A new look at influenza vaccination

Since the first influenza vaccine was licensed over 50 years ago, vaccination has consistently demonstrated its health benefits in preventing influenza and influenza vaccination is playing an increasingly important role in public health programmes in many countries. Individuals for whom vaccination is most commonly recommended include elderly persons over 65 years of age, persons with high risk medical conditions and children.

The increase in acceptance of influenza vaccination serves to highlight the challenges that must be met to provide adequate annual supplies. Two types of influenza viruses, Influenza A and B, cause epidemic human disease. Influenza viruses vary on the basis of two surface antigens, haemagglutinin (HA) and neuraminidase (NA). Immunity to the surface antigens, particularly HA, reduces the likelihood of infection and severity of disease if infection occurs. However, antibody against one influenza virus type or subtype confers limited or no protection against another type or subtype. Frequent emergence of antigenic variants through antigenic drift is the virologic basis for seasonal epidemics.

Every year the available vaccines are reformulated to contain three virus strains selected following consideration of the most recent epidemiologic surveillance data. Recommendations for the antigenic composition of influenza vaccines, for both the northern and the southern hemispheres, are issued twice a year by the World Health Organization (WHO). Reference strains of influenza viruses, obtained from original isolates by the WHO surveillance network, are provided to vaccine manufacturers in March for the northern hemisphere and in September for the southern hemisphere. Manufacturers then have only 6 months to produce and license the new season’s trivalent influenza vaccines and distribute them to health care providers. Timing the delivery of vaccines is crucial because annual vaccination programmes are conducted during the few months preceding the influenza season.

It is therefore not surprising that ongoing influenza vaccine developments are aimed at continuing improvement of vaccine effectiveness. The continually changing antigenic nature of influenza through ‘drift’ or ‘shift’ support the need for vaccines that provide broader cross-protection. This paper provides a brief summary of technical developments to increase vaccine effectiveness.

Improving efficacy

Currently available trivalent inactivated vaccines (TIV) are safe and effective in preventing influenza. Studies have demonstrated clinical efficacy is between 70-90% for laboratory confirmed influenza in healthy adults, 60-90% in children and 50-60% in the community dwelling elderly. Clinical effectiveness data show, amongst other clinical endpoints, that influenza vaccines reduce all cause mortality by 50% in the community dwelling elderly. However, three factors point to the need for vaccines that provide greater efficacy within strains and also across strains, and also highlight the need for vaccines that can stimulate both systemic and local immune responses and ultimately both humoral and cell-mediated immunity:

- Immunosenescence, an age-related decline in immune competence in the elderly, can lead to a diminished immune response to vaccination (and infection).
- Two doses of vaccine are needed to promote a protective immune response in immunologically naive subjects, especially the very young individuals that are receiving the vaccine for the very first time.
- Constant antigenic ‘drift’ (and more dramatically ‘shift’) necessitate annual matching of antigens in TIV with those of circulating strains.

TIV types

Adjuvants

Adjuvants are not routinely used in most current TIV. However, adjuvant use may potentially optimise the immune response, especially in the naïve population or the known lower responders. Aluminium is the most commonly used and well studied adjuvant. Recent human trials of vaccines against various non-circulating human and avian strains (H2N2, H9N2, H5N1) have shown enhanced immunogenicity with alum adjuvants. MF59, a proprietary oil-in-water emulsion adjuvant, has also shown promise in enhancing immunogenicity in these immunologically naïve individuals.

Immune stimulating complexes (ISCOMS), combinations of the immunogen with phospholipids, cholesterol and saponin that form a cage-like molecular structure, have been shown to both promote antibody responses to the immunogen and also to introduce antigens into the major histocompatibility complex (MHC) class 1 presentation pathway, inducing CD8+ cytotoxic lymphocyte (CTL) activity. Other adjuvant developments include derivatives from bacterial toxins such as cholera toxin and Escherichia coli heat-labile toxin (LT) and outer membrane protein of Neisseria meningitidis for mucosal use, and cytokines or synthetic immunostimulants.

Virosomes

Virosomes are virus-like particles that consist of reconstituted influenza viral envelope proteins, basically HA and NA. Virosomes retain the virus to cell binding and membrane fusion capacities but lack internal genetic material. Although...
theoretically carrying adjuvantage properties (e.g. priming for CTL seen in animal models), they have not shown so far improved immune response as compared to TIV vaccines in humans. A licensed virosome influenza vaccine is presently available in Europe as LAIV.

**Live attenuated influenza vaccine**

Live attenuated influenza vaccine (LAIV) is produced by reassembly of a temperature sensitive, cold-adapted (ca) master strain with the epidemic wild-type virus. The reassortant carries the HA and NA genes from the epidemic virus and the remaining six non-surface genes from the master strain. Replication of the ca virus occurs in the upper respiratory tract, but is inhibited at core body temperature, i.e. 37°C as in the lungs. Mucosal delivery of LAIV induces both local mucosal immunity and cell-mediated responses and may provide greater hetero-subtypic immunity than is seen with inactivated vaccines. LAIV is registered in the US for use in healthy individuals 5-49 years of age.

**DNA vaccination**

The DNA vaccination principle consists of the in situ synthesis of protein antigens of interest (e.g. HA, NA, M1) after the introduction of the plasmid DNA vaccines coding for these proteins into host cells. The subsequent expression of these antigens on the host cell surface elicits both a humoral and cellular immune response. Response to homologous and hetero-subtypic challenge with influenza virus has been promising in various animal models, but immunogenicity, efficacy and safety are yet to be demonstrated in human subjects.

**Conserved viral antigens**

The idea of a 'universal vaccine' for influenza has focussed on utilising conserved viral antigens such as the M2 protein. M2 forms an ion channel across the membrane of a virus particle or infected cell. The extracellular portion of M2 (M2e) comprises 23 amino acids that show little variation between strains. Pre-clinical vaccines have been developed using M2e fused to the hepatitis B core protein, and also using M1 and NP in the form of DNA vaccines.

**Alternative routes of administration**

Alternative routes of administration to traditional intramuscular (IM) injection have been also investigated. These other routes aim at delivering antigen directly to dendritic cells by intradermal or epidermal routes, or directly to the nasal mucosa by inhalation. Intradermal injection practice is well established for BCG and used in some countries for rabies post-exposure treatment.

Recent studies have shown that in tradermal administration of influenza vaccine could induce similar antibody responses with smaller doses of antigen. Alternatively, nasal administration of influenza vaccine using either LAIV (described above) or inactivated adjuvanted vaccines have been shown to induce both serum antibody levels and local IgA responses. The adjuvants tested have included Viessera meningitidis OMP, and E. coli heat labile toxin (subsequently withdrawn due to an association with Bell’s palsy). None of these approaches is currently licensed.

**Alternative production techniques**

Present egg-based production involves the growth of the vaccine viruses in the allantoic cavity of embryonated chicken eggs and subsequent harvesting, inactivation, purification and splitting of the virus using ether and/or non-ionic detergent. Current global production capacity of around 300 million doses annually has evolved to meet the increasing demand.

However, the existing capacity falls far short of what would be required in case of a pandemic. This critical issue, in addition to the potential vulnerability of egg supply, has led the WHO to recognise the potential of cell-culture systems as a supplement or alternative to traditional egg-based systems. However, no cell culture influenza vaccine has been commercialised yet and egg-based production remains the best understood, proven method of influenza vaccine manufacture.

**Mammalian cell culture**

Mammalian cell culture systems include Vero (African Green monkey kidney), MDCK (Madin Darby canine kidney) and PerC6 (human) continuous cell lines as alternatives to eggs for virus vaccine production. While vero cell culture has advantages of viral growth in a closed bioreactor system, and a secure supply of host cells, the problem of adaptation of the influenza flu virus to growth in the cultured cells remains. There is a long history of Vero cell use in production of both polio virus and rabies vaccines.

All the major producers of influenza vaccine are actively conducting research in influenza vaccine production in mammalian cell culture. Currently two influenza vaccines in Vero and MDCK cells respectively are under development in Europe.

**Conclusion**

Increasing acceptance of the benefits of vaccination in annual epidemics combined with the threat of pandemic influenza have clearly accelerated research and development for improved influenza vaccines that are safe, and potentially of greater effectiveness, including enhanced hetero-subtypic immunity.

**References**