

Live Attenuated Vaccines: Influenza, Rotavirus and Varicella Zoster Virus

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Abstract Since vaccinia virus was first used to protect against smallpox in the eighteenth century, live attenuated vaccines have proved to be highly effective in reducing the morbidity and mortality caused by many human viral pathogens. Contemporary live viral vaccines are designed using several different strategies to achieve attenuation. These basic principles and approaches are illustrated by vaccines to prevent rotavirus, influenza and varicella-zoster virus infections that are described in this chapter. As shown from the experience with these three vaccines, contemporary live attenuated viral vaccines have had a major impact on disease caused by these ubiquitous human pathogens.

1 Introduction

The value of live viral vaccines was established historically by the recognition that inoculation with vaccinia virus protected against smallpox. In this case, a closely related but much less virulent pathogen of cattle elicited protective immunity in people. Contemporary live viral vaccines are designed using several different strategies to achieve attenuation. These basic principles and approaches are illustrated by vaccines to prevent rotavirus, influenza and varicella zoster virus (VZV) infections that are described in this chapter. In the case of VZV, influenza and one of the current rotavirus vaccines, attenuation is accomplished through laboratory

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manipulations of a naturally occurring “wild type” parental strain recovered from an infected individual. For one of the rotavirus vaccines, a “Jennerian” approach, similar to that used for smallpox, has been used. In all cases the goal of attenuation is to preserve sufficient replicative capacity so that the vaccine virus will induce a robust and broad adaptive immune response similar to the natural infection, but not the illness expected, after inoculation of the wild type virus. For this purpose, the attenuated virus must retain infectivity at the site of inoculation, whether the vaccine is given by oral or intranasal mucosal inoculation, as is the case for the live attenuated rotavirus and influenza virus vaccines, or by subcutaneous injection of VZV vaccines. Some live attenuated viral vaccines are associated with mild symptoms, such as fever or rash in some recipients but the tropisms of the parent virus that typically damage host cells are disrupted. VZV vaccines are derived from a clinical isolate that was attenuated by the traditional approach of sequential passage in human and nonhuman cells and by adapting the virus to grow at low temperature. The rotavirus vaccine made by GSK was also attenuated by multiple passage of wild type human rotavirus in cell culture. The other rotavirus vaccine and the influenza vaccine’s attenuation relies on the inherent capacity of these viruses to undergo reassortment. This strategy can be adapted to achieve recombinants that have genes from a related, nonhuman virus as in the case of rotavirus or in which genes from virulent strains are inserted into a “backbone” consisting of genes that have acquired attenuating mutations by cold adaptation or other methods, as was done to create live attenuated influenza vaccines. The ability of the vaccine virus to replicate in the human host becomes restricted as a consequence of these laboratory manipulations. However, it is critical that live attenuated vaccine viruses retain their genetic stability, to assure both that adaptive immunity comparable to that elicited by the wild type virus is maintained and that replication in the host does not produce a reversion to the virulence of the wild type virus. Since live attenuated viral vaccine strains may be transmissible, genetic stability must also be retained despite replication in secondary contacts. Once attenuation has been achieved, the development of live attenuated viral vaccines requires defining the optimal infectious dose and dosage regimen to elicit adaptive immunity against the pathogen. Finally, their potential to provide protection against infection must be confirmed in large-scale efficacy trials. As shown from the experience with rotavirus, influenza and VZV vaccines, live attenuated viral vaccines have major benefits for reducing the morbidity and mortality caused by these ubiquitous human pathogens.

2 Influenza

2.1 Introduction

Influenza is the major cause of epidemic and pandemic severe respiratory disease in people of all ages in all areas of the world. Influenza is also an important natural pathogen of other animal species, including birds, horses and pigs. Natural infection

with wild-type influenza virus elicits a long-lasting immune response that protects the individual from influenza illness following re-exposure to the same, or very similar, strain of influenza but not from influenza strains that are antigenically distinct from the infecting strain. As influenza virus evolves it undergoes genetic changes in all its genes, including those encoding the major antigens on the virion surface [the hemagglutinin (HA) and neuraminidase (NA) glycoproteins], which are targets of protective immunity. Because the virus can undergo antigenic drift and shift, it may cause multiple symptomatic infections throughout a lifetime. Inactivated influenza vaccines were first put into use over 50 years ago for military personnel and have been in general use for more than 30 years. Although inactivated vaccines are generally safe and effective, there is room for improvement, especially in very young children and elderly adults and in situations where the vaccine strain is “antigenically mismatched” with the circulating strain. In order to address some of the deficiencies of the inactivated influenza vaccine, live attenuated influenza vaccines (LAIV) have been developed.

2.2 Virology, Epidemiology and Pathogenesis

The influenza viruses are members of the Orthomyxoviridae family, characterized by a negative sense, single-stranded, eight segment RNA genome surrounded by a lipid membrane. The two major surface glycoproteins, the HA and NA, are inserted into the outer lipid membrane and determine the serologic classification of the specific viral strain. Three genera of influenza virus (A, B, and C) circulate in humans but types A and B cause most of the morbidity and mortality and these two types are those that are currently incorporated into the various licensed vaccines. Influenza naturally infects humans as well as several other animal species including avian species including poultry, pigs and horses. These animal hosts often serve as a reservoir for the evolution of new pandemic strains via gene reassortment interactions.

Worldwide, influenza viruses cause considerable morbidity and mortality every year, with an estimated 35,000 deaths in adults >50 years old and an average of 114,000 excess hospitalizations each year in the United States [1]. The seasonal winter outbreaks of influenza result from antigenic drifts that occur every few years in each of the three (or four) major influenza viruses, the two A strains (H3N2, H1N1), and one or more B strains, that are now circulating and the introduction of new susceptibles into the population. Influenza viruses are spread by inhalation of viral particles aerosolized by coughing and sneezing [2]. The pathogenesis of influenza virus infection begins with infection of the respiratory mucosal epithelium. Influenza is an acute febrile illness associated with myalgias, headache, cough, rhinitis, and otitis media, which is usually self-limited but can progress to pneumonia. Generally, the risk of influenza morbidity and mortality is highest in persons >65 years old, young children under 5 years, and persons with chronic cardiac or pulmonary disease or immunocompromising conditions. As was seen in

the recent pandemic of variant H1N1 virus, pandemic strains can occasionally cause increased morbidity or mortality in healthy young adults as opposed to the elderly. Because first encounters with influenza often cause lower respiratory tract infection, the hospitalization rate for influenza is 100 per 100,000 children aged 0–4 years [1]. High infection rates in children of school age also facilitate influenza spread. Influenza pandemics result from antigenic shifts associated with reassortment events or emergence of new strains from avian reservoirs, as occurred in 1918, 1957, 1968 and 2009. Under these conditions, an influenza virus with an HA and/or NA that had not previously infected humans and that can infect, cause disease and be transmitted efficiently, is introduced into a large naïve population. At any given time, the potential risks of a new pandemic are virtually impossible to estimate accurately but such a pandemic constitutes a major public health emergency as occurred with the recent emergence of the novel variant H1N1 strain in 2009.

2.3 *Immunology*

Immune responses to a wild-type influenza infection are robust and leave the individual with a strong immunological memory that prevents the same or an antigenically similar variant from causing disease for decades. The response can be measured in many different compartments, including IgG and IgA antibodies in the serum, secretory IgA in the nasal secretions, and T, B and NK cells in the peripheral blood as well as various lymphoid tissues, especially those in the respiratory tract. Functional antibodies that neutralize the virus or prevent it from binding its cognate receptor are designated hemagglutination inhibiting (HAI) antibodies and can be found in the serum and occasionally in nasal secretions. Cellular immune responses and additional antibody responses target a variety of regions on the viral HA and NA surface glycoproteins and other proteins encoded by the virus, particularly M, NP, and NS. The quantity of HAI and the amount of neutralizing antibody in serum have been correlated with the extent of protection from disease; some evidence indicates that the serum titer of antibodies to NA is also correlated with protection. Despite the presence of these multiple components of immunological memory and effector function and substantial information correlating some of these responses with protection, the fundamental role each has in preventing illness following re-exposure to influenza remains to be elucidated.

The immune response to inactivated influenza vaccine has been extensively studied [2]. The immune response to vaccination with LAIV has been studied in several different settings and the immune response is qualitatively similar but quantitatively less than that elicited by natural infection. After LAIV immunization, mucosal IgA, serum HAI, and neutralizing antibodies and cellular T and B cell responses are observed. Lower responses are not surprising given that the vaccine stimulates immunity by replication in the upper respiratory tract, the site of replication of the wild-type virus. The level of replication of the LAIV strain is significantly reduced compared to that of wild-type virus. Despite evidence for

vaccine-induced immunity in both local and systemic compartments, the specific functional role of any particular immune response and validated correlates of LAIV-induced protection from influenza disease in vaccinated individuals have not been defined.

LAIV elicits the most robust immune response in young children, particularly those that are seronegative for influenza at the time of vaccination [3–5]. Seroconversion rates, measured by the presence of HAI antibody, are as high as 80–90% in young children after two doses of vaccine. Seroconversion rates are lower for children or adults that have preexisting antibody at the time of vaccination. The presence of antibody at the time of immunization may limit the extent of replication of the vaccine in the upper airways, evidenced by lower rates of shedding, and may mask the boosting of the immune response using relatively crude measures of immunogenicity such as HAI antibody in the serum. In children, LAIV induces nasal secretion of IgA and production of circulating IgG and IgA antibody secreting cells (ASC) 7–10 days after immunization [6, 7]. Children 6–36 months of age have measurable IFN γ -secreting cells in their PBMCs by 13 days after LAIV; these responses were not evident in children vaccinated intranasally with heat-inactivated LAIV or intramuscularly with inactivated vaccine [8]. In a study of children aged 5–9 years, blood was collected at 10 and 28 days post vaccination and stimulated with the A/H3N2 strain *ex vivo*. Both CD4 and CD8 influenza-specific T cell frequencies were increased in these children compared to their prevaccination values. These increases were greater than those observed for children vaccinated with the seasonal trivalent inactivated influenza vaccine (TIV) in the same study and the CD8⁺ T cells induced by LAIV underwent a number of specific phenotypic changes [9–11].

Vaccine studies often rely on correlate markers to demonstrate that the vaccine will perform as expected under the conditions being studied. A robust correlate of protection is an immunological marker that when present coincides with protection from disease upon subsequent exposure to the wild-type virus and the lack of which correlates with susceptibility to illness. Due to high rates of efficacy demonstrated for LAIV combined with the difficulty in using traditional serum-based influenza assays to measure an immune response in adults, these markers have been difficult to identify for LAIV. Virtually all adults have had multiple encounters with wild-type influenza and/or have been vaccinated for influenza. Thus, most adults have readily measurable levels of influenza antibody in their serum prior to vaccination. In contrast to studies in young seronegative children, vaccination of adults with LAIV does not usually produce a measurable increase in serum HAI antibody titers. Recent studies on T cell immunity following vaccination showed similar results [9, 11]. The levels of prevaccination influenza-specific CD4 and CD8 cells increase with age of the subjects and adults have significantly higher baseline quantities than children [11]. The level of prevaccination influenza specific CD4 T cells seems to be a critical determinant of whether or not vaccinees experience a subsequent rise in either CD4 or CD8 T cells. In contrast to the T cell and HAI responses, adults generally have increased influenza-specific antibody-secreting B cells in the blood 7–10 days post LAIV vaccination. Although only 16% of adults have a serological

response measured by a fourfold or greater increase of HAI antibody following immunization, approximately 80% of the subjects have a measurable increase in the number of influenza-specific IgG-secreting antibody-secreting cells in the peripheral blood. This was true for individuals who had been vaccinated in the prior year as well as those who were not [6, 7]. These data demonstrated that LAIV elicits a readily detectable B cell response in most adults, which is consistent with the clinical experience that LAIV is highly efficacious in an adult population aged 18–49 [12, 13].

2.4 Vaccine Development, Composition, and Mechanism of Attenuation

Development of live, attenuated vaccines based on the cold-adapted (*ca*), attenuated *ca* A/Ann Arbor/6/60 and *ca* B/Ann Arbor/1/66 backbones has spanned several decades. The vaccine contains three vaccine strains, two attenuated influenza A strains and one attenuated influenza B strain. These vaccine strains are genetic reassortants each harboring two gene segments from the currently circulating wild-type virus conferring the appropriate antigens (e.g., A/H3N2, A/H1N1, or B) and six gene segments of live, attenuated influenza A or influenza B donor virus or master donor virus (MDV). The resulting 6:2 genetic reassortant combines the attenuation inherent to the MDVs with the antigens needed to elicit a neutralizing immune response that should prevent disease caused by currently circulating strains of influenza. LAIV is used for active immunization of subjects from ages 2 through 49 and is currently manufactured in specific pathogen-free embryonated chicken eggs. The three constituent attenuated influenza A and B strains are blended and filled into sprayer devices used to deliver the vaccine liquid into the nasal passages.

In the 1960s, investigators set out to attenuate influenza virus for vaccine use through a process designated as cold-adaptation. Forcing the virus to replicate efficiently at lower than normal temperatures resulted in changes to its genetic makeup making it less fit to replicate at normal and elevated body temperatures, thereby attenuating the strain. Biological characterization of *ca* A/Ann Arbor/6/60 and *ca* B/Ann Arbor/1/66 demonstrated that the resulting viruses are cold adapted, as defined by ability to replicate to titers at 25°C that were similar to titers obtained at 33°C. The strains are also temperature sensitive (*ts*), as defined by replication of the virus at 39°C that was debilitated compared to its replication at 33°C [14]. The spectrum of temperatures at which the *ca* virus replicated well was lower than the wild-type viruses that caused disease. Further characterization of *ca* A and B/Ann Arbor strains in the highly susceptible ferret model demonstrated that these strains were attenuated (*att*) compared to wild-type influenza viruses and were unable to replicate in the lung tissues of ferrets or elicit signs of influenza-like illness (ILI) [15]. These two strains provide the genetic background of all LAIV strains, imparting their *ca*, *ts*, and *att* properties to the vaccine.

Sequence analysis and comparison of the genomes of *ca* A/Ann Arbor/6/60 and *ca* B/Ann Arbor/1/66 to their respective parental strains confirmed that a number of changes had accumulated during cold passage. The introduction of reverse genetics enabled biological traits to be associated with specific nucleotides without having to account for potential problems caused by constellation effects. Five nucleotide positions distributed between the PB1, PB2, and NP gene segments of A/Ann Arbor/6/60 controlled both the *ts* and *att* properties [16]. Studies with B/Ann Arbor/1/66 revealed that three positions (two in PA and one in NP) control the *ts* phenotype, an additional two nucleotides in M control the *att* phenotype, and another subset of three changes in PA and PB2 are responsible for the *ca* phenotype [17, 18]. When the minimal set of mutations are made in divergent influenza strains the biological traits transferred; thus, the fundamental mechanisms restricting the replication of these vaccine strains at elevated temperatures are a result of the complex genetic signatures that affect multiple points of the replication cycle to provide a robust and stable set of attenuating changes to the viruses.

Following intranasal administration, the vaccine virus infects and replicates in epithelial cells of the upper respiratory tract resulting in an immune response. Characterizing the genetic stability of the vaccine in humans was important for understanding the properties of the vaccine. In a study of genetic stability, 98 children, 9–36 months of age, were vaccinated with LAIV and nasal swab samples were taken at frequent and regular intervals. Of the children in the study, 86% shed at least one of the three strains in the vaccine. The *ca* and *ts* phenotypes were preserved in all the shed viruses tested [19]. Of the isolates, 54 were chosen at random and their genomes sequenced in their entirety and compared to the sequences of the strains used to vaccinate the children. These analyses revealed that some genetic changes occurred in a majority of shed isolates and in some cases the mutations were shared by multiple isolates [20]. Interestingly, in most cases, the change(s) evident in the virus that was shed were representative of changes that existed in the bulk vaccine material. Despite the presence of these mutations, all isolates invariably retained their characteristic biological attenuated properties.

A corollary concern associated with genetic stability and vaccine shedding is the potential for person-to-person transmission of the virus. The study of the genetic stability of the vaccine in children was also designed to assess the probability of transmission of the vaccine virus. In addition to the 98 children vaccinated with LAIV, 97 children attending the same day care center received placebo. Nasal swabs were obtained at frequent intervals from each child and the presence of vaccine virus was assessed. Vaccine virus was recovered from 80% of the vaccinated children and from only one placebo recipient [19]. The influenza B vaccine virus recovered from the placebo recipient was shed on only 1 day. The transmitted vaccine isolate was shown to retain its characteristic *ca* and *ts* properties and exhibited the attenuation phenotype in ferrets and the placebo child from whom LAIV was recovered had no symptoms of influenza. These results indicated a 0.58% probability of vaccine transmission occurring from a single contact of a vaccinated young child with an unvaccinated young child [19].

The likelihood of transmission from a vaccinated adult is expected to be substantially lower because LAIV shedding is much less than in children.

2.5 *Safety and Efficacy*

Vaccines derived from *ca* A/Ann Arbor/6/60 and *ca* B/Ann Arbor/1/66 have been extensively characterized in clinical studies. Prior to the mid 1990s, monovalent and bivalent forms of these vaccines were evaluated in over 15,000 subjects in a number of different clinical studies, many sponsored by the NIH [21]. Studies of commercially produced, frozen and refrigerator stable, trivalent formulations of LAIV (Flumist[®], MedImmune) have been conducted in a wide range of settings in individuals from 6 months to over 80 years of age. These studies have been done both before and after licensure.

LAIV has reproducibly prevented ILI caused by all three currently circulating influenza types. A meta-analysis of placebo-controlled studies showed that the mean efficacy of two doses in previously unvaccinated young children was 77%, with efficacies of 85%, 76%, and 73% against A/H1N1, A/H3N2, and B, respectively. The mean efficacy of one dose in previously vaccinated children was 87% [8, 22–30]. A single dose of vaccine, while not optimal, has been shown to provide a high degree of clinical efficacy among previously unvaccinated young children [26, 31].

Three studies were conducted in which LAIV and TIV were compared. The largest of these included over 8,000 children. LAIV was shown to reduce the burden of illness by nearly 55% compared to TIV. Of note, the A/H3N2 strains circulating in this study were antigenically mismatched to the two vaccines and the children vaccinated with LAIV had 79% fewer cases of modified ILI compared to the TIV group [24]. In two other studies, one conducted in children with recurrent respiratory illness and the other in older children with asthma, LAIV was also shown to be more efficacious than TIV [22, 27]. Immunity elicited by LAIV may provide for a larger margin of error for antigenic mismatching than occurs after inactivated vaccine administration. LAIV has been shown to provide protection against significantly antigenically drifted variants in several clinical settings. In 1997–1998, children were immunized with a trivalent blend of LAIV containing the A/Wuhan/359/95 (H3N2) strain. The H3N2 virus that subsequently circulated was A/Sydney/05/97, which has an H3 that is quite distinct from the antigen contained in the vaccine. Despite this mismatch, the vaccine conferred efficacy greater than 85% against the A/Sydney/05/97 virus [24]. During the same season, LAIV was also shown to protect adults against the drifted H3 strain [12]. In a direct comparison study of LAIV and TIV in children, LAIV reduced modified ILI caused by an antigenically drifted A/H3N2 strain by 79% compared to TIV [23].

Two placebo controlled field studies in adults have been reported using either effectiveness endpoints [12] or culture confirmed prevention of ILI in adults 60 years or older [32]. In a series of field studies in young adults, TIV was more

efficacious than LAIV; however, both groups suffered less illness than observed in the placebo group. In a study conducted in the 2007–2008 influenza season with 1,952 subjects, the inactivated vaccine was shown to have an efficacy of 72% compared to a placebo and LAIV had an efficacy of 29% compared to a placebo [33–35]. These two vaccines have also been studied in military personnel. In a retrospective cohort analysis, LAIV was more effective than TIV at preventing influenza illness in recruits and TIV was slightly more effective in nonrecruits [36]. In an analysis of more than a million nonrecruits, TIV was more effective at lowering health care encounters for pneumonia and influenza than LAIV and the latter was shown effective in only one of the three seasons analyzed. However, LAIV was effective in the subset of vaccine-naïve service members at levels similar to TIV [37]. The less robust results of these studies in adults compared to studies in children, where LAIV has appeared to be more efficacious than TIV, may reflect the interaction of LAIV with the already flu-experienced immune system of the adult host. Presumably the higher level of preexisting immunity in adults is more restrictive for immune responses to the replication dependent LAIV than for the parenterally administered, non replicating TIV.

Safety is obviously a critical issue when evaluating a live viral vaccine. In controlled studies, the most common adverse events in children 2 years of age or older given LAIV were runny nose or nasal congestion, low-grade fever, decreased activity and decreased appetite. In the youngest children, who received two doses of vaccine, no significant differences were observed following the second dose. In adults, the most common adverse events were runny nose/nasal congestion, cough, and sore throat, which were all short lived. In a large safety database study using the Northern California Kaiser Hospital system, a 3.5-fold increase in asthma events was noted within 42 days of vaccination in the prespecified age stratum of 18–35 months [38]. The observation was further investigated in the large efficacy study of LAIV and TIV in young children. In the age stratum less than 24 months of age (6–23 months), 3.2% of children in the LAIV group had medically attended wheezing events within 42 days of vaccination compared to 2.0% in the TIV group. This difference was significant. There was no significant difference in rates after 42 days in this age group or in the children 24 months of age or older [1, 23]. These findings led to the licensure of LAIV for children over the age of 2 years.

2.6 Issues for the Future

The utilization of the live virus vaccine technology continues to be refined and improved. Recent studies in children should encourage greater use of this vaccine in this highly susceptible and vulnerable population. The current manufacturing methods used to make LAIV, like those used to produce the inactivated vaccine, are based on production technologies that are over 50 years old. More modern production methods, including manufacturing in cell culture substrates, are needed

and are being developed. In addition, the generation of the 6:2 reassortant viruses used to initiate vaccine seed strain production is being refined and integrated with the use of reverse genetics technology. Finally, the attributes that make this vaccine effective in young children are being further explored and LAIV strategies are being developed for pandemic solutions where the advantages over inactivated TIV for producing large amounts of vaccine are substantial.

3 Rotavirus

3.1 Introduction

Rotaviruses are the most frequent cause of severe diarrheal disease in young children worldwide and are also ubiquitous enteric pathogens of many other mammalian and avian species. By 5 years of age, 1 in 50 children worldwide will have been hospitalized and 1 in 205 will have died from rotavirus-associated causes. Virtually all of these deaths occur in children living in developing countries. The worldwide morbidity and mortality associated with rotavirus makes it one of major vaccine-preventable causes of infant mortality. Natural infection with wild-type rotavirus elicits immunity that efficiently protects from subsequent severe illness, irrespective of the rotavirus serotype. In the past decade, two live attenuated, orally administered rotavirus vaccines have been developed and introduced in many countries; both have proven safe and effective. Several third generation candidates are in late-stage development. In the USA, the introduction of one of the currently licensed rotavirus vaccines has been associated with a remarkable decline in rotavirus illness. However, the overall impact of the licensed rotavirus vaccines on disease in extremely poor countries, where they are needed the most, remains to be determined.

3.2 Virology, Epidemiology and Pathogenesis

Although human rotaviruses (RVs) were discovered in the intestines of children with diarrhea only 36 years ago [39], substantial progress has been made in our understanding of their role in human disease. Rotaviruses are the most important cause of severe watery diarrhea in all regions of the developed and less developed world. During diarrheal illness, rotaviruses are shed in the stool in great quantity (in amounts as high as 10^{10-11} particles per gram of stool), which allowed the rapid development of sensitive and specific antigen detection diagnostics. Based on results from these simple diagnostics tests, it soon became clear that RVs were the cause of approximately 20–30% of severe diarrheal disease requiring hospitalization in children under the age of 5, worldwide [40]. Recent estimates indicate that

there are over 114 million rotavirus diarrheal episodes annually; these lead to approximately 24 million clinic visits, 2.4 million hospitalizations (40% of all diarrheal hospitalizations), and over 500,000 deaths in children under 5 years of age [41].

The burden of disease from rotavirus infection is not restricted to the less developed world. Studies from Europe indicate that approximately half of severe gastroenteritis in children less than 5 years of age is caused by rotavirus. In studies from the US, 50% of children hospitalized or treated in the emergency department for gastroenteritis were infected with rotavirus [42]. These data lead to the estimate that one of every 150 children under 3 years of age will be hospitalized and 1 of 11 will be seen as an outpatient in an emergency department for treatment of rotavirus disease. In the US, rotavirus is estimated to cause 20–40 deaths, 55,000–70,000 hospitalizations, and 410,000 physician visits annually [43]. The overall costs of rotavirus disease in the US are thought to exceed a billion dollars annually.

Rotavirus disease occurs with high frequency around the world, in both temperate and tropical climates and in both developed and less developed countries. The large quantity of virus that is shed probably explains why improvements in hygiene in the developed world have not reduced the incidence of infection. In temperate climates, prior to the introduction of vaccines (see below), rotavirus disease occurred seasonally in the cooler dryer months of the year [44]. In the US, waves of rotavirus infection tend to start in the southwest in the fall and end in the northeast in the spring, whereas in Europe infections tend to spread from south to north over generally the same time frame [45]. Of note, a recent study predicts that wide-spread vaccination will alter this seasonal trend [46]. The seasonality of rotavirus infections fluctuates far less in tropical climates but the highest numbers of infections occur in the coolest and driest months of the year [47].

Rotaviruses, like other members of the Reoviridae family, have a double stranded (11 segment) RNA genome and icosahedral symmetry. The viral serotype is determined by its two surface proteins, VP4 (P type) and VP7 (G type) [48]. Both of these proteins are the targets of neutralizing and protective antibodies. Due to its segmented RNA genome, the genes encoding VP4 and VP7 segregate relatively independently. At least eleven distinct human VP4 P types and ten VP7 G types have been isolated [49]. However, only a small number of P and G type combinations are encountered with any significant frequency in people and just four combinations, P(8)G1, P(8)G2, P(8)G3, and P(4)G2, account for over 90% of all isolates. Serotypic diversity does change over time and based on geography, especially in the less developed world. In the last decade, isolation of P(8)G9 and viruses has been more frequent. The relationship between serotypic diversity and protective immunity is still not well understood but it seems clear that a significant level of heterotypic immunity is produced following an initial rotavirus infection. Because of this immunity, severe episodes of illness after the primary infection are relatively uncommon [50].

From studies of animals and experimental infection of adult volunteers, the incubation period for rotavirus is usually less than 48 h. It was thought that in immunocompetent children, rotavirus infection was restricted to the mature enterocytes

on the tips of the small intestinal villi. However, recent studies in humans and animals indicate that this paradigm is not correct; most rotavirus infections are associated with some level of viremia and systemic replication [51]. The clinical relevance of the findings of extraintestinal spread and replication of rotavirus is still unclear and the great bulk of rotavirus replication clearly occurs in the mature villus tip cells of the small bowel. The pathologic changes in the intestines of children infected with rotavirus include shortening and atrophy of the villi, mononuclear infiltration in the lamina propria and distended cisternae of the endoplasmic reticulum. A direct relationship between the extent of enteric histopathologic changes and disease severity has not been demonstrated. In a mouse model, rotavirus disease is associated with very modest histopathology.

3.3 Immunology

Studies of natural rotavirus infection in man and animals demonstrated the existence of acquired immunity both to recurrent disease and, to a lesser extent, reinfection following primary infection [50]. Passive transfer studies of monoclonal antibodies in mice demonstrated that neutralizing antibody to either VP4 or VP7 could transfer either homotypic or heterotypic protection, depending on the antibody specificity in vitro [40]. Interestingly, other studies in mice have shown that non-neutralizing IgA antibodies to the antigenically conserved VP6 protein can also mediate protection, apparently via an antiviral effect occurring during transcytosis [52]. This novel intracellular neutralization event could help explain the well-documented clinical observation of heterotypic immunity following primary infection with a single rotavirus serotype. Several studies in the mouse model indicated that that B cells were the critical determinant of protection from reinfection after infection whereas CD8⁺ T cells were responsible for restricting the course of viral shedding during primary infection [53]. CD4⁺ T cells aid CD8⁺ T cells and B cells and apparently can mediate active protection via an IFN γ -dependent pathway after immunization with recombinant VP6.

Rotavirus-specific fecal IgA responses occur in the majority of children after a symptomatic infection. They peak from 1 to 4 weeks after infection and then decline rapidly. It is reasonable to hypothesize that the transient presence of mucosal immunity after primary infection contributes to the absence of sterilizing immunity to reinfection. Secondary and subsequent rotavirus infections tend to boost the fecal IgA response and in many children eventually induce sustained, protective fecal anti-rotavirus IgA levels [54]. Studies of human neutralizing antibody responses against rotavirus have shown that upon first exposure to rotavirus, children develop higher homotypic than heterotypic antibody levels [53], although both types of response are usually present. Studies in animal models and humans indicate that the presence of intestinal antibodies is probably the primary protective effector mechanism against rotavirus. Protective humoral immunity in several, but not all, animal models is associated with the presence of neutralizing antibodies

directed at VP4 and/or VP7. In studies performed in day care centers and orphanages where antibodies to rotavirus were measured very shortly before a rotavirus outbreak, intestinal and/or serum antibody levels correlated with protection against rotavirus reinfection [55]. Levels of rotavirus-specific antibodies (stool IgA in particular) were correlated with protection in some but not all studies involving naturally infected as well as vaccinated children. In vaccine studies, some investigators [56] found a correlation between the presence of neutralizing antibodies and protection; however, the percentage of children with detectable serotype specific neutralizing antibody titers is always significantly less than the percentage of children protected by vaccination [57]. Thus, although serotype specific neutralizing antibodies seem to play a role in protection, it seems likely that heterotypic antibodies or against other proteins or other mechanisms also play a role in immunity.

Recent studies have also drawn attention to the possible importance of the innate immune response and interferon in regulating rotavirus immunity. In the gnotobiotic porcine model, probiotic treatment with *Lactobacillus acidophilus* significantly enhanced both B and T cell responses to attenuated live virus infection [58]. It has been shown that levels of type I and II IFN are elevated in rotavirus-infected children and animals [59, 60]. Both type I and II interferon are able to limit rotavirus infection in vitro and, in early studies, IFN α administration successfully alleviated RV diarrhea in cattle and pigs. The IFN-regulatory factor 3 (IRF3) interacts with the RV protein NSP1, clearly linking RV infection to innate immunity [61]. NSP1 also inhibits activation of NF κ B by a novel mechanism involving targeted degradation of an F-box protein of the E3 ligase complex [62, 63]. Studies in vivo demonstrated that the systemic virulence of selected strains of rotavirus was enhanced and a lethal biliary and pancreatic disease induced when interferon signaling was abrogated during rotavirus infection [64]. Hence, innate immunity plays a critical role in modulating rotavirus infection in vitro and in animal model systems but the role in humans remains largely unexplored.

3.4 Vaccines Development, Composition and Mechanism of Attenuation

Two live attenuated, orally administered rotaviral vaccines are currently licensed and in use in many countries around the world [48, 65–67]. Both vaccines have been shown to be safe and effective as well as cost-effective in developed and developing countries. The aim of anti-rotavirus vaccination strategies is to reproduce the level of immunity induced following natural infection. Natural infection, either symptomatic or asymptomatic, efficiently prevents subsequent severe rotavirus disease but does not necessarily prevent reinfection or mild illness. Based on the observation that animal rotaviruses appear to be substantially restricted for growth, pathogenicity, and transmission in heterologous hosts such as humans (host range restriction), the initial strategy for rotavirus vaccine development was a modified Jennerian approach using either live simian/human or bovine/human rotavirus

reassortants as vaccines. In this approach, an animal origin rotavirus that is restricted for growth in humans is reassorted with a human rotavirus and reassortants are isolated with genomes that are primarily animal in origin but which contain genes encoding VP4 or VP7 from the human parent. Such reassortants are expected to induce protective immunity to human rotaviruses because of the presence of human surface proteins (VP4 or VP7) but not to cause severe disease because most of their genome was derived from the animal rotavirus parent which is restricted for growth in the human host. Of note, these vaccines are fully replication competent in cell culture and they are derived from potentially virulent animal strains but they have undergone considerable cell culture passage which, in itself, could also be a cause of attenuation. The first modified Jennerian vaccine of this type was a quadrivalent rhesus vaccine (RotashieldTM, Wyeth/Lederle) consisting of four monoreassortants between human G types 1 through 4 and the simian RRV strain [68]. The vaccine contained components for expression of these G types based on the assumption that immunity to the four most common human rotavirus types would be needed to induce a high level of efficacy. This vaccine was shown to be highly immunogenic and efficacious in multiple phase III studies in the USA, Finland and Venezuela and was licensed for use in the United States. Of note, the level of efficacy substantially exceeded the level expected based on the type-specific neutralizing antibody response induced by the immunization and this finding later was repeated with the bovine rotavirus based modified Jennerian vaccine. Shedding studies of the RRV-based vaccine indicated that it was shed in moderate quantity and was able to transmit with reasonable efficiency to unvaccinated children in the environment. The consequences of this shedding and transmission were never evaluated but it did not appear that the virus gained virulence during passage in humans. Unfortunately, after licensure and administration to almost one million American children, the RRV-based vaccine was withdrawn from the market because of its strong temporal association of the first dose of vaccine (which was licensed to be given orally at 2, 4, and 6 months of age) with intussusception in older (over 3 months of age) children receiving their first dose. Interestingly, the impact of this vaccine on the total attributable risk of intussusception, especially if initially administered to children only at 2 months of age or below, remains unknown but was likely to be very small. At the time the vaccine was withdrawn from manufacture, it was undergoing efficacy evaluation in less developed countries which were never completed. However, immunogenicity studies carried out in Bangladesh indicated that the RRV-based vaccine appears to have been more immunogenic in this setting than either of the two currently licensed second generation vaccines.

Subsequently, a second generation pentavalent modified Jennerian vaccine called RotaTeqTM (Merck), based on mono or di-reassortants derived from the mixed infection of a bovine rotavirus (WC3) and human rotavirus strains which provided human G1, G2, G3, G4 (VP7), and P[8] (VP4) was introduced [65]. This vaccine contained five (rather than four) separate reassortants based on the theoretical consideration that immunity to all five serotypic species would be most efficient at inducing protective immunity. The basis of attenuation for this vaccine, like the

preceding RotashieldTM product, is presumed to be the inherent host range restriction linked to its primarily bovine-origin genome. The specific genetic and mechanistic basis for host range restriction of the two reassortant-based vaccines is not well understood, although several studies indicate that this restriction is linked, at least in part, to rotaviral protein NSP1 and host specific inhibition of the innate immune system [69]. Studies of viral shedding have demonstrated that, in general, the bovine-based reassortant vaccine is highly restricted for replication in humans and is shed in a very limited fashion compared to wild-type human rotavirus or to the RRV-based vaccine although no head-to-head comparison was ever done. Nevertheless, recent studies have demonstrated that severely immunodeficient children can become chronically and symptomatically infected with this vaccine [70]. As with the RRV vaccine, this vaccine is administered orally in a three dose regimen.

The other currently licensed vaccine (RotarixTM, GSK) is a more traditionally constructed, live attenuated vaccine. A virulent G1P human rotavirus strain (89-12) was multiple passaged in monkey kidney cell culture in order to acquire a suitable level of attenuation to reduce virulence [66]. The rationale underlying the development of this monovalent vaccine was that a single natural rotavirus infection, either symptomatic or asymptomatic, very effectively provides protective immunity to subsequent severe disease, irrespective of serotype. Therefore, it seemed logical that a single attenuated human rotavirus strain might do the same. As with the Merck vaccine, the molecular basis for attenuation of this vaccine candidate is unknown although presumably, in this case, point mutations introduced during multiple passage in cell culture are responsible for the attenuation. Since the actual molecular basis for attenuation of the two currently licensed vaccines remains unknown, it is difficult to comment definitively on their level of genetic stability except to say that, to date, evidence for reversion to virulence has not been found. This vaccine is also administered orally, but in this case only two doses of vaccine are given.

3.5 Safety and Efficacy

The two second generation vaccines have been evaluated for safety and efficacy in studies representing a wide variety of socioeconomic conditions in several countries. In very large field studies both were shown to be safe and effective. Protection rates in developed or moderately developed countries provided by both vaccines are very similar; rates vary from 70–80% against any rotavirus disease to 90–100% against severe gastroenteritis. Interestingly, to date, no appreciable advantage of the multivalent over the monovalent vaccine has been observed. Large pre- and post-licensure studies have shown that these vaccines are not associated with intussusception if the first dose is administered to children under the age of 3 months [71]. The effectiveness of the pentavalent Merck vaccine in preventing rotavirus-associated gastroenteritis and hospitalizations was demonstrated in the United States and the monovalent vaccine effectively prevented rotavirus-associated

deaths in Mexico [72–74]. Since successful efficacy and/or immunogenicity trials in a variety of countries around the world have been completed, a general WHO global recommendation for the use of these vaccines was issued in June 2009 [75]. Of note is the fact that recent studies of the two new vaccines in very poor areas of Africa and Asia indicate that these vaccines are less effective (49.5–76.9% protection rates against severe disease) in these countries than has been observed in developed countries [76]. However, they are still very cost-effective in terms of number of severe rotavirus-induced diarrheas prevented: Overall in African trials, Rotarix prevented 3 out of 5 episodes of severe rotavirus-induced disease per 100 vaccinated children [75].

3.6 Issues for the Future

Although two safe and effective live attenuated rotavirus vaccines are now available, several important practical issues are not yet resolved. Exactly how important the moderate decrease in efficacy of these vaccines in very poor countries is and whether it can be circumvented in some straightforward way is not known. Second, the two current vaccines cost too much to be affordable without substantial subsidies and cheaper vaccine products will be required to serve the global needs in the long term. The two vaccines are currently highly effective but rotaviruses certainly have the ability to evolve serotypically and it remains to be seen if new strains of human rotavirus will emerge that are less effectively countered by immunity elicited by the current vaccines. Finally, as with all vaccines, rare and unexpected adverse events are always possible and continued vigilance regarding safety, especially in very immunosuppressed children is necessary.

Several third generation rotavirus vaccines are in various stages of development. A pentavalent live attenuated reassortant vaccine based on another bovine strain (UK) is currently undergoing evaluation in several less developed countries including China, India and Brazil. This vaccine has been shown to be highly efficacious in phase two trials in Finland. Monovalent human rotavirus vaccines derived from naturally attenuated strains recovered from human infants in India and Australia are in various stages of evaluation. The Indian strain (116E) appears to be highly immunogenic in preliminary studies [77]. Several groups have proposed that some form of parenterally administered inactivated vaccine might be safer vis-à-vis the risk of intussusception. Such a vaccine might be more immunogenic and hence more effective in some less developed regions where efficacy of the live virus vaccines seems to be restricted [76]. No data from human studies is currently available to evaluate the utility of this strategy. Finally, the quadravalent rhesus rotavirus-based vaccine is currently undergoing a re-evaluation in a phase three efficacy trial in Africa based on the early data indicating that this vaccine appeared to be more immunogenic than the current two commercial vaccines and hence, might be substantially more efficacious in a less developed setting. The results of this interesting trial should be available in the next year or two.

4 Varicella Zoster Virus

4.1 Introduction

VZV is a human alphaherpesvirus, most closely related to herpes simplex viruses (HSV) 1 and 2. VZV has the typical morphology of herpes virus particles and while its double stranded DNA genome is the smallest of the human herpesviruses, at least 70 gene products are encoded [78]. VZV causes varicella (chickenpox) as the primary infection and establishes life-long persistence in sensory ganglia; reactivation from latency produces the clinical syndrome referred to as zoster (shingles) [79, 80].

4.2 Epidemiology

The molecular epidemiology of VZV has been investigated extensively based on the detection of single nucleotide polymorphisms in selected VZV open reading frames and by whole VZV genome sequencing of more than 20 isolates [81, 82]. VZV circulates as five distinct clades that exhibit varying predominance in different geographical areas but overall, the genetic diversity of the virus is limited. While clades 1 and 2 are the most divergent, genome sequences were 99.83% identical, with only 188 site differences [83]. Sequences of nine clade 1 viruses showed 99.9% identity compared with Dumas strain, which is the first VZV genome that was sequenced [84]. Clade 1 viruses are most common in Europe and North America, clade 2 viruses are predominant in Asia and clade 5 is most prevalent in Africa. The Oka virus used to derive VZV vaccines is a clade 2 strain. As expected, immigration has redistributed European, African and Asian clades.

VZV skin lesions contain high concentrations of infectious virus during both primary and recurrent infections, which results in a highly successful strategy for persistence in the human population. Whereas the prevalence of other human herpesviruses has declined in developed countries, varicella epidemics continue to produce high infection rates. Episodes of zoster in older individuals provide a constant mechanism for reintroducing the virus, causing varicella in naïve individuals who are in close contact and who then spread the virus to other susceptibles. In temperate climates, VZV is acquired almost universally during childhood; attack rates are substantially lower in tropical areas. Before varicella vaccine was introduced, the incidence of varicella in the United States was ~four million cases per year, reflecting the number of children in the annual birth cohort. Secondary bacterial infections and VZV encephalitis were the most common morbidities; hospitalization rates were estimated to be 2–5 per 1,000 cases and approximately 100 fatal cases were reported annually [85]. The estimated incidence of herpes zoster is >1 million cases per year in the United States and complications, especially post-herpetic neuralgia, are frequent in older individuals [80]. While the

morbidity caused by VZV in healthy children and adults is significant, illness associated with this ubiquitous pathogen can be much more severe in immunocompromised patients. Children who are immunodeficient because of underlying disease or immunosuppressive therapies may develop progressive varicella; the risk of VZV reactivation is much higher in immunocompromised children and adults, and whether or not it is manifest as cutaneous zoster, reactivation may cause life-threatening disseminated VZV infection.

4.3 Pathogenesis of Primary and Recurrent VZV Infection

As defined clinically, the events in primary VZV infection include respiratory inoculation, viremia and the appearance of vesicular skin lesions. Studies of VZV pathogenesis using the severe combined immunodeficiency (SCID) mouse model show that VZV exhibits a marked tropism for T cells in human thymus/liver xenografts in vivo; VZV is also highly infectious for human tonsil T cells, particularly those in the subpopulation of activated, memory CD4 T cells, in vitro [86, 87]. VZV is readily transferred into skin xenografts when infected tonsil T cells are injected into the circulation of SCID mice. Infected T cells exit capillaries and initiate replication in epidermal cells, which progresses over a 10–21 day period until the lesion reaches the skin surface; cell–cell spread of VZV in skin is modulated by a potent innate response of the epidermal cells surrounding the newly forming lesion [88]. Induction of the IFN pathway and upregulation of NFkB signaling are prominent in adjacent cells. These observations suggest a model of primary VZV pathogenesis in which the virus infects respiratory epithelial cells, enters T cells in tonsils and other lymphoid tissues of the Waldeyer's ring and initiates a T cell-associated viremia which transports the virus to skin sites of replication; after a period of subclinical lesion formation during the incubation period, the characteristic varicella exanthem appears.

In the course of primary infection, VZV gains access to cranial nerve and dorsal root ganglia and as suggested by recent evidence, to autonomic enteric ganglia as well [89]. Access is presumed to occur via retrograde transfer along neuronal axons from skin lesions, T cell viremia or both. VZV-infected T cells transport the virus into DRG xenografts in the SCID mouse model [90]. Persistent infection is established in neurons for the life of the host. Abortive replication limited to ganglia or to subclinical skin replication may occur; however, when VZV reactivation causes zoster, VZ virions are presumed to move by anterograde axonal transport to the skin dermatome where vesicular lesions appear. In contrast to HSV, VZV reactivation can destroy neurons and satellite cells in the affected ganglia [80]; cell–cell spread with fusion of neurons and surrounding satellite cells is observed in VZV-infected DRG xenografts in the SCID model [91]. Recurrent VZV may also lead to viremia. Migrating T cells may become infected during trafficking through skin or ganglion sites of VZV replication, allowing viral transport to lungs, liver, brain and other organs in immunocompromised patients.

4.4 *Host Response*

In addition to innate cellular responses, NK cells appear to be critical since NK cell deficiencies are associated with severe, often fatal primary VZV infection. In the healthy host, VZV-specific immunity emerges in parallel with the appearance of skin lesions during primary VZV infection; both VZV antibodies and VZV-specific CD4 and CD8 T cells are induced [92]. However, cell-mediated immunity is necessary to resolve varicella, as shown by the risk of progressive infection in immunocompromised children who developed VZV IgG and IgM antibodies but failed to mount a VZV-specific T cell response [93]. Adaptive CD4 and CD8 T cells and IgG antibodies that recognize various VZV proteins persist but their peptide specificity is not well-characterized. VZV antibodies that bind envelope glycoproteins exhibit neutralizing activity. The extent of both antibody and T cell mediated memory immunity to VZV may be determined by the initial clonal expansion or by secondary stimulation from varicella exposures or subclinical reactivations, or by all of these mechanisms. Circulating VZV memory T cell frequencies are ~0.1–0.2% in immune adults [94].

While reported, symptomatic second episodes of varicella are rare, even among severely immunodeficient patients. A clinical history of varicella is often unreliable; individuals with apparent second cases had no evidence of prior infection when specimens obtained before the episode were available [95]. Protection from varicella illness appears to be induced regardless of the clade that caused the initial infection, which can be explained by the highly conserved VZV genome. An important caveat is that the incidence of subclinical VZV reinfection is not known although it has been proved, using molecular methods, that VZV reactivations in the same individual were caused by viruses of different VZV clades [96]. Protection from clinically apparent reinfection may be mediated by neutralizing antibodies present at respiratory sites of inoculation or by a rapid humoral and T cell response if replication is initiated. Adults in close contact with children who have varicella exhibit boosts in both VZV antibody and cell-mediated responses. Administering passive antibodies to VZV within 4 days after exposure of a naïve host can prevent varicella or modify its severity, as demonstrated by varicella zoster immune globulin prophylaxis in immunocompromised children and newborns. Experiments in the SCID mouse model show that infection may be blocked when antibody to the glycoprotein, gH, which has potent neutralizing activity, is given shortly after inoculation of skin xenografts [97].

In one of the most well-established immunologic correlates known, zoster in older adults and immunocompromised patients is associated with reduced T cell proliferation and production of IFN- γ and other cytokines by peripheral blood mononuclear cells stimulated with VZV antigen and with fewer circulating VZV-specific CD4 and CD8 T cells [92, 98]. In contrast, VZV IgG antibody titers are not related to the risk of reactivation; passive antibody administration did not alter zoster severity in clinical studies done before antiviral drugs were available. However, antibodies may contribute to modulating cell–cell spread of VZV in

skin and possibly in the affected ganglion. Symptomatic zoster is associated with a dramatic increase in the VZV-specific T response; IgG, IgM and IgA antibody titers are also boosted but the resolution of zoster, like varicella, requires cellular immunity. Of interest, hematopoietic cell transplant recipients may have subclinical reactivation, detected by the presence of VZV DNA in peripheral blood mononuclear cells and recover VZV-specific T cell responses without clinical zoster.

4.5 VZV Vaccines

VZV is the only human herpesvirus for which vaccines are licensed. The live attenuated varicella and zoster vaccines are made from the attenuated Oka virus [99, 100]. Inactivated Oka-derived vaccines have also been evaluated in immunocompromised and healthy patients [101].

Composition. The VZV Oka vaccine seed stock was derived from a clinical isolate, the parent Oka (pOka) virus, which was recovered from a varicella skin lesion; pOka was passaged in guinea pig and human fibroblasts at low temperature. VZV vaccines contain infectious VZ virions made in cells approved for manufacturing live viral vaccines; Oka-derived vaccines also contain viral and host cell proteins and DNA because VZV replication is very highly cell-associated. Oka vaccines are currently manufactured by Biken, Merck and GlaxoSmithKline. Not surprisingly, because of the extreme cell association of VZV, Oka vaccines made by all three manufacturers represent mixtures of VZV genomes. Multiple single nucleotide polymorphisms are identified; some are shared in the various vaccine preparations but others are not and wild type markers are also present [102–104]. The pediatric vaccines, Varivax (Merck) Varilrix (Glaxo) and Okavax (Biken) contain approximately 1,300 pfu of Oka vaccine virus.

Mechanism of attenuation. Attenuation of pOka was achieved empirically by tissue culture passage and verified clinically by the administration of Oka vaccine preparations to susceptible children in Japan [100]. The experience showing attenuation of the Biken Oka vaccine was confirmed in trials of varicella vaccines made from vaccine Oka seed stocks by Merck in the U.S. and by GlaxoSmithKline in Europe.

Investigations in the SCID mouse model demonstrate that vaccine Oka has reduced virulence in skin compared to pOka. In contrast, pOka and vaccine Oka do not differ in their infectivity for T cells and DRG xenografts in vivo [79, 86, 90, 105]. These experiments suggest that attenuation of vaccine Oka in skin is intrinsic, resulting from genetic changes accumulated during tissue culture passage in fibroblasts rather than simply because the vaccine is given by a subcutaneous route of inoculation. The evidence that this attenuation is tissue/cell type specific for skin but not T cells or DRG is consistent with the capacity of Oka vaccine to cause a varicella-like rash in immunocompromised patients and its potential to establish latency in the sensory ganglia of healthy vaccinees [106, 107]. Experiments with pOka/vOka chimeric viruses showed that attenuation in skin was conferred by

different segments of vaccine Oka in the chimera, suggesting that multiple VZV genes have relevant mutations [108]. Identifying mutations that might contribute to attenuation by full genome sequencing is challenging because as noted, varicella vaccines contain mixtures of variants that have various genetic differences [109]. Vaccine Oka mutations do not alter its susceptibility to inhibition by acyclovir and related antiviral drugs.

Measles–mumps–rubella–varicella (MMR-V) multivalent vaccines. Vaccine Oka is also used as a component of a multivalent vaccine containing live attenuated measles, mumps and rubella (MMR-V) [110, 111]. This formulation requires a higher titer of vaccine Oka than the single component vaccine. The Merck vaccine (ProQuad) contains not less than 3.99 log₁₀ pfu of vaccine Oka; the GlaxoSmith-Kline vaccine (PriorixTetra) contains not less than 3.3 log₁₀ pfu.

Higher potency vaccines for zoster. Higher potency live attenuated Oka vaccines have been developed and evaluated for their potential to increase VZV cellular immunity in healthy older adults in the U.S. [112]. Dose-finding studies were done using VZV-specific T cell proliferation and responder CD4 T cell frequencies as the endpoint before a large scale efficacy study was undertaken [98]. High potency vaccines boosted VZV T cell responses among 55–87-year-old subjects to ranges observed in younger adults, ages 35–40 years, who had naturally acquired VZV immunity. The infectious virus content of the high potency zoster vaccine manufactured by Merck Inc. is ~20,000 pfu, which is more than 14-fold more than Oka/Merck pediatric vaccines. This higher infectious virus content is presumed to be necessary because zoster vaccine recipients have pre-existing VZV immunity and because immunosenescence diminishes the antiviral T cell responses of older individuals.

Inactivated VZV vaccines. Heat inactivation can reduce the infectious virus content of varicella vaccine to undetectable levels. Heat inactivated vaccine was used to assess effects on T cell responses in healthy elderly individuals [113] and for immune reconstitution and zoster prevention in hematopoietic cell transplant patients [101].

Clinical experience with the efficacy and safety of varicella vaccines. The development of live attenuated VZV vaccines in the U.S. and Europe was first undertaken to protect children with leukemia from varicella [99]. The capacity of vaccine Oka to cause varicella-like illnesses has limited use in immunocompromised children. However, trials of live attenuated varicella vaccines in healthy children led to their introduction as a routine childhood vaccine in North America, Australia and some Europe and Asian countries [99, 114]. Pre-licensure evaluations demonstrated that these vaccines induced both humoral and cell-mediated immunity against VZV, with antibody titers and VZV T cell proliferation responses in the range of those observed after natural VZV infection in childhood. Immunogenicity, measured by serologic and cell-mediated responses, correlated with infectious virus and antigen content. Age was also a factor; a two dose regimen was required to achieve >90% seroconversion rates in those over 12 years old.

The efficacy of varicella vaccine was demonstrated in a small placebo controlled trial in the U.S., leading to licensure in 1995 [99]. Extensive post-licensure

surveillance has supported that the vaccine is effective and safe in healthy children and adults. The recommendation to vaccinate all children at 12–18 months of age and all susceptible older children and adults has had a major impact on varicella incidence, hospitalizations and deaths among the pediatric population and also among persons in older age groups, most of whom benefit indirectly [85, 115]. The annual incidence of varicella decreased by more than 80% when a coverage rate of ~90% was achieved in 2005; hospitalization rates for varicella complications decreased by 88% and age-adjusted mortality was reduced by 66%.

Although the initial recommendation was to give a single dose to children under 12, reports of breakthrough infection in vaccinated children remained relatively common. Efficacy analyses during outbreaks and in surveillance sites showed a single dose vaccine effectiveness rate of ~85%. Although breakthrough varicella cases were typically mild, these cases were a source of VZV transmission to other susceptibles and interfered with the public health objective of varicella control. Therefore, a two dose regimen for all age groups was implemented in the U.S. in 2007 [116]. Whether this pattern reflects waning immunity is debated [117, 118]. However, the single dose regimen is associated with lower seroconversion rates by the most sensitive assay for VZV IgG antibodies, suggesting that primary vaccine failure accounts for many cases of apparent breakthrough varicella in vaccine recipients [119].

Reports about varicella vaccine adverse events to the U.S. vaccine adverse event reporting system (VAERS) showed a rate of 2.6/100,000 doses during the first 10 years after licensure [120, 121]. Varicella vaccine can cause a mild, self-limiting rash in healthy recipients within the first 6 weeks [122, 123]. Some children with severe undiagnosed immunodeficiencies have developed progressive infection caused by Oka vaccine virus; however, treatment with acyclovir has been effective in most cases.

Zoster after vaccination. Zoster can occur in vaccinated individuals. Using sequence differences between Oka and most North American VZV isolates, it has been possible to demonstrate that these cases can be due to either vaccine or wild type VZV [109]. Zoster caused by wild type VZV has been reported in vaccine recipients with no history of breakthrough varicella, indicating that infection can be acquired subclinically, reach neurons and establish latency in sensory ganglia [106]. When evaluated in vaccinated immunocompromised children, zoster was significantly less common than zoster following natural infection. More recently, prospective studies in healthy children demonstrated that the incidence of zoster was 4–12 fold less in vaccinated children under 10 years of age compared to those with natural infection [124] and zoster was rare (27.4 cases/100,000 person years) in 170,000 vaccinated children [125]. Vaccine-related cases of zoster have been mild although cases of meningitis and meningoencephalitis have been reported [107].

Information about how commonly Oka vaccine virus establishes latency in sensory ganglia is limited and consists of VZV DNA sequence analysis of skin lesion specimens from vaccinated people with clinical zoster. However, recent evidence from a postmortem study suggests that vaccine Oka persists in multiple

ganglia for years after vaccination, as observed with wild type VZV and latency was established without vaccine-associated skin lesions [89]. Whether viral load is reduced compared to wild type VZV is not known. These observations are consistent with the capacity of vaccine Oka virus to cause viremia in immunocompromised children and with the evidence that its T cell tropism is intact in the SCID mouse model. The potential for super-infection with wild type VZV in vaccinated individuals along with the persistence of the vaccine virus in ganglia may also permit genetic recombination of wild type and vaccine viruses. Recombination of Oka and wild type VZV has been demonstrated by direct sequencing of VZV DNA from zoster lesions in a previously vaccinated individual.

MMR-V. MMR-V vaccine was licensed in the U.S. based on comparability of the VZV antibody titers against viral glycoproteins, as measured by ELISA. Although the mechanism is not known, MMR-V has been associated with an increased incidence of febrile seizures from 7 to 14 days after the first vaccine dose; rates were 4/10,000 for MMR and 9/10,000 for MMR-V [126]. These observations led to the recommendation to offer an option of giving MMR and varicella vaccines at different sites or to give MMR-V, along with counseling parents about the rare possibility of febrile seizures within 2 weeks after vaccination.

Live attenuated varicella vaccine in high risk patients. Varicella vaccine has been used in clinical practice to immunize children with HIV infection against severe varicella and zoster when their CD4 T cell counts were >15–25%; a recent report found an 82% effectiveness against varicella and 100% effectiveness against zoster in a review of carefully monitored children with HIV [107, 127]. Varicella vaccine has also been given to children with leukemia in remission and solid organ transplant recipients as a safer option than risking natural infection [98]. The rationale is that antiviral therapy can be given if varicella-like illness occurs.

Varicella vaccine issues for the future. Whether the two dose regimen introduced in 2007 will reduce the incidence of breakthrough varicella in childhood requires continued surveillance. Like many viral infections, varicella is more severe in adults. Therefore, as has been true for other childhood viral vaccines, it is important to maintain active surveillance programs to be sure that protection is sustained. Whether those with vaccine-induced immunity will need booster doses is difficult to predict; robust immune responses elicited by a two dose regimen in early childhood may prove to be as long-lasting as natural immunity. Whether intermittent re-exposure to varicella is necessary to maintain natural immunity is not known but interrupting varicella epidemics will obviously reduce such contacts. Based on the assumption that some boosting of memory immunity is required, whether by exogenous re-exposure or endogenous restimulation through subclinical reactivation, some models predict a higher incidence of zoster among those with natural infection, as a consequence of varicella vaccine programs. However, surveillance studies show no increase in zoster [100]. New information indicating that Oka vaccine latency occurs frequently may mean that vaccine-related zoster will be a concern as vaccine recipients become older. If so, as described below, zoster can also be prevented by vaccination. Oka vaccine latency may also result in recombination with wild type

VZV in vaccine recipients who are super-infected. However, it seems unlikely that reversion to wild type patterns of VZV virulence will occur.

Clinical experience with the efficacy and safety of zoster vaccine. The association of zoster-related morbidity in older adults and immunocompromised patients with declining memory T cell immunity along with experience confirming the efficacy and safety of live attenuated varicella vaccines set the stage for developing zoster vaccines [112]. In an early proof of concept study, immunization with a heat-inactivated Oka/Merck vaccine preparation was associated with a reduction in the incidence of zoster from 33 to 13% during the first year after autologous hematopoietic cell transplantation when the vaccine was given as one dose before and three doses after transplantation [101]. Comparing vaccine recipients with matched controls who were unvaccinated showed that VZV specific CD4 T cell responses were reconstituted much earlier among vaccinees, despite their severely immunocompromised state. No vaccine-related adverse effects were observed in recipients of this heat inactivated VZV vaccine. By showing a correlation between restoring VZV T cell immunity and reduced zoster incidence, this study provided direct evidence of the role of cell-mediated immunity in preventing the progression of VZV reactivation to symptomatic zoster.

After dose finding studies, a large placebo-controlled trial was done to evaluate high potency live attenuated Oka vaccine preparations, ranging from 18,700 to 60,000 pfu (median 24,600 pfu) for effects on zoster incidence and severity [128]. Enrolment targeted healthy adults who were >60 years old. Among the 38,546 participants, the median age was 69 in both the vaccine and placebo cohorts; 6.6% of vaccine and 6.9% of placebo recipients were ≥80 years old. Intensive surveillance for zoster was carried out for an average of 3 years; cases were determined by laboratory confirmation and each episode was assessed using pre-established criteria for zoster severity, post-herpetic neuralgia, and health quality of life. The primary endpoint of the study was a zoster burden-of-illness score, representing a composite index reflecting the incidence and severity of zoster. This score was significantly lower in the vaccine cohort compared to the placebo cohort ($P < 0.001$); the effect was independent of sex or age <70 vs. >70 years. Post-herpetic neuralgia (PHN) is the most common debilitating complication of zoster in older individuals. PHN rates were 0.46 cases per 1,000 person-years in the vaccine cohort and 1.38 cases in the placebo cohort ($P < 0.001$); the effect of vaccination on PHN rates was also independent of sex and age stratification. The study was designed with the incidence of zoster per 1,000 person-years as a secondary endpoint. The zoster incidence was 5.42 in the vaccine group and 11.12 per 1,000 person-years in the placebo group ($P < 0.001$). This difference represents a 51.3% efficacy of the high potency vaccine for zoster prevention among individuals >60 years. When the data was stratified by age cohorts, vaccine efficacy for zoster prevention was 63.9% among those <70 years old vs. 37.6% in those who were >70 years old ($P < 0.001$). Thus vaccine recipients in the older cohort were more likely to develop zoster despite immunization. Nevertheless, participants in the vaccine group who were >70 years old experienced less severe zoster than those >70 years in the placebo group. The impact of vaccination on zoster severity was

greater among those >70 years, who were at a higher risk for a more severe episode. Although the vaccine was less effective for zoster prevention in the older age cohort, the benefit of reduced zoster severity maintained vaccine efficacy, assessed from the burden of illness, at 55.4% in healthy adults >70 years old. Overall, the burden of illness score was reduced by 61.1% and PHN incidence was 66.5% lower in men and women >60 years old who were vaccinated.

Serious adverse events were uncommon and rates were equivalent in the vaccine and placebo cohorts. Even though the vaccine contained high concentrations of infectious vaccine Oka, all episodes of zoster were confirmed to be wild type VZV when lesion specimens were tested by PCR and sequencing.

Immunogenicity of zoster vaccine. Zoster vaccine given to healthy older individuals in dose finding studies boosted VZV T cell responses above baseline, with a half life of at least five years [112]. High potency Oka vaccine corrected deficiencies in CD4 T cells that produced IFN- γ or IL-2 and frequencies of CD4 and CD8 effector memory T cells that responded to VZV antigen [94]. Some participants in the efficacy study of zoster vaccine were also evaluated for effects on VZV cellular immunity as measured by responder cell frequencies and ELISPOT assay. Responses were higher within the first 6 weeks when vaccine and placebo recipients were compared and were higher in those who were less than 70 years old when vaccinated compared to those over 70 in the vaccine cohort. Despite a decline by 12 months, VZV T cell responses continued to be above baseline in the vaccine group for the 3 year follow-up period [94].

Zoster vaccine issues for the future. The experience with high potency Oka vaccine demonstrates that symptomatic VZV reactivation can be prevented or its consequences minimized in healthy older people by enhancing their VZV-specific T cell responses. Importantly, safety was maintained despite the high inoculum of vaccine virus. That this intervention diminishes the risk and consequences of VZV reactivation is relevant to the potential for vaccine control of other herpesviruses that also persist for the life of the host and although the effect is on reactivation rather than active replication of a chronic infection, the zoster vaccine can be viewed as a proof of principle that therapeutic vaccination is feasible. Among the unresolved questions are the optimal age for giving zoster vaccine and how long protection against zoster and post herpetic neuralgia will be maintained. More information about protection in the very old is also needed. Whether the vaccine virus reaches sensory ganglia when given to individuals with pre-existing VZV immunity is not known. Given the evidence that super-infection can occur with wild type VZV strains and in varicella vaccine recipients, it is possible that zoster vaccination could lead to recombination events as has been observed in a few instances after varicella vaccination. Whether inactivated Oka vaccine will reduce zoster morbidity in immunocompromised patients in a larger placebo-controlled trial is being evaluated.

Opportunities to improve live attenuated VZV vaccines. The VZV genome can be mutated readily using cosmid and bacterial artificial chromosome methods and the consequences of targeted mutations on VZV virulence in skin, T cells and DRG can be evaluated in the SCID mouse model of VZV pathogenesis. Insights gained

about the molecular mechanisms of VZV pathogenesis have relevance to designing “second generation” live attenuated VZV vaccines that have reduced potential to infect T cells and neurons and are safer for immunocompromised patients. Disrupting T cell tropism would be predicted to prevent vaccine virus delivery to neuronal cells, which would block the establishment of vaccine virus latency in sensory ganglia and eliminate the potential for vaccine virus recombination with wild type VZV as well as vaccine-related zoster.

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