Chapter 6
Influenza in the Elderly

Caterina Hatzifoti and Andrew William Heath

Introduction

Influenza is a highly contagious upper respiratory tract disease caused by the influenza (flu) viruses types A, B, and C. Worldwide, 20% of children and 5% of adults develop symptomatic infections due to influenza A or B viruses each year.[1] The virus causes asymptomatic disease as well as lung, brain, heart, kidney, and muscle disorders and predisposes patients of all age groups to bacterial pneumonia. Illness development depends on the patient's age, pre-existing immunity, immune competence, virus properties, smoking, and pregnancy. Rates of serious infection and death are highest among people aged >65 years and people with serious medical conditions. The virus is most commonly spread among humans by respiratory droplets containing virus via coughing and sneezing but can sometimes also be transmitted directly to humans by avian or swine species.

Virology and Epidemiology

Flu virus is a member of the family Orthomyxoviridae and there are three types of the virus A, B, and C, but only the first two cause widespread outbreaks. All types of influenza viruses have segmented genomes (eight single-stranded segments of RNA) enclosed within a lipid envelope derived from the host cell membrane (Fig. 6.1) and show great antigenic diversity, mainly resulting from single but accumulating nucleotide changes, known as *antigenic drift*. Mutation rates in RNA viruses such as influenza viruses and HIV are much higher than in eukaryotes or DNA viruses owing to the lack of repair mechanisms for RNA that exist for DNA replication.

Changes that replace entire genes through reassortment of RNA fragments in a cell infected with two or more virus strains, a process called *antigenic shift*, are less common, but can have a dramatic effect in enabling complete viral evasion of the immune system (Fig. 6.2). Antigenic shift often results in worldwide epidemics, or pandemics such as those that occurred in 1957 and 1968. Detailed molecular

C. Hatzifoti and A.W. Heath
University of Sheffield Medical School, Beech Hill Road, Sheffield, UK

S.L. Percival (ed.), *Microbiology and Aging*.
DOI: 10.1007/978-1-59745-327-1_6,

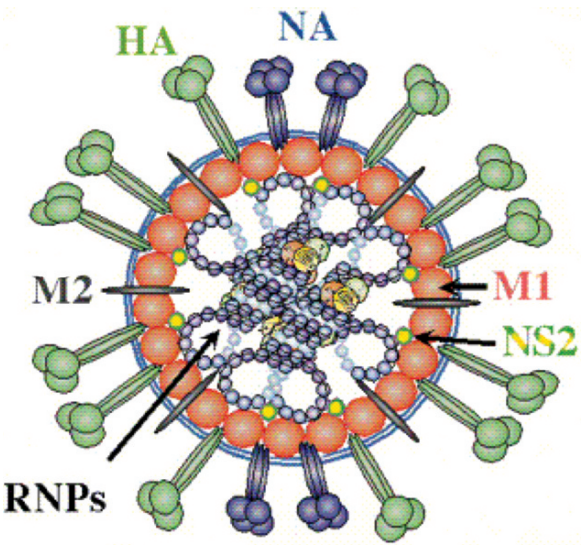

Fig. 6.1 Schematic structure presentation of an Influenza virus particle. (Courtesy of Dr. Paul Digard, Pathology Department, University of Cambridge.) The outer surface consists of a lipid envelope consisting of glycoprotein spikes of two types, hemagglutinin (HA) and neuraminidase (NA). The inner side of the envelope is lined by the matrix protein and the genome segments are packaged into the ribonucleoprotein (RNP) core (See Color Plates)

analysis of different flu strains is important for comprehending the evolution of influenza pandemic viruses.

The two surface glycoprotein antigens, hemagglutinin and neuraminidase (Fig. 6.1), are involved in flu virus attachment and pathogenesis, and it is largely changes in these surface antigens through antigenic drift or shift that allow a new viral strain to evade pre-existing immunity. Influenza B viruses have only 1 subtype of hemagglutinin and 1 type of neuraminidase and therefore do not undergo antigenic shift, whereas influenza A viruses have 15 different possible subtypes of hemagglutinin (H1–H15) and 9 potential neuraminidase subtypes (N1–N9). Birds can be infected by influenza A viruses with any combination of the 15 HA and 9 NA genes forming a global reservoir of virus. While human and swine pandemic influenza viruses have so far been largely restricted to a few surface antigens (H1, H2, H3, N1, N2; at least as far as can be ascertained, which is going back only a century or so!), there are potentially a large number of possible new surface antigen combinations that could arise and infect humans. It is thought that close association of birds, such as ducks, with mammals, such as pigs, in agriculture allows coinfection with avian and mammalian influenza virus strains, which occasionally leads to the production of a virus with different surface glycopoteins which is still able to infect humans[2] (Fig. 6.2).

In recent years, there have been a number of outbreaks of variously shifted strains that have so far, luckily, failed to transmit from human to human. For instance, between May 1997 and early 1998, there were 18 confirmed human cases of an H5N1 virus (similar to an avian strain that killed many thousands of chickens) and 6 of those 18 cases were fatal.[3] Overall, H5N1 virus has since this

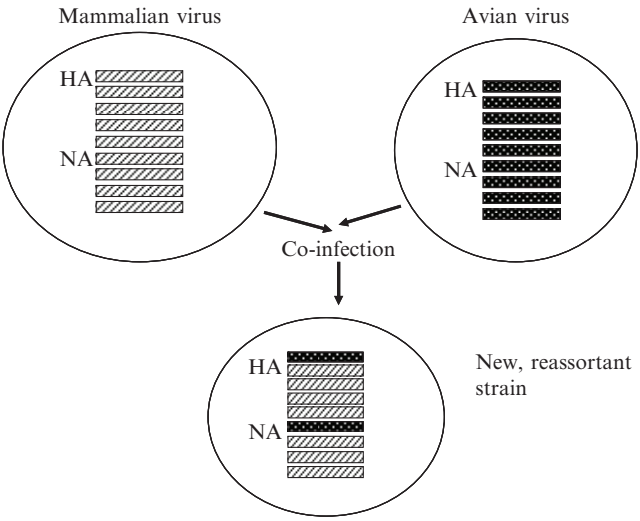

Fig. 6.2 Cartoon illustrating reassortment of influenza genes to produce a novel virus. Coinfection with a mammalian strain and an avian virus strain with different genes, including HA and NA, results in a virus with many internal proteins encoded by the original mammalian genes, but with new HA and NA derived from the avian strain. The new virus is able to replicate well in mammalian cells and is able to evade immune responses generated against earlier circulating strains. The rectangular blocks represent viral genes

chapter was prepared caused 79 human infections in Vietnam, Cambodia, and Thailand, of which 46 were fatal.[4] The danger posed to the world should these strains become capable of human to human transmission is obvious. During the production of the final draft of this manuscript, news has emerged from WHO indicating that the pattern of avian flu infections in northern Vietnam is now consistent with human-to-human spread.

In nonpandemic years (that is, most of the time), epidemics of influenza mainly occur during the winter months in temperate regions and are caused by drifted strains of virus related to those that had circulated in previous years. These epidemics are, on average, responsible for approximately 36,000 excess deaths annually in the United States alone. An estimated 90% of these deaths occur in persons aged >65 years.[5]

The timing and magnitude of influenza virus activity is unpredictable, but using efficient surveillance data, and assessing levels of activity in a timely manner using defined "threshold values," epidemiologists can indicate when sufficient flu activity is occurring in a population to warrant the use of interventions such as the prophylactic use of antiviral drugs.[6] Antiviral chemotherapy is discussed in the section "Antiviral Treatment."

Symptoms and Related Illnesses

The incubation period for influenza is 1–4 days with an average of 2 days,[7] although cough and malaise can persist for more than 2 weeks. Adults typically are infectious from the day before symptoms occur through approximately 5 days after illness.

Uncomplicated influenza illness is characterized by the abrupt onset of constitutional and respiratory signs and symptoms such as fever, myalgia, headache, nonproductive cough, sore throat, and rhinitis.[8] These symptoms are of course common to many viral infections, and respiratory viruses other than influenza are known to contribute to lower respiratory tract complications and deaths in elderly people during the winter months while producing symptoms very similar to those of influenza virus infection. In 1997, Nicholson and colleagues[9] studied the causes of respiratory infections in elderly people living at home in Leicestershire, UK, and concluded that 52% of the diseases were caused by rhinoviruses, 26% by coronaviruses, 9.5% by influenza, and 7% by respiratory syncytial virus (RSV).

Complications in the lower respiratory tract can occur following influenza virus infection in the elderly. The most common serious complication of influenza is pneumonia, which may occur at the same time as the influenza-like illness or up to 2 weeks afterwards. Viral pneumonia accompanied by toxemia can develop within 24 h following the onset of influenza, usually influenza Type A infection. The pneumonia is an interstitial pneumonitis with severe hyperemia and broadening of the alveolar walls together with a mononuclear cell infiltration, capillary dilatation, and thrombosis.[10] The symptoms include tachypneoa, tachycardia, high fever, and hypotension. Hypoxemia and death may follow between 1 and 4 days later. Initial improvement in those who survive occurs 5–16 days after onset of the pneumonia. Generally, there are no lasting complications after severe influenza infection, although a few patients develop a diffuse interstitial fibrosis accompanied by impaired lung function.[10]

Pneumonia and secondary infections, which commence after apparent recovery from the influenza infection, are usually caused by a bacterial superinfection with organisms such as *Streptococcus pneumoniae*, *Staphylococcus aureus* or *Hemophilus influenzae*. Infection with *S. aureus* affects the lung by causing oedema, hyperemia, hemorrhaging, consolidation, and formation of pus. When secondary bacterial infections are associated with type A influenza viruses they can be particularly harmful. During influenza A infections, there is apoptosis of leukocytes recruited into the airways, resulting in reduced efficiency of bacterial phagocytosis and destruction.[11] Many other immune functions may be compromised during influenza virus infection, and influenza virus infection of epithelial cells has been shown to directly enhance bacterial adherence to the cells.[12]

Antivirals and Influenza Vaccines for the Elderly

Flu hemagglutinin is the major component of current influenza vaccines and neuraminidase is the primary target for antiviral drug activity. The receptor for hemagglutinin is the terminal sialic acid residue on host cell surface sialyloligosaccharides, while the viral enzyme neuraminidase (sialidase) catalyzes the hydrolysis of sialic acid residues from sialyloligosaccharides. Most of the recently developed anti-influenza drugs inhibit sialidase of influenza viruses A and B.

Antiviral Treatment

Well-defined and validated antiviral drugs have proven to be curative in many cases and have a critical advantage over vaccine therapy. Flu vaccines need to be redesigned annually to immunize against particular strains or group of strains, a process that can take several months. Chemoprophylaxis should be considered for people at high risk during the time from vaccination until immunity has developed, since antibody responses in adults develop approximately 2-weeks post vaccination.[13] Prophylactic use of antiviral agents is also an option for preventing influenza among persons with anaphylactic hypersensitivity to eggs or other components of the influenza vaccine, or in "at-risk" individuals (including those aged over 65) recently exposed to a person with an influenza like illness, or for those same individuals during an epidemic. Indeed, the U.K. National Institute for Clinical and Health Excellence (NICE) recently published guidelines indicating that prophylactic postexposure use of ostelamivir (see below) is recommended for use in at-risk groups in residential care during periods when the virus is known to be circulating, or in unprotected at-risk people exposed to someone with an influenza-like illness. Unprotected people in the cases above means people not vaccinated since the previous flu season, or when the vaccine strain does not closely match the circulating strains of virus, or during the period before the vaccination takes effect.

Many experts consider that the best way of preparing for a flu pandemic (which would likely occur in the absence of any appropriate vaccine) is to prepare a stock of sufficient of doses of antiviral drugs, in order reduce the symptoms and possibly to slow transmission of the pandemic strain for long enough to allow the development and production of strain-specific vaccines. This policy has been adopted by a number of countries; however, only a fraction of the approximately 30 million doses needed in the UK are so far available. The most practical influenza medication is considered to be Tamiflu (ostelamivir-phosphate) produced by Roche. Although Tamiflu is available on the National Health Service (NHS) for treating high-risk groups, supply may be limited.

Zanamivir and ostelamivir belong to the neuraminidase inhibitor group of antiviral compounds and they are up to 84% (zanamavir) and 87% (ostelamivir) effective in preventing laboratory confirmed influenza illness.[14,15] Ostelamivir prophylaxis in particular led to a 92% reduction in influenza illness among nursing home residents during a 6-week study.[16] Zanamavir is not recommended for treatment for patients with underlying airway disease, since cases of respiratory dysfunction have been reported after inhalation together with allergic reactions such as oropharyngeal or facial edema.[17] Administration of ostelamivir has presented with fewer side effects such as nausea and/or vomiting[18,19] and these can be controlled if the drug is taken with food.[15]

Amantadine and rimantadine belong to another group of antiviral agents, which prevent the symptoms of influenza A illness by blocking of the M2 ion channel and altering optimal pH conditions to inhibit virus uncoating and replication. They are

not effective against influenza B infection, since influenza B viruses lack the M2 protein. When used as prophylactic agent, amantadine can prevent illness while permitting subclinical infection and development of a protective antibody response, which does not interfere with the antibody response to the vaccine.[20]

Side effects related to amantadine and rimantadine are usually mild and stop immediately after treatment, but serious side effects have been reported such as central nervous system (CNS) symptoms, behavioral changes, hallucinations, agitation, and seizures.[21,22] These more severe side effects have been observed among older persons who have been taking amantadine as prophylaxis at a dosage of 200 mg per day[23] and can be reduced by lowering the dosage of the drug.

A novel antiviral agent, NA cyclopentane inhibitor RWJ-270201 was shown to have potent inhibitory activity against NAs of influenza A and B viruses and a unique pattern of activity against resistant variants. It proved to be approximately threefold more potent than zanamavir in inhibiting NA activity of A/H1N1 clinical isolates, approximately fourfold more potent than zanamavir in inhibiting NA activity of A/H3N2 clinical isolates, and approximately sixfold more potent than ostelamivir carboxylate in inhibiting NA activity of influenza B virus clinical isolates.[24] To test the commercial prospects of the drug, phase III trials commenced in North America and Europe in February 2000. RWJ-270201 significantly reduced viral titers in infected patients during phase II studies, without causing any side effects. Under a worldwide influenza collaboration formed in September 1998, Johnson & Johnson has received exclusive worldwide rights to RWJ-270201.[25]

Antiviral chemotherapy (choice of drug, dosage, and duration of therapy) depends on patient's age, weight, renal function, health problems, and related medication and should be taken only during the period of peak influenza activity in a community, in order to be more cost effective and reduce the risk of the appearance of resistant viral strains.[26] In a laboratory (ferret) model of infection, resistance of influenza virus A/LosAngeles/1/87 (H3N2) to amantadine was generated within 6 days, during a single course of treatment, similar to the situation in humans.[27]

Influenza surveillance information and diagnostic testing can guide treatment decisions. Early diagnosis of influenza could theoretically reduce the inappropriate use of antibiotics and exclude possible bacterial infections, which can produce symptoms similar to influenza as mentioned in the previous section. Diagnostic tests available for influenza include viral culture, serology, rapid antigen testing, polymerase chain reaction (PCR), and immunofluorescence-based assays.[28]

Current Influenza Vaccines

Flu vaccination has been a valuable means in protecting vulnerable groups such as children, the elderly, and people with chronic respiratory, heart, renal diseases, diabetes, and immunosuppression.

Current flu vaccines are generally produced from virus grown in fertile hens' eggs and then inactivated by formaldehyde or β-propiolactone (whole-killed vaccine). Other variations consist of detergent split virus, in which the viral envelope

has been disrupted using detergents (split vaccine), and purified hemagglutin and neuraminidase antigens (subunit vaccine).[29,30]

The Trivalent Inactivated Influenza (TIV) vaccine currently consists of two influenza A strains (one H3N2, one H1N1) and an influenza B strain. It provides some protection against influenza complications in the elderly including pneumonia and death when given shortly before the beginning of flu season. Each year, the vaccine is reformulated, based on assessment of which viruses have been circulating globally. Not surprisingly, protection appears to vary depending upon how closely the challenge viruses are matched with the vaccine strains. A reduction of 61% in influenza-related deaths was seen when the vaccine and circulatory strains were well matched and only 35% when they were not well matched.[31]

Inactivated influenza vaccine administered to the elderly and other high-risk groups for the year 2004–2005 contained the formaldehyde inactivated strains: A/New Caledonia/20/99 (H3N2), A/Wyoming/3/2003 (H1N1), and B/Jiangsu/10/2003 (0.5 ml intramuscular dose). Common side effects of the vaccine found in less than one in ten persons are redness, bruising around the injection site, sweating, fever, headache, tiredness, or joint and muscular pain, but these symptoms usually disappear within 1–2 days without treatment. In general, healthy people in the age group of 65–74 years present minimal systemic side effects and only a low incidence of local side effects after influenza vaccination.[32] Subvirion and purified surface antigen preparations of the inactivated vaccine, as described above, are also available.

The vaccine can prevent hospitalizations, which constitute the principal direct cost of influenza, and studies from a number of countries with differing healthcare systems have shown vaccination of older and high-risk populations to be cost effective.[33] Vaccination of healthcare workers in nursing homes and hospitals is also associated with a substantial decline in mortality among patients.[34] The Institute of Medicine recently produced a report on future vaccines,[35] which ranked potential vaccines and vaccination strategies into four groups depending upon the projected cost of the program per quality adjusted life year (QALY) saved. Influenza vaccination for one-fifth of the population per year (or once every 5 years per individual) was put into the top group with the most favorable vaccines, those for which a vaccination strategy would save money as well as QALYs.

While the trivalent influenza vaccine is approximately 70–90% effective in preventing illness in healthy younger people, in older populations protection can be as low as 30%,[36] with the average from a number of studies being around 50%.[37–40] This low efficacy of influenza vaccination in the elderly is of great importance, as this group is among the most susceptible to the serious consequences of the infection.

Immune Responses in the Elderly

The reduced efficacy of influenza vaccines in the elderly mentioned in the section "Current Influenza Vaccines" is attributed to immunosenescence, the deterioration of immune responses to immunization, or infection associated with aging. Some ways in which immune responses are impaired in the elderly are summarized in Table 6.1.

Table 6.1 Summary of impaired immune responses in the elderly

Immunity component	Impact of aging	Reference
Macrophages	Decreased number, inefficient presentation of Ags to T cells, reduced phagocytosis, reduced generation of nitrous oxide and superoxide, delayed wound healing, decline in TLRs, cytokine and chemokine expression	41
NK cells	Decreased proliferation, cytokine secretion, and CD69 expression	42
Neutrophils	Impaired chemotaxis, degranulation, and phagocytosis	43, 44
Ag-specific T and B cells	Altered clonal expansion, diminished ability to generate high antibody titer	41
Naïve, mature T cells	Decreased number, reduced expression of MHC II	45
APC function (DCs, LCs)	Poor hypersensitivity to allergens	46
TLRs	Decline in the secretion of antimicrobial peptides and pro-inflammatory cytokines	47

Abbreviations: Ag antigen; *TLR* Toll-like receptor; *NK* natural killer; *MHC II* major histocompatibility complex class II; *APC* antigen presenting cell; *DCs* dendritic cells; *LC* Langerhans' cells

Innate Immune Responses

The initial site of influenza virus replication is thought to be the tracheobronchial ciliated epithelium, but the whole respiratory tract may be involved. Pulmonary infection is strongly related with mortality associated with influenza virus infection either because of viral pneumonia or because of bacterial superinfection. Aging is associated with a progressive decline in lung performance due to alterations in lung parenchyma and elastic recoil[48] and a decrease in tracheal mucus development[49] important for pathogen clearance. Lower sensitivity of the respiratory system to acute disease and infection delays important clinical symptoms such as dyspnoea and tachypnoea, which are important for diagnosis of influenza-associated diseases.

The first line of defense against pathogens, the innate immune functions of macrophages, natural killer (NK) cells, and neutrophils, are impaired with aging leading to lack of early protective immunity to influenza and bacterial infection, thus making the elderly susceptible to viral and bacterial pneumonia and skin and gastrointestinal tract infections.

Macrophages are present in the lungs as well as in other parts of the body and function as pathogen scavengers by initiating inflammatory responses and phagocytosis to eliminate pathogens. Their adherence, opsonization, and phagocytic ability have shown an age-related decline in several murine models.[50,51] Expression of the adhesion molecules VCAM-1 and ICAM-1 was delayed in the elderly[52] and the wound healing process was found to be delayed in older humans and rodents[53] because of delayed re-epithelialization, angiogenesis, collagen deposition, wound strength, and delayed infiltration of macrophages.

Table 6.2 Mammalian Toll-like receptor activity

Receptor	Ligand PAMP	Known activation cascades
TLR 1	Triacetylated lipoproteins	Unknown
TLR 2	Lipoproteins, peptidoglycan (Gram-positive bacteria), lipoteichoid acids, fungal structures	MyD88-dependent TIRAP
TLR 3	Double-stranded RNA	MyD88-independent TRIF
TLR 4	Lipopolysaccharide membrane (Gram-negative bacteria), HSP60, mBD2, fungal structures	MyD88-dependent TIRAP MyD88 independent TRIF/TICAM/TRAM
TLR 5	Flagellin	MyD88-dependent IRAK
TLR 6	Diacetylated lipoproteins	Unknown
TLR 7	Small synthetic compounds, immiquinod, imidazoquinoline, ss RNA	MyD88-dependent IRAK
TLR 8	ssRNA	MyD88-dependent IRAK
TLR 9	Unmethylated CpG DNA	MyD88-dependent IRAK
TLR 10	None defined	Unknown
TLR 11	Uropathogenic bacteria	MyD88-dependent IRAK

Abbreviations: PAMP pathogen associated molecular patterns; *TLR* Toll-like receptor; *MyD88* adaptor protein in the Toll IL-1 receptor family signaling; *TIRAP* Toll-IL-1 receptor domain-containing adaptor protein; *TRIF* TIR domain containing adaptor inducing IFN-β; *HSP 60* 60-kDa heat shock chaperonin protein; *mBD2* mouse β defensin 2; *TRAM* thyroid hormone receptor activator molecule; *IRAK* IL-1 receptor-associated kinase; *CpG* cytosine preceding a guanosine pattern

Innate immune responses are frequently initiated via Toll-like-receptors (TLRs), a set of conserved molecules that recognize pathogen-associated molecular patterns (PAMPs) and endogenous proteins associated with danger and stress signals. Upon TLR stimulation, a variety of antimicrobial peptides and proinflammatory cytokines (IL-6, TNF-α, etc.) are synthesized to assist in the clearance of the invading pathogen. The 11 TLRs recognized to date and their ligands, as well as their signaling activation pathways that may be altered during aging, are summarized in Table 6.2. Clearly, responses to viral infection are influenced by TLRs, such as TLR-3. This receptor is constitutively expressed in human alveolar and bronchial epithelial cells. Its ligand is double-stranded RNA, which is produced during influenza and other viral infections, and its expression was found to be positively regulated by the influenza A virus via the secretion of the cytokines IL-8, IL-6, RANTES, and interferon-beta, and the upregulation of the major adhesion molecule ICAM-1.[54] In a recent study, Renshaw and colleagues[47] assessed TLR expression on splenic and peritoneal macrophages of aged mice and concluded that decreased expression and function of TLRs resulting from aging may partially contribute to the increased susceptibility of the elderly population to bacterial, viral, and yeast infections.

The secretion of cytokines such as IL-6, TNF-α, and chemokines such as MIP-1a, CCL5 is also dysregulated in the aged population[47] (Table 6.1). Increased levels of prostaglandin E(2) have been linked to suppression of IL-12 and class II MHC

expression on antigen presenting cells (APCs), an effect that can be reversed by vitamin E supplementation.[55] Both cytokine and chemokine molecules are involved in immune responses to inflammation such as fever. Thus the poor inflammatory response and the lack of presentation of clinical signs in the elderly may delay diagnosis and may contribute to the higher mortality rates seen in older people.

Adaptive Immunity

In relation to the impaired innate immune functions mentioned above, humoral and mainly cellular immune responses decline with age because of the limited generation of high-affinity, protective antibodies against pathogens and of thymus atrophy, respectively. The latter limits the quantity of naïve T cells against infectious agents and new antigens. Inefficient aged T-cell cooperation and limited production of cytokines may lead to the decline in specific antibody responses. Frail, elderly subjects exhibit a blunted and somewhat delayed type 1 T-cell response to influenza vaccination, which is correlated positively with the reduced IgG 1 subclass and the total antibody response.[56] On the other hand, an imbalance in the production of pro- and anti-inflammatory cytokines and the accumulation of $CD8^+$ $CD28^-$ IFN-γ producing T cells in the aging immune system could diminish the likelihood of elderly persons producing specific Abs of sufficient titer following influenza vaccination.[57] Also, an increase in self-reactive antibodies has been observed in older vaccinated patients.[58]

Influenza Vaccine Research and Future Prospects

Newer Methods of Inactivated Vaccine Production

The components of inactivated influenza virus vaccines are produced in embryonated hen's eggs and this presents some practical difficulties. First, the egg supply is often limited, and eggs must be ordered a long time in advance. Secondly, many people are allergic to egg proteins and therefore cannot receive the vaccine, and thirdly, not all strains grow well in eggs, and therefore sometimes the virus strain chosen for use in the vaccine is a compromise based upon antigenic similarity to circulating strains and ability to grow in eggs. Because of these problems with egg growth of the virus, there is interest in producing vaccine virus in tissue culture cells.

Madin Darby Canine Kidney (MDCK) cells are widely used for the isolation of the virus, and Vero cells derived from African green monkey kidney have been recently authorized by the WHO for vaccine production.[59] The safety and immunogenicity of an MDCK-cell-grown influenza vaccine was confirmed in a phase II clinical trial.[60]

Another potential means of avoiding production in eggs would be to use a recombinant subunit vaccine wherein components such as hemagglutinin and neuraminidase are produced in a heterologous system from cDNA introduced into the expression vector. To this end, Baculovirus-based production of intact hemagglutinin in insect cells has been demonstrated, and its immunogenicity has been proven.[61]

Generating Broadly Cross-Reactive Responses

The selection of stable antigenic targets is critical in the design of an influenza vaccine. To date, this area is somewhat under-researched, with the majority of studies focusing on the HA and NA antigens. A universal influenza virus vaccine that does not require frequent updates and/or annual immunizations would offer significant advantages over current seasonal flu vaccines, including, of course, protection against pandemic strains.

Influenza matrix protein, which lines the inside of the lipoprotein envelope enclosing the virus RNA (Fig. 6.1), is a promising antigenic target. It is a multifunctional protein that plays an important role in virus replication by regulating the bidirectional transport of ribonucleoprotein (RNP) into and out of the nucleus, inhibiting viral RNA polymerase activity by binding to RNP and mediating the association of RNP with viral envelope glycoproteins on the inner surface of the cytoplasmic membrane for virion formation and budding. Influenza-matrix-protein-derived peptide GILGFVFTL was found to be 100–1,000 times more effective than commonly used peptides in sensitizing HLA-$A2^+$ target cells to lysis by influenza-virus-specific cytotoxic T lymphocytes.[62]

The highly conserved M2 integral membrane protein encoded by influenza A viruses has also been suggested as a potential antigen for a universal vaccine. M2 protein possesses an ion channel activity that is required for efficient virus entry into host cells. The M2 cytoplasmic tail, in particular, plays a role in infectious virus production by coordinating the efficient packaging of genome segments into influenza virus particles.[63]

Synthetic peptides of M2 extracellular domain conjugated to keyhole limpet hemocyanin or *Neisseria meningitidis* outer membrane protein complex were found to be highly immunogenic in mice, ferrets, and rhesus monkeys and were able to confer protection against lethal challenge with either H1N1 or H3N2 virus in mice.[64,65] Disappointingly, antibody induced by the M2 vaccine did not cross-react with the H5N1 virus, which could be related to the next pandemic strain.[65]

Another conserved influenza protein, the nucleoprotein (NP), may be a potentially valuable vaccine because of its cross-reactivity against even distantly related virus subtypes. This antigen in combination with small amounts of IL-2 was shown to induce strong proliferation of resting $CD4^+$ and $CD8^+$ T cells from young and elderly donors.[66]

Antigen delivery systems can influence the immune response quantitatively as well as qualitatively and the route of administration might drastically affect the

success of a vaccine. In the case of influenza, antigen delivery can be critically important, as it might be necessary to stimulate substantial levels of mucosal immunity, which is considered helpful in protection against mucosal infections, in the absence of side effects. New improved intervention strategies through immunization and/or vaccine delivery are therefore needed to reduce morbidity and mortality in the elderly due to influenza and related complications and provide adequate protection during influenza virus epidemics.[10]

Live Attenuated Vaccines

The intranasal vaccine FluMist, a cold-adapted, live-attenuated, trivalent influenza virus vaccine (LAIV) developed by MedImmune was approved by the U.S. Food and Drug Administration on June 17, 2003 only for administration to healthy persons aged 5–49 years.[32] The ''cold adaptation'' process encourages replication in the nasal passages to induce immunity but restricts replication in the increased temperatures of the lower respiratory tract and lungs.[67] Live attenuated virus vaccine has perceived advantages over killed vaccines in that administration is by the intranasal route. This may be considered preferable to injection by vaccines, and these vaccines may generate stronger mucosal-cell-mediated immune responses than conventional killed vaccines. FluMist's role in the general prevention of influenza is yet to become clear, and there have been some problems associated with distribution, as the vaccine had to remain frozen. In order to circumvent this problem, a next-generation live vaccine was recently produced by MedImmune named CAIV-T, assessed in clinical trials and shown to be immunogenic and safe in healthy and at-risk populations.[67]

Preclinical Vaccine Research and Development

Other methods of mucosal vaccine delivery have been investigated including the use of heterologous viral systems. In a recent report, Abe and colleagues[68] demonstrated protection against lethal influenza virus infection in mice immunized intranasally with a recombinant baculovirus expressing the hemagglutinin gene of the A/PR/8/34 (H1N1) flu virus. Protection was later linked to activation of immune cells by bAcNPV via the Toll-like receptor 9 (TLR9)/MyD88-dependent signaling pathway.[69] Similar to these findings, vaccination with influenza virosomes has shown to elicit high titer of influenza-specific antibodies and T-helper cell and cytotoxic T-cell responses against encapsulated antigens due to the intrinsic adjuvant activity of virosomal formulations.[70]

Improved cell-mediated immune responses have long been considered a desirable attribute of influenza vaccines. Novel pH-triggered microparticles encapsulating a model MHC class I-restricted peptide Ag from the influenza A

matrix protein were efficiently phagocytosed by human monocytes and dendritic cells, and led to increased antigen presentation and primed CTL responses with minimal cellular toxicity and no functional impairment.[71]

As described above, TLR stimulation may be important in immune responses to vaccines and is certainly important in the action of adjuvants designed to enhance vaccine responses. TLR expression and function may also decline in older people.[47] Binding of the cell surface protein CD154 on activated T cells to CD40 on B cells, dendritic cells, macrophages, and other cell types leads to B-cell activation, proliferation, and antibody production independently of Toll receptor recognition and signaling. We have described work showing that conjugates of anti-CD40 mAbs with antigens are very potent immunogens[72,73] and we have recently observed that CD40 adjuvant conjugates with influenza virus antigens were successful in inducing specific anti-influenza antibody and cellular responses.[74]

Similar approaches have been employed with anti-CD40 mAb and liposomally encapsulated nuclear protein peptide NP366-374, corresponding to a CTL epitope on NP. Intranasal immunization of this formulation effectively induced mucosal immunity to reduce virus replication in the lung, suggesting that anti-CD40 mAb also functioned as a mucosal adjuvant through MHC class I- and class II-dependent pathways.[75]

Another interesting approach compared the immunogenicity and safety of a novel, interleukin-2 (IL-2)-supplemented trivalent liposomal influenza vaccine (INFLUSOME-VAC) with that of a commercial trivalent split virion vaccine in community-residing elderly volunteers of a mean age 81 years. At 1-month post vaccination, hemagglutination inhibition for the A/New Caledonia (H1N1) and A/Moscow (H3N2) strains was significantly higher in the INFLUSOME-VAC group. INFLUSOME-VAC also induced a greater anti-neuraminidase (NA–N2) response without the detection of IL-2 antibodies and no increase in anti-phospholipid IgG antibodies, while the adverse reactions were similar in both the liposomal and split virion vaccine.[30]

Similar delivery systems developed for mucosal immunization include immune-stimulating complexes (ISCOM), cage-like structures about 30–40 nm in diameter composed of glycosides, cholesterol, immunizing protein antigen, and phospholipids. ISCOM influenza vaccines have been shown to be more immunogenic than conventional vaccines in humans[76] but failed to protect monkeys against distant drift variants of influenza A (H3N2) viruses.[77]

Conclusion

The combination of vaccination with new antiviral agents in high-risk groups can be powerful tools in the fight against influenza. However, emphasis must be given to improvement in the efficiency of use of these tools. Influenza vaccination levels should hopefully continue to increase owing to greater acceptance of preventive medicine by physicians, increased administration of the vaccine by healthcare providers other than practitioners, and new information regarding vaccine effectiveness,

cost effectiveness, and safety.[78] The Advisory Committee on Immunization Practices (ACIP) in the US recommends using strategies to improve vaccination levels in the elderly, including using reminder/recall systems and standing order programs.[79] They recommend that inpatient influenza immunization programs are practiced to target high-risk, hospitalized individuals >65 years who might otherwise have not received influenza vaccination.[80] Additional strategies are also needed to achieve the Healthy People 2010 objectives among all racial and ethnic groups, since vaccination levels among blacks and Hispanics lag behind those among whites.[81]

References

1. Turner D, Wailoo A, Nicholson K, Cooper N, Sutton A, Abrams K. Systematic review and economic decision modelling for the prevention and treatment of influenza A and B. Health Technol Assess 2003;7:1–170.
2. Wuethrich B. Infectious disease. An avian flu jumps to people. Science 2003;299:1504.
3. Hatta M, Neumann G, et al. Reverse genetics approach towards understanding pathogenesis of H5N1 Hong Kong influenza A virus infection. Philos Trans R Soc Lond B Biol Sci 2001;356:1841–3.
4. Bulletin of the WHO. May 1, 2005.
5. Bridges CB, Fukuda K, Uyeki TM, Cox NJ, Singleton JA; Centers for Disease Control and Prevention, Advisory Committee on Immunization Practices. Prevention and control of influenza. Recommendations of the Advisory Committee on Immunization Practices (ACIP). MMWR Recomm Rep 2002;51:1–31.
6. Goddard NL, Kyncl J, Watson JM. Appropriateness of thresholds currently used to describe influenza activity in England. Commun Dis Public Health 2003;6:238–45.
7. Clements ML, Betts RF, Tierney EL, Murphy BR. Serum and nasal wash antibodies associated with resistance to experimental challenge with influenza A wild-type virus. J Clin Microbiol 1986;24:157–60.
8. Nicholson KG, Baker DJ, Chakraverty P, Farquhar A, Hurd D, Kent J, Litton PA, Smith SH. Immunogenicity of inactivated influenza vaccine in residential homes for elderly people. Age Ageing 1992;21:182–8.
9. Nicholson KG, Kent J, Hammersley V, Cancio E. Acute viral infections of upper respiratory tract in elderly people living in the community: comparative, prospective, population based study of disease burden. BMJ 1997;315:1060–4.
10. Jennings R, Read R. Clinical Assessment. Influenza in Practice. The Royal Society of Medicine, London, 2005, pp. 19–27.
11. Colamussi ML, White MR, Crouch E, Hartshorn KL. Influenza A virus accelerates neutrophil apoptosis and markedly potentiates apoptotic effects of bacteria. Blood 1999;93:2395–403.
12. Okamoto S, Kawabata S, Nakagawa I, Okuno Y, Goto T, Sano K, Hamada S. Influenza A virus-infected hosts boost an invasive type of *Streptococcus pyogenes* infection in mice. J Virol 2003;77:4104–12.
13. Gross PA, Russo C, Dran S, Cataruozolo P, Munk G, Lancey SC. Time to earliest peak serum antibody response to influenza vaccine in the elderly. Clin Diagn Lab Immunol 1997;4:491–2.
14. Monto AS, Robinson DP, Herlocher ML, Hinson JM Jr, Elliott MJ, Crisp A. Zanamivir in the prevention of influenza among healthy adults: a randomized controlled trial. JAMA 1999;282:31–5.

15. Hayden FG, Atmar RL, Schilling M, Johnson C, Poretz D, Paar D, Huson L, Ward P, Mills RG. Use of the selective oral neuraminidase inhibitor ostelamivir to prevent influenza. N Engl J Med 1999;341:1336–43.
16. Peters PH Jr, Gravenstein S, Norwood P, De Bock V, Van Couter A, Gibbens M, von Planta TA, Ward P. Long-term use of ostelamivir for the prophylaxis of influenza in a vaccinated frail older population. J Am Geriatr Soc 2001;49:1025–31.
17. Gravenstein S, Johnston SL, Loeschel E, Webster A. Zanamivir: a review of clinical safety in individuals at high risk of developing influenza-related complications. Drug Saf 2001;24:1113–25.
18. Nicholson KG, Aoki FY, Osterhaus AD, Trottier S, Carewicz O, Mercier CH, Rode A, Kinnersley N, Ward P. Efficacy and safety of the oral neuraminidase inhibitor ostelamivir in treating acute influenza: a randomized controlled trial. US Oral Neuraminidase Study Group. JAMA 2000;283:1016–24.
19. Nicholson KG, Aoki FY, Osterhaus AD, Trottier S, Carewicz O, Mercier CH, Rode A, Kinnersley N, Ward P. Efficacy and safety of ostelamivir in treatment of acute influenza: a randomised controlled trial. Neuraminidase Inhibitor Flu Treatment Investigator Group. Lancet 2000;355:1845–50.
20. Tominack RL, Hayden FG. Rimantadine hydrochloride and amantadine hydrochloride use in influenza A virus infections. Infect Dis Clin North Am 1987;1:459–78.
21. Dayton N. Symmetrel Package Insert. Endo Pharmaceuticals, Inc., Chadds Ford, PA, 2000.
22. Atkinson WL, Arden NH, Patriarca PA, Leslie N, Lui KJ, Gohd R. Amantadine prophylaxis during an institutional outbreak of type A (H1N1) influenza. Arch Intern Med 1986;146:1751–6.
23. Guay DR. Amantadine and rimantadine prophylaxis of influenza A in nursing homes. A tolerability perspective. Drugs Aging 1994;5:8–19.
24. Gubareva LV, Webster RG, Hayden FG. Comparison of the activities of zanamivir, ostelamivir, and RWJ-270201 against clinical isolates of influenza virus and neuraminidase inhibitor-resistant variants. Antimicrob Agents Chemother 2001;45:3403–8.
25. Barnard DL. RWJ-270201 BioCryst Pharmaceuticals/Johnson & Johnson. Curr Opin Investig Drugs 2000;1:421–4.
26. Patriarca PA, Arden NH, Koplan JP, Goodman RA. Prevention and control of type A influenza infections in nursing homes. Benefits and costs of four approaches using vaccination and amantadine. Ann Intern Med 1987;107:732–40.
27. Herlocher ML, Truscon R, Fenton R, Klimov A, Elias S, Ohmit SE, Monto AS. Assessment of development of resistance to antivirals in the ferret model of influenza virus infection. J Infect Dis 2003;188:1355–61.
28. Cox NJ, Subbarao K. Influenza. Lancet 1999;354:1277–82.
29. Boyce TG, Hsu HH, Sannella EC, Coleman-Dockery SD, Baylis E, Zhu Y, Barchfeld G, DiFrancesco A, Paranandi M, Culley B, Neuzil KM, Wright PF. Safety and immunogenicity of adjuvanted and unadjuvanted subunit influenza vaccines administered intranasally to healthy adults. Vaccine 2000;19:217–26.
30. Ben-Yehuda A, Joseph A, Barenholz Y, Zeira E, Even-Chen S, Louria-Hayon I, Babai I, Zakay-Rones Z, Greenbaum E, Galprin I, Glück R, Zurbriggen R, Kedar E. Immunogenicity and safety of a novel IL-2-supplemented liposomal influenza vaccine (INFLUSOME-VAC) in nursing-home residents. Vaccine 2003;21:3169–78.
31. Nordin J, Mullooly J, Poblete S, Strikas R, Petrucci R, Wei F, Rush B, Safirstein B, Wheeler D, Nichol KL. Influenza vaccine effectiveness in preventing hospitalizations and deaths in persons 65 years or older in Minnesota, New York, and Oregon: data from 3 health plans. J Infect Dis 2001;184:665–70.
32. Harper SA, Fukuda K, Uyeki TM, Cox NJ, Bridges CB; Advisory Committee on Immunization Practices (ACIP), Centers for Disease Control and Prevention (CDC). Prevention and control of influenza: recommendations of the Advisory Committee on Immunization Practices (ACIP). MMWR Recomm Rep 2004;53:1–40.
33. Office of Technology Assessment. Cost Effectiveness if Influenza Vaccination. Washington, DC, 1981.

34. Carman WF, Elder AG, Wallace LA, McAulay K, Walker A, Murray GD, Stott DJ. Effects of influenza vaccination of health-care workers on mortality of elderly people in long-term care: a randomised controlled trial. Lancet 2000;355:93–7.
35. Stratton KR, Durch JS, Lawrence RS. Vaccines for the 21st Century; A Tool for Decision Making. Editors Institute of Medicine of the National Academies, Washington, DC, 1999.
36. Ginaldi L, Loreto MF, Corsi MP, Modesti M, De Martinis M. Immunosenescence and infectious diseases. Microbes Infect 2001;3:851–7.
37. Powers DC, Hanscome PJ, Pietrobon PJ. In previously immunized elderly adults inactivated influenza A (H1N1) virus vaccines induce poor antibody responses that are not enhanced by liposome adjuvant. Vaccine 1995;13:1330–5.
38. Beyer WE, Palache AM, Sprenger MJ, Hendriksen E, Tukker JJ, Darioli R, van der Water GL, Masurel N, Osterhaus AD. Effects of repeated annual influenza vaccination on vaccine seroresponse in young and elderly adults. Vaccine 1996;14:1331–9.
39. Gluck R, Wegmann A. Influenza vaccination in the elderly. Dev Comp Immunol 1997; 21:501–7.
40. Crocetti E, Arniani S, Bordoni F, Maciocco G, Zappa M, Buiatti E. Effectiveness of influenza vaccination in the elderly in a community in Italy. Eur J Epidemiol 2001;17:163–8.
41. Plowden J, Renshaw-Hoelscher M, Engleman C, Katz J, Sambhara S. Innate immunity in aging: impact on macrophage function. Aging Cell 2004;3:161–7.
42. Solana R, Mariani E. NK and NK/T cells in human senescence. Vaccine 2000;18:1613–20.
43. Butcher SK, Chahal H, Nayak L, Sinclair A, Henriquez NV, Sapey E, O'Mahony D, Lord JM. Senescence in innate immune responses: reduced neutrophil phagocytic capacity and CD16 expression in elderly humans. J Leukoc Biol 2001;70:881–6.
44. Mancuso P, McNish RW, Peters-Golden M, Brock TG. Evaluation of phagocytosis and arachidonate metabolism by alveolar macrophages and recruited neutrophils from F344xBN rats of different ages. Mech Ageing Dev 2001;122:1899–913.
45. Herrero C, Sebastián C, Marqués L, Comalada M, Xaus J, Valledor AF, Lloberas J, Celada A. Immunosenescence of macrophages: reduced MHC class II gene expression. Exp Gerontol 2002;37:389–94.
46. Bhushan M, Cumberbatch M, Dearman RJ, Andrew SM, Kimber I, Griffiths CE. Tumour necrosis factor-alpha-induced migration of human Langerhans cells: the influence of ageing. Br J Dermatol 2002;146:32–40.
47. Renshaw M, Rockwell J, Engleman C, Gewirtz A, Katz J, Sambhara S. Cutting edge: impaired Toll-like receptor expression and function in aging. J Immunol 2002;169:4697–701.
48. Janssens JP, Krause KH. Pneumonia in the very old. Lancet Infect Dis 2004;4:112–24.
49. Ho PL, Yam WC, Cheung TK, Ng WW, Que TL, Tsang DN, Ng TK, Seto WH. Fluoroquinolone resistance among *Streptococcus pneumoniae* in Hong Kong linked to the Spanish 23F clone. Emerg Infect Dis 2001;7:906–8.
50. Khare V, Sodhi A, Singh SM. Effect of aging on the tumoricidal functions of murine peritoneal macrophages. Nat Immun 1996;15:285–94.
51. De la Fuente M, Medina S, Del Rio M, Ferrández MD, Hernanz A. Effect of aging on the modulation of macrophage functions by neuropeptides. Life Sci 2000;67:2125–35.
52. Ashcroft GS, Horan MA, Ferguson MW. Aging alters the inflammatory and endothelial cell adhesion molecule profiles during human cutaneous wound healing. Lab Invest 1998; 78:47–58.
53. Gosain A, DiPietro LA. Aging and wound healing. World J Surg 2004;28:321–6.
54. Guillot L, Le Goffic R, Bloch S, Escriou N, Akira S, Chignard M, Si-Tahar M. Involvement of Toll-like receptor 3 in the immune response of lung epithelial cells to double-stranded RNA and influenza A virus. J Biol Chem 2005;280:5571–80.
55. Wu D, Mura C, Beharka AA, Han SN, Paulson KE, Hwang D, Meydani SN. Age-associated increase in PGE2 synthesis and COX activity in murine macrophages is reversed by vitamin E. Am J Physiol 1998;275:C661–8.

56. Deng Y, Jing Y, Campbell AE, Gravenstein S. Age-related impaired type 1 T cell responses to influenza: reduced activation *ex vivo*, decreased expansion in CTL culture in vitro, and blunted response to influenza vaccination *in vivo* in the elderly. J Immunol 2004;172:3437–46.
57. Saurwein-Teissl M, Schönitzer D, Grubeck-Loebenstein B. Dendritic cell responsiveness to stimulation with influenza vaccine is unimpaired in old age. Exp Gerontol 1998;33:625–31.
58. Huang YP, Gauthey L, Michel M, Loreto M, Paccaud M, Pechere JC, Michel JP. The relationship between influenza vaccine-induced specific antibody responses and vaccine-induced nonspecific autoantibody responses in healthy older women. J Gerontol 1992;47: M50–5.
59. Kistner O, Barrett PN, Mundt W, Reiter M, Schober-Bendixen S, Dorner F. Development of a mammalian cell (Vero) derived candidate influenza virus vaccine. Vaccine 1998;16:960–8.
60. Halperin SA, Nestruck AC, Eastwood BJ. Safety and immunogenicity of a new influenza vaccine grown in mammalian cell culture. Vaccine 1998;16:1331–5.
61. Treanor JJ, Wilkinson BE, Masseoud F, Hu-Primmer J, Battaglia R, O'Brien D, Wolff M, Rabinovich G, Blackwelder W, Katz JM. Safety and immunogenicity of a recombinant hemagglutinin vaccine for H5 influenza in humans. Vaccine 2001;19:1732–7.
62. Trojan A, Urosevic M, Hummerjohann J, Giger R, Schanz U, Stahel RA. Immune reactivity against a novel HLA-A3-restricted influenza virus peptide identified by predictive algorithms and interferon-gamma quantitative PCR. J Immunother 2003;26:41–6.
63. McCown MF, Pekosz A. The influenza A virus M2 cytoplasmic tail is required for infectious virus production and efficient genome packaging. J Virol 2005;79:3595–605.
64. Neirynck S, Deroo T, Saelens X, Vanlandschoot P, Jou WM, Fiers W. A universal influenza A vaccine based on the extracellular domain of the M2 protein. Nat Med 1999;5:1157–63
65. Fan J, Liang X, et al. Preclinical study of influenza virus A M2 peptide conjugate vaccines in mice, ferrets, and rhesus monkeys. Vaccine 2004;22:2993–3003.
66. Gschoesser C, Almanzar G, Hainz U, Ortin J, Schonitzer D, Schild H, Saurwein-Teissl M, Grubeck-Loebenstein B. $CD4^+$ and $CD8^+$ mediated cellular immune response to recombinant influenza nucleoprotein. Vaccine 2002;20:3731–8.
67. Filmore D. Flu trials. Mod Drug Discov 2004;7:44–48.
68. Abe T, Takahashi H, Hamazaki H, Miyano-Kurosaki N, Matsuura Y, Takaku H. Baculovirus induces an innate immune response and confers protection from lethal influenza virus infection in mice. J Immunol 2003;171:1133–9.
69. Abe T, Hemmi H, Miyamoto H, Moriishi K, Tamura S, Takaku H, Akira S, Matsuura Y. Involvement of the Toll-like receptor 9 signaling pathway in the induction of innate immunity by baculovirus. J Virol 2005;79:2847–58.
70. Huckriede A, Bungener L, Daemen T, Wilschut J. Influenza virosomes in vaccine development. Methods Enzymol 2003;373:74–91.
71. Haining WN, Anderson DG, Little SR, von Bergwelt-Baildon MS, Cardoso AA, Alves P, Kosmatopoulos K, Nadler LM, Langer R, Kohane DS. pH-triggered microparticles for peptide vaccination. J Immunol 2004;173:2578–85.
72. Barr TA, McCormick AL, et al. A potent adjuvant effect of CD40 antibody attached to antigen. Immunology 2003;109:87–92.
73. Barr TA, Carlring J, et al. Antibodies against cell surface antigens as very potent immunological adjuvants. Vaccine 2006;24S2:S20–21.
74. Hatzifoti C, Heath AW. CD40-mediated enhancement of immune responses against three forms of influenza vaccine. Immunology 2007;122:98–106.
75. Ninomiya A, Ogasawara K. Intranasal administration of a synthetic peptide vaccine encapsulated in liposome together with an anti-CD40 antibody induces protective immunity against influenza A virus in mice. Vaccine 2002;20:3123–9.
76. Ennis FA, Cruz J, Jameson J, Klein M, Burt D, Thipphawong J. Augmentation of human influenza A virus-specific cytotoxic T lymphocyte memory by influenza vaccine and adjuvanted carriers (ISCOMS). Virology 1999;259:256–61.

77. Rimmelzwaan GF, Baars M, van Beek R, van Amerongen G, Lövgren-Bengtsson K, Claas EC, Osterhaus AD. Induction of protective immunity against influenza virus in a macaque model: comparison of conventional and iscom vaccines. J Gen Virol 1997;78:757–65.
78. Singleton JA, Greby SM, Wooten KG, Walker FJ, Strikas R. Influenza, pneumococcal, and tetanus toxoid vaccination of adults – United States, 1993–7. MMWR CDC Surveill Summ 2000;49:39–62.
79. McKibben LJ, Stange PV, Sneller VP, Strikas RA, Rodewald LE; Advisory Committee on Immunization Practices. Use of standing orders programs to increase adult vaccination rates. MMWR Recomm Rep 2000;49:15–6.
80. Lawson F, Baker V, Au D, McElhaney JE. Standing orders for influenza vaccination increased vaccination rates in inpatient settings compared with community rates. J Gerontol A Biol Sci Med Sci 2000;55:M522–6.
81. CDC. Racial/ethnic disparities in influenza and pneumococcal vaccination levels among persons aged >65 years. United States 1989–2001. MMWR Morb Mortal Wkly Rep 2003;52:958–62.