied and represent the vaccines that, hopefully, will bring about important improvements in the near future.
Keywords: Influenza, vaccination

Die Grippeerkrankung stellt eine schwere Belastung für die Gesellschaft dar. Sie bewirkt ein kollektives Leiden, das sich in Schwierigkeiten des familiären Managements, in Arbeits- und Schulausfällen sowie in Beeinträchtigungen der Freiwilligenarbeit abzeichnet. Überdies sind die Kenntnisse der Grippeabwehrkräfte bisher noch nicht vollständig, auch wenn, Dank der Molekularbiologie, der Aufbau, die chemische Zusammensetzung und die Genetik des Grippevirus weitgehend bekannt sind. Die größte Schwierigkeit in der Entwicklung eines Impfstoffs wird durch die Variabilität des Grippevirus hervorgerufen. Die wichtigsten Grippeviren sind A und B, wobei der Influenza-Virus A sich in weitere Subtypen aufgeteilt, von denen H3N2 und H1N1 heute am bedeutendsten sind. Der gegenwärtige Grippeimpfstoff wird jährlich mit den Subtypen aktualisiert, die die größte Wahrscheinlichkeit aufweisen, in der Bevölkerung während der folgenden Grippesaison aufzutreten. Neue Grippeimpfstoffe werden zurzeit auf der Basis der Molekularbiologie, als rekombinierte DNA und als nackte DNA Impfstoffe entwickelt und repräsentieren die Impfstoffe, die hoffentlich wichtige Verbesserungen für die nahe Zukunft bringen werden.
Stichworte: Grippe, Schutzimpfung

Introduction

In the United States influenza causes more than 100,000 hospitalisations and an average of 20,000 deaths per year (NPI, 2002). It is envisaged that during the 2002/03 season in the USA approximately 114,000 patients will develop serious complications, such as pneumonia (Treanor 2002). In Italy, at the height of the 2001/02 winter epidemic (lasting 8 weeks) the cases, as estimated by the method of sentinel paediatrician and physicians, were 2,610,611, with a sizeable damage of more than 1,300,000 Euros (Gasparini in press). The sever-

ity of the disease is greater at extreme ages. In the elderly, because of an often precarious state of health, the disease may well worsen some of the underlying pathologies, especially those related to the respiratory and cardiovascular system.

The social distress resulting from the disease translates into difficulties in family management as well as absence from work, school and social work. During the epidemics many public utility services, like hospitals, schools and transport are subject to serious consequences (inefficiency, reduced performance by staff on duty, etc).

The best way to fight the disease is through vaccination. Unfortunately, the complex biology of the virus does not allow the preparation of a constantly effective vaccine. Moreover, there is still some uncertainty on anti-influenza immunity facts. The greatest difficulty of the vaccine lies in the great variability of the influenza virus. It is in fact well known that there are 3 types of influenza viruses, A, B and C. From an epidemiological viewpoint the A and B viruses are the most important ones. The A viruses include several subtypes, H3N2 and H1N1 being currently the most important ones. The B viruses and the A virus subtypes undergo minor variations, which make the viral population very diversified, even though only some strains are actually prevalent in the population. One hundred and ten (110) peripheral surveillance centres in all the continents carry out the current updating of predominant viral strains world-wide.

Historically, the know-how for the preparation of influenza vaccines became a scientific asset ever since Smith was able to isolate the virus (Smith 1935) in embryonated chicken eggs. Since then vaccines have been constantly improved up to the current split and subunit vaccines. The latter are the ones currently used in most industrialised countries, including Italy.

Since the viral reservoir is not exclusively human, the vaccination strategies adopted during the last 50 years aim at controlling the disease, especially in at-risk subjects, such as the elderly. Japan is the only country to have adopted an extensive vaccination strategy. New influenza vaccines based on molecular biotechnology are being currently widely studied.

Biological and antigenic characteristics of the influenza virus

Under the electron microscope the influenza virus reveals a slight pleiomorphism. The most typical shape is that of a chestnut husk. Two types of spicules emerge from the viral envelope. They are responsible for the haemagglutination activity (recognition of the specific receptor on the respiratory mucosal cells) and neuroaminidase activity (which promotes the liberation of the virus after its replication). The virus has a helical structure and its genome is located on 8 RNA segments.

The virus adapts well to its hosts, which are many: man, swine, bird, etc. In fact, from one viral generation to the next the rate of spontaneous mutation is very high (Kinnunen et al. 1992). Furthermore, animal and human A viruses have the opportunity to form hybrids among them. According to Scholtissek et al. (1983) the *in vivo* hybridisation phenomenon occurs in swine, particularly in China, where these animals live in very close conditions with poultry (ducks) and man.

The strains that have undergone modifications constitute the viral populations circulating in the human population. Many different strains circulate within the child population whereas within adult and elderly populations, which have experience of previous contacts, only some viruses have the possibility to circulate.

Anti-influenza immunity

These are the immune mechanisms protecting from influenza: innate and adaptive (humoral and cellular) immunity. The most important viral antigens are: haemagglutinin, neuroaminidase, the membrane protein M2 and the nucleoprotein. The antibodies against these antigens perform different functions: for example, neutralisation, antibody dependent cell-mediated cytotoxicity (ADCC), etc. The antibodies against H aggregate the virus, prevent its linkage to the receptor and preclude its fusion with the cellular membrane. Antibodies against neuroaminidase show partial protection in man while those against the M2 protein have been shown to reduce the numerousness of virions in the experimentally infected animal model.

The cellular immunity mechanisms are especially mediated by T CD8 and CD4 lymphocytes. The former kill the infected epithelial cells whereas the latter promote the T-cell-dependent response as well as the antibody response. The mucosal immunity also carries out an important role in protecting against infection. It is mainly a function of secretory IgA from the upper respiratory tract. Both infection and nasal vaccines stimulate the production of these antibodies (Treanor 2002).

Past, present and future of vaccines

The first influenza vaccines were prepared after Smith in 1935 managed to culture the virus in embryonated chicken eggs. Subsequently much remarkable advancement was achieved. The crude extraction and purification techniques of the initial vaccines meant that a certain amount of egg proteins were administered with the actual vaccine.

During the Seventies, the use of more sophisticated techniques, such as saccharose gradient and continuous flow ultracentrifugation, allowed to reduce the amount of protein nitrogen / pro dose below 50 μg. However, children and young people sometimes still poorly tolerated the whole vaccine. Especially after 1976, when the H1N1 subtype of the A virus (A/New Jersey/76) was isolated and, after extensive vaccination imposed by President Carter, the split and subunit vaccines were definitely optimised.

In the winter of 1978-79 it became necessary to introduce the trivalent vaccines. During the Eighties a new standard was introduced. We went from the International Haemagglutination Units to the international haemoagglutinin standard, with the immunodiffusion dosage. In the winter of 1993-94 the antigenic content pro dose was increased and the European quality control on vaccines became effective. During the second half of the Nineties, vaccines strengthened by oil-water emulsions and by virosomes were authorised in Italy. The latter represented the answer to the need for better protection for the most important category of at-risk subjects, namely the elderly, who show the well-known phenomenon of immuno-senescence (Saririan et al. 1993). In this per-

spective, other substances with adjuvant action have been studied, such as: the cholera toxin-B (Tamura 1992), the heat labile enterotoxin from *Escherichia coli* (Katz 1992), etc. Other researchers (Moldoveanu et al. 1992) have studied an anti-influenza vaccine characterised by slow antigen release from microspheres of lactic and glycolic acid polymers.

More recently, researchers have tried to strengthen the action of vaccines through the simultaneous administration of cytokines (interferon b and g, interleukin 2, etc.) (Cao et al. 1992; Provinciali et al. 1994; Mc Elhaney et al. 1996). Arulanandam et al. (1999) studied on the murine model a soluble anti-influenza vaccine combined with interleukin 12. The following year the same authors also showed the importance of interleukin 12 administration to newborn mice in amplifying the response to vaccines in general. Hagiwara et al. (1999) experimented in the animal model some anti-influenza vaccines containing as adjuvant different non-toxic mutants of cholera toxin administered intranasally. The results obtained by these authors showed good tolerability and immunogenicity (with the induction of satisfactory levels of secretory IgA [SIgA] in the mucosa).

The research area of intranasal anti-influenza vaccines is currently the object of specific in-depth studies. Thus Muszkat et al. (2000) tested an inactive intranasal vaccine in elderly subjects and observed the secretion of SIgA in 50% of those receiving it compared with 20% of the subjects being administered the intramuscular vaccine.

The American National Institute of Allergy and Infections Disease (NIAID) currently funds many studies on intranasal spray vaccines containing live attenuated viruses. As part of related projects, during a study carried out in 1997-98 (Belshe et al. 2000) in children, the vaccine showed high levels of efficacy even against heterologous strains (A/Sidney-like). The study of intranasal anti-influenza vaccines containing live cold-adapted attenuated viruses represents a line of development of anti-influenza vaccines that has been pursued for the last 25 years. Nichol & Goodman (1999) studied their effects against placebo by intranasal administration in 3041 healthy adults (18-64 years). Treanor et al. (1999) compared it to the conventional vaccine. Such vaccines seem to be very suitable for children, who are most affected during epidemics and who often present important complications, such as otitis media. Furthermore, the non-parenteral administration should increase compliance for this vaccine at all ages.

These are some of the other research lines being currently pursued:

- –) Preparation of permissive vaccines containing only neuroaminidase (Johansson/Kilburne 1991), which could be particularly useful during an epidemic.
- –) Use of substrates such as *in vitro* cell cultures for virus amplification instead of embryonated chicken eggs (Kistner et al. 1999);
- –) Optimisation of anti-influenza vaccines that give lasting protection through the identification of stable epitopes, invariable or slightly variable areas, of glycoproteins on the surface of the influenza virus, (Ben-Yedidie et al. 1999).

Finally, we should pay special attention to future vaccines, which can be obtained with molecular biotechnology. We have already mentioned the epitopes of surface glycoproteins, which can be "constructed" with DNA-recombinant

techniques. Recently Neirynck et al. (1999) have isolated a gene coding for the M2 protein, almost stable in all A-type influenza viruses, and for the core antigen of the hepatitis-B virus (HBcAg) obtaining excellent results in the protection spectrum of the murine model. Johansson (1999) using gene recombinant methodologies studied and obtained the expression of haemagglutinins and neuroaminidases of the A/Nanchang/933/95 virus through recombinant baculoviruses infecting insect cell lines.

Other researchers (Roberts et al. 1999) studied, in the animal model, recombinant viral vectors, such as the vesicular stomatitis virus (VSV), obtaining good protection results. Finally, there is a proliferation of animal studies on DNA vaccines. These studies entail the intramuscular administration, actually by "shooting" them over the cutaneous surface, of plasmids in which genes or microgenes of the influenza virus have been inserted. Thus the cells of the recipient animal express these genes and induce the onset of immunity. They seem to be very effective for influenza vaccination in different animal models such as mice (Iwasaki et al. 1999) and horses (Lunn et al. 1999). The usefulness of naked DNA vaccines is, however, controversial, as there are many doubts on their potential efficacy in man (Gluek 2003)

Vaccination strategies

Vaccines currently used in the western world are the split or subunit types. Most of the industries that produce vaccines have or are in the process of discontinuing the production of inactivated whole vaccines. In split vaccines there is the virus broken down by surface-active agents and only some whole viral parts. In subunit vaccines only the surface glycoproteins are present. These subviral products are both immunogenic and well tolerated by both children and asthmatics.

Ever since the first vaccines became available, production and purification problems oriented Public Health Services towards targeted vaccinations. Today countries like the USA, Canada, Germany England, France and Italy encourage the vaccination of at-risk subjects like the elderly, nursing home residents, adults and children suffering from chronic diseases of the cardiovascular and respiratory system, subjects with metabolic diseases, renal dysfunctions, haemoglobinopathy. After careful evaluation of each case, immunisation is also advisable for people suffering from an altered production of antibodies. Vaccination is recommended for children under treatment with salicylates and who are thus at risk of Reye's syndrome. Vaccination is also recommended for pregnant women (2nd-3rd month) during winter. Furthermore, vaccination is strongly recommended for those people who may transmit the viral infection to high-risk subjects (such as general healthcare and nursing home personnel). To decrease the socio-economic impact of the disease, it would also be useful to vaccinate all subjects working in public services. The dosage includes the administration of two reduced doses (0.25 ml) at 4 weeks' interval for children from 6 to 35 months; two doses (0.5 ml) at the same interval for children up to 8 years and a single dose (0.5 ml) for subjects who are 9 years and older (American Academy of Paediatrics 2000).

Most industrialised countries offer vaccination to all those subjects that are at risk of serious complications and, during the last few years, have encouraged vaccination particularly in elderly subjects.

Relatively recent estimates (Ambrosch 1999) seem to indicate that 70% of the subjects aged >70 years are vaccinated annually in France and that 50% of the people aged >65 years are vaccinated every autumn in Belgium. In the USA, in the year 2000, 64.9% of the people were vaccinated (CDC, 2002). In Italy, the vaccination coverage of these at-risk subjects was greatly encouraged, so much so that it went from an average of 30% in 1995 to rates of approximately 50% for the 2000-01 season (Vellucci et al. 2001).

Japan is the only country that experimented with a different strategy by adopting a mass immunisation program in school children during the 1962-1994 period. Only after the interruption of this extensive vaccination program it became obvious how sharply it had affected the mortality rate for respiratory diseases in the elderly (Reichert et al. 2001; Sugaya 2002).

In fact, as Francis (1967) had hypothesised as far back as 1967, as the studies carried out by Tecumsech and Adrian in 1969 (Beveridge 1982) and as we have also recently observed (Ansaldi et al. in press), children are the prime targets of the virus; they then infect other family members, including the elderly. During a study that we carried out on approximately 4,000 subjects aged 0-14 years, we noted that many strains of influenza viruses circulate simultaneously in children. It is then probable that they are extremely important for diversified influenza strains, of which only one part will have the possibility to circulate among adults and the elderly by virtue of their immunity experience (Gasparini in press).

Recently (CDC 2002), the Advisory Committee on Immunization Practices (ACIP) has recognised the usefulness of recommending influenza vaccination even for children aged 6-23 months.

Obviously, vaccination strategies should be based on health economics studies. These types of studies have been carried out in several countries. For instance, during a survey carried out in Italy we were able to estimate that the net economic benefit for each vaccinated elderly person is 110.20 Euros (Gasparini 2002). Similar studies carried out in the USA by Nichol et al. (1999) showed variable estimates of the net economic benefit for each vaccinated elderly person from 39.11 to 88.30 Euros.

Vaccination against influenza represents, therefore, not only a particularly important health intervention but also a considerable economic benefit.

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