The Immunological Basis for Immunization Series

Module 23: Influenza Vaccines

Immunization, Vaccines and Biologicals

World Health Organization

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Contents

Abbreviations and acronyms

APC	Antigen presenting cells
att	Attenuated (property of live-attenuated influenza vaccines)
ca	Cold-adapted (property of live-attenuated influenza vaccines)
CI	Confidence interval
CMI	Cell-mediated immunity
EID	Egg infectious dose
FFU	Fluorescent focus units
GBS	Guillain-Barré syndrome
GISRS	Global Influenza Surveillance and Response System
HA	Hemagglutinin
HA1	Globular head region of the hemagglutinin protein
HAI	Hemagglutination inhibition
HGR	High-growth reassortant
HLA	Human leukocyte antigen
IIV	Inactivated influenza vaccine
ILI	Influenza-like illness
LAIV	Live attenuated influenza vaccine
M	Matrix protein
MDCK	Madin-Darby canine kidney
MDV	Master donor virus
µg	Microgram
MN	Microneutralization
MP	Matrix protein
M2	Matrix protein 2
NA	Neuraminidase
NIC	National Influenza Centre
NP	Nucleoprotein
OR	Odds ratio
ORS	Ocular respiratory syndrome
PA	Polymerase acidic protein
PAMPs	Pathogen-associated molecular patterns
PB1	Polymerase basic protein 1
PB2	Polymerase basic protein 2
PRRs	Pattern recognition receptors
RCT	Randomized controlled trial
RIDT	Rapid influenza diagnostic test
RT-PCR	Reverse transcription-polymerase chain reaction
SAGE	Strategic Advisory Group of Experts on immunization
SRID	Single radial immunodiffusion
SV	Sub-virion (includes both split and purified surface antigen vaccines)
ts	Temperature-sensitive (property of live-attenuated influenza vaccines)
VAERS	Vaccine Adverse Event Reporting System
VE	Vaccine effectiveness
WHO	World Health Organization

Preface

This module is part of the WHO series *The Immunological Basis for Immunization*, which was initially developed in 1993 as a set of eight modules, comprising one module on general immunology and seven modules each devoted to one of the vaccines recommended for the Expanded Programme on Immunization, i.e. vaccines against diphtheria, measles, pertussis, polio, tetanus, tuberculosis and yellow fever. Since then, this series has been updated and extended to include other vaccines of international importance.

The main purpose of the modules is to provide national immunization managers and vaccination professionals with an overview of the scientific basis of vaccination against a range of important infectious diseases. The modules developed since 1993 continue to be vaccine-specific, reflecting the biological differences in immune responses to the individual pathogens and the differing strategies employed to create the best possible level of protection that can be provided by vaccination. The modules also serve as a record of the immunological basis for the WHO recommendations on vaccine use, published in the WHO vaccine position papers.[1]

This module concerns vaccines against influenza, an infectious disease of worldwide public health importance which presents unique immunological challenges. The vaccines are also unique, their content necessitating reformulation prior to each annual influenza season and requiring annual re-vaccination. The module answers the questions that stem from the exceptional nature of the influenza viruses and their capacity for rapid mutation and antigenic change, and the need to align vaccine development with those characteristics, now and in the future. The existing types of influenza vaccines and the immune responses to them are described, and future needs and prospects are outlined.

[1] See: *http://www.who.int/immunization/documents/positionpapers_intro/en/index.html*, accessed 10 Aug 2017.

1. Influenza virus and disease

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Influenza is a contagious, acute respiratory illness that appears to have caused outbreaks, epidemics and pandemics for centuries. Many early historical accounts of what are now believed to have been influenza epidemics or pandemics describe recognizable features including the typical clinical symptoms, a short incubation period, high attack rates in multiple age groups, and rapid progression of disease through the population, often with the greatest mortality in the elderly. The search for the pathogen responsible for influenza intensified after the devastating influenza pandemic of 1918–1919, believed to have caused about 50 million deaths worldwide and a dramatic decline in life expectancy in many countries.[1] In 1933, a British team successfully isolated the first influenza virus from an ill patient, and researchers almost immediately began working to develop a vaccine against influenza. The majority of influenza vaccines in use today are similar to those developed during the 1930s and 1940s in that they are inactivated, parenterally administered and depend on the induction of virus-specific immune responses. These inactivated vaccines along with newer recombinant inactivated and live attenuated influenza vaccines remain the cornerstone of global influenza prevention and control efforts.

1.1 Influenza epidemiology

Influenza is an acute febrile viral respiratory disease of global public health importance because of the substantial morbidity and mortality that occurs during both annual epidemics and relatively infrequent pandemics. Influenza epidemics are caused by both type A (H3N2 and H1N1) and B/Yamagata and B/Victoria lineages viruses; pandemics have been caused only by influenza A viruses. Annual influenza epidemics occur because influenza A and B viruses circulating globally are evolving and changing their antigenic properties over time such that immunity conferred by previous infection or vaccination no longer provides protection. The continuous transmission of epidemic influenza viruses is primarily due to this antigenic variation or so-called antigenic "drift" that takes place in the two major virus surface proteins, the hemagglutinin (HA) and the neuraminidase (NA) (*Figure 1*). During antigenic drift, circulating influenza A or B viruses are displaced when a corresponding new antigenic variant emerges, spreads and replaces its previously circulating precursor. When a new variant becomes predominant and circulates globally, immunity to it increases in the population to a point at which another antigenic variant emerges

and the cycle of increasing immunity and antigenic escape continues.[2,3] The process of ongoing antigenic drift periodically provides a new pool of susceptible human hosts which in turn fuels the next influenza epidemic. In contrast, antigenic "shift" results from major changes in the HA antigen of influenza type A virus, often with changes also in the NA antigen, caused by reassortment between different influenza A subtypes, such as between animal and human subtypes. The resulting viruses can potentially cause regional outbreaks or a global pandemic.

Annual epidemics of influenza are associated with substantial national and global disease burdens and are responsible for far more accumulated morbidity and mortality than result from pandemics. For example, in the United States of America (USA) it has been estimated that from thousands to tens of thousands of deaths and hundreds of thousands of hospitalizations occur because of annual influenza epidemics.[4,5] These disease impact studies also demonstrated that influenza A(H3N2) viruses are often associated with the highest overall morbidity and mortality, particularly during years when a new antigenic variant emerges and spreads through the population, as occurred during the 2014–2015 influenza season in the northern hemisphere.

The timing, intensity and duration of influenza activity vary considerably from year to year and are not predictable. The predominant influenza viruses circulating may differ geographically and temporally within and between countries. During annual influenza epidemics in temperate regions of the world, sharp seasonal rises in acute influenza illness result in increased physician visits, increased hospitalizations for management of lower respiratory tract complications and excess influenza-related deaths during the winter months; however, sporadic cases and institutional outbreaks are also detected out of season. In countries with tropical and subtropical climates, influenza seasonality is less obvious than in those with temperate climates and influenza can occur throughout the year with one or more small peaks of activity that may be associated with the rainy season or other environmental and social factors.[6] Some countries in tropical or subtropical regions of the northern hemisphere may have a summertime peak of influenza activity.[7] Furthermore, large countries such as India, China and Brazil have two or more distinct epidemic patterns in geographically and climatically different regions.[6] Although the factors affecting influenza seasonality are not fully understood, temperature and humidity have significant effects on virus survival and circulation, and seasonal differences in behaviour, such as school attendance and indoor congregation during inclement weather, are also believed to affect virus transmission.

Influenza attack rates vary by age group, by season, by geographic location, by the predominant virus type/subtype and by setting (community or institutional). In general, influenza A has the greatest severity of disease, while influenza B has been shown to have major impacts on health, particularly in pa age groups.[8] Community studies have shown that attack rates are typically higher in children than in adults. Overall, annual influenza virus infection rates have been estimated at 2–10% in adults and 20–30% in children aged 5 years and younger.[9,10] Attack rates during institutional

outbreaks can be much higher with rates of up to 80–90% reported in settings such as boarding schools and military training camps and an average attack rate of over 40% reported in a review of outbreaks in nursing home residents.[11]

Influenza pandemics occur at irregular intervals and spread rapidly around the world. To cause pandemics, pandemic viruses must be transmitted efficiently from person to person and must have an HA or both HA and NA proteins that differ enough from those of the previously circulating epidemic influenza A viruses that there is little immunity to the pandemic virus in the human population. Attack rates during influenza pandemics are typically higher in most age groups than during epidemics. For example, illness rates among school children were reported to be as high as 40% for the 1918-19 pandemic and over 50% for the pandemics of 1957 and 1968.[12] For reasons that are not fully understood, the 1918 influenza pandemic caused particularly high mortality rates in young healthy adults even though the overall attack rates were not substantially different from those in other pandemics. The high mortality rates in young adults had numerous health, social and political consequences, including an overall decrease in life expectancy. The less severe 1957 and 1968 pandemics both emerged and spread first in China. The most recent influenza pandemic was caused by a novel H1N1 virus which spread first in Mexico, subsequently spread to the USA and then swept around the globe during 2009 and 2010.[13,14] Importantly, two or more genes of each of these four pandemic viruses were traced back to influenza A viruses circulating in birds or pigs which harbour influenza viruses that are substantially different from those circulating in humans and serve as sources of genes for new emerging pandemic influenza viruses.

1.2 Influenza disease and pathogenesis

Influenza A and B virus transmission occurs through both large and small droplet aerosols produced when an infected person coughs or sneezes, as well as through direct contact with infected respiratory secretions including fomites. The relative importance for each of these modes of transmission is unknown but recent data have confirmed that aerosol transmission of fine droplets can be a source of infection.[15,16] The primary sites of influenza infection are epithelial cells in the upper respiratory tract where most viral replication usually occurs, although replication can also take place in other parts of the respiratory tract leading to bronchitis, tracheitis, and pulmonary parenchymal involvement in more severe cases.[17,18] Viraemia is detected rarely in epidemic influenza virus infections and there is no evidence for transmission via blood. The quantity of virus present in the upper respiratory tract has been found to correlate with the severity of symptoms. Infection by epidemic influenza A and B viruses causes similar disease and viral pathology.[19-21]

Typical influenza illness is characterized by sudden onset of symptoms that often include fever, sore throat, dry cough, headache, chills, muscle aches, fatigue and loss of appetite.[21,22] Gastrointestinal symptoms, including abdominal pain, vomiting and diarrhoea, are observed among children but are less common in adults. Otitis media

is also common in children. During periods of influenza circulation, cough and fever are the best predictors of influenza in adults and adolescents.[22] Fever usually lasts for about 3–5 days, with the temperature most often between 38 °C and 40 °C but may be higher, particularly in children. Children with higher fevers can present with febrile seizures while young infants may present only with unexplained fever or sepsis-like syndrome.[23] Older adults may present with loss of appetite, fatigue and confusion but without fever.[24,25] In uncomplicated influenza, fever and body aches typically last for 3–5 days while cough and fatigue may persist for 2 weeks or longer. The majority of influenza virus infections resolve with non-specific therapies. Asymptomatic influenza virus infections are quite common and estimated to comprise 30% or more of all influenza virus infections.[26,27] Virus shedding may occur during these infections with spread of influenza to others.

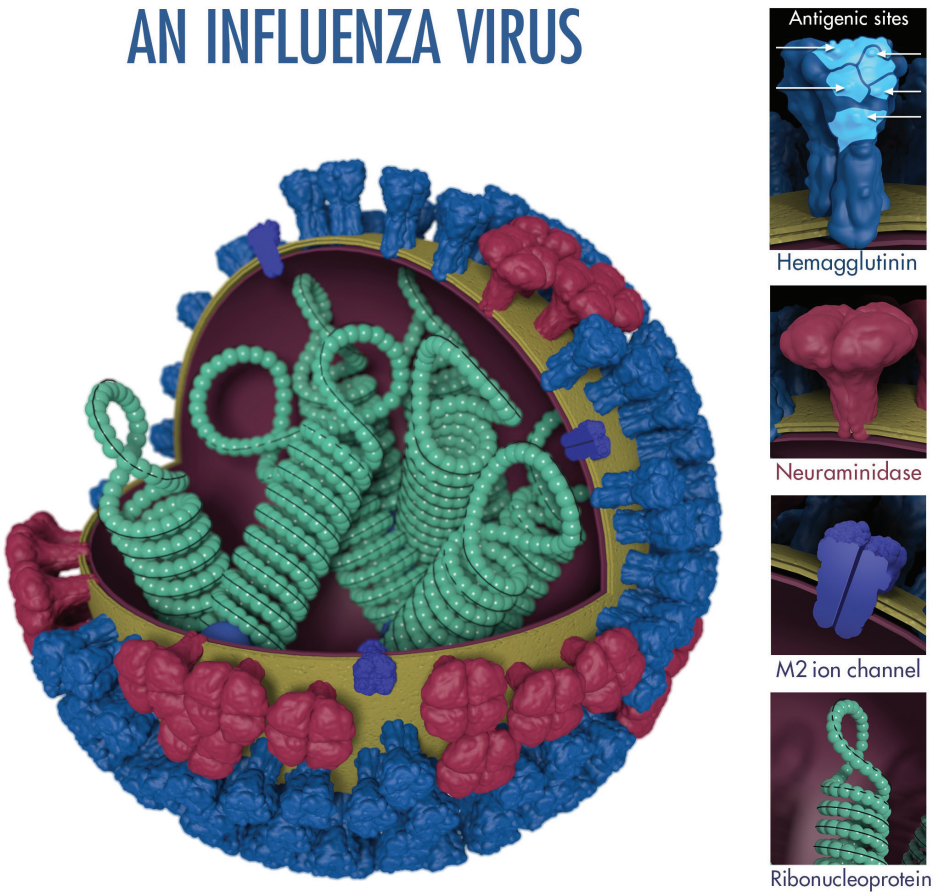
Several population groups are at increased risk for more severe influenza illness and influenza-related mortality, including persons with certain underlying medical conditions,[28-30] infants and young children,[31-33] and elderly adults.[34,35] In addition, pregnant women have higher rates of hospitalization during influenza epidemics[36,37] and increased influenza-related hospitalization and mortality during pandemics.[38,39] Analyses of risk factors for severe influenza have generally been consistent in their findings, however they come mainly from high-income country settings. Influenza is also associated with worsening of chronic lung and heart disease;[4,40] patients with asthma, chronic pulmonary disease and congestive heart failure have increased clinic and hospital visits during influenza seasons compared to persons without these conditions. Worsening of diabetes, renal diseases and blood disorders have also been reported.[41-44] Those who are immunocompromised (including cancer, organ transplant and HIV patients) are also at higher risk for more severe influenza disease.[45-48] Children with neurologic and neuromuscular diseases have increased risks of influenza-related hospitalization and death.[43,49] Primary viral pneumonia and secondary bacterial pneumonia also result from influenza virus infection. In both developed and developing countries, influenza infection has been detected in about 5–10% of hospitalized patients with lower respiratory tract infections.[23,50-55] However, influenza complications, hospitalization and death may occur even among those with no known risk factors, although much less frequently.

1.3 Influenza viruses

Influenza viruses circulating in humans are classified in the Orthomyxoviridae family and belong to three genera based on their biological and immunological properties: influenza A, B and C. Influenza A viruses cause human epidemics and pandemics and were first isolated in 1933 by Smith, Andrewes and Laidlaw,[56] while influenza B viruses are also responsible for epidemic, but not pandemic, disease and were first isolated in 1940 by Thomas Francis.[57] Influenza C viruses generally cause localized outbreaks of mild upper respiratory disease in children but do not cause epidemic disease in multiple age groups. Influenza C viruses are not discussed in more detail as they have relatively little public health impact and vaccines have not been developed against them.

Currently two influenza A subtypes (H1N1pdm09 and H3N2) and two genetic lineages of influenza B viruses (B/Yamagata and B/Victoria) are co-circulating in humans and causing epidemic influenza disease. Influenza A and B viruses appear in electron micrographs as pleomorphic or spherical 80–120nm particles with surface spike-like projections about 10–14 nm long. Influenza A and B viruses have segmented, negative sense RNA genomes which allow exchange of viral genes (genetic reassortment) when two different influenza A or two different influenza B viruses infect the same host (*Figure 2*). Genetic reassortment between influenza A and B viruses does not occur. Eight RNA gene segments are present in viral particles and these RNA segments are associated with viral nucleoprotein (NP) and three viral polymerase proteins (PB1, PB2 and PA) that together make up the viral replication complex (*Figure 1*). Replication complexes are surrounded by matrix (M) protein and a lipid envelope that is derived from host cell membranes from which a surface layer of small virally encoded "spikes" project.[58] These spike-like projections are the viral HA and NA proteins responsible for attachment to host cells and for release of mature virus from infected cells, respectively.

Figure 1. Three-dimensional illustration showing the different features of an influenza virus, including the surface proteins hemagglutinin (HA) and neuraminidase (NA).



Note: Public domain image courtesy of CDC/ Douglas Jordan, Ruben Donis, James Stevens, Jerry Tokars; CDC Influenza Division. *https://phil.cdc.gov/phil/home.asp*

The key to understanding the immunological basis of current influenza immunization lies mainly in the two major viral surface proteins, HA and NA. The HA is the most important and abundant surface protein and is the viral antigen against which protective (neutralizing) antibodies are directed. The functional HA trimeric molecule has two regions, a highly variable globular head region called the HA1, and a more conserved stem region termed HA2. The NA is less abundant than HA and is present in tetrameric form. Antibodies to the NA decrease disease severity and contribute to protection from infection. Variation does not occur synchronously in HA and NA proteins. Genetic changes among influenza viruses are monitored over time by sequencing the genes encoding these two proteins. Thus, the central problem for influenza immunization is that both the HA1 region of the HA and the NA proteins of influenza viruses are highly variable due to errors in replication followed by immune selection. Although antibodies to both proteins play a role in protection, it is the amount and immunogenicity of HA protein, but not of NA, that are measured in current vaccines.[59]

Because the two key proteins, HA and NA, of circulating influenza A (H1N1 and H3N2) and influenza B viruses evolve and change over time, year-round global surveillance efforts are undertaken by the WHO Global Influenza Surveillance and Response System (GISRS).[60] GISRS is a network of public health laboratories initiated after the Second World War to monitor the influenza viruses circulating globally. This network has grown to include over 140 National Influenza Centres (NICs) in over 110 countries which isolate and identity influenza viruses causing disease within these countries. The NICs are nominated by their respective ministries of health, are designated by WHO and are the cornerstone of global influenza surveillance. They also work closely with six WHO Collaborating Centres which perform complex antigenic, molecular and serologic assays to characterize the viruses sent to them by the NICs. These coordinated, highly technical activities of the GISRS are necessary because influenza surveillance is conducted all year round due to the potential for antigenic drift of circulating viruses and the need to include antigenically well-matched vaccine viruses for optimal vaccine effectiveness. The challenge for GISRS is to detect new antigenic variants of epidemic influenza viruses and to identify candidate vaccine viruses in time to recommend them for inclusion in the vaccine formulation for the coming influenza season.[61]

WHO issues recommendations for the composition of influenza vaccines twice each year, in February or March and in September or October, in time to enable vaccine production prior to the influenza seasons in the northern and southern hemispheres, respectively. These vaccine virus recommendations are updated if one or more new antigenic variants that are not be well covered by the corresponding vaccine virus have emerged and are spreading in association with influenza outbreaks or epidemics. Once an important antigenic variant has been identified, the WHO Collaborating Centres along with the Essential Regulatory Laboratories select suitable candidate vaccine viruses, prepare high growth reassortants for vaccine production by manufacturers, and produce corresponding reagents for vaccine standardization. These vaccine composition recommendations provide crucial guidance to national

regulatory authorities and vaccine manufacturers for the development and production of contemporary influenza vaccines.[60] National authorities should select the WHO recommended February/March or September/October vaccine formulation based on vaccine availability and the timing of epidemic disease peaks in their specific countries or regions.

In addition to the influenza A H1N1 and H3N2 subtype viruses circulating in humans, at least 16 HA and 9 NA influenza A subtypes are circulating globally in aquatic birds which serve as an influenza reservoir and a source of genes for future pandemic viruses.[62,63] At least two additional subtypes of influenza A viruses infect bats; however, so far no public health problems have been associated with these particular viruses.[64] It is known that influenza A viruses from the aquatic bird reservoir infect poultry relatively frequently and have infected pigs, horses, cats, dogs and marine mammals. Influenza A viruses of different subtypes (e.g. H1N1, H3N2 and H1N2 in pigs and H9N2, H5N1, H5N6 and H7N9 in poultry) have become established in these secondary hosts. This explains how influenza A viruses of different subtypes than those currently circulating in humans are occasionally transmitted across the host-species barrier from domesticated birds (mainly poultry) or pigs to humans. Fortunately, onward transmission from the index case to other humans is relatively rare. A notable exception occurred in 2009 when a novel H1N1 virus that contained genes of swine, avian and human influenza virus origin was the cause of the most recent worldwide influenza pandemic. This virus, named H1N1pdm09, likely originated in pigs because all of the eight gene segments of the 2009 pandemic virus had been detected previously among influenza viruses circulating in pigs.[13] During the 2009 pandemic, the H1N1pdm09 virus displaced the previously circulating H1N1 virus and became established in the human population; it is currently circulating as one of the four groups of influenza viruses that cause epidemics worldwide. This event provided a clear example of a pandemic virus that emerged from an animal reservoir; it also provided strong evidence for the role of pigs as a source of new human pandemic influenza viruses, a hypothesis that had been proposed a number of years previously.[65] The emergence of the H1N1pm09 virus re-emphasized the importance of ongoing vigilance and surveillance for human infections caused by animal influenza viruses and for surveillance in pigs as well as birds.

The ability of a particular influenza virus to infect a given host (e.g. humans, pigs, or birds) efficiently is determined largely by whether it can efficiently attach to, replicate in, and be released from infected cells in that host. The HA proteins on the surface of virus particles attach to sialic acid receptors on the surface of susceptible cells of the respiratory tract and this is the essential first step in infection. Importantly, there are different molecular arrangements or linkages for sialic acid on host cell receptors in birds compared to those in the upper respiratory tract of humans. These differences in the types of receptors in humans and birds restrict the number of human infections by influenza viruses that typically infect birds and vice versa. In particular, human influenza viruses bind preferentially to cells with sialic acid with an alpha-2,6 linkage to galactose and this type of cell is present in the upper respiratory tract of humans.[66] In contrast, avian influenza viruses bind best to cells which have sialic acid with an

alpha-2,3 linkage to galactose and this type of cell is present in abundance in the intestinal tract of birds and in the lower respiratory tract of humans. The ability of certain avian influenza viruses to attach to and replicate in the lower respiratory tract of humans helps to explain why infections caused by highly pathogenic avian influenza viruses of the H5 subtype and those caused by H7N9 viruses can be extremely severe.[67] Unlike HA, the NA has enzymatic activity which removes the viral receptor, sialic acid, from viral and host proteins and is thus responsible for release of mature virus particles from the surface of infected cells.[68] This enzymatic release of virus from infected cells is critical for influenza viruses to spread from cell to cell and from person to person.

1.4 Influenza diagnosis

Early diagnosis of influenza can reduce the inappropriate use of antibiotics and provide the option of using influenza antiviral medications in certain situations. Respiratory infections caused by a variety of other viral or bacterial pathogens can also result in illnesses resembling influenza, so that individual cases of influenza can be difficult to identify reliably by clinical examination alone. Therefore, influenza-specific laboratory diagnostic tests including virus culture, rapid antigen detection, immunofluorescence assays, and molecular methods have evolved over the years.[69,70] One molecular method, the reverse-transcription-polymerase chain reaction (RT-PCR), has been used to detect influenza in respiratory specimens and has become an important and sensitive tool for clinicians and for public health surveillance efforts[71,72] and for conducting studies of influenza vaccine effectiveness.[73] In the case of community-wide outbreaks or institutional outbreaks of febrile respiratory disease during winter months, once it has been confirmed that influenza is circulating, a diagnosis based on clinical evaluation alone is more accurate than during periods of low levels of influenza activity. Nevertheless, because a number of other respiratory pathogens have quite similar epidemiologic patterns (e.g. respiratory syncytial virus) the use of diagnostic tests to guide individual treatment or institutional actions to limit spread is useful when multiple pathogens are circulating in the community. For accurate laboratory diagnosis, the preferred clinical samples are nasopharyngeal swabs, throat swabs, nasal swabs, nasal washes or nasal aspirates. Clinical samples should be collected as early as possible after onset of illness and preferably within the first three to four days for best results.[74]

A number of commercially available point-of-care rapid influenza diagnostic tests (RIDTs) detect the presence of influenza virus proteins (most commonly viral NP) in clinical specimens using an immunoassay. Some of these tests detect influenza A and B viruses and distinguish between them while others detect only influenza A, or detect both A and B without distinguishing between them. Typically, these tests are relatively easy to perform and results are available in approximately 15 minutes. Because RIDTs provide results within a clinically relevant time-span, they may be used to help with decision-making about treatment and to determine whether influenza virus is the cause of institutional respiratory disease outbreaks. If RIDT results are positive, this information can guide institutional actions on treatment of

patients with antivirals to reduce spread of infection. An important limitation of the RIDTs is that they generally have lower sensitivity (50–70%) compared to RT-PCR and virus culture and therefore false negative results are possible, particularly during times when influenza is circulating widely in the community.[74,75] In contrast, the specificities of RIDTs are generally high (90–95%) which means that false positive test results are less likely than false negatives but false positives can occur, especially during times when influenza activity is low. For accurate results, it is important to handle respiratory samples as described in the manufacturers' instructions in the package inserts. The commercial RIDTs that are available vary from country to country.

Molecular methods are now used to diagnose influenza virus infections in clinical and research laboratories. RT-PCR assays detect the presence of influenza viral RNA in respiratory specimens and have become the most commonly used molecular diagnostic tests for influenza. During recent years, RT-PCR methods have become the gold standard for influenza diagnosis in many public health laboratories. They are also being used more commonly in clinical settings such as hospitals, particularly in high-income settings. In general, RT-PCR methods for influenza are highly sensitive and specific.[71] While some of these assays only detect and differentiate influenza A and B viruses, other available assays can identify the influenza virus type (A or B) as well as the subtype (H1N1 or H3N2) and lineage (B/Yamagata or B/Victoria). Results for molecular assays may be available from between 30 minutes and 8 hours, depending on the assay used.[76] Multiplex molecular panels used to detect other respiratory viruses concurrently with influenza are available in clinical laboratories in some countries.

It remains important for some laboratories to continue to use virus culture so that influenza viruses can be fully characterized in specialized laboratories. Virus culture is necessary to determine whether antigenic drift has occurred, which may signal a need to update the viruses contained in the influenza vaccines used worldwide. Virus culture is also essential as it currently serves as the source of the vaccine viruses themselves.

If respiratory specimens are not available, influenza virus infections can be identified by measuring increases in serum influenza antibodies of infected individuals. The level of these antibodies is measured most accurately by the haemagglutinin inhibition (HAI) and microneutralization (MN) assays.[77] In order to confirm that an influenza virus infection has occurred, blood samples must be taken both early in the acute (first 7 days) and later in the convalescent (3 weeks or more after onset of symptoms) stages of illness. Antibody levels in the convalescent serum must be at least 4-fold higher than in the acute phase serum to confirm infection. It is important to note that these serological assays may underestimate infections in vaccinated individuals.[78] Serologic confirmations of infection most often takes place within a research setting due to the complexity of the assay and the inability to act on the results for clinical care. Serological assays are less sensitive for detection of influenza virus infections than the molecular methods that are now in common use.

1.5 Immunological responses to natural infection by influenza viruses

Unlike many other natural viral infections (e.g. measles, mumps, chicken pox, etc.), infection by influenza viruses does not provide life-long protective immunity against subsequent infections with related viruses. This lack of life-long protection is remarkable because robust innate, humoral (antibody), and cell-mediated (T cell) responses to influenza can be measured in humans after infection. In essence, it is currently understood that HA-specific antibodies in serum and in the respiratory tract neutralize the virus and prevent infection, while the T cell response is important for viral clearance and host recovery. The absence of life-long protective immunity has been attributed primarily to the extensive antigenic diversity that exists among the influenza viruses to which a person is exposed to during a lifetime. This diversity is due to the exceptional ability of influenza viruses to change over time through both mutation and genetic reassortment. The fact that natural infection does not confer long lasting protection provides an important clue as to why efforts to prevent and control this disease through immunization are so challenging. Nevertheless, studying the immune responses to natural influenza virus infection provides valuable information for understanding the correlates of protection and for potential future approaches to improve influenza vaccines.

1.5.1 Innate immune responses to infection

The innate immune system detects infection by microbial pathogens, including influenza viruses, through recognition of distinct pathogen-associated molecular patterns (PAMPs).[79,80] Cells of the innate immune system include epithelial cells, dendritic cells, and macrophages which recognize the PAMPs of influenza viruses through the use of pattern recognition receptors (PRRs).[81] Once PAMPs of a pathogen are recognized, the PRRs trigger a signalling cascade that results in the secretion of type I interferons and other defence molecules such as cytokines; these in turn activate many anti-viral genes that make adjacent uninfected cells more resistant to infection.[81] In addition, natural killer T cells, monocytes, macrophages and neutrophils move to the site of infection to deliver natural defence molecules locally.[80] Importantly, co-stimulatory molecules are also expressed after virus detection by the PRRs on antigen presenting cells (APC) and these molecules activate the adaptive immune responses, resulting in rapid production of antibody and T cell responses.

While innate immune responses are critical components of the first line of host defence against influenza, it has been found that systemic symptoms such as fever, muscle aches and fatigue are, at least in part, a result of the innate immune response. It has also been found that higher levels of certain cytokines are present in patients with more severe influenza disease that in those with milder illness. These findings, taken together with studies in animal models, indicate that innate immunity is the first line of host defence and is important for recovery from infection but can also contribute to disease pathology if cytokine levels become too high, such as has been reported with infections caused by highly pathogenic avian H5N1 viruses.[82]

1.5.2 Humoral (B cell) immune responses to infection

Infection by influenza viruses rapidly induces production of specific antibodies capable of neutralizing the infecting virus and reducing virus replication and spread in the host and the eventual clearance of the virus.[83] These antibodies are directed against both highly variable portions of the immune-dominant globular head regions of the HA and highly conserved regions of the stalk of the HA. The HA-specific antibodies that bind to the globular head region (HA1) of the molecule inhibit virus attachment and entry into the host cell. These antibodies bind to four or five distinct antibody combining sites on the head region of the HA, depending on the virus type and subtype, and effectively neutralize the virus. Anti-HA neutralizing antibodies contribute to the complete clearance of influenza virus infection, which usually takes place within one or two weeks of infection. After virus clearance, memory B cells and long-lived plasma cells are produced which protect against future infections with the same or antigenically similar influenza viruses. While antibody-mediated protection against the infecting virus is strong, protection against viruses that have undergone significant antigenic drift in the HA is reduced. This explains why individuals can be successively infected by antigenically distinct influenza viruses of a given type or subtype throughout life.

It has been discovered recently that influenza virus infection also induces relatively low levels of cross-reactive and cross-protective antibodies directed against the highly conserved stalk region of the HA protein and against certain conserved regions in the HA globular head. These antibodies block entry of many antigenically distinct influenza viruses into the host cell or block other key functions of influenza viruses that are necessary for virus replication. It is hoped that a greater understanding of the HA stalk antibodies will lead to development of more broadly cross-reactive influenza vaccines and of therapeutic monoclonal antibodies that can be used to treat severely ill patients.[84]

For decades the level of anti-HA antibodies in human serum has been measured by the HAI and/or MN assays. A serum HAI antibody titre of 1:40 or 1:32 has been established as a level that provides protection from infection in about 50% of those with this level of antibody[85] but higher titres may be required in children less than 6 years of age.[86] In general, the higher the titre of anti-HA antibody the less susceptible the individual is to infection. The levels of IgG antibodies against the HA in serum of previously infected or vaccinated individuals has been widely viewed as the best indicator of protection against influenza disease, but it is not the only one identified. Infection-blocking secretory IgA antibodies against the HA are also present on mucosal surfaces of the upper respiratory tract but it has been more difficult to obtain reliable IgA antibody measurements in nasal secretions.

In contrast to antibodies to the HA, those directed against the NA act to reduce virus spread from cell to cell, thus helping to reduce disease severity.[87] Antibodies to the NA bind to NA and block its enzymatic activity causing virus particles to aggregate or clump together on the infected cell surface thereby reducing the amount of virus

that is released.[87,88] The level of anti-NA serum antibody has also been shown to correlate with protection from infection.[87-89] Antibodies to the more conserved NP and M2 proteins are also detected after infection in humans and in animal models but do not appear to prevent infection.[90,91] However, antibodies to the NP and modified M2 proteins have been demonstrated to provide protection from severe disease and death in some animal models[92-94] and these proteins have been identified as targets for development of more broadly protective next-generation influenza vaccines.

1.5.3 Cell-mediated (T cell) immune responses to infection

Although the role of T cell immunity is not yet as clear as the role of the B cell antibody response in protection from influenza virus infection, data supporting its importance have accumulated over time. Studies have sometimes produced conflicting results regarding the role of the T cell response in protection from disease. However, a few studies have demonstrated an even better correlation of protection for influenza-specific CMI responses than for neutralizing antibody titres. The CMI responses are mediated by CD4+ and CD8+ T lymphocytes produced in the thymus and directed at virus-infected and antigen-presenting cells. In brief, naive CD4+ and CD8+ T cells circulate throughout the body's lymphatic tissues where they detect foreign antigens displayed on the surface of antigen-presenting cells (APC) such as dendritic cells.[95] Once activated by recognition of the presence of a foreign antigen, CD8+ T cells migrate to the site of virus infection where their main function is to eliminate virus-infected cells. The most important function of CD4+ T cells is to promote an effective antibody response, while also promoting effective CD8+ T cell responses.

Natural influenza virus infection induces T cell responses primarily directed against the more conserved so-called "internal" (NP, M and polymerase) viral proteins. These T cell responses are therefore more cross-reactive against a diversity of influenza viruses than the more strain-specific antibody responses directed against the highly variable portions of the HA and NA proteins. Unlike antibody recognition which relies on binding to larger discontinuous epitopes, T cell immunity works through immune recognition by activated CD4+ and CD8+ lymphocytes of short linear fragments of about 8–9 (CD8+) or 15–20 (CD4+) amino acids (epitopes) of the "internal proteins". The ability of an individual to respond to a specific T cell target epitope in these internal proteins is dependent on their human leukocyte antigen (HLA) phenotype and there is considerable variation among HLA phenotypes in the global population. This variation creates a diversity of responses in individuals to the various T cell epitopes present in the internal influenza virus proteins, which complicates the study of human T cell responses. Nevertheless, T cells from adults exposed to seasonal influenza are responsive to NP, M1 and polymerase basic protein 2 (PB2) implying that pre-existing T cell responses in humans can contribute to immunity across influenza A subtypes. But despite these interesting and important observations indicating that T cell responses can protect within and even across subtypes, sequential infection with antigenically drifted human influenza viruses and with pandemic viruses still occurs, leaving questions about how and when T cell responses are most effective.[96]

In many viral infections, including influenza, CD8+ T cells have been shown to play a vital role in clearing virus and enabling host recovery. CD8+ T cells are killer cells which find and kill virus-infected cells in the body.[95] An early challenge study in humans provided indirect evidence for a role of T cell activity in reducing viral shedding by demonstrating that all subjects with pre-existing influenza-specific T cells cleared the challenge virus effectively whether or not they had detectable anti-HA antibodies.[97] Subsequent studies during the 2009 influenza pandemic showed that in the absence of detectable serum antibody to the HA of the virus, high numbers of influenza-specific CD8+ T cells were correlated with less severe disease in patients.[98] Recent studies have also demonstrated that CD8+ T cells induced by seasonal virus infection cross-react with a diversity of viruses, including the distantly related HPAI, H5N1 and H7N9 viruses,[99-101] thus providing solid evidence of sub-type cross-reactivity of these T cells. Once the infecting influenza virus has been cleared, the majority of virus-specific CD8+ T cells die while the remaining 5–10% form a stable, long-lived pool of memory T cells that can be called into action rapidly in the face of another influenza virus infection.

The role of CD4+ T cells is less well understood than that of CD8+ T cells but they have important functions in the immune response to influenza viruses. CD4+ T cells promote an effective immune response both by supporting the antibody response and by producing antiviral and pro-inflammatory cytokines, along with other functions.[102] The most important role of immune CD4+ T cells appears to be in providing the necessary signals, via production of cytokines and other immune signalling molecules, for optimal antibody production.[102] A second important role for CD4+ T cells is to provide help to CD8+ T cells through the production of cytokines that support the establishment of optimal CD8+ T cell memory after infection. In addition, some CD4+ T cells can directly kill virus-infected cells via responses to portions of the relatively conserved NP and M1 proteins. CD4+ T cells also produce cytokines that are important for an overall effective immune response.[102] A recent study has shown that the presence of memory CD4+ T cells specific for influenza in humans correlated with decreased virus shedding.[103] These memory CD4+ T cells could both lyse infected cells and cross-react with peptides from variant influenza viruses, providing evidence for heterologous protection. Taken together, these human studies emphasize the importance of CD4+ T cells in recovery from virus infections and in reducing the severity of illness.

2. Influenza vaccines

Immunization is the primary and most effective approach for the prevention and control of influenza. This is true despite the fact that ongoing evolution of influenza viruses and the consequent antigenic changes pose a challenge for the production of influenza vaccines that are efficacious against currently circulating viruses, and require updating periodically to keep pace with virus evolution. The first inactivated influenza A vaccines were developed soon after the discovery in 1933 that a virus was the causative agent of influenza. Influenza vaccine development accelerated during the late 1930s and 1940s, prompted by memories of the severity of the 1918 influenza pandemic and fear that a similar outbreak might occur during the Second World War. These early vaccines were produced from purified influenza A and B viruses grown in embryonated hens' eggs, then chemically inactivated, and were administered intramuscularly.[104,105] While these inactivated whole-virus vaccines were initially developed for use in military personnel, they were licensed for use in civilian populations in 1945 in the USA after their efficacy had been demonstrated in military recruits, college students and other populations.

Currently, both inactivated influenza vaccines (IIVs) and live attenuated influenza vaccines (LAIVs) have been developed and approved for use. Most IIVs are administered either intramuscularly or intradermally, and LAIVs are administered intranasally. Immunization with all currently approved IIVs is based primarily on inducing an antibody response to the antigenically variable HA protein. Although the immune response to LAIVs is more complex and involves both T cell and antibody responses, these vaccines also work best when the HAs in the vaccine match those of circulating viruses. A vaccine mismatch occurs if the antigenic properties of circulating viruses are significantly different from those of the corresponding vaccine virus, i.e. when an 8-fold or greater reduction in HAI titre is observed for circulating viruses of a given type or subtype compared with the homologous titre for the corresponding vaccine virus. Thus, all seasonal vaccines must be reformulated regularly so that the viruses contained in vaccines are antigenically matched as closely as possible with the currently circulating viruses because the vaccines lose effectiveness in the face of antigenic variation in the HA and NA proteins. The formulation of influenza vaccines is therefore modified routinely to include the viruses that are expected to be predominant during the next influenza season.

While most of the influenza vaccines manufactured worldwide continue to be produced using virus cultivated in eggs, then inactivated, and administered intramuscularly, other types of influenza vaccines have been approved in more recent years. Newer vaccines include live attenuated as well as recombinant, adjuvanted, and intra-dermally administered IIVs. The most important limitations of current influenza vaccines are

the need for annual vaccination, and their strain-specificity, providing protection only against specific antigenic variants of the virus type/subtypes included in the vaccine. Recent vaccine development efforts have therefore focused on increasing the breadth of protection against antigenic variants and on increasing the duration of immunity beyond one year.

For many years both IIVs and LAIVs have contained three vaccine virus components. These trivalent (3-valent) vaccines contain representative influenza A viruses of both the H3N2 and H1N1 subtypes and a relevant influenza B virus. Because two antigenically distinct lineages of influenza B viruses are currently circulating, some countries have recently approved the use of quadrivalent (4-valent) vaccines containing representative viruses of both the antigenically distinct B/Victoria and B/Yamagata lineages as well as those representing the two circulating influenza A virus subtypes. The viruses included in influenza vaccines are recommended by WHO and approved by national authorities six to nine months prior to the next influenza season, in order to allow vaccine manufacturers sufficient time to produce the hundreds of millions of vaccine doses required to meet global demand.[106]

2.1 Inactivated influenza vaccines (IIVs)

The first IIVs were developed by growing large quantities of influenza viruses in embryonated hens' eggs, collecting the virus-containing allantoic fluid, and then purifying and inactivating the whole virus preparations. The subsequent use of zonal centrifugation and chromatographic methods has provided vaccines with greater purity and lower levels of reactogenicity than the early vaccines.[107,108] Purified vaccine virus preparations for each recommended vaccine virus are chemically inactivated with formalin or beta-propriolactone, and then mixed together to produce trivalent or quadrivalent vaccine; the viral envelope is disrupted by detergents to ensure virus inactivation. The majority of inactivated vaccines currently in use are detergent-disrupted sub-virion (SV) vaccines of two types: split virus vaccines and purified surface antigen vaccines. Whole virus preparations are now used in only a few countries. IIVs have been administered by intramuscular, subcutaneous, intradermal, intranasal and oral routes, but are now usually injected intramuscularly.

Because many wild-type influenza viruses do not grow to high titres in eggs, the development of high-growth reassortant (HGR) vaccine viruses has been important for ensuring vaccine supply for egg-based vaccines since the 1970s. These HGR vaccine viruses are produced by genetic reassortment between a wild-type virus that has the desired HA and NA and the laboratory-adapted A/Puerto Rico/8/34 virus that grows to high titres in eggs.[109,110] During reassortment, progeny viruses which replicate well in eggs and have the appropriate antigenic properties are selected. Such HGR vaccine viruses often have a more spherical morphology than that of the typically filamentous wild-type viruses, which facilitates greater recovery of virus during the various purification steps. The manufacturing advantage of using these HGR vaccine viruses is so great that the majority of egg-based vaccines have been manufactured using influenza A HGR, and in recent years some manufacturers have also used HGR influenza B viruses.

Most IIV formulations continue to be produced using eggs, although some licensed influenza vaccines are now produced in approved mammalian cell lines or in insect cells.[111] Mammalian cell culture is a well-established substrate for production of a wide variety of viral vaccines. A vaccine made by propagating influenza virus in Madin-Darby canine kidney (MDCK) cells was first approved in the European Union[112] and more recently in the USA.[113] This vaccine is produced by growing each recommended vaccine virus in suspended MDCK cells, and then recovering, purifying, inactivating and disrupting the virus using processes similar to those used for egg-based vaccines. As with egg-based vaccines, the vaccine viruses are processed separately, purified and inactivated before being mixed together to formulate the trivalent or quadrivalent product. Another IIV approved for use in the USA and Mexico uses recombinant DNA technology to produce large quantities of HA proteins of the recommended vaccine viruses in insect cell cultures. The HA proteins are then purified and used in a vaccine that, unlike egg-based vaccines, does not contain neuraminidase or other viral proteins.[114] These newer non-egg based approaches to production of IIVs have the potential to reduce the time necessary to produce vaccines, and enable production of a product with higher purity and without egg components; such vaccines can be used in individuals who have severe egg allergies.

The amount of HA antigen per vaccine dose has been correlated with immunogenicity of IIVs and is standardized by using the single radial immunodiffusion (SRID) test. SRID is currently the only test that is fully validated by national regulatory authorities, but its performance requires standard antigen and specific polyclonal sera, which can be time consuming to produce. For the majority of IIVs the amount of HA antigen per vaccine component is 15 µg for older children and adults (45 µg of total HA for trivalent vaccines and 60 µg for quadrivalent vaccines), and 7.5 µg of HA per vaccine dose for children aged 6–35 months.[115] IIVs have not been approved for use in infants less than 6 months of age; historically, this has been due to concerns regarding the reactogenicity of whole virus vaccines, and possible interference by maternal antibody. However, contemporary vaccines have been shown to be safe and well tolerated when given to infants younger than 6 months in clinical trial settings.[116] While a single dose is recommended for most age groups, two doses of vaccine at one-month intervals are recommended for previously unvaccinated children less than 9 years of age in some countries.[117,118] It has been found that adults aged 65 and older benefit from higher amounts of antigen per dose, or formulations containing an adjuvant. IIV formulations containing 60 µg of HA antigen per dose (high-dose), or containing a standard amount of antigen plus an adjuvant, are approved for use in these older adults in some countries.[115] A large clinical trial conducted over several years demonstrated superior vaccine effectiveness of the high-dose vaccine in adults aged 65 years and older.[119] In contrast, the recombinant HA vaccine is formulated to contain 45 µg of HA per vaccine virus and this vaccine was shown to have similar levels of reactogenicity, immunogenicity and efficacy to those of standard-dose vaccines.[120,121] Although several available influenza vaccines have different amounts of antigen per dose, most countries do not preferentially recommend one product over another. However, health-care providers should make sure that the formulation administered is appropriate for the age and health status of the person to be vaccinated.

Some approved influenza vaccines contain an oil-in-water adjuvant, MF-59 or alum, which is added to vaccines to improve the level and breadth of the immune response. The adjuvant AS03 has been previously used with vaccines against 2009 pandemic H1N1 influenza. As of 2017, it is not present in seasonal influenza vaccines. Adjuvanted vaccines are of particular interest for populations which typically respond less well to IIVs, such as elderly adults. MF-59 adjuvant-containing vaccine was first approved for elderly populations in Italy in 1997 and since then vaccines containing MF-59 have been approved for use in several European countries and Canada.[122] Vaccines containing MF-59 are also approved for use in children aged 6–23 months in Canada.[123] In 2015, an MF-59-adjuvanted vaccine was approved for use in adults 65 years of age and older in the USA.

2.1.1 Measurement of protection after immunization with IIV

Vaccination with IIVs primarily induces antibodies to the HA and NA proteins although antibody responses to the NP and M proteins can also be detected in some individuals. Because antibody levels to the HA are the best available correlate of protection, assays that measure antibody to the HA are most often used to assess protection after immunization. While both the HAI and neutralization assays measure the levels of strain-specific antibodies in the serum, the HAI assay is used most commonly because it is easier to perform. The basis for the HAI assay is that the HA proteins of influenza viruses naturally bind to red blood cells of some species and link the red cells together, causing hemagglutination.[124] In the HAI assay, antibodies to the HA protein in the serum of infected or vaccinated persons bind to the viral HA antigens included in the assay and prevent red blood cells from binding to the virus.[124] This antibody competition with red blood cells for virus binding inhibits the ability of the virus particles to bind and agglutinate the red cells, a process termed hemagglutination inhibition. Different patterns of agglutination occur based on the amount of HA antibody present in the serum. The HAI assay uses red blood cells from turkeys, chickens, guinea pigs or humans, depending on availability and properties of the viruses being tested.[125] One complexity of this assay is that non-specific inhibitors may be present in some human sera and interfere with its performance. Such inhibitors are removed by treating all sera with receptor-destroying enzyme prior to performance of the assay. While higher amounts of antibody to the HA are correlated with higher levels of protection in the population, there is no specific titre that can guarantee protection in every individual. Nevertheless, a titre of 1:40 has been used as a standard seroprotective titre in vaccine trials for many years.

Neutralization assays have two advantages over HAI assays: they are often more sensitive than HAI assays in detecting antibody and they measure functional neutralizing antibody capable of preventing virus infection.[77] The drawback is that the titration of test viruses, an early step in performance of neutralization assays, is time consuming and cumbersome. There are several types of neutralization assays, including neutralization-enzyme immunoassays, plaque reduction neutralization assays and focus reduction neutralization assays that can measure antibodies in small amounts of serum.[125] Studies using both HAI and neutralization assays performed in

parallel have demonstrated that there is good correlation between the two methods. These studies have shown that the seroprotective HAI titre of 1:40 generally corresponds to a neutralization titre of approximately 1:80, although the latter is not generally considered to be a regulatory standard.

2.1.2 Antibody responses to IIV

Serum antibody titre to the HA is the immune response most commonly measured in influenza vaccine studies, mainly because serum antibody has been shown to correlate well with protection in human infection, vaccine efficacy and challenge studies.[85,126] Rises in serum antibody titres to the NA and other virus proteins, anti-HA mucosal antibodies, and rises in T cell responses can also be detected after IIV administration, but the roles of these immune responses in protection after vaccination are less well understood. The magnitude of the anti-HA serum antibody response to IIVs is dependent on age and health status of the person vaccinated and on the levels of pre-existing antibody. In primed (previously infected or vaccinated) healthy adults under 65 years of age, one intramuscular dose of IIV containing 15 µg of HA per HA antigen stimulates good HI antibody responses in individuals with low pre-existing HA antibody levels. However the antibody titre increases less in individuals with high levels of pre-existing HA antibody.[107] In primed individuals, a rise in anti-HA serum antibody response can be detected as early as 2–6 days post vaccination with IIV. Typically this immune response is defined by a 4-fold increase in anti-HA antibody titre. Serum antibody levels peak 2–4 weeks after vaccination, and decline by about 50% by 6–12 months later.[107]

The serum antibody response consists mainly of anti-HA IgG, with lower amounts of IgM and IgA. Rises in antibodies specific to the vaccine virus as well as antibodies that cross-react with earlier circulating, antigenically related viruses can be detected in primed individuals post vaccination.[127] Serum antibody and other immune responses have been shown to be equivalent in pregnant women compared with non-pregnant women.[128] While only a single dose of IIV is required to mount an effective immune response in influenza-primed older children and adults, young unprimed children generally require two doses of IIV given at least 4 weeks apart to stimulate a protective antibody response.[117] Adults aged 65 and older often have less vigorous serum antibody responses than younger vaccine recipients, reflecting the lower vaccine effectiveness often observed in elderly adults. Underlying medical conditions and age may both contribute to lower antibody responses in this group. Antibody responses in elderly adults can be enhanced by using adjuvanted vaccines or high-dose vaccines. Adjuvanted vaccines have been demonstrated to increase the antibody responses to both vaccine and drifted viruses.[122] High-dose vaccine, which contains 60 µg of HA antigen per vaccine virus and increases antibody titres by from 1.5-fold to 2-fold compared with standard-dose vaccine, has been licensed for use in older adults.

2.1.3 Cell-mediated immune responses to IIV

Cell-mediated immune responses to inactivated influenza vaccines have been studied much less extensively in humans than the corresponding antibody responses, but there has been an effort in recent years to begin to fill this gap. Among the reasons for the relative lack of understanding of the T cell response to influenza vaccines in humans are that they depend on the HLA phenotype of the individual and T cell responses are more cumbersome to measure. Consequently, many of the assumptions regarding the T cell response to influenza vaccination are extrapolations from natural infection or immunization studies in mice or in other animal models. Because of the importance of the T cell response for recovery from infection and for protection from serious disease (see section 1.5.3) it is thought that priming these responses may affect outcomes such as disease severity, hospitalization and death. It is therefore important to elucidate the T cell response, which primarily targets the more conserved "internal" antigens of the virus. In spite of renewed efforts to study T cell responses after vaccination with IIV, the roles of these responses in protection from disease remain unclear, largely because reports from human clinical trials have provided mixed results due to differences in the age and immune status of the populations tested, differences in vaccines used for immunization, and other factors. A simplified overview is provided below.

An early study designed to examine the T cell response after vaccination of healthy primed adults with IIV demonstrated that whole-virus IIV induced peripheral blood T cell responses while subunit vaccines did not.[129] This measureable T cell response to whole-virus IIV was of variable duration with responses declining to baseline after a year or earlier in most vaccine recipients. These findings were in general agreement with studies done previously in mice. It has been found that the amount of antigen per dose and the number of doses of whole-virus vaccine administered prior to influenza activity have an effect on the magnitude of the T cell response, and that addition of certain adjuvants can increase the T cell responses in adults. The 2009 pandemic H1N1 vaccine with AS03 adjuvant enhanced both antibody and CD4+ T cell responses in adults compared with non-adjuvanted vaccine[130] and a similar effect has also been shown for adjuvanted influenza vaccine in elderly adults.[131,132]

In a study comparing CD8+ T cell responses in children and adults after vaccination with IIV and LAIV, it was found that responses were variable and dependent on both vaccine type and age.[133] In this study, trivalent IIV induced a significant increase in influenza-specific CD8+ T cells in children aged 6 months to 4 years, but not in those aged 5 to 9 years,[133] reflecting the diversity of the immune response to IIV in different age groups and the need for more research. Although the number of virus-specific CD8+ T cells decreases in people aged 65 years and older, there is evidence that T cell responses may provide a degree of protection after IIV immunization in this group. Studies in older adults with pre-existing medical conditions showed that IIV induced higher levels of granzyme B – which mediates virus-infected cell killing and is a surrogate measure for CD8+ T cell response – was a better indicator of protection than antibody titre.[134] These results along with those in other age groups emphasize

the need for additional studies focused on cell-mediated immune responses to IIVs. Because of the heterogeneity of the immunological responses to IIVs, researchers are adopting a systems biology approach to understand the complexity of the immune response to influenza vaccine in different age groups.[135]

2.1.4 Availability of IIVs

There are many different IIV products and product presentations approved for human use by national regulatory authorities worldwide but their availability varies from country to country. Preferential recommendations for one product over another are rarely provided when multiple products are available and considered equivalent. Importantly, age indications for various IIVs differ and only age-appropriate vaccine should be used for vaccination. The majority of IIV doses available globally are trivalent, containing two influenza A viruses and one B virus, but quadrivalent vaccines containing two B viruses are becoming more widely available. The larger IIV manufacturers produce vaccines for the global market, however certain countries (e.g. China and Japan) have their own domestic IIV manufacturers and some of their vaccines are licensed only in one or a few countries. The categories and general characteristics of approved IIVs are listed in *Table 1*.

Table 1. General characteristics and administration routes of influenza vaccines licensed for prevention of epidemic influenza

Vaccine Type	Preparation Type	Production Substrate	Adjuvant	Route of Administration
Inactivated	Whole Virus	Eggs	None	intramuscular
Inactivated	Whole Virus	Cell culture	None	intramuscular
Inactivated	Sub-virion	Eggs	None	intramuscular, intradermal
Inactivated	Sub-virion	Cell culture (MDCK cells)	None	intramuscular
Inactivated	Whole virus	Eggs	Alum	intramuscular
Inactivated	Sub-virion	Eggs	MF-59	intramuscular
Recombinant	Expressed HA protein	Cell culture (insect cells)	None	intramuscular
Live Attenuated	Influenza A and B Ann Arbor-based	Eggs	None	intranasal
Live Attenuated	Influenza A and B, Leningrad-based	Eggs	None	intranasal

Note: Each type of influenza vaccine may be available only as a trivalent formulation or as both trivalent and quadrivalent formulations, depending on country-specific regulatory approval, vaccine recommendations, cost and other considerations.

2.1.5 IIV safety and contraindications

Hundreds of millions of people are vaccinated with IIV globally each year, providing a very large body of information on safety, indicating that these vaccines are generally well tolerated and safe in both adults and children. The most common adverse

events after vaccination are tenderness, pain, redness or swelling at the injection site occurring within 24 hours post-vaccination and usually resolving within a few days. Local reactions observed after administration of IIV are similar to those associated with all injectable vaccines. In addition, fever, fatigue and muscle aches and other systemic events have been reported, primarily in those receiving vaccine for the first time, such as young children.[136,137]

Whole-virus IIVs were used until the 1970s and 1980s when they were largely replaced by SV IIVs, mainly because of the greater reactogenicity of the whole-virus vaccines, particularly in children.[138] In a study of SV IIV in over 900 healthy children aged 1–15 years, post-vaccination fever occurred in 12% of children aged 1–5 years and in 5% of children in older age groups.[139] More recent randomized controlled trials (RCTs) using contemporary formulations of vaccines found differences in local reactions such as sore arm and redness at the injection site, but no differences in systemic side effects.[140] Large post-licensure SV IIV studies in the USA in children <18 years of age, including one study of 45 356 infants aged 6–23 months, revealed no increases in medically attended events except self-limiting vomiting and diarrhoea after vaccination in one study.[141,142] Another large study of 66 283 children from 24 through 59 months of age showed an increase in fever and gastrointestinal tract symptoms in vaccine recipients.[143]

An increased incidence of febrile seizures associated with use of one particular IIV product produced in Australia for the 2010-2011 influenza season was first reported in Australia. Follow-up studies revealed that, compared to other IIV products, this vaccine caused higher rates of fever as well as febrile seizures.[144-146] The manufacturer attributed the increase in febrile reactions to an increase in lipid-mediated delivery of RNA fragments of some antigens due to the detergent splitting process.[146,147] This IIV product is no longer recommended for use in children under 9 years of age in the USA and under 5 years in Australia.[147,148]

Following the Australian episode, other countries have enhanced influenza vaccine safety monitoring, especially in young children. In studies in the USA, an increased risk of febrile seizures was observed in children 6–24 months of age who received pneumococcal conjugate vaccine at the same time as the IIV product approved for use in this age group, but not following IIV alone.[149] It is important to stress that the overall risk of febrile seizures following IIV is low, and that no increase in risk has been observed in children over 4 years of age. A comprehensive recent review of IIV vaccine safety in children has been published.[147]

A number of placebo-controlled studies using adjuvanted and non-adjuvanted vaccines have shown that 10–64% of adults may experience injection-site reactions, with rates being higher for vaccines containing adjuvants than for non-adjuvanted formulations. In these studies, injection-site reactions were typically mild and rarely interfered with daily activities.[150-152] Systemic reactions in adults who receive standard-dose IIVs are uncommon and generally appear as mild to moderate fevers in less than 1% of recipients. Headache, myalgia and fatigue also have been reported in adults, but in placebo-controlled trials these symptoms were reported with equal frequency

in vaccine and placebo recipients.[152,153] Importantly, no new IIV safety concerns were identified in a comprehensive review of post-licensure surveillance data including almost 750 million vaccinations in the USA.[154] In a head-to-head comparative study it was found that injection site reactions and systemic events were reported more frequently in the elderly after receipt of a high-dose vaccine containing 180 µg than after receipt of standard-dose vaccine, but these reactions were generally mild and transient.[155] In a larger randomized study of high-dose vs standard-dose IIV, no difference was found in the occurrence of serious adverse events in the two groups.[156] In studies comparing intramuscular and intradermal administration of IIV, the intradermal route has been reported to be associated with higher rates of injection-site reactions including redness and swelling, but with similar frequency of local pain and systemic reactions.[157]

Guillain-Barré syndrome (GBS) is a an autoimmune-mediated demyelinating disorder characterized by rapidly progressive symmetrical weakness of the extremities; it is triggered by an antigenic stimulus that results in an abnormal immune response which damages self-proteins on nerves of the peripheral motor system. GBS usually presents as an acute flaccid paralysis and occurs at a background rate of approximately one case in 100 000 person-years. Most patients with GBS have had a recent gastrointestinal infection (particularly caused by *Campylobacter jejuni*) or a respiratory virus infection, including influenza.[158-160] Because of the association of antecedent infections with GBS it has been suggested that molecular mimicry may play a role in disease pathogenesis. The strongest evidence for molecular mimicry comes from observations that certain molecules on cell walls of *C. jejuni* share antigenic properties with gangliosides present on human peripheral nerves and that these bacterial cellular molecules can induce antibodies against GM1 gangliosides, resulting in damage to neurons.[161,162]

The first vaccine causally associated with GBS was the 1976 swine whole-virus influenza vaccine that was administered to approximately 35 million people in the USA during 1976–1977.[147] This vaccine was produced in response to a swine influenza outbreak on a military base and the finding that the causative virus had similarities to the virus which caused the deadly 1918 pandemic. While the feared pandemic never materialized, recognition of two clusters of GBS in recipients of the swine influenza vaccine resulted in cessation of the vaccination campaign followed by careful epidemiological studies of the relationship between GBS and the vaccine. In these studies, it was found that those vaccinated with the 1976 swine influenza vaccine were 9.5 (95% CI: 8.2–10.3) times more likely to develop GBS than unvaccinated counterparts. This corresponds to an estimated attributable risk of approximately one additional case for every 100 000 vaccinated persons.[163] Additional epidemiological studies were carried out between 1977 and 2008; most did not find a statistically significant association between influenza vaccination and GBS.[147] Only two such studies found a small significant increased risk, in the order of one additional GBS case per million people vaccinated.[164,165] No subsequent study of IIV has demonstrated an increased risk similar to that observed for the 1976 swine influenza vaccine deployed in the USA.

During 2009 and 2010, intensive studies, often using modern epidemiological approaches, were undertaken to examine a possible association between receipt of the 2009 H1N1 pandemic vaccine and GBS in a number of countries in Asia, Europe and North America. As millions of people were vaccinated, these studies offered a unique opportunity to examine vaccine safety. In Europe, a case-control study that included participants from Denmark, France, Netherlands, Sweden and the United Kingdom (UK) who had received adjuvanted monovalent vaccines, found no statistically significant increased risk of GBS after adjusting for potential confounders such as influenza-like illness (ILI) and receipt of seasonal influenza vaccine.[166] In the USA. a meta-analysis of data from six active surveillance systems, using self-controlled case series methods, found 54 cases in the vaccine exposure period compared with 23 cases in the control period, resulting in an incident rate ratio of 2.35 (95% CI: 1.53–3.68).[167] The authors conducted a number of sensitivity analyses with results remaining approximately consistent. There was a consistent relative risk ratio across ages, however statistical significance was not reached in children, likely due to smaller numbers in this age group. Expressed in a different way, this study found that the attributable risk of GBS after influenza vaccine receipt ranged from one additional case per million vaccinated children to three cases per million for persons aged 65 and older, reflecting the higher background rate in older individuals.[167]

Researchers from Australia, Canada, China, Denmark, Finland, Netherlands, Singapore, Spain, UK and USA participated in an international collaboration using two self-controlled case series methods: (1) data were pooled across all sites (pooled analysis) and (2) estimates from each site were weighted based on within and between study errors and then merged using a meta-analytic approach.[168] In this study, receipt of H1N1 vaccines was associated with increased relative incidence of GBS of 2.86 (95% CI: 1.88–4.34) for the pooled analysis and of 2.42 (95% CI: 1.58–3.72) for the meta-analytic approach. These results were consistent with those obtained in the previous study in the USA and across a variety of sensitivity analyses. Although this study suggested a higher risk of GBS in recipients of adjuvanted than non-adjuvanted H1N1 vaccines, the results were not statistically significant. While many other individual studies have been published on the association of GBS and the H1N1 pandemic vaccine and previous seasonal vaccines, the USA meta-analysis[167] and the international collaboration[168] appear to have the strongest methodologies and were adequately powered to detect a small increased risk. These and other studies[169,170] suggest a doubling of the risk of GBS, which means between one and three cases per million people vaccinated, depending on the age group. This small increased risk of GBS post-vaccination appears to be far lower than the risk of GBS after influenza infection.[160,171] Thus the benefits of influenza vaccination, including prevention of GBS caused by influenza infection, outweigh this very small risk of GBS after receipt of IIV. Nevertheless, having had onset of GBS within 6 weeks following a previous dose of influenza vaccine is a precaution with respect to further influenza vaccination.[115]

Ocular respiratory syndrome (ORS) is an acute, self-limited reaction to IIV and with symptoms including red eyes, facial swelling and/or respiratory symptoms such as coughing, sore throat, wheezing, hoarseness and difficulty in breathing. Typically

the symptoms are mild and resolve within 24 hours, and affected vaccine recipients usually do not seek medical care.[28] ORS was first described in Canada and was associated with one particular vaccine formulation.[172] ORS has also been reported in the USA and Europe.[173-175] The specific cause of ORS in some vaccine recipients is not known. It has been found that the risk of ORS is low after subsequent vaccination.[176]

There is considerable evidence that pregnant women are at increased risk for complications of both epidemic and pandemic influenza, especially during the second and third trimesters, and that influenza disease can be more severe in pregnant women with underlying medical conditions.[177] Vaccination of pregnant women is now considered safe for mother and fetus during all trimesters, although data on safety of vaccine administered during the first trimester are somewhat limited.[177-180] In a study in the USA, it was found that between 1990 and 2009, in the Vaccine Adverse Event Reporting System (VAERS) database only 20 serious adverse events were reported among 11.8 million pregnant women after receipt of trivalent IIV.[181] In a follow-up study of reports for the period 2010–2016 using the same database, no new or unexpected maternal or fetal adverse events were recorded.[182] Studies in children of women who had received seasonal IIV during pregnancy have found no increase in infant or early childhood malignancies.[183] In other studies, no serious adverse events and no significant differences in pregnancy outcomes were observed in women vaccinated during pregnancy compared with those in unvaccinated pregnant women.[184-186] In addition, recent reviews and systematic reviews examining safety of seasonal and monovalent 2009 H1N1 IIVs in pregnant women found no evidence of harm to them or the fetus.[187-190] A large study in Denmark found no risk for early childhood morbidity among children who had been exposed in utero to inactivated AS03-adjuvanted pandemic influenza vaccine.[191]

Allergic reactions after receipt of vaccines are generally mediated by IgE and typically occur soon (within minutes to a few hours) after vaccination. Symptoms of allergic reactions following vaccination range from those that are more common and relatively minor, including itching, erythema, rhinitis, rhinoconjunctivitis, gastrointestinal symptoms, urticaria and angioedema, to those that are rare and life threatening such as anaphylaxis, which is the most severe form of IgE-mediated reaction.[180,192] Anaphylaxis after vaccine receipt is estimated to occur at a rate of about one per million vaccine doses.[192] For example, a large study using data in the Vaccine Safety Datalink in the USA examined reports of anaphylaxis in adults and children who had received over 25.1 million doses of vaccines of various types; it was found that among the 7.4 million recipients of IIV, 10 cases of anaphylaxis were reported, representing a rate of 1.35 cases (95% CI: 0.67–2.47) per million doses administered.[193] Like other vaccines, IIV contains various components that might cause allergic reactions, including vaccine antigens, residual media used to grow the virus, preservatives and other excipients.

Because the vaccine viruses in most current IIVs are grown in eggs and contain small but measurable amounts of the egg protein ovalbumin, the possibility of reactions to influenza vaccines in egg-allergic individuals could be of concern to them and

their health-care providers.[180,192] In a large Canadian review of 4172 egg-allergic patients, 513 of whom reported severe allergic reactions, no cases of anaphylaxis were reported in IIV recipients.[194] In a review of 28 studies in North America and Europe that evaluated 4315 subjects including 656 with a history of anaphylaxis after eating eggs, it was found that reaction rates after IIV receipt were similar in those with and without a severe allergy to eggs.[171,195] This is probably because vaccines that are available in North America and Europe contain less than 1 µg of ovalbumin per dose and it has been demonstrated that it takes substantially higher quantities of egg protein to trigger reactions in egg-allergic individuals.[180,192] Unfortunately, the egg protein content for vaccines produced in other parts of the world is often unknown. In summary, while in the past IIVs were contraindicated for persons with egg allergy, it is now known that these individuals can safely receive influenza vaccines containing low amounts of egg protein.

Narcolepsy is a chronic disorder characterized by extreme daytime sleepiness, associated with loss of hypocretin secreting cells in the hypothalamus and absence of hypocretin in the cerebrospinal fluid, along with cataplexy.[196] In response to a signal of increased numbers of cases of narcolepsy in Sweden and Finland in the summer after the 2009 influenza pandemic, a number of epidemiological studies were conducted in Europe where the AS03-adjuvanted monovalent 2009 H1N1 pandemic vaccine Pandemrix was used widely during the pandemic. Of note, monovalent influenza vaccines containing AS03 adjuvant were widely used in many countries in Europe during the 2009 pandemic as an antigen-sparing strategy to enable production of additional doses of vaccine.[197] The post-pandemic studies identified strong evidence of an association between receipt of AS03-containing monovalent pandemic vaccines and occurrence of narcolepsy, with the greatest risk in children and adolescents.[198-200] Some of the country-level studies were part of the European CDC and the Vaccine Adverse Events Surveillance and Communication consortium (VAESCO) that included case-control studies in Denmark, Finland, Italy, Netherlands, Norway, Sweden and the UK. It was found that in Finland and Sweden, where early signals for narcolepsy were first reported, a highly statistically significant association was seen after vaccination with AS03 adjuvant-containing vaccine (OR 14.2; 95% CI: 2.5–infinity) for children. In addition, a large study examined age-, gender- and country-specific rates of narcolepsy in the 9 years prior to the 2009 H1N1 vaccination campaign and in the year following that campaign in Denmark, Finland, Italy, Netherlands, Sweden and the UK. Increased rates of narcolepsy were recorded in Finland and Sweden in children and adolescents between 5 and 19 years of age after September 2009 compared with previous periods; in both countries pandemic vaccine coverage was relatively high in most age groups.[201] A much lesser association with narcolepsy was observed for Arepanrix, an AS03-containing vaccine that was used in Canada and produced in a different facility.[202]

The mechanism by which narcolepsy occurs in children vaccinated with Pandemrix is not fully understood but it is associated with presence of the HLA-DQB1*06:02 allele, the prevalence of which varies across populations.[196,203] No similar association has been observed for 2009 H1N1 pandemic vaccines containing the adjuvant MF-59,

or in the small number of persons studied who received seasonal vaccines containing AS03. No association has been found between narcolepsy and seasonal vaccines without the AS03 adjuvant. An increase in diagnoses of narcolepsy temporally related to the H1N1 pandemic was noted at one centre in northern China where pandemic vaccine was not used.[204,205] No association between the H1N1 pandemic and narcolepsy has been found in other countries.

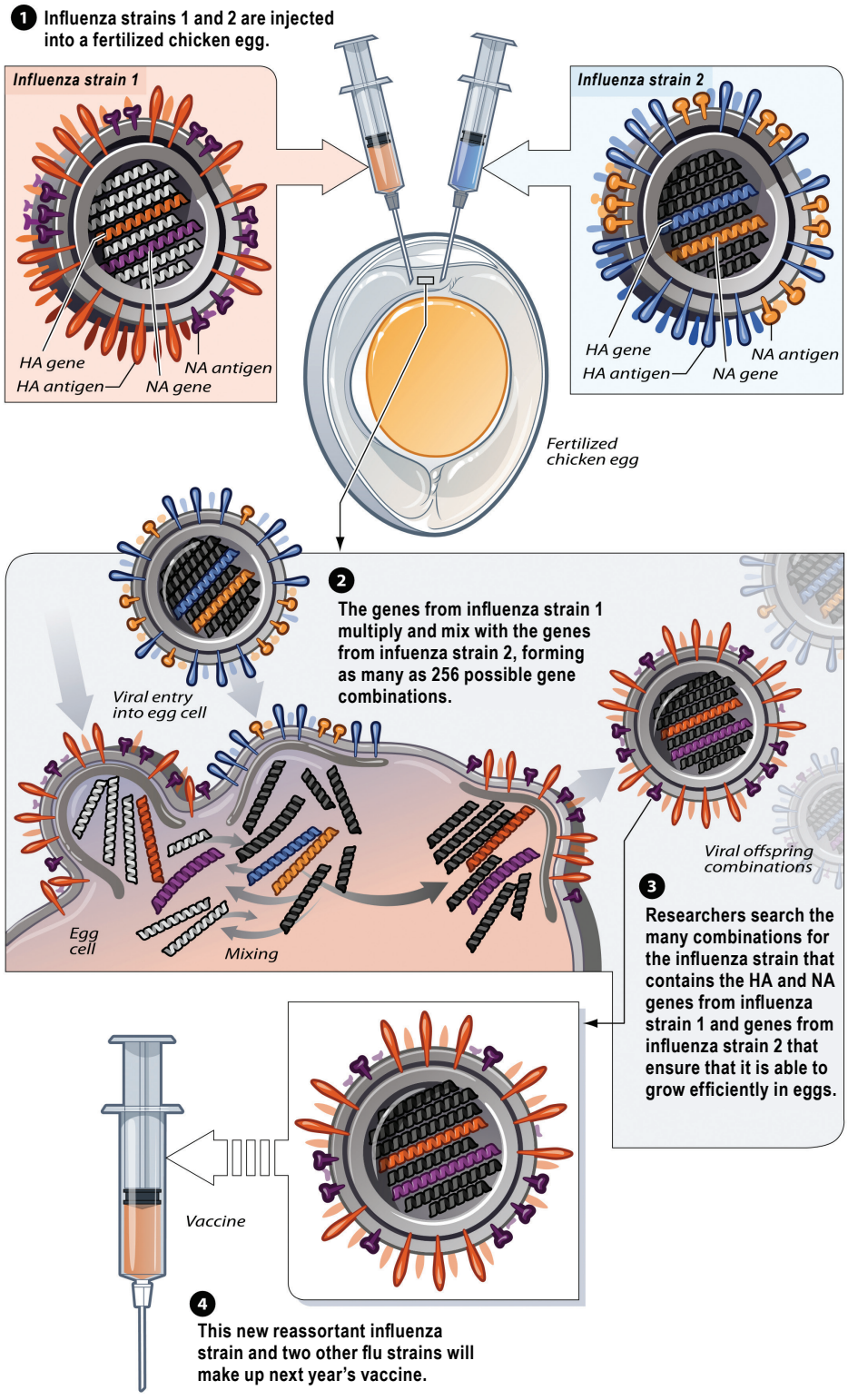
2.2 Live attenuated influenza vaccines (LAIVs)

Development of LAIVs for prevention of influenza, as for the development of IIVs, was initiated shortly after influenza viruses were first identified.[206,207] LAIVs were developed, in part, because of their potential to more closely mimic natural infection and to stimulate more durable immunity than inactivated vaccines. Some other attractive features of LAIVs are the ease of delivery (needle-free), potential for lower production costs, potential for cross-reactivity and protection from drifted strains, and earlier availability during a public health crisis such as a pandemic. However the LAIVs also have disadvantages: they are not recommended for use in pregnant women, in children under 2 years of age, in the elderly or in those who are immunosuppressed, and are also contraindicated for persons with severe asthma and children on long-term aspirin therapy.[28]

A number of different approaches have been used to develop potential live attenuated influenza vaccine viruses, including: sequential passage of wild-type viruses in animals, eggs or cell culture; mutation of influenza viruses induced by ultraviolet light or chemical means; and sequential passage at decreasing temperatures until attenuation is achieved. Importantly, only the latter approach was used to develop the currently licensed LAIVs for which stable attenuation of influenza A and B master donor viruses (MDVs) was achieved by making these strains "cold-adapted" (*ca*). In addition to being attenuated (*att*) and *ca*, these LAIV MDVs are also temperature-sensitive (*ts*), meaning that they are unable to replicate at the higher temperatures (38–39 °C) present in the lower respiratory tract. It has been shown that multiple gene mutations in the genomes of MDVs are responsible for their *att*, *ca* and *ts* properties and that these key mutations are located on the six "internal" (non-HA and NA) gene segments of the MDVs. This makes genetic reversion of vaccine viruses to the wild-type phenotype unlikely and explains why the attenuation of these viruses has proved to be stable.[208-210] This stability allows the rapid development of updated attenuated vaccine viruses either through classical reassortment between the MDVs and a WHO-recommended epidemic virus, or through the molecular approach of reverse genetics using cloned "internal" genes from MDVs and HA and NA genes from recommended wild-type viruses. With both of these reassortment approaches, the resulting vaccine viruses contain HA and NA gene segments from the relevant recommended wild-type virus and the other six attenuating gene segments from the MDVs (*Figure 2*).

Figure 2. Illustration showing genetic reassortment in the production of influenza vaccine

An influenza virus contains eight gene segments. The goal is to combine the desired HA and HA genes from influenza strain 1 with genes from influenza strain 2, which grows well in eggs and is harmless in humans.



Note: Adapted public domain image courtesy of the National Institutes of Health of the United States Department of Health and Human Services.

Two different, independently developed LAIVs are licensed and available for use in different countries around the world. For each of these two approved LAIVs, the approach was to develop one live attenuated cold-adapted MDV for influenza A and another for influenza B vaccine virus preparation. The first approved LAIV was developed at the Institute for Experimental Medicine in St. Petersburg, Russia (former USSR), and was licensed for use in children and adults in 1987.[206,211] This LAIV is manufactured in eggs and is approved for use in persons aged 3 years and older in Russia; approval was based on clinical trial results demonstrating that this vaccine was safe, immunogenic and protective in children, and in adults including the elderly.[206] The two *att*, *ca*, *ts* MDVs for the Russian LAIV are A/Leningrad/134/17/57 and B/USSR/60/69 viruses for the A and B vaccine components, respectively. In 2009, the Russian LAIV technology was licensed to WHO for use in developing countries, particularly for pandemic preparedness.[212] Importantly, WHO subsequently granted sub-licenses to vaccine manufacturers in low and middle income countries and as a result, the Russian MDVs were provided to vaccine manufacturers in China, India, and Thailand for vaccine manufacture. In addition, the Institute for Experimental Medicine regularly provides pandemic and seasonal candidate vaccine viruses to the sub-licensees consistent with WHO recommendations on influenza vaccine composition. This has led to regulatory approval in India of a seasonal trivalent formulation of LAIV using the Russian MDVs.[206]

A second LAIV was developed in the USA at the University of Michigan.[213] This LAIV is based on the two *ca*, *att* and *ts* MDVs, A/Ann Arbor/6/60 and B/Ann Arbor/2/66 for the influenza A and B vaccine virus components, respectively. After extensive clinical trials, a trivalent frozen form of this LAIV was first licensed in the USA in 2003 for prevention of influenza in healthy persons aged 5–49 years. In 2007, a liquid formulation was approved in the USA for those aged 2–49 years. Instead of classical reassortment, reverse genetics technology is now used for accelerated production of reassortant vaccine viruses. After approval in the USA, the trivalent formulation was also approved for those aged 2–49 years in Israel, Mexico, Macau (China), Republic of Korea and the United Arab Emirates, for those aged 2–59 years in Canada, and for children aged 2–17 years in the European Union.[207] A quadrivalent LAIV based on the Ann Arbor MDVs and containing two influenza A viruses and both B/Yamagata and B/Victoria lineage viruses has been approved in some countries.

2.2.1 Routes of administration and dose

LAIVs based on the Ann Arbor MDVs are given intranasally (as drops or spray) and require either one or two doses, depending on national approvals and recommendations. In the UK one dose is recommended for all children except those with clinical risk conditions for whom two doses are recommended. Two doses given one month apart are recommended in the USA and Canada for children less than 9 years of age who have not been vaccinated against influenza previously; one dose is recommended for children who have been vaccinated previously and those aged 9 years and older.[28]

For the two LAIVs based on Russian MDVs, vaccine administration is intranasal, and age-specific recommendations for this LAIV also vary by country. One dose of this LAIV is recommended for persons aged 2 years and older in India, and for those aged 3 years and older (including elderly adults) in the Russian Federation.[206]

The LAIV developed in the USA contains 107 fluorescent focus units (FFU) for each of the 3 or 4 vaccine components in a 0.2 mL nasal sprayer[207] while that developed in Russia contains 10^7 EID_{50} for influenza A and $10^{6.5}$ EID_{50} for influenza B components. Small safety studies are conducted prior to final release of the vaccine for administration in years when one or more of the vaccine viruses have been changed.

2.2.2. Immunological responses to LAIVs

LAIVs were developed in the hope that the response to infection by an attenuated live virus would mimic more closely the immunity acquired after natural infection. In recent decades, it has been suggested that LAIVs, like natural influenza infections, might induce a longer lasting and broader immune response than immunization by IIVs. This hypothesis was based largely on the fact that natural infection produces longer lasting protection than IIV and the likelihood that a replicating live attenuated influenza vaccine would induce better T cell and mucosal immunity than IIVs, due to their ability to replicate in the upper respiratory tract without causing disease. Immunological studies have shown that LAIVs induce not only some serum antibodies to HA and NA but also mucosal IgA and T cell mediated immune responses.[206,214-216] Although no single correlate of protection for LAIVs has been identified, it has been found that each of the above immune responses is likely to contribute to the protection afforded by LAIVs.

2.2.3 Antibody responses to LAIV

Serum antibody titre to the HA has been the most commonly measured immune response in influenza vaccine studies. However assessing protective immunity after administration of LAIV is more complicated than for IIV, primarily because a measurable increase in anti-HA serum antibodies following vaccination with LAIV occurs less frequently among previously primed individuals. In order to induce an immune response, LAIV vaccine viruses must replicate in the upper respiratory tract and if the vaccine recipient has pre-existing anti-influenza antibodies, replication may be inhibited. In several studies in adults including the elderly, less than 35% of LAIV recipients had detectable serum antibody responses to the A(H3N2) and B vaccine components with somewhat higher but variable responses to the A(H1N1) component.[214,217] It is believed that LAIV immunity is the result of a combination of a variety of different types of immune responses in addition to humoral antibody.[218] In this regard, it is important to note that anti-HA serum antibody titres in adults vaccinated with LAIV have been found to be similar to those of placebo recipients, even in studies where significant protection from infection was afforded by LAIV. While serum anti-HA serum antibody responses in healthy adults after receipt of LAIV are low compared with responses after IIV receipt, anti-HA IgA titres in nasal washes were found to be higher in LAIV than in IIV adult recipients.[214,219] Higher

levels of IgA in nasal wash specimens of vaccinees correlated with protection from a wild-type virus challenge, providing evidence that local secretory IgA antibody is important for protection from infection.[220]

In contrast to adults, LAIV induces a more robust serum antibody response in young children who are immunologically naive to influenza. Early studies demonstrated that approximately 90% of seronegative children developed an adequate vaccine response to influenza A(H3N2) and B viruses and 60% developed a response to the A(H1N1) component after two doses of the Ann Arbor-based vaccine.[217,221] These early studies also demonstrated that seroconversion rates in unprimed children vary by virus strain[222] and that serum antibody responses are less frequent in children with pre-existing antibody.[216] Serum antibody levels remained high in these vaccinated children for 5–8 months after receipt of LAIV.[217] There have been fewer studies measuring nasal than serum antibody responses, due to the difficulties in sampling and assaying for increases in nasal antibody. However early studies conducted in small numbers of immunologically naive children showed that IgG and IgA antibodies against the HA were present in nasal wash specimens within two weeks after LAIV vaccination and persisted for up to a year in about half of those vaccinated.[216] Furthermore, the antibody response after LAIV administration in naive young children was characteristic for that expected after a primary viral infection.[223] In addition, a small challenge study in children showed that receipt of LAIV 12 months prior to challenge significantly reduced homologous LAIV shedding and that the presence of nasal IgA before challenge was correlated with protection.[224]

2.2.4 Cell-mediated immune responses to LAIV

The main targets of the cell-mediated immune response to influenza are the conserved internal proteins of the virus, as outlined in section 1.5.3. It is considered that vaccine-induced robust T cell responses might protect against complications or severe disease even if they do not protect against infection; consequently, LAIVs are candidates for vaccine probe studies with severe illness outcomes. Since LAIV replicates in cells in the upper respiratory tract, it induces both CD4+ and CD8+ T cell responses. While T cell responses after LAIV receipt have been studied less extensively than T cell responses after natural infection or IIV receipt, it has been found that there are increases in CD4+ and CD8+ T cells producing influenza-specific gamma-interferon (INF-gamma) as well as increases in NK T cells in both adults and children.[214,222,225] In one large field trial of LAIV in children aged 6–35 months, it was found that the post-vaccination level of T cells producing influenza virus-specific IFN-gamma was the best correlate of protection, although the T cell type responsible was not identified.[225]

2.2.5 Availability of LAIVs

Two LAIV formulations are currently approved for use, one based on the MDVs used in Russia and Asia and the other based on MDVs used in North America and Europe. The countries in which these two vaccines are licensed and indications for their use are discussed in sections 2.2 and 2.2.1.

2.2.6 LAIV safety, virus shedding, stability, transmission and adverse events post-immunization

LAIVs have been shown to be generally well tolerated and safe in the target populations.[206,226] These live vaccines are administered by spraying into the nose in order to induce immunity by replicating in cells lining the nasal passages. The temperature-sensitive and attenuation properties of LAIVs prevent them from replicating in the lower respiratory tract and causing serious disease. The most commonly reported adverse events after vaccine receipt are mild respiratory symptoms such as runny nose and nasal congestion in vaccinees aged 2–49 years, sore throat in those aged 18–49 years, and fever >38 °C in children aged 2–6 years.[28] While some studies have reported comparable levels of adverse events in LAIV and placebo recipients, other studies have shown an increase in these adverse events, most often after receipt of the first dose of LAIV in young children.[227-232] Of note, one study in the USA showed an increase in hospitalization among children aged 6–11 months and an increase in medically attended wheezing in children aged 6–23 months;[233] as a result LAIV developed in the USA has not been approved for children less than 2 years of age. The current trivalent Russian LAIV has not been studied in children less than 2 years of age.[206]

While both children and adults can shed the attenuated vaccine viruses after vaccination, the duration of shedding is longer in children than adults, as is also seen after natural influenza virus infection. In addition, shedding of vaccine virus in those immunized with LAIV is shorter than in individuals naturally infected with seasonal viruses.[207] In one study of 345 LAIV recipients aged 5–49 years it was found that vaccine viruses could be detected by virus culture in approximately 30% of vaccinees.[234] The highest amounts of virus shedding were present within 2 days of vaccination and the amount and duration of shedding correlated inversely with age. The viruses in LAIV are genetically and phenotypically stable, including after shedding.[207,208,210,235]

LAIV transmission to close contacts including spouses, room-mates and household members has been detected very rarely.[236] In one study designed to detect transmission of LAIV from vaccinated to unvaccinated children aged 8–36 months in a child-care centre it was found that an influenza B LAIV virus was recovered from one unvaccinated child who was in contact with vaccinated children. Importantly, the virus isolated from the unvaccinated child retained the attenuated phenotype of the parent vaccine virus, demonstrating vaccine virus stability after transmission.[235] The presence of LAIV viruses has not been reported among public health influenza surveillance samples collected from unvaccinated individuals, which is another indication that transmission from vaccine recipients to their contacts is rare, and that the attenuating mutations present in the LAIV MDVs developed in Russia and in the USA are both stable.

3. Influenza vaccine performance

There have been many clinical studies to assess the performance of influenza vaccines in preventing influenza. These studies have had different designs and been conducted in different influenza seasons and age groups, and have had different endpoints. Because of these important differences in study design, the estimates of influenza vaccine efficacy and effectiveness have differed significantly from study to study. Vaccine efficacy studies examine reduction in the risk of disease in vaccinated persons under ideal circumstances, in which the vaccine recipients and placebo recipients are matched as well as possible for age, underlying medical conditions and other important factors, in order to reduce the chance of study bias. RCTs are the gold standard for clinical trials of vaccine efficacy but are very expensive, and most often conducted by pharmaceutical companies in support of the initial vaccine approval by regulatory authorities. They may also be performed if regulatory authorities require manufacturers to continue such studies post-licensure for verification of vaccine performance over time in subsequent influenza seasons.

Post-licensure performance of influenza vaccines is usually assessed in vaccine effectiveness (VE) studies which examine the reduction of risk of disease among vaccinated persons in real-world conditions. VE studies are often non-randomized cohort studies that are carried out in the community. They are less expensive than RCTs, are somewhat easier to conduct, and do not pose ethical concerns in situations where vaccination policies have made vaccination the standard of care. However, because the participants in these observational studies are not randomized, the analysis of data requires statistical adjustments to take into account important differences in the characteristics of individuals who were vaccinated and those who were not. WHO has published a guide on the evaluation of influenza vaccine effectiveness to encourage and facilitate the use of standard methods.[237] Currently, influenza VE studies are performed annually in a number of countries around the globe including countries in the northern hemisphere (e.g. Canada, USA and several countries in Europe) and in the southern hemisphere (e.g. Australia and New Zealand). Because results of individual studies of the performance of influenza vaccines vary considerably, meta-analyses of larger groups of studies in similar age groups are referenced preferentially in the discussions below.

Laboratory-confirmed infection is the most specific outcome for both influenza vaccine efficacy and effectiveness studies, and RT-PCR is now widely used for this purpose. However, virus culture remains essential in order to provide an assessment of

the antigenic match between circulating viruses and the current vaccine formulation. Many earlier RCTs incorporated a serological definition of influenza virus infection which required a 4-fold rise in anti-HA antibodies, but it has been shown more recently that such studies may have overestimated vaccine efficacy because of difficulties in detecting a serological rise after infection in individuals who had been vaccinated.[78] These earlier studies are therefore excluded from meta-analyses of VE studies conducted in recent years.

In years when circulating viruses are antigenically distinct from the corresponding vaccine viruses, influenza vaccines generally perform relatively poorly. This was well illustrated in a 2-year study in working-age adults in which an antigenic variant of the H3N2 subtype emerged and predominated in the 1997–1998 northern hemisphere influenza season and antigenically well-matched vaccine viruses were included in the vaccine for the subsequent year of the study.[238] In this study vaccine efficacy was higher (86%) and statistically significant during the second season when vaccine and circulating viruses were more closely matched antigenically than in the earlier mismatched year when vaccine efficacy was estimated at 50% but was not statistically significant. Furthermore, a meta-analysis of vaccine efficacy against antigenically matched and mismatched strains demonstrated that protection against disease was better in years when the circulating strains were antigenically well matched with the vaccine viruses. Nevertheless, benefit of vaccination was apparent even in years when vaccine viruses were not optimally matched with circulating viruses.[239]

3.1 IIV vaccine efficacy and effectiveness

Adults

An important meta-analysis published in 2011 included only studies with medically attended laboratory-confirmed influenza using virus culture or RT-PCR, but not those with a serological endpoint due to the methodological concern mentioned above. This meta-analysis identified eight studies of IIV in healthy adults aged 18–64 years conducted over nine influenza seasons that met the study criteria, and found a pooled vaccine efficacy of 59% (CI: 51–67%) and a mean vaccine efficacy of 62%.[126] The potential role of vaccine mismatches in this meta-analysis was not clear. In a RCT in healthy adults, where infection was confirmed by RT-PCR or cell culture, it was demonstrated that vaccine efficacy estimates for egg-based and cell culture-based trivalent IIVs were comparable.[240] In that study, it was found that vaccine efficacy was about 60% against all circulating influenza viruses, but higher against well-matched strains.

Older adults

IIV efficacy studies in adults aged 65 years and older have not been conducted in high-income countries during recent decades because immunization policies in such countries include recommendations that older adults should receive influenza vaccine annually and this is considered the standard of care. As a result, there have been no recently published RCTs measuring vaccine efficacy against RT-PCR or culture-confirmed infections in older populations. However, an early RCT in which serology

was used as the basis for laboratory confirmation reported vaccine efficacy of 58% against serologically confirmed symptomatic influenza in community-dwelling older adults.[153] Due to a lack of recent RCTs in older adults, it is important to examine results from observational studies. One recent meta-analysis of published vaccine effectiveness studies in elderly adults demonstrated an average VE of 50% against symptomatic influenza with confirmed influenza virus infection.[55] Importantly, this study also demonstrated no effectiveness during times when influenza was not circulating, and lower average VE when vaccine match was suboptimal than in seasons where vaccines were well-matched, as would be expected from studies in other population groups. In studies of community-dwelling elderly persons, it has been found that IIV may reduce secondary complications, hospitalizations and death, both among those with and without chronic medical conditions.[241-245] It should be noted however that these studies were conducted using medical record databases and, with one exception,[245] did not use laboratory-confirmed influenza as an endpoint or take into account the fact that healthier elderly persons are more likely to be vaccinated than their more vulnerable counterparts. IIV VE in elderly nursing home residents is most often lower than in healthy younger adults and has been estimated to range from 20% to 40%.[246-248] In conclusion, the preponderance of evidence indicates that while the effectiveness of IIV is lower in elderly adults than in healthy younger adults, vaccination with IIV reduces the risk of influenza virus infection and its complications in this population.

Children

Children over 2 years of age typically have a good immunological response to IIV after the second dose of vaccine, particularly to the influenza A virus components, but the immunological response in children between 6 months and 2 years of age has been less well studied. As observed in other age groups, the protective effect of influenza vaccine in children varies by year depending on the intensity of virus circulation, vaccine match, sensitivity of the defined endpoints and by age subset. While a considerable number of vaccine efficacy studies have been conducted in young children, relatively few of the earlier studies used laboratory confirmation of infection by RT-PCR or virus culture, as has been done in more recent studies.[249] As for the adult age groups discussed above, several meta-analyses have been made of vaccine effectiveness and efficacy studies in children aged 2–17 years in order to summarize results that have varied by season and age group.[250-253] These meta-analyses found that in RCTs of IIV there was an estimated vaccine efficacy of between 40% and 90% in years when there was a significant amount of influenza activity and a good antigenic vaccine match. However, efficacy of IIV was lower or not detectable in some years when there was limited influenza activity or a poor vaccine match.

Maternal immunization

The strategy of maternal influenza immunization can protect pregnant women and newborn children with a single vaccination.[254] RCTs of maternal influenza immunization have demonstrated that pregnant women typically have a good immunological response to IIV, and that anti-HA antibody titres in newborn infants can exceed maternal titres due to active antibody transport mechanisms across the

placenta. Clinical trials have shown vaccine efficacy against laboratory-confirmed influenza in pregnant women ranging from 31% to 70%,[255,256] with vaccine efficacy against the same outcome in infants during the first 6 months ranging from 30% to 49%.[256,257] Inactivated influenza vaccines elicited a lower immunological response in women with HIV; nevertheless, in the same study, vaccine efficacy against laboratory-confirmed influenza was 58%.[257]

3.2 LAIV vaccine efficacy and effectiveness

Adults

There have been fewer LAIV efficacy and effectiveness studies in adults than in children. The largest and most often cited RCT of LAIV in adults involved over 4500 healthy working adults aged 18–64 years and examined reductions in self-reported respiratory illness, missed days of work, health-care visits and antibiotic use during the time that influenza viruses were circulating, but did not include laboratory-confirmed influenza endpoints.[258] Although this study was conducted during the 1997–1998 influenza season when there was a poor match between the influenza A(H3N2) vaccine virus and the predominant circulating viruses, it was found that LAIV vaccine recipients had 24% fewer febrile upper respiratory tract illnesses, and significant reductions in days of lost work, seeking health care, and prescriptions for antibiotics, compared to unvaccinated subjects.[258] A subsequent RCT comparing IIV and LAIV in healthy adults aged 18–49 years was conducted during the 2007–2008 influenza season when influenza A(H3N2) viruses predominated (90%) with only a minor influenza B component (9%) circulating.[259] This study used laboratory-confirmed influenza endpoints with virus isolation in cell culture, virus identification using RT-PCR, or both. The efficacy of IIV against both types of influenza was 68% (95% CI: 46–81) and 36% (95% CI: 0–59) for the LAIV. IIV efficacy against influenza A was 72% (95% CI: 49–84) and was 29% (95% CI: -14–55) for LAIV, with a relative efficacy of 60% (95% CI: 33–77) for IIV.[259] This study indicated that IIVs are more effective than LAIVs in young healthy adult populations.

Children

A limited number of RCTs with culture-confirmed influenza as an endpoint have been conducted with LAIV in children of various ages. In earlier LAIV studies, vaccine efficacy was generally high and ranged from 60% to 90%.[28,232,253] In addition to studies that used LAIV and a control in the study population, a more limited number of direct head-to-head comparisons of LAIV and IIV in children have been conducted. These studies also demonstrated high levels of vaccine efficacy and improved performance of 35% to 55% above efficacy seen with IIV.[126,250,252,260] These studies spanned years with varying degrees of match between a vaccine virus and the corresponding circulating virus, and indicated that LAIV provided better protection than IIV during study years in which a poor vaccine match occurred due to antigenic drift. These earlier studies led to recommendations for preferential LAIV administration to young children of various age groups in countries including Canada, Germany, Israel, Sweden, the UK and the USA. In more recent years, RTCs of LAIVs based on the Russian MDV backbone were conducted in resource-poor

settings in Bangladesh[261] and Senegal.[262] The study in Bangladesh was in children aged 2–4 years who received a trivalent influenza vaccine containing lyophilized LAIV (Nasovac-S, Serum Institute of India) containing A/California/7/2009 (H1N1)-like, A/Victoria/361/2011 (H3N2)-like, and B/Wisconsin/1/2010 (Yamagata lineage)-like vaccine viruses or a placebo. The outcome monitored was symptomatic laboratory-confirmed influenza using RT-PCR for well-matched viruses. H1N1 pdm09 and H3N2 viruses predominated in this study with attack rates of 3.6% for H1N1pdm09 and 12.3% for H3N2 in the placebo group and vaccine efficacy of 50% (95% CI: 9.2–72.5) against H1N1pdm09 and 60.4% (95% CI: 44.8–71.6) against H3N2. The study in Senegal included 1761 healthy children aged 2–5 years, with laboratory-confirmed symptomatic influenza as the primary outcome. Influenza was laboratory confirmed in (18%) of LAIV in both vaccine and placebo recipients, giving a vaccine efficacy of 0.0% (95% CI: -26.4–20.9). In this study, LAIV was well tolerated in young children but did not provide protection against influenza. The reason for the absence of LAIV efficacy in young children in Senegal for the same season in which a significant efficacy was observed using a similar endpoint and dose of LAIV (Nasovac) among young children in Bangladesh is unclear.

A recent observational study in the USA in children aged 2–17 years indicated that the effectiveness of the H1N1pdm09 component of seasonal LAIV was lower than that for the corresponding component of IIV.[263] It was suggested that this unexpected result for the LAIV H1N1pdm09 component during four seasons after the 2009 pandemic might have resulted from thermal instability of the vaccine virus.[263-265] In addition, this study did not find superior LAIV effectiveness compared with IIV against influenza A(H3N2) or B viruses in these same seasons. In other countries using the same LAIV, or LAIVs using the Russian MDVs, different results were obtained. The reasons for the poor effectiveness of LAIV in the USA are not yet clear but based on these results the ACIP recommended that LAIV should not be used in the USA during the 2016-17 and 2017-18 influenza seasons.[180]

4. Influenza vaccine use

4.1 Influenza vaccine policy and programmatic use

In accordance with its mandate to provide guidance to Member States on health policy matters, WHO publishes vaccine position papers providing recommendations on vaccines and immunization for diseases that have an international public health impact. The papers summarize essential background information on the respective diseases and vaccines, and conclude with the current WHO position concerning their use in the global context. The influenza vaccine position paper is available on the WHO website.[266]

Current influenza vaccines are usually administered annually because of the relatively short duration of protection and because the vaccine formulations are updated annually. In most countries, influenza vaccination policies and programmes focus primarily on efforts to protect those who are most vulnerable to influenza-related severe disease, hospitalization and death.

WHO encourages countries to make their own policy decisions based on national needs and priorities, disease burden, cost-effectiveness, programmatic feasibility and other appropriate country-specific considerations. As of 2014, 115 of 193 WHO Member States reported that they have a national influenza vaccine programme.[267] Most high-income countries (92%) reported having an influenza vaccination policy, while only four of 49 low-income countries reported having such a policy.

While the WHO Global Action Plan for Influenza Vaccines has made progress in increasing potential global vaccine production capacity and in expanding production of seasonal influenza vaccine in low and middle-income countries,[268,269] most of the influenza vaccine is manufactured in industrialized countries in the WHO Region of the Americas and the WHO European and Western Pacific Regions. Recent vaccine distribution surveys have highlighted the uneven distribution of influenza vaccine across the globe. Most of the influenza vaccine doses distributed go to the Americas and Europe, with less than 5% of all doses distributed in the WHO South-East Asia, African, and Eastern Mediterranean Regions.

4.2 Future prospects for improving immune responses with new influenza vaccines

Influenza vaccines have a long history of proven safety and effectiveness but current inactivated and live vaccines have some limitations that make prevention and control an ongoing global challenge. This is due to the ever-changing nature of influenza viruses, the under-utilization of influenza vaccines, the need to vaccinate annually, and relatively poor vaccine effectiveness especially in elderly adults. These limitations could be overcome if a definitive universal influenza vaccine were designed and developed to provide lifetime protection against all influenza A and B viruses. The design of such a vaccine is extremely complex and a universal vaccine is not expected to be available soon. The extent of the challenge for designing this type of vaccine is illustrated by the fact that even successive influenza virus infections with different influenza A virus subtypes and B virus lineages do not provide durable protection. Nevertheless, recent advances in understanding the immune response to influenza virus infection and vaccination along with advances in understanding virus protein structure have stimulated renewed efforts in the search for new approaches for next-generation influenza vaccines that would provide broader and/or more long lasting protection.

WHO has convened meetings in recent years to review progress towards developing improved influenza vaccines.[270,271] Many new approaches which might improve the protection conferred by influenza vaccines were reviewed, including: new approaches in vaccine design, e.g. targeting antibody responses to conserved epitopes such as the HA stalk, use of viral vectors, and improving cell-mediated immune responses; new approaches in vaccine production, e.g. use of LAIVs, use of adjuvants, cell-based and recombinant protein-based manufacturing; and novel administration routes such as microneedle array skin patches. Discussions concerning programmatic issues and challenges in low-resource countries, the costs of developing new vaccines, and regulatory challenges faced by industry have also taken place.[271] WHO has developed a guidance document to highlight the preferred product characteristics for next-generation influenza vaccines and data needs for policy makers, particularly in low and middle-income countries.[272]

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