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REVIEW

A review of licensed viral vaccines, some of their safety concerns, and the advances in the development of investigational viral vaccines

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Summary Viral vaccines could be considered among the most important medical achievements of the 20th century. They have prevented much suffering and saved many lives. Although some curative antiviral drugs exist, we desperately depend on efforts by academic, governmental and industrial scientists in the advancement of viral vaccines in the prevention and control of infectious diseases. In the next decade, we hope to see advancement in the development of current and investigational viral vaccines against childhood and adult infections. In this article, we will review the licensed viral vaccines, some of their safety concerns, and the advances in the development of investigational viral vaccines.

Introduction

An inadequate number of antiviral drugs are available for many viruses. Many available antiviral drugs do not provide cures for infections but merely alter the clinical course of disease. Therefore, the prevention of these infections is all the more crucial. The answer lies in the immunization and education of the public, especially those individuals at highest risk for each respective virus (Table 1). The significant impact of immunization against viral agents, such as smallpox, cannot be overstated. Other viral vaccines have led to notable decreases in infections and complications. Since the introduction of poliovirus vaccine, the poliovirus has been eradicated from the Western Hemisphere and is estimated for global eradication by the year 2005.[1] The measles vaccine is another example. After years of widespread measles vaccination, a record low 37 measles cases were reported in the United States (U.S.) in 2002.[2] Available viral vaccines also serve to provide a basic framework of knowledge and experience with which other viral vaccines can be developed.

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Table 1 Licensed and Investigational Viral Vaccine Characteristics as of 2003

Licensed vaccines

	Smallpox	MMR	VZV	Influenza
Antigenic form	Live-attenuated	Live-attenuated	Live-attenuated	Inactivated
Application	Subcutaneous (multiple-puncture)	Subcutaneous	Subcutaneous	Parenteral/intranasal
Schedule	One dose	First dose at 12–15 months, second dose at 4–6 years	12 months–12 years	Annually from September to November
Protection efficacy	≥95%	93–99%	≥90%	70–90%
Duration	3–5 years	≥30 years	-	1 year
Adverse reactions	Fever, anaphylaxis, cardiac related events[a], eczema vaccinatum, ocular vaccinia, vaccinia necrosum, postvaccinial encephalitis, CNS abnormalities[b], death	Anaphylaxis, fever, exanthems, encephalitis, parotitis, lymphadenopathy, arthralgia	Rash, MVLS, fever, local AEs	Fever, headache, arthralgia, myalgia, GBS, hypersensitivity
	HAV	HBV	Rabies virus	Poliovirus
Antigenic form	Inactivated	Recombinant	Inactivated	Live attenuated/inactivated (IPV)
Application	Parenteral	Parenteral	Parenteral	Oral/parenteral
Schedule	Two doses 6–12 months apart	Three doses at 0, 1 and 6 months	Three doses on days 0, 7 and 21 or 28 for preexposure	Four doses of IPV at 2, 4, 6–18 months and between 4 and 6 years
Protection efficacy	≥97%	50–99%	100%	≥95%
Duration	≥20 years	Lifelong	≥2 years	≥25 years
Adverse reactions	Headache, malaise fever, feeding problems	Fatigue, headache, pain, headache	Nausea, abdominal reaction, headache, dizziness, myalgia, systemic allergic reactions, neurologic complications	VAPP, hypersensitivity
	Yellow fever	JEV		
Antigenic form	Inactivated	Inactivated		
Application	Parenteral	Parenteral		
Schedule	Every 10 years with travel to endemic regions	Prior to travel to endemic regions; 3 doses on day 0, 7 and 30		
Protection efficacy	≥95–98%	78% after 2 doses, 99% after 3 doses	-	
Duration	≥30 years	-	-	
Adverse reactions	Fevers, headaches, myalgias, anaphylactic reactions	Fever, headaches, malaise, nausea, abdominal pain, dizziness, rash, myalgia, neurologic complications		

Investigational vaccines

	Rotavirus	HIV	HSV	HPV	CMV
Antigenic form	Live-attenuated	Recombinant	Inactivated	Inactivated	Live-attenuated
Application	Oral	Parenteral	Parenteral	Parenteral	Parenteral
Schedule	Discontinued; 3 doses at 2, 4 and 6 months of age	Every 3 months for 3 years (gp 160 subunit vaccine)	-	3 doses at day 0, month 2 and month 6	Three doses
Protection efficacy	49–57%	-	73% in women serologically negative for both HSV-1 and HSV-2 from acquiring HSV-2	100%	-
Duration	-	-	-	-	-
Adverse reactions	Intussception, fever, irritability, decreased appetite and activity	Local AEs, nausea, malaise, myalgia, arthralgia, headache, fever	-	Local AES	

	RSV	Parainfluenza virus	Adenovirus
Antigenic form	Live-attenuated	Live-attenuated	Live
Application	Intranasal/parenteral	Intranasal	Oral
Schedule	1 dose	1 dose	1 Dose
Protection efficacy	-	-	-
Duration	-	-	-
Adverse reactions	Nasal congestion, local AES	-	-

Abbreviations: MMR, measles, mumps, rubella; VZV, varicella zoster virus; MVLS, modified varicella like syndrome; GBS, Guillain–Barré syndrome; HAV, hepatitis A virus; HBV, hepatitis B virus; VAPP, vaccine-associated paralytic poliomyelitis; HIV, human immunodeficiency virus; HSV, herpes simplex virus; HPV, human papillomavirus; CMV, cytomegaolvirus; RSV, respiratory syncytial virus.

[a] Cardiac related events include myocarditis, pericarditis, myocardial infarction and angina.

[b] CNS abnormalities include postvaccinial encephalopathy and encephalomyelitis.

New vaccines are needed and the progress with finding new vaccines cannot be rapid enough. The incidence of many viral infections is increasing despite available antiviral agents. With threats of bioterrorism and many viruses as potential agents, the development of viral vaccines will be extremely important in the protection of certain members of the population such as public health and healthcare response teams, laboratory workers working with viruses and military personnel. The risks of vaccination must also be considered with the administration of a vaccine. Safety concerns exist for some of the viral vaccines and these vaccines should be given with consideration to host factors such as age, health status (immunocompetent vs. immunocompromised or other illnesses), viral exposures, pregnant women, and allergies. Prospective vaccines generally take decades before they are developed and are marked by small increments of advancement before the final product becomes available. In this article, we will provide a review of licensed viral vaccines, some of their safety concerns, and the recent advances in the development of investigational viral vaccines by the year introduced into the U.S.

Smallpox

Edward Jenner was the first to demonstrate that inoculation of cowpox virus into human skin could lead to protection from subsequent smallpox infection.[3] The inoculation substance was named *vaccine*, based on the Latin word *vacca*, meaning cow. Vaccines used for smallpox vaccination are derived from vaccinia virus, a species similar to cowpox. The virus that causes smallpox is variola virus. This virus belongs to the *Poxviridae* family and *Orthopoxvirus* genera which include the vaccinia, cowpox and monkeypox viruses. The smallpox vaccine consists of several strains of the live attenuated vaccinia virus and has served as the prototype of a successful viral vaccine. Prior to immunization, smallpox infection killed hundreds of millions of people. The eradication of this disease has been considered one of the greatest accomplishments in medicine. Because of recent concerns that smallpox may be used for potential biological warfare, the threat of this virus has not been completely eliminated. Renewed interest has developed in the production of smallpox vaccines.

Two smallpox vaccines will be available in the future.[4] Both are administered by direct inoculation into the superficial layers of the skin. The virus is able to grow and induce an immunological response, which serves to protect the host against smallpox. Dryvax (Wyeth Laboratories Inc., Marietta, Pennsylvania) is licensed for immunization of smallpox public health and healthcare response teams and laboratory workers who are involved with research activities involving the vaccinia virus. An emergency vaccination strategy has been developed in the event of a smallpox outbreak to fulfill the recommendations of the national Advisory Committee on Immunization Practice (ACIP).[5,6] Smallpox vaccination priority will be given to those with early diagnosis of cases, all who had been in contact with the patient since onset of fever, all household members of the contacts, healthcare workers, public health personnel, first responders and other personnel who will assist with outbreak control measures and emergency response activities.[5,6] Dryvax, a stored lyophilized calf-lymph vaccine, is freeze dried and reconstituted before use with a diluent that contains 50% glycerin and 0.25% phenol. When reconstituted, the lyophilized undiluted vaccine contains $\sim$100 million living vaccinia virus/mL. In the absence of circulating smallpox, this vaccine is contraindicated in individuals with allergies to polymyxin B sulfate, streptomycin sulfate, chlortetracycline hydrochloride and neomycin sulfate. Those individuals, who have allergic symptoms to the above compounds and have contact with individuals with smallpox or the presence of smallpox, should be concurrently given antihistamine or glucocorticoids. The smallpox vaccine is also contraindicated in persons: with a history or presence of eczema or atopic dermatitis; who have other acute, chronic, or exfoliative skin conditions; who have conditions associated with immunosuppression such as persons infected with human immunodeficiency virus (HIV); using topical ocular steroid medications; are $<$18 year of age; pregnant or intend to become pregnant during the next 4 weeks; or breastfeeding.[7] Eczema vaccinatum (Fig. 1), a serious form of disseminated vaccinia infection, can occur among persons with atopic dermatitis and other dermatologic conditions. Persons reporting atopic dermatitis or other dermatologic conditions in themselves or household members should not be vaccinated, unless a healthcare provider determines that the rash is not eczema or atopic dermatitis.[7]

The second smallpox vaccine (Acambis/Baxter Laboatories) is a tissue culture cell vaccine which involves the use of two cell lines for the propagation of vaccinia virus, the Vero monkey kidney cell line and the human fibroblast cell line MRC5. The tissue culture cell vaccine is being developed in hopes of supplanting the calf-lymph vaccine if a more extensive vaccination program is needed.[8]

Both vaccines are able to elicit humoral and cell-mediated immunity. Greater than 95% of individuals

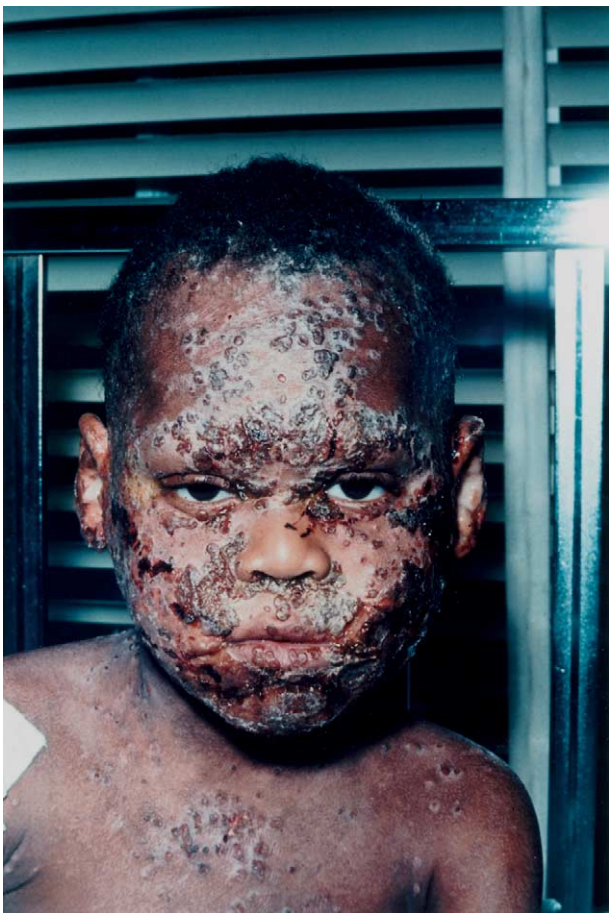

Figure 1 Eczema vaccinatum. (Reprinted from Mucocutaneous Manifestations of Viral Diseases, 2002, Figs. 3-13, page 47 by courtesy of Marcel Dekker, Inc.).

develop a successful vaccination, defined as an antibody titer of 1:10 or greater, within 1-2 weeks of immunization.[9] Although there is controversy about the duration of immunity to smallpox vaccination, two studies have shown that vaccine protection duration is 3-5 years and residual immunity may last 30 years or greater in persons who have undergone revaccination with smallpox.[6,10,11] In more than 90% of volunteers vaccinated against smallpox 25-75 years ago, a substantial humoral or cellular immunity (or both) against vaccinia persisted, whereas antiviral T-cell responses declined slowly, with a half-life of 8-15 years.[11] Individuals undergoing postexposure vaccination should receive the smallpox vaccination within 3 days of exposure.[12] Postvaccination may prevent the natural history of the disease, alter the severity of the disease, and provide protection from mortality. Some epidemiological evidence suggests that postexposure vaccination can be done up to 4-7 days.[9]

A third smallpox vaccine is under development. The modified *Vaccinia* Ankara (MVA) is derived from the Ankara *Vaccinia* strain. It is one of the most highly attenuated strains. With more than 570 passages in chicken embryo fibroblasts, it is host restricted and unable to replicate in human and other mammalian cells.[13] The MVA was developed to serve as an attenuated smallpox vaccine for primary vaccinations in persons residing in regions where smallpox was not endemic. The advantage of the MVA lies in its safety profile as no adverse reactions have been observed in clinical trials in persons at high risk with skin lesions.[14] The vaccine has been safely used to vaccinate $>$120 000 persons in Turkey and Germany; however, its effectiveness against smallpox is unknown.

Smallpox vaccination is generally safe and effective for prevention of smallpox.[15] Based on a series of studies conducted in the 1960s, estimates of the frequency for the adverse events associated with smallpox vaccination among adults 20 years of age and older were reanalyzed and included generalized rashes (250 per one million primary vaccinees), eczema vaccinatum (30 per one million primary vaccinees), vaccinia necrosum ($>$10 per one million primary vaccinees), postvaccinial encephalitis (four per one million primary vaccinees), and death (five per one million primary vaccinees).[15] Ocular vaccinia (Fig. 2) may also occur secondary to autoinoculation. Myocarditis has been increasingly reported with administration of the New York City Board of Health (NYCBOH) smallpox vaccinia strain among the U.S. military, occurring in 7.8 per 100,000 primary vaccinees which was 3.6 times that of unvaccinated personnel.[16] This data is in contrast to older Finnish data which suggested that myocarditis occurred once in every 10,000

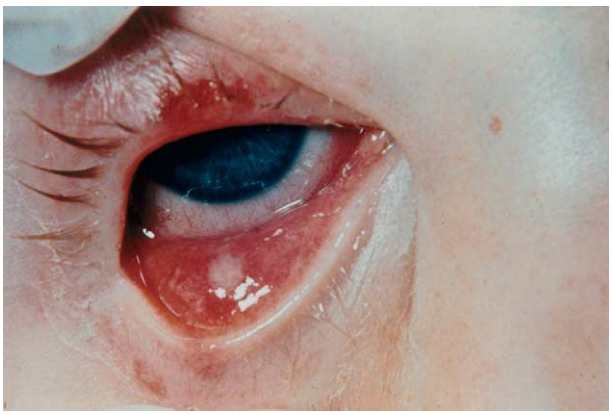

Figure 2 Pustule on the lower eyelid secondary to autoinoculation of vaccinia. (Photograph courtesy of Roberto Arenas, MD, Mexico City, Mexico) (Reprinted from Mucocutaneous Manifestations of Viral Diseases, 2002, Figs. 3-11, page 47 by courtesy of Marcel Dekker, Inc.).

military recruits vaccinated with a non-NYCBOH vaccinia strain.[17]

The European Union has set up a rapid alert system for biological–chemical attack, but only a few European nations have smallpox vaccine stockpiles, and developing countries have made almost no preparations.[18] The United Kingdom decided to use the Lister Elstree strain for the smallpox vaccine by PowderJect (Oxford, UK), which is buying the vaccine from Bavarian Nordic (Copenhagen, Denmark and Munich, Germany).[19] This is the seed virus most commonly used to produce vaccine during the worldwide smallpox eradication campaign. Compared to the MVA, the gold standard of recombinant vaccinia viruses, the Lister Elstree strain compared favorably in terms of safety and immunogenicity.[20]

The Dutch Ministry of Health set April 2003 as the contingency deadline for mass smallpox vaccination.[21] The country's disease control centre felt that ring vaccination was the best option before mass vaccination was implemented. Ring vaccination entails isolating a confirmed or suspected case of smallpox by vaccinating people in a specific area and anyone who has come within 2 m of an infected person. If there were many overlapping rings or if ring vaccination failed, then mass vaccination could follow. The Dutch Institute of Public Health and the Environment has already manufactured 20 million smallpox vaccines.[21]

Licensed vaccines

Influenza

The inactivated influenza vaccine consists of three virus strains (generally two type A and one type B). This vaccine was federal drug administration (FDA) approved in 1945, and is prepared each year based on the viruses most likely to circulate in our region of the world. The vaccine virus is grown in embryonated hen eggs and is then purified and inactivated. Trivalent inactivated vaccines are currently available as subvirion (split), purified surface antigen (subunit), and whole virus preparations. Whole virus influenza vaccines should not be given to children $\leq$12 years of age, due to the increased potential for febrile reactions.[22]

More than 80% of children and young adults who receive influenza vaccination develop high levels of antibody titers.[23] Persons with chronic disease or the elderly may develop lower immune responses and remain susceptible to infection. However, vaccination in these individuals has been shown to decrease the risk of complications, hospitalization and death.[24,25] In children and young adults, the influenza vaccine has been 70–90% effective in preventing influenza during controlled trials with a good match between the vaccine and circulating influenza strains.[26,27]

A study of vaccination in low-risk elderly persons demonstrated a 58% efficacy in preventing laboratory-confirmed influenza.[28] When studied in elderly nursing home residents, the influenza vaccine is 30–40% effective in preventing influenza illness, but is also 50–60% effective in preventing pneumonia or hospitalization and 80% effective in preventing death.[29,30] Immunity following influenza vaccination begins within 1–2 weeks and rarely persists beyond 1 year.[31] Protective antibody levels may only last 4 months or less in certain elderly patients. In addition, the strains of influenza may differ significantly from one season to the next, thus increasing the need for annual vaccinations.

Influenza immunization is indicated for individuals $\geq$6 months of age and who are at increased risk for complications of influenza or are in contact with those individuals (i.e., caregivers, medical personnel). With the early 2003–4 flu season in the United States and increased reports of morbidity associated with influenza in the paediatric population, the ACIP has updated their recommendations to include a focus on children including those 6–23 months of age because young, otherwise healthy children are at increased risk for influenza-related hospitalization, and studies suggest that the use of the influenza vaccine among children is cost saving.[32] It is likely that ACIP will recommend universal immunization in children in the near future.

Other at-risk populations include persons $\geq$65 years of age, residents of nursing homes, those with chronic pulmonary or cardiovascular disorders, and persons with HIV. Vaccination is also indicated for any individual who desires to decrease their risk for influenza infection. The immunization regimen consists of one dose given each year, from September through mid-November. Administration of the vaccine is still recommended after mid-November if influenza activity has not peaked in the community. Previously unvaccinated children $<$9 years of age should receive two vaccine doses at least 1 month apart to develop sufficient antibody levels.[22]

Other than local reactions, adverse effects of influenza vaccination may include fever, malaise, headache, arthralgia and myalgia. These symptoms typically begin within 6–12 h and persist for 1–2 days. In one clinical trial, the incidence of adverse effects did not differ between the vaccinated group

and placebo.[33] Guillain–Barré syndrome (GBS) has been associated with influenza vaccination. A significantly greater frequency of GBS was found with the 1976 swine influenza vaccine,[34] but more recent investigations show an extremely small risk of GBS with the current vaccines, which is slightly more than one extra case per million vaccinees.[22] Immediate allergic reactions, with hives, angioedema, or systemic anaphylaxis, rarely occur after influenza vaccination.[35] These hypersensitivity reactions are most likely due to residual egg protein exposure to sensitive patients. The majority of egg-allergic subjects can safely receive immunization, but those with a history of anaphylactic reaction to eggs or previous influenza vaccines should discuss their history of such allergies with their physician before a decision is made regarding vaccination.[22]

A promising new intranasal vaccine has been approved by the FDA as an alternative form of influenza vaccination. The cold-adapted, live attenuated, trivalent influenza virus vaccine (FluMist) is able to replicate in the cooler nasal passages and stimulate mucosal as well as systemic immunity, similar to natural infection. However, the altered virus is unable to grow in the warmer temperatures of the lower respiratory tract. Placebo, controlled clinical studies in children 15–71 months of age have shown the vaccine to be 93% effective in preventing culture-positive influenza A and B infections.[36] Also, the vaccinated group had 21% fewer febrile illnesses and 30% fewer cases of febrile otitis media when compared with placebo. Adverse reactions were mild and included rhinorrhoea, fever, and lethargy. A similar study in 4561 adults demonstrated 23% fewer days of severe febrile illness and 25% fever days of febrile upper respiratory tract illness resulting in 28% fewer missed work days and 41% fewer physician visits.[37] This needle-free vaccine is conveniently administered and is recommended to prevent influenza in healthy people 5–49 years of age. The disadvantage of Flumist is the cost. It is at least $60 per dose (and not covered by Medicare or most insurance plans), compared to the inactivated influenza vaccine at the usual cost of $10–20 per dose (which is covered by Medicare Part B and Medicaid and most insurance plans).[38] Healthy individuals aged 9–49 years are administered a single dose annually before the winter, and children aged 5–8 years, are recommended to have two doses the first year they are immunized with cold-adapted, trivalent influenza vaccine to ensure protection against all strains contained in the vaccine.[39] Thereafter, a single annual revaccination for the child is sufficient.

In Switzerland, a licensed inactivated virosomal-subunit influenza vaccine (Nasalflu, Berna Biotech) containing *Escherichia coli* heat-labile toxin as a mucosal adjuvant existed. During four winter seasons (1996–1999), no serious adverse events were reported in the prelicensure trials conducted among 1218 volunteers.[40,41] In October 2000, it was introduced to the Swiss market as the first licensed intranasal influenza vaccine in the world. However, 7 months after its release, the Swiss Drug Monitoring Center and various University of Zurich institutions received 46 case reports of Bell's palsy among recipients of the vaccine. Berna Biotech suspended distribution of the vaccine, and a clinical investigation into the association was started. From October 1, 2000 to April 30, 2001, a total of 773 patients with Bell's palsy were identified in Switzerland. Of the 412 who could be evaluated, 250 (60.7%) were enrolled and matched with 722 control patients. In this case–control study, 68 patients with Bell's palsy (27.2%) and eight controls (1.1%) had received the intranasal vaccine ($P < 0.001$).[42] Compared to parenteral vaccines, this intranasal vaccine was associated with a significantly increased risk of Bell's palsy (adjusted odds ratio, 84.0; 95% confidence interval, 20.1–351.9), 19 times the risk in the controls, which corresponds to 13 excess cases per 10 000 vaccines. It was found that the period of highest risk was 31–60 days after vaccination. The vaccine has since been taken off the market. It should be mentioned that Bell's palsy has not been reported with FluMist, another intranasal influenza vaccine.

In Europe, a new MF59-adjuvanted influenza subunit vaccine named Fluad has been shown to be very immunogenic.[43–45] However, this vaccine has caused significantly higher rates of transient and mild local reactions compared with conventional subunit vaccines.[43,45] An inactivated, split virion influenza vaccine named Vaxigrip given to children, adults, and the elderly has been shown to be safe and immunogenic.[46] As recommended by the WHO for the 1998–1999 influenza season, both vaccines contained the three strains: A/Beijing/262/95 (H1N1), A/Sydney/5/97 (H3N2), and B/Beijing/184/93. A randomized controlled trial compared the two vaccines in a total of 2150 subjects.[47] The subjects receiving Fluad experienced more local reactions compared to those in the Vaxigrip group. Both vaccines were immunogenic for the three strains of influenza. In subjects 75 years of age or older, Fluad was more immunogenic than Vaxigrip for all three virus strains. This conclusion is consistent with a meta-analysis of 20 trials in elderly subjects that showed a greater immune response for the adjuvanted vaccine compared with non-adjuvanted subunit and split vaccines.[48]

Yellow fever

Yellow fever is considered the original viral haemorrhagic fever. Although most individuals experience only mild illness, approximately 15% of infected persons develop serious disease, with hepatorenal dysfunction, myocardial injury, and haemorrhage.[49] Twenty percent to 80% of serious infections with yellow fever result in death.

The incidence of yellow fever has been increasing dramatically in the past two decades.[50] Between 1985 and 1996, 23 543 cases and 6421 deaths were reported to the WHO, although many more cases are believed to go unreported.[49] Yellow fever is found in tropical South America and sub-Saharan Africa.[51] Two clinically identical forms of yellow fever exist—urban and jungle. The urban form is transmitted from human to human by the *Aedes aegypti* mosquitoes. The jungle form is transmitted among non-human primates by various mosquitoes, and humans are incidentally infected.

The live attenuated yellow fever vaccine, FDA approved in 1953, is produced by growing the 17D virus strain in chick embryos. Seroconversion rates with the vaccine are 95–98% in both adults and children.[50,52] Immunity has been documented for at least 30–35 years and is thought to be lifelong.[53] Regardless, a certificate of yellow fever immunization for international travel to certain countries is only valid for 10 years, requiring revaccination thereafter. Immunization for yellow fever is indicated for anyone $\geq$9 months of age living or traveling in endemic areas (tropical South America or sub-Saharan Africa).[51] Vaccination may also be required for entry into particular countries, and current information is available from health departments.

The yellow fever vaccine is known to be extremely safe with few side effects. Two percent to 5% of vaccine recipients may develop low-grade fevers, mild headaches, myalgia, or other mild symptoms, generally lasting 5–10 days. Immediate hypersensitivity reactions have been reported in less than one per one million vaccine doses.[51] The affected vaccinee typically develops rash, urticaria, and/or asthma symptoms, and usually has a history of egg allergy. Of greater than 200 million vaccine doses worldwide, only 22 cases of encephalitis with the yellow fever vaccine have been reported.[54] The majority of these cases occurred in children under 4 months of age, prompting the recommended delay of vaccination until 9 months of age. Concerns about the yellow fever vaccine have been reported in persons with advanced aged. In 1998, the Centers for Disease Control and Prevention (CDC) was notified of severe illnesses and one death in elderly U.S. residents temporally associated with yellow fever vaccination.[55] The rate of reported adverse events among elderly vaccinees has been found to be higher than vaccinees 25–44 years of age. As for now, recommendations for elderly travelers should include balancing the risks for severe illness and death due to yellow fever infection against the risk for systemic illness due to yellow fever vaccine.[55] Yellow fever immunization should be withheld from pregnant women and immunosuppressed individuals. In the U.S., asymptomatic infection with HIV is not considered a contraindication.[51]

Poliovirus

Since the introduction and widespread use of two polio vaccines, the number of poliovirus infections and complications has dramatically decreased. In 1994, the Western Hemisphere was certified to be free of indigenous wild poliovirus.[56] The last case of indigenously acquired wild poliovirus infection in the U.S. occurred in 1979.[57] Since that time, an average of 8–9 cases of paralytic polio have been reported each year in the U.S. due to the use of the oral, live attenuated polio vaccine (OPV).[58] This vaccine-associated paralytic poliomyelitis (VAPP) occurs in one case per 2.4 million doses, but is more common after the first vaccine dose (one case per 750,000 first OPV doses).[58] VAPP is believed to occur because of a mutation, or reversion, of the vaccine virus to a more neurotropic form. These mutated viruses are called revertants and are believed to occur in almost all vaccine recipients, but it only rarely results in paralytic disease. The VAPP that results is identical to that caused by wild virus, and may be permanent.[58] In 2002, the World Health Organization (WHO) certified Europe 'free of poliomyelitis'.[59] The last European case of indigenous wild poliomyelitis occurred in eastern Turkey in 1998, when a 2-year-old unvaccinated boy was paralyzed by the virus. However, poliovirus imported from polio-endemic countries continues to be a risk.

The WHO developed a strategy for global eradication of poliomyelitis by the end of the year 2000, which unfortunately was not met. Significant progress has been achieved toward that goal, with a 90% reduction of poliomyelitis cases between 1988 and 1996.[60] In 1988, poliovirus was found on every continent other than Australia. However, by 1998, only three major foci of disease remained, including the regions of South Asia, West Africa, and Central Africa.[61]

The inactivated poliovirus vaccine (IPV) was

developed by Jonas Salk in the early 1950s and was introduced for use in the U.S. in 1955. Although this vaccine was shown to be safe and efficacious, its use quickly declined after introduction of the OPV in the early 1960s. An enhanced version of the inactivated vaccine was developed in 1978[62] and later licensed in the U.S. in 1987. This more potent formulation results in improved immunity in children and adults.[63] In children given the three dose regimen, 99–100% develop antibody responses to all three types of poliovirus 2 months after receiving the second dose.[64] Significant increases in antibody concentrations are observed after administration of the third dose. In separate clinical studies, 99–100% of subjects developed protective antibodies after three doses.[65,66] Although the use of IPV results in less gastrointestinal immunity than OPV,[67] various combinations of the two poliovirus have been shown to provide optimal gastrointestinal immunity.[65,68] The duration of immunity induced by IPV is unknown, but is thought to be long-term. A study in Sweden using four doses of less potent IPV indicated that over 90% of vaccine recipients had persistent antibodies after 25 years.[69]

The OPV was first licensed in the U.S. in 1963. OPV consists of live attenuated strains of the three serotypes of poliovirus, all grown in monkey kidney cell culture. In the 1960s, OPV quickly became the favored vaccine because of its ease of oral administration, consistent production of gastrointestinal immunity, expected long-lasting immunity, and spread of the vaccine virus to unvaccinated contacts.[70] After three doses of OPV, over 95% of recipients produce immunity to all three serotypes of poliovirus.[64] This immunity is considered to be long lasting, and likely lifelong. Because of fecal shedding of the vaccine virus after OPV administration, this vaccine can immunize unvaccinated contacts.[71] However, viral shedding of mutated virus may also lead to VAPP in unvaccinated contacts, particularly the immunosuppressed. The risk of VAPP is almost 7000 times higher for persons with certain types of immunodeficiencies, particularly B lymphocyte disorders which reduce the synthesis of immune globulins with these conditions.[58]

Between 1980 and 1994, 125 cases of VAPP were reported in the U.S. Forty-nine (39.2%) of these cases occurred in healthy vaccine recipients. Forty cases developed in healthy contacts of the vaccine recipient. Twenty-three cases occurred in immunodeficient vaccinees, and seven cases developed in immunodeficient contacts of vaccine recipients. The remaining six cases developed in community contacts.[58] VAPP occurs more frequently in adults, immunodeficient persons, and those receiving their first dose of OPV.[67] Because of the diminished risk for wild poliovirus disease in the U.S., the risk for VAPP is now considered to be less acceptable.[58] It is now recommended that to eliminate the risk for VAPP, an all-IPV schedule be used for routine childhood vaccination in the United States. All children should receive four doses of IPV: at age 2 months, age 4 months, between ages 6 and 18 months, and between ages 4 and 6 years. OPV, if available, may be used only for the following special circumstances: (1) mass vaccination campaigns to control outbreaks of paralytic polio; (2) unvaccinated children who will be traveling within 4 weeks to areas where polio is endemic or epidemic; and (3) children of parents who do not accept the recommended number of vaccine injections; these children may receive OPV only for the third or fourth dose or both. In this situation, healthcare providers should administer OPV only after discussing the risk of VAPP with parents or caregivers. As a result, OPV supplies are expected to be very limited in the United States after inventories are depleted.[72]

No serious adverse effects have been reported with IPV. This vaccine contains trace amounts of polymyxin B, neomycin, and streptomycin, and may cause hypersensitivity reactions in persons allergic to these substances. OPV has no serious adverse effects other than VAPP. Evidence indicates that neither OPV nor IPV increases the risk for Guillain–Barré syndrome (GBS).[58]

Measles, mumps and rubella

A combination measles, mumps, rubella (MMR) live virus vaccine against measles, mumps, and rubella was introduced in the1960s, and annual reported cases of these infections have declined by more than 98%.[73] This decline is largely attributable to the recommendation that all states implement a two dose MMR vaccination as a requirement for children to enter school. The CDC suggests vaccination with the first MMR dose at 12–15 months and the second dose at 4–6 years of age.[74] Immunization produces a mild subclinical infection that is non-communicable. These live attenuated viruses are not recommended for pregnant women or women considering conception within the next 3 months. Immunization is contraindicated in those with immunosuppression. Individuals with asymptomatic HIV and persons with mild immunosuppression may still be considered for vaccination with MMR. Ultimately, the patient's healthcare provider will determine the degree of immunosuppression based on the patient's severity of condition, laboratory assessment and treatment, and whether

or not the patient is able to receive the MMR. Another contraindication to the MMR includes individuals with a history of anaphylactic hypersensitivity to neomycin. Healthy individuals with minor illnesses with or without fever and in persons with an allergy to eggs should not be excluded from receiving the vaccine. In persons with a history of allergy to eggs, the risk for severe anaphylactic reactions is exceedingly low.[74] These patients should be observed for 90 min after immunization for possible adverse events.[75]

Other safety issues about the MMR have been questioned, especially a possible link between the use of this vaccine and autism. In 1998, a study by Wakefield and colleagues found that eight of 12 children with chronic enterocolitis had onset of regressive developmental disorders, mostly autism with the administration of the MMR vaccination.[76] This study, however, had a number of limitations: too few cases were provided for any generalization, no healthy control children were provided for comparison, no identification of time period during which cases were identified, and inability to reproduce these results by others. In response to concerns about the MMR vaccine, in 2001, the CDC and the National Institutes of Health asked the National Academy of Sciences—Institute of Medicine (IOM) to establish an independent expert committee to review hypotheses about existing and emerging immunization safety concerns. The conclusion of the Committee was that the vast majority of cases of autism could not be caused by the MMR vaccine. Since this report, a number of epidemiologic studies have shown the lack of association between the administration of the MMR vaccine and autism.[77–80] The Committee concluded that there was no need to review the existing recommendations for universal use of MMR at 12–15 months of age and 4–6 years of age. The Committee's conclusion upholds the current policy of giving the MMR vaccine as one instead of three separate injections.

In Europe, there are three major MMR vaccines: Vac triple MSD (Aventis Pasteur MSD), which contains Enders Edmonston hyper-attenuated (measles), Jeryl-Lynn (mumps), and Wistar RA 27/3 (rubella); Triviraten (Berna), which contains Schwarz, RIT4385, which is derived from the Jeryl Lynn, and Wistar RA 27/3; and Priorix (Glaxo SmithKline), which contains Edmonston-Zagreb, Rubini (mumps), and Wistar RA 27/3. The Jeryl Lynn strain used in Vac triple MSD is utilized in most industrialized countries. The Triviraten vaccine has been widely utilized in various European nations, and all these countries report sporadic outbreaks of mumps, which has been attributed to the failure of the Rubini strain to confer protection.[81–83]

Measles

Measles has a very efficient transmission rate.[84] Early epidemiological studies estimated that a case of measles could cause 75% of susceptible family contacts to develop the disease.[85] The first measles vaccine was licensed in 1963. With the development of universal childhood immunization in the U.S., the incidence of measles has decreased by greater than 99%. Measles is no longer considered an indigenous disease in this country.[86] In 2002, a record low of 37 cases of measles were confirmed.[87] Even with these encouraging results, lack of compliance with routine MMR vaccination in the U.S. remains problematic, and greater than one million children die of measles each year in developing countries.[88]

The measles vaccine is a further attenuated version of the previous Enders-Edmonston virus strain. Fewer adverse reactions occur with this further attenuated version. The measles vaccine is produced by culturing the Moraten virus strain in chick embryo cells. The measles vaccination produces both a humoral and cellular immune response.[89] After receiving a two dose MMR vaccine, 95–99% of recipients develop serologic evidence of immunity to measles.[90,91] Immunity is believed to be life-long, and similar to an acquired infection with the wild-type virus.[92] Although extremely rare, measles infection has been reported in patients with previously documented postimmunization seroconversion.[93,94] From 1985 to 1990, epidemic of measles cases were found to be occurring among unvaccinated children and much less frequently in vaccinated children, mostly children under 5 years of age.[93,94] Vaccinated children who received only one dose were not always protected from measles, thus, leading to the recommendation of a second dose for children between the ages of 5 and 19 years of age to ensure protection for those children who did not develop immunity to the first dose. Since 1990, sporadic outbreaks of measles have occurred in populations that refuse vaccination or only received one dose of the measles vaccination, including communities in Utah and Nevada, Christian Scientist schools in Missouri and Illinois, and adult and student communities.[93,94]

Adverse effects after measles vaccination include fever (5–15%),[74] transient viral exanthems,[73] and less commonly encephalitis or encephalopathy (less than one per one million vaccines).[95] A small number of reports have described the occurrence of subacute sclerosing pancencephalitis (SSPE) in persons with a history of

vaccination but no known history of infection.[96-98] A more careful review of those individuals suggest that some cases had unrecognized natural measles infection prior to vaccination, and the SSPE was directly related to the infection.[74] It is generally thought that with the widespread use of the MMR vaccine, SSPE has been eliminated in the U.S., and the live measles vaccine does not increase the risk for this complication.[74]

Mumps

The live attenuated mumps vaccine was first introduced in 1967. From 1989 to 2001, a decrease from 5712 cases of mumps to 231 were reported, the lowest annual total ever reported.[99-102] As more children, adolescents, and adults receive two doses of the MMR vaccine, the annual incidence of mumps has steadily decreased by 99%. In Europe, large serological surveys for mumps for six countries (Denmark, England and Wales, France, Germany, Italy, and the Netherlands), conducted in the mid-1990s, showed low incidence of disease where mumps vaccine coverage was high (e.g., Netherlands) and a high incidence of disease in countries where vaccine coverage was poor (e.g., Italy).[103]

All children and adults born in 1957 or later who do not have a medical contraindication should receive the MMR vaccine unless they have documentation of immunization or serological evidence of immunity to measles, mumps, and rubella. Children should receive the mumps vaccine, as part of the MMR, when they are at least 12 months of age. However, the second dose of MMR is not generally considered a booster dose because a primary immune response to the first dose provides long-term protection against developing mumps. Persons born before 1957 are generally considered to be immune to mumps.

The mumps vaccine, the Jeryl-Lynn strain, is prepared in chick embryo cell culture. Clinical efficacy studies have shown that 97% of children and 93% of adults develop serological evidence of immunity after vaccination.[104-106] Lower protection rates, ranging from 75 to 95%, have been reported with outbreak studies.[107-110] Serologic and epidemiological evidence suggests that immunity persists for at least 30 years after immunization.[99-102]

Adverse reactions to the mumps vaccine include low-grade fever, mild parotitis, and viral exanthemas. Adverse neurological effects are extremely rare and have not been causally associated with the mumps vaccine.[111]

Rubella

In unvaccinated individuals, infection with rubella can have significant morbidity and mortality. The largest annual total cases of rubella and congenital rubella syndrome (cataracts, congenital heart disease, loss of hearing, hepatosplenomegaly, jaundice, microcephaly, developmental delay) occurred in the U.S. in 1969, when 57,686 cases were reported (58 cases per 100,000 population). Following vaccine licensure in 1969, rubella incidence fell rapidly, with fewer than 1000 cases per year reported (<0.5 cases per 100 000 population) in 1989. In 2002, a record low annual total of 18 cases of rubella were reported. Similar to the mumps vaccine, at least one dose of rubella vaccine, as combination MMR vaccine, is routinely recommended for all children and adults born in 1957 or later.

Two different live attenuated rubella vaccine strains, HPV-77 and Cendehill, were initially developed and licensed in the U.S. in 1969. In 1979, these two vaccine strains were replaced by the RA 27/3 (rubella abortus 27, explant 3) vaccine which is grown in human diploid fibroblast cell culture. Nasal antibodies are higher, and serum antibody titers are more persistent with the RA 27/3 vaccine.[112,113] This vaccine induces an antibody response in more than 97% of recipients.[106,114] Immunity is thought to be lifelong and has been shown to be persistent for at least 16 years.[115,116] RA 27/3 is also associated with fewer adverse events compared to the previous rubella vaccines.

Adverse effects after rubella vaccination include fever, lymphadenopathy, and viral exanthemas, typically between 5 and 12 days after vaccination.[111,117] Arthralgias and arthritis occur more commonly in adult vaccines, especially women (up to 40%).[118-120] Joint symptoms are less common with children (0.5%).[118] In both adults and children, joint symptoms typically begin within the first 3 weeks after vaccination and remit within 11 days.[121] Although any joint may be affected, the knees and the fingers are most frequently involved.[117]

Rabies virus

In 1885, Louis Pasteur developed the first vaccine for postexposure treatment of rabies.[122] This and several other rabies vaccines that followed, contained brain or nerve tissue, posed a serious risk of neurological complications. In addition, some of these vaccines led to pathogenic infections because of incomplete inactivation of the vaccine virus. Safer duck embryo vaccines were later introduced, but proved to be less immunogenic. After years of

development and studies, cell culture-derived vaccines have become the gold standard for rabies immunization. The human diploid cell vaccine (HDCV), licensed in the U.S. in 1980, contains concentrated and purified inactivated rabies virus from the Pitman-Moore strain. Compared with previous rabies vaccines, the HDCV induced higher levels of antibody response at an earlier time. Multiple studies have shown HDCV to be effective for both preexposure and postexposure immunization.[123–125]

In several clinical studies of the rabies vaccine regimens for pre and postexposure prophylaxis, all subjects develop antibody responses within 2–4 weeks.[126–128] The antibody response typically develops within 7–10 days and lasts for at least 2 years.[129] Preexposure prophylaxis is intended for those at high risk of contracting rabies (bites by carnivorous wild animals or bats, bites by dogs or cats that develop symptoms during 10 days of observation or are rabid, suspected rabid or unknown (i.e., escaped) or any bite from an unprovoked attack), and is given in three doses on days 0, 7 and 21 or 28. CDC recommends that postexposure prophylaxis be given to those who are exposed to suspected or confirmed rabid animals.[129] This regimen is given in conjunction with rabies immune globulin and consists of five vaccinations given on days 0, 3, 7, 14 and 28. Previously immunized individuals who have been exposed to rabid animals require only vaccination given in two doses 3 days apart.

Boosters are recommended for persons who have frequent exposure to rabies virus (i.e., persons working in a research laboratory or vaccine production of rabies virus) are at the highest risk for inapparent occupational exposures.[129] These persons should have a serum sample submitted for antibody testing every 6 months, and administered a booster to maintain a complete neutralization at a 1.5 serum dilution by rapid fluorescent focus inhibition test (RFFIT).[129] Other individuals who are at frequent risk (i.e., veterinarians and staff, animal control, wild-life officers, and spelunkers) should have their serum sampled every 2 years and administered a booster to maintain a complete neutralization at a 1:5 serum dilution by RFFIT.[129] Those with low frequency exposures do not require routine preexposure boosters after the completion of primary preexposure vaccination.[129]

Adverse reactions are less common with the HDCV when compared with previously available rabies vaccines. Twenty percent of recipients report mild systemic effects, such as nausea, abdominal pain, headache, dizziness and muscle aches.[129] Systemic allergic reactions, such as hives and anaphylactic shock, have rarely been reported.[130] Neurologic complications, including three cases of GBS, have rarely been reported.[129] Approximately 6% of individuals who receive a booster dose of HDCV develop an immune complex-like reaction in the 2–21 days that follow.[131,132] These cases are characterized by generalized urticaria and may include angioedema, nausea, vomiting, fever, malaise, arthralgia or arthritis. These reactions have been associated with the presence of betapropiolactone altered human albumin contained in the HDCV.[133,134]

Because of the high cost associated with the HDCV, the development of other cell culture vaccines have been pursued. The purified chick embryo cell culture vaccine (PCECV) has been licensed in the U.S. for both prophylactic and postexposure immunization. This vaccine is produced by the growth of fixed rabies virus strain Flury LEP in chicken embryo fibroblast culture. Clinical studies have shown it to be as effective and well-tolerated as HDCV, with antibody responses in over 99% of recipients.[135,136] Compared with HDCV, no type III hypersensitivity reactions have been observed with PCECV,[137] but serious anaphylactic reactions or neuroparalytic events have been reported. Rabies vaccine absorbed (RVA) is another available rabies vaccine, which is produced by growth of the Kissling strain of Challenge Virus Standard rabies virus in fetal rhesus lung diploid cell culture. All three types of the inactivated rabies vaccine are considered comparable in safety and efficacy.[129]

Hepatitis B virus

Prior to the development of the hepatitis B vaccine, the annual incidence of individuals infected with HBV in the U.S. was estimated to be 200,000–300,000.[138] In 1981, a plasma-derived hepatitis B vaccine was licensed in the U.S. This vaccine was highly effective in inducing immunity, but a few limitations existed. Large scale production was not feasible because of the limited supply of a suitable carrier plasma. Despite the chemical treatment of plasma products for safety, there was also some concern about the risk, albeit small, of HIV transmission.[139] In 1986, both of these issues were addressed with the licensure of the yeast recombinant hepatitis B vaccine.

This vaccine was the first ever licensed recombinant viral vaccine prototype as well as the first effective viral vaccine for a sexually transmitted disease. This vaccine is produced by recombinant DNA technology which inserts the gene for the hepatitis B surface antigen into the yeast

Saccharomyces cerevisiae (baker's yeast). In high-risk homosexual men, clinical studies have demonstrated a three-dose vaccine efficacy of 82–93% in preventing acute hepatitis B.[140,141] Approximately 95% of immunocompetent adults develop significant antibody titers after a three dose hepatitis B vaccination. Nearly 99% of children respond to vaccination.[142] The level and duration of protection decrease with advancing age, such that only 50–70% of those over age 60 acquire immunity.[143,144] Other factors associated with a lower likelihood of seroconversion include immunosuppression, renal failure, prematurity with low birth weight, age older than 40 years, obesity, and smoking.[145–148] In these specific individuals, annual antibody testing should be assessed, and a booster dose administered for those persons with anti-HBs levels <10 mIU/mL.

Alternate delivery systems (adenovirus and vaccinia virus), adjuvants,[148,149] and several different types of vaccines (DNA vaccines[150] and PreS vaccines[151–153]) are under evaluation in hopes of finding ways to increase the immunogenicity of the hepatitis B vaccine. This is especially important in the populations with lower rates of seroconversion. Few studies are available on the duration of immunity afforded by the hepatitis B vaccine. The available studies suggest that long-term efficacy is expected.[147] In the first year after vaccination, antibody levels decline rapidly. Thereafter, the antibody levels decline at a slower pace.[154] The loss of detectable antibodies to hepatitis B years after vaccination does not necessarily indicate a lack of immunity. The majority of individuals are protected by immunological memory in B lymphocytes, which mount an anamnestic response to natural infection.[155] There are, however, case reports of individuals developing hepatitis B infection after being previously vaccinated.[156,157] These individuals generally have subclinical disease. None have developed chronic infection or serious complications.[147]

The hepatitis B vaccine includes three doses, given at months 0, 1 and 6. This vaccination is recommended for: persons living in or traveling to areas of high endemicity of hepatitis B; healthcare personnel; morticians; persons engaging in high risk sexual activity; persons with chronic liver disease due to causes other than hepatitis B; prisoners; users of illicit injectable drugs; police and fire department personnel who render first aid; and all children aged 0–18 years. Because of the widespread use in children, a thimerosal-free vaccine was recently approved by the FDA. Thimerosal is a mercury-containing preservative, which has prompted the limitation of its use in children.[158]

For postexposure prophylaxis, unvaccinated persons who have had exposure to persons with acute hepatitis B (e.g., sexual contact, following needlestick or splash accident, birth of a neonate from a HBsAg-postive women) should be administered the hepatitis B vaccine. In both the unvaccinated and vaccinated exposed person, the hepatitis B immunoglobulin is given as soon as possible and the hepatitis B antigen and antibody level checked within a month of exposure. Postexposure prophylaxis leads to increased survival and decreased serologic recurrence.[159,160]

The adverse effects of the hepatitis B vaccination are generally mild and well-tolerated. The most common effects include fatigue (15%), headache (9%) and fever (1–9%).[161,162] A postmarketing clinical surveillance of 4.5 million doses of hepatitis B vaccine over 5 years revealed no serious or severe reactions attributable to the vaccine.[163] Large-scale hepatitis B vaccination programs have been unable to establish any association between the vaccine and severe adverse effects other than rare episodes of anaphylaxis.[162,164] There are rare reports of individuals developing thrombocytopenic purpura,[165–167] vasculitis,[168,169] rheumatoid arthritis,[170] lichen planus,[171] and a lichenoid reaction.[172] However, these conditions do not occur at a higher rate than in the unvaccinated population. Reports of a causal relationship between the hepatitis B vaccine and a variety of autoimmune diseases have been disproven. This vaccine does not increase the risk of multiple sclerosis,[173] nor does it cause a relapse of preexisting multiple sclerosis.[174]

Twinrix, a new combination vaccine, protects people at least 18 years of age against hepatitis A and hepatitis B virus. This vaccine was FDA licensed on May 11, 2001. It combines two already approved vaccines, Havrix and Engerix-B, so that persons at high risk for exposure to both viruses can be immunized against both at the same time. The advantage of this vaccine is the reduced number of injections from 5 to 3. This vaccine is administered at months 0, 1 and 6. Preliminary data from 11 clinical trials indicate that 99.9% of vaccinees develop seroconversion against hepatitis A virus and 98.5% against hepatitis B surface antigen, with persistence up to 4 years (GlaxoSmithKline Biologicals, unpublished data, 2001). Adverse effects of this vaccine are similar in type and frequency to the monovalent hepatitis A and hepatitis B vaccines.

AmBirix is a combined hepatitis A and B vaccine that contains a purified, inactivated strain of hepatitis A virus and a recombinant, yeast-derived hepatitis B surface antigen used in Europe.[175] In an open-label study, it was shown to be safe and immunogenic when administered at 0 and 6 months

in children ages 1–11 years.[176] When compared to the 3-dose Twinrix, AmBirix elicited similar reactogenicity and immunogenicity profiles in healthy adolescents.[177] The reduction in the number of doses from the current three dose schedule makes vaccination against hepatitis A and B more convenient to the patient, reduces healthcare staff time, and may lower the overall vaccination costs. An open-label, randomized study showed that AmBirix given at either 0 and 6 months vs. 0 and 12 months resulted in similar reactogenicity and immunogenicity profiles in 12–15 year old healthy adolescents.[178]

Japanese encephalitis

Japanese encephalitis is an endemic arboviral infection transmitted by various *Culex* mosquitoes in parts of Asia. Although the majority of infections are subclinical, this infection causes an average of 35,000 reported cases and 10,000 deaths each year.[179] One out of 250 infections leads to symptomatic disease.[180] The resulting encephalitis is typically severe, with a 25–40% case fatality rate.[179,180] Residual neurologic sequelae are evident in 10–30% of cases.[52]

An inactivated Japanese encephalitis virus vaccine was developed several decades ago, and was licensed in the U.S. in 1992 for persons $\geq$1 year of age.[181,182] Since the risk to short-term tourists and business travelers is very low ($<$0.1/100,000), the vaccine is recommended for travelers to Asia who will be spending a month or longer in endemic areas during the transmission season of the virus (which varies according to geographic region).[183] From 1978 to 1999, only 24 cases of Japanese encephalitis worldwide have been reported. Of these cases, many were travelers or military personnel who resided in Asia for $>$1 month.

Ten percent of vaccines develop systemic side effects, including fever, headache, malaise, chills, dizziness, rash, myalgia, abdominal pain, and nausea/vomiting. Adverse neurologic events, such as encephalitis or peripheral neuropathy, occur in one to 2.3 cases per one million vaccinations.[184] Therefore, a 10 day period following vaccination is recommended before traveling.

Hepatitis A virus

Crowded living areas and poor sanitation are reasons for developing hepatitis A, especially in the developing world. Epidemiolgoic studies show that in non-immune persons, the incidence of hepatitis A is three cases per 1000 individuals that reside in endemic areas and is as high as 20 per 1000 individuals facing unfavorable hygienic situations (e.g., backpackers, aid workers in remote areas, and missionaries). The WHO and most experts agree that travelers visiting endemic areas should be vaccinated, and these regions include Africa, Asia except Japan and Singapore, the Caribbean, Mexican border, and remote parts of Eastern Europe.[185–188] The hepatitis A vaccine, licensed in 1995, has also been considered to be included in routine childhood immunizations, but due to most children experiencing asymptomatic or mild infection, this recommendation of universal immunization of children with hepatitis A is not currently implemented. Two inactivated hepatitis A vaccines, Havrix and Vaqta, are licensed and available in the U.S. Both vaccines are propagated in human diploid fibroblast culture and inactivated by formalin. These vaccines are administered in two doses given at 6–12 months apart in adults and children 2 years and older. Both of these inactivated vaccines show excellent as well as comparable immunogenicity and efficacy rates. Over 97% of recipients develop protective levels of antibodies within 1 month after the first dose, and over 99% of recipients are protected 1 month after the second dose.[185–190] In placebo-controlled clinical trials in Thailand (which has high rates of hepatitis A), two doses of the inactivated hepatitis A vaccine were 94% effective in protecting against hepatitis A infection.[191] A similar study showed 100% efficacy in children in New York after a single dose of vaccine.[192]

Limited long-term data and duration of immunity has yet to be determined for this vaccine. In a study using a three dose series in adults, detectable antibodies were found in all subjects 4 years after immunization.[193] Kinetic models of antibody concentration decline have estimated that protective levels of hepatitis A antibodies can be expected to persist for 20 years,[194] and likely up to 30 years.[195] Other mathematical evaluations of long-term immunity after a primary and booster dose for hepatitis A have calculated that protective antibody levels should persist for 24–47 years.[196] Some authorities believe that immunity will persist beyond the loss of detectable antibody levels,[196] as occurs with hepatitis B immunization. This view, however, has not been validated.

Epaxal is an aluminium-free, virosome-formulated hepatitis A vaccine in Europe. Virosomes are safe, efficient, and easily prepared carrier systems for small virions such as hepatitis A virus.[197] The surface of the virosomes contains the hemagglutinin antigen from the influenza virus, which augments the immune response to the inactivated hepatitis A virus.[197] Given at months 0 and 12,

Epaxal has been shown to be safe, well tolerated and highly immunogenic in adults,[198,199] children and infants greater than 6 months of age.[200] Epaxal may be a better option than Havrix for infants, as studies have shown that infants administered Havrix had either no response or late response to the vaccine.[201,202] Patients who received the second dose of the vaccine 18–54 months after the first were shown to have no loss of immunogenicity.[203] In adults, protection against hepatitis A virus after two doses of Epaxal has been estimated to be at least 20 years.[204]

Recommendations for the use of the hepatitis A vaccine include: persons at least 2 years of age living in or traveling to areas of high endemicity for hepatitis A; persons with chronic liver diseases due to causes other than hepatitis A; persons engaging in high risk sexual activity; residents of a community experiencing an outbreak of hepatitis A; users of illicit injectable drugs; and routine paediatric use in some states and regions. Limited data exists for the use of this vaccine for postexposure prophylaxis.

Immunoglobulins offer immediate protection in approximately 85% of recipients. Immunoglobulins may diminish the antibody responses to live vaccines and, therefore, live vaccines should be administered 14 days or more before or greater than 6 weeks after immunoglobulin administration. Immunoglobulins may also be considered in travelers to high risk areas within 4 weeks after the initial dose of the hepatitis A vaccine since protection may not be complete until 4 weeks after the administration of the hepatitis A vaccine.

No serious side effects have been attributed to the hepatitis A vaccine in clinical trials.[193] Mild adverse effects can occur. These effects include soreness at the injection site for both adults and children. In adults, headache (14%) and malaise (7%) occur most commonly, and in children this vaccine has been associated with feeding problems (8%) and headache (4%).

Varicella zoster virus

Prior to the widespread use of varicella vaccine, annual U.S. figures for varicella infection included approximately 4 million cases, 11,000 hospitalizations, and 100 deaths.[205] The annual incidence of varicella today has decreased markedly since the introduction of this vaccine. The varicella vaccine, developed by Takahashi in 1974 and approved in 1995, is a live-attenuated Oka strain vaccine. This vaccine has been shown to be very safe and effective.[206–208] All susceptible children who are at least 12 months of age through 18 years should receive the varicella vaccine. The varicella vaccine is recommended as part of the routine childhood immunization schedule at 12–18 months of age. The ACIP recommends varicella vaccine for all susceptible children who are at least 12 months old to prevent disease due to and transmission of varicella. Healthcare workers with unknown immunization status with the varicella zoster vaccine, should be screened by serologic tests, and immunized if they are found to be seronegative to varicella zoster virus (VZV). As for the geriatric population, a few studies have shown restoration of VZV cell-mediated immunity on administration of live-attenuated varicella vaccine which lasts up to 4 years after vaccination.[209–213] Various studies report serum anti-VZV antibody concentrations, and production of interferon-gamma to be increased following vaccination.[214] From these studies, it is likely that enhancement of cell-mediated immune response in elderly individuals through vaccination with live-attenuated varicella vaccine will protect this population from herpes zoster and attenuate its complications.[212] Large, placebo, controlled-clinical trials are underway to examine the potential efficacy of this vaccine in the geriatric population.

The varicella zoster vaccine was developed in Japan by the attenuation of virus isolated from the vesicular fluid of a healthy boy (with the surname Oka) with natural varicella infection.[215] Clinical studies with the live attenuated Oka strain vaccine have showed a 90% seroconversion rate 4 weeks after vaccination with few adverse reactions.[216] Long-term follow-up studies have shown protection against chickenpox for at least 17–19 years. All of these subjects continue to have persistent antibodies and delayed-type skin reactions to the varicella-zoster antigen.[217] In a double-blind, placebo-controlled study of the Oka vaccine in 914 U.S. children, the varicella vaccine was shown to have an efficacy of 100% at 9 months.[218] At a 7-year follow-up, 95% of the subjects remained free of clinical disease with chickenpox.[219] Other studies have shown that the Oka vaccine induces humoral and cell-mediated immunity in healthy children[220–222] with protection for at least 8 years, while other studies suggest effectiveness decreases significantly after 1 year post vaccination.[223] Delayed-type hypersensitivity skin reactions to varicella-zoster virus antigens have been shown to occur for at least 10 years after vaccination.[224] Case series studies demonstrate less severe varicella (i.e., afebrile, $<$50 lesions, and shorter duration of illness) in vaccinated persons than those unvaccinated.[225,226]

In children, the Oka vaccine should be given as a

single dose at 12 months to 12 years of age. Individuals over the age of 13 should receive two doses, 4–8 weeks apart. The duration of protection is unknown at this time, and the need for a booster immunization is uncertain. It has been observed that vaccinees, who are exposed to natural varicella have a boost in antibody levels. However, it is postulated that in a highly vaccinated population, a lack of exposure to natural varicella may result in waning immunity.

In adolescents and adults, two doses 4–8 weeks apart are necessary to produce seroconversion rates and antibody responses similar to those obtained in healthy children.[206–208] The varicella-zoster vaccine is recommended for susceptible adults, particularly those in high-risk situations (i.e., healthcare personnel); children who have no history of chickenpox and are required to attend school; and immunosuppressed individuals, particularly those with acute lymphocytic leukaemia (ALL).[227–229] The VZV vaccination can be safely administered to ALL patients if the patient's lymphocyte counts are $>700/mm^3$. Two doses separated by 3 months are given to these individuals since their immune response does not provide a protective seroconversion rate with only one dose.

If a vaccine rash develops in the vaccinee, varicella transmission can occur at about one-fourth the rate of natural varicella (20–25% vs. 87%).[230] The incidence is between 18 and 77 per 1,000,000 person years of follow-up in children.[231] Herpes zoster can later develop either from this vaccine-type virus or from natural wild-type VZV.[232,233] The incidence is less than that seen in children with prior chickenpox,[226] such that vaccinated children may have a decreased risk for herpes zoster.

A modified varicella-like syndrome (MVLS) may occur in some vaccinated children after exposure to the natural wild-type varicella virus.[219,234,235] The average rate of MVLS varies from 0 to 2.72% of vaccinated children each year after vaccination with the U.S. licensed Oka strain vaccine. These children typically develop a milder form of disease. Most children do not have associated fever, and only 50% of them develop vesicular lesions (typically <50 lesions).[236] Children with MVLS have not been shown to have associated systemic or serious disease. These children who develop MVLS often develop a more complete and longer-lasting antibody response to varicella vaccination.[234]

Adverse effects of the Oka vaccine are generally well tolerated. The most common side effects in both children and adults include mild tenderness, erythema, or induration at the injection site (19.3–24.4%); fever (10.2–14.7%); and a localized or generalized varicella-like rash (3.8–5.5%). The transmission of the varicella vaccine virus from a healthy vaccinee is low, but may be more likely if a rash develops after vaccination, especially among those who are immunocompromised. Individuals receiving vaccination should avoid close association with susceptible high-risk individuals for up to 6 weeks. This vaccination is contraindicated in pregnancy or any woman planning to become pregnant within 3 months, since this is a live attenuated virus and natural varicella is known to cause fetal harm. Recent data indicates that the varicella vaccine effectiveness is $>95\%$ for preventing disease and 100% for preventing moderate or severe disease in susceptible contacts when given within 36 h of exposure.[237]

Investigational vaccines

All of the vaccines available up to the end of the 20th century have been used solely to prevent disease. Some FDA approved viral vaccines have been removed from the market because of safety issues such as the rotavirus vaccine. Also, new candidate vaccines have been developed and are being evaluated for the treatment of already acquired viral infections. Table 1 lists the predominant diseases for which candidate vaccines are presently under investigation.

Adenovirus

Adenovirus is a common cause of significant respiratory illness in military groups, and immunization is thus indicated in the U.S. military population. Adenovirus is also a cause of pneumonia in hospitalized children as well as gastroenteritis in infants and children, although immunization is not recommended for this population. Vaccines, approved in 1980, have been available as live, oral, enteric-coated tablets, available in two different strains—type 4 and type 7 adenovirus vaccines. Several studies of vaccine recipients demonstrated a significant decrease, a 94–100% reduction, in acute respiratory disease due to adenovirus.[238] Unfortunately, production of these vaccines was discontinued in 1996, and the Department of Defense is currently searching for an alternate source of the product.[239]

Rotavirus

Rotavirus causes more than 125 million cases of diarrhoea annually in children less than 5 years of

age, with approximately 600,000 deaths, worldwide.[240,241] In the U.S., rotavirus is responsible for approximately 50,000 hospitalizations and 20–40 deaths each year. In 1998, the rhesus-human reassortant tetravalent (RRV-TV) rotavirus vaccine (Rotashield) was licensed for use in the U.S. This oral vaccine consists of live attenuated rhesus rotavirus serotype 3 and human-rhesus reassortants, which express serotypes 1, 2 and 4. In clinical trials, three doses of rotavirus vaccine resulted in 49–57% efficacy against disease.[240,242,243] The vaccine also prevented dehydration in 100% of recipients and reduced physician visits by 73%.[240,241] Twenty percent of infants develop fever after rotavirus vaccination, generally 3–5 days after the first dose. Older infants had a higher incidence of febrile reactions, which restricted the use of this vaccine to the first 6 months of life. Irritability and decreased appetite and activity have been reported as adverse effects in some trials.

The rotavirus vaccine was FDA-approved for administration of three doses at 2, 4 and 6 months of age. Soon after public availability of the vaccine, several cases of intussusception in vaccinees were reported, with approximately 220–300 cases per 100,000 infant-years compared with 45–50 cases per 100,000 infant-years in unvaccinated infants.[244] According to the Vaccine Adverse Event Reporting System, the majority of infants developed this complication after the first vaccine dose and developed symptoms within 1 week of immunization. Because of concerns over the possible association between the rhesus-based rotavirus vaccine and intussusception, the CDC recommended postponement of rotavirus vaccination until further studies are complete, and this vaccine is no longer available.[244]

Another human-animal reassortant vaccine is undergoing clinical trials.[241] It is based on a bovine rotavirus parent strain (WC-3), and has thus far proved to be safe and effective. A different live rotavirus vaccine has also shown promising efficacy rates in phase II clinical trials. The human rotavirus vaccine 89–12 is a live, attenuated G1 strain derived from a fecal specimen from a rotavirus-infected child in Cincinnati, Ohio.[245] This vaccine is 89% effective in preventing disease in infants after only two doses.[246] Serologic evidence of immunity is demonstrated in 94% of recipients. Mild fever has been the only adverse reaction experienced to date.

Herpes simplex virus

Infection with herpes simplex virus can be cosmetically disfiguring with substantial morbidity and mortality. A herpes simplex virus (HSV1 and HSV 2) vaccine has been sought for the past eight decades. Initial studies in 1920 unsuccessfully used untreated vesicular fluid from herpes lesions to attempt to induce immunity.[52] In the 1930s, inactivated whole virus vaccines made from HSV-infected animal tissues were developed.[247] Many inactivated whole virus vaccines have been developed over the past years, but none of the candidate vaccines have proved to be sufficiently immunogenic.

Several other approaches for HSV vaccines are currently under development and evaluation. Two recombinant subunit vaccines have been investigated in phase III trials. A vaccine developed by Chiron contains HSV-2 surface glycoproteins gB and gD and the adjuvant MF59. This vaccine did not provide protective efficacy for preventive or therapeutic use and development of this vaccine was subsequently halted.[248,249] The other recombinant vaccine developed by SmithKline Beecham contains glycoprotein gD and the adjuvant monophosphoryl lipid A immunostimulant (MPL).[250] In clinical trials, this vaccine had a clinical efficacy of 73% in protecting women who are serologically negative for both HSV-1 and HSV-2 from acquiring HSV-2 disease.[251] Two multicenter, double-blind, randomized, controlled studies of an HSV-2 glycoprotein-D-subunit vaccine formulated with a new adjuvant (AS04) containing aluminum hydroxide (alum) and MPL to prevent genital herpes have shown some efficacy in subgroup analysis.[251] The first study was a phase 3, double-blind, randomized efficacy trial involving 847 randomized subjects who were seronegative for both HSV-1 and HSV-2. The second study was a phase 3, double-blind, randomized trial designed to evaluate the safety of the vaccine in subjects of any HSV serologic status. This study randomized 2491 patients, of which 1867 of them were seronegative for HSV-2, and 710 of them HSV-2 negative women.

In Study 1, the efficacy of the vaccine was 38% (95% confidence interval, −18–68%; 15 cases occurred in the vaccine group and 24 in the control group), and in Study 2, the efficacy in female subjects was 42% (95% confidence interval, −31–74%; nine cases occurred in the vaccine group and 16 in the control group).[251] However, in women who were seronegative for both HSV-1 and HSV-2, the efficacy in Study 1 was 73% (95% confidence interval, 19–91%; $P = 0.01$), and the efficacy in Study 2 was 74% (95% confidence interval, 9–93%; $P = 0.02$). The vaccine was not shown to be effective in women who were seropositive for HSV-1 or in men.

This efficacy difference between women and men may be explained by biologic and immunological

factors. The acquisition of HSV in women is likely to occur through the vaginal-cervical mucous membrane, which has no stratum corneum. Secretions that constantly wash this membrane contain antibodies and white blood cells. Vaccination could provide an immunologic barrier to HSV acquisition at this mucosal site that is not applicable to men.[251] There is evidence that with some autoimmune disorders, infections, and vaccinations, female subjects (both human and animal), have enhanced immune responses by type 1 helper T (Th1) cells as compared with male subjects.[252,253] Th1-type responses, especially interferon-secretion, have been shown to be important for the control of HSV infection.[254]

The HSV-2 glycoprotein-D-subunit vaccine is being further studied in a phase III, double-blinded, randomized controlled trial sponsored by GlaxoSmithKline and the National Institutes of Health. The enrollment goal is 7550 women who are seronegative for HSV-1 and HSV-2, and they will receive the investigational herpes vaccine or the investigational formulation and schedule of Havrix given in a dosing schedule of 0, 1 and 6 months. The primary endpoint is to evaluate vaccine efficacy in the prevention of genital herpes caused by either HSV-1 or HSV-2 between months 2 (post second dose) and 20 in healthy women who are initially seronegative for HSV-1 and HSV-2. As of March 2004, over 1000 women have been enrolled, but no cases of genital herpes have been reported.

Another approach combines the safety profile of a killed vaccine with the immunogenic potential of a live virus vaccine.[255] The disabled infectious single cycle (DISC) vaccine lacks the glycoprotein H (gH) gene necessary for virus entry into cells. Thus, the herpes simplex virus is unable to spread to surrounding cells after a single replication cycle and essentially remains non-infectious. In animal studies, this approach has provided encouraging results for both preventive and therapeutic treatment.[255,256] However, this candidate vaccine failed in phase II trials. Clinical trials for this vaccine are planned to evaluate the efficacy in preventing infection in seronegative partners of discordant couples.[52]

DNA vaccines are also being studied for potential HSV immunization. These vaccines are only able to express 1 or 2 viral antigens at a time. In the absence of adjuvants, a strong cell-mediated immunity can be induced. In animal studies, inoculations of plasmid DNA carrying the desired viral genes have shown promising results for the prevention of infection.[257,258] A DNA vaccine encoding for the glycoprotein D2 (gD2) is currently in phase I clinical trials, and several others are in preclinical development.

Live attenuated HSV vaccines have also been attempted. Viruses that are the safest and most attenuated tend to lack immunogenicity, therefore, causing difficulty in developing this vaccine. Past research has shown that stable attenuation of HSV is not achieved after passage in cell culture. The vaccine strain has the potential to revert to its virulent state and cause disease after immunization. A genetically engineered HSV mutant vaccine has been found to be safe and effective in animal studies,[259] but in humans, the strain is overly attenuated and lacks sufficient immunogenicity.[260]

Human papillomavirus

More than 30 subtypes of human papillomavirus (HPV) are known to be sexually transmittable. Certain subtypes are associated with malignancy (16, 18, 31, 33, 45, 52, 58) and other subtypes are associated with condylomata (6 and 11). The few subtypes allow for more focused strategies for immunization.

In the past, vaccine development had been hampered due to the inability to culture HPV. An in vitro culture system for HPV has been developed, furthering the prospect for advancements in this field.[261] Virus-like particles (VLPs) are designed to self-assemble into conformations that resemble natural HPV. VLPs have been designed for all of the major HPV subtypes. Clinical trials are underway for HPV-11 L1 VLP,[262] HPV-6 L1 VLP,[263] and HPV-16 L1 VLP.[264] In a double-blind, placebo controlled study, individuals who were HPV-16 negative and received the HPV-16 vaccine had a reduced incidence of both HPV-16 infection and related cervical intraepithelial neoplasia at a median follow-up of 17 months.[265]

Fusion protein vaccines are under evaluation for the immunotherapy of cervical cancer and genital warts. A live recombinant vaccinia virus, TA-HPV, has been engineered to express the E6 and E7 protein genes for HPV 16 and 18 as a treatment for cervical cancer.[52] The vaccinia virus serves as the viral vector for this vaccine. Phase I and II clinical trials of TA-HPV[266] have provided encouraging results, and further studies are underway. A recombinant fusion protein vaccine, TA-GW, consisting of HPV-6 L2 and E7 proteins, is under investigation for the treatment of genital warts. A phase II clinical trial has shown the vaccine to be immunogenic, with encouraging clinical responses.[267] A third fusion protein vaccine, TA-CIN, is in preclinical development for the treatment of cervical dysplasia.[52]

Although the T-cell repertoires in mice and humans differ, peptide-based vaccines have been shown to be protective against HPV-induced tumors in mice. Early-stage human clinical trials are underway. One clinical trial involves HLA-A*0201 binding to HPV16-E7 peptides.[268] Other investigational approaches to HPV immunization include DNA vaccines,[268] bacterial vectors,[269,270] and dendritic cells pulsed with HPV epitopes.[261]

Respiratory syncytial virus

Respiratory syncytial virus (RSV) is the most common cause of severe lower respiratory tract infection in infants and young children, resulting in approximately 90,000 hospitalizations and 4500 deaths in the U.S. each year. Early attempts for vaccine development were thwarted when clinical trials of a formalin-inactivated vaccine in the 1960s led to severe and unexpected illness upon subsequent natural RSV infection.[271,272] Since that time, multiple vaccine approaches have been evaluated.

There are many concerns about the use of inactivated RSV vaccines. The first RSV vaccine tested in the mid-1960s was an intramuscular, formalin-inactivated and aluminum-precipitated vaccine.[271,272] In children less than 2 years of age, it was observed that after the administration of the vaccine, subsequent natural infection with RSV was associated with a more severe respiratory disease.[271,272] The most widely accepted theory for this phenomena is that an imbalance occurred in the Th1 and Th2 lymphocyte responses to the vaccine. Mice immunized with the formalin-inactivated RSV vaccine had pulmonary histologic findings consistent with a Th2-type response with an ineffective immune response to RSV.[28] Due to these past events with the formalin-inactivated RSV vaccine, no inactivated RSV vaccines are being researched.

There is no licensed vaccine for the prevention of RSV, but there has been significant progress in live attenuated RSV vaccine candidates.[273,274] Several live attenuated, cold-passaged, temperature-sensitive (cpts) RSV mutants have been evaluated in RSV-naive infants, seropositive children, and healthy adults. In adults and RSV-seropositive children the cpts 530/1009, cpts 248/955 and cpts 248/404 mutants have been shown to be safe.[274] However, these cpts mutants have been underattenuated in young infants and hence are not sufficiently safe vaccine candidates to proceed further in vaccine trials in infants.[275,276] In infants, the cpts 248/955 and cpts 530/1009 mutants produced prolonged viral replication and were insufficiently attenuated, as the virus was transmitted to almost a quarter of placebo recipients. The cpts 248/404 vaccine was associated with an increased frequency of upper respiratory symptoms and little evidence of lower respiratory illness.[276] However, there was a reduction in reinfectivity with the second dose, implying that the first dose was somewhat protective. Recent developments in reverse genetic technology have allowed for the genetically engineered mutant virus (rABcp 248/404/1030, rA2cp 248/404/1030[ε]SH), which recently has been evaluated in infants and appears to be promising as a vaccine candidate.[274]

Live attenuated vaccines for RSV have the advantage of intranasal administration and the potential to protect against both upper and lower respiratory tract disease. Early trials with cold-passage strains demonstrated poor infectivity or immunogenicity.[277,278] Further research has provided improved cold-passaged, temperature-sensitive mutants. One particular candidate, cpts 248/404 has been shown to be safe and immunogenic in children older than 6 months, but led to nasal congestion in infants 1–2 months of age.[275] Additional live attenuated vaccine candidates are currently under evaluation in animal models with promising results.[52] Advanced technology may also provide improved live attenuated vaccines which are genetically engineered.[279]

Subunit vaccines consisting of purified RSV glycoproteins are other promising candidates for RSV immunization. Two separate purified F subunit protein vaccines have been shown to be safe and immunogenic in clinical trials involving healthy adults, elderly subjects, RSV-seropositive children over 12 months of age, and children with pulmonary disease.[280–286] Further clinical studies including a subunit vaccine with the G protein fragment of RSV-A are being planned.[52]

Parainfluenza virus

Human parainfluenza virus (PIV) type 3 infections are the second most common cause of serious respiratory tract disease in infants and children (after RSV). Each year, approximately 25% of children < 5 years of age experience a clinically significant PIV infection and 2% of them require hospitalization. This virus subtype commonly causes bronchopneumonia and bronchiolitis in young children. Two separate live attenuated vaccines have been under evaluation. The cold-passaged (cp) HPIV-3 vaccines are cold-adapted, temperature-sensitive prospects. In early studies, the cp-18 strain was not sufficiently attenuated for children, but the cp-45 strain showed promising

results. When the cp-45 strain was given intranasally to children, the vaccine candidate was found to be immunogenic and safe.[287] The antigenically-related bovine parainfluenza-3 (BPIV-3) vaccine has also been evaluated in early clinical trials.[288,289] Results indicate that this vaccine is safe, immunogenic, and poorly transmittable. In addition, serum hemagglutination-inhibition antibody responses are increased with BPIV-3 when compared with those induced by cold-passaged HPIV-3. Trivalent subunit vaccines[290] as well as recombinant vaccines[291,292] are also under evaluation as potential parainfluenza vaccine candidates.

Cytomegalovirus

Although cytomegalovirus (CMV) produces an uncommon mononucleosis-like syndrome in immunocompetent patients, its potential effects in the newborn and immunocompromised patient can be devastating. Congenital CMV is the most common intrauterine infection in the U.S. An estimated 8000 American infants develop neurological or fatal complications each year because of this disease.[293]

Several types of CMV vaccines are under evaluation. The first of these is the live attenuated Towne strain vaccine, which was first developed in the mid-1970s. Clinical studies in seronegative renal allograft recipients showed that the vaccine did not prevent infection, but significantly reduced the incidence of severe disease by approximately 85%.[294,295] Another study evaluated the effect of CMV vaccine in preventing child-to-mother transmission of CMV acquired in day-care centres.[296] The infection rate for vaccinated mothers was no different than placebo, while naturally seropositive mothers were protected. These disappointing results showed that the Towne strain vaccine did not induce immunity as effectively as natural infection. Work is underway to develop improved versions of the Towne strain vaccine.[297,298]

Subunit glycoprotein B (gB) vaccines have also been evaluated for CMV immunization. Clinical studies of the vaccine in healthy toddlers and adults have shown good immune responses, but neutralizing antibodies rapidly declined in the 6 months following the third dose.[299,300] A fourth dose in adults led to higher antibody levels, though titers declined again in 6 months.[299] Further long-term data on this study are not yet available. A clinical vaccine efficacy study in mothers is underway to evaluate the effects of the antibody response.[301]

The canarypox-gB recombinant vaccine has been developed and evaluated as a candidate CMV vaccine. Initial trials have demonstrated a weak antibody response after multiple doses, but additional studies are currently evaluating its potential as a primer for boosting of subsequent Towne strain injections.[302] Other potentially hopeful avenues for CMV vaccines include DNA plasmids[303] and HLA restricted peptide-based vaccines.[304]

Human immunodeficiency virus

Developing an effective HIV vaccine is critical. Millions of people have been infected and are dying worldwide. Currently, over 74 HIV vaccine candidates are reported to be in research and development or preclinical testing in animals.[52,123] Since 1987, at least 34 different HIV candidate vaccines have begun phase I trials, and only a few have progressed to phase II trials.[52,305,306] Only VaxGen AIDS product, AIDSVAX, a recombinant gp 120 subunit vaccine, advanced to phase III trials, but it failed.[307] A potential obstacle to the successful development of an effective HIV vaccine is genetic variation, specifically the envelope protein.

Recombinant subunit HIV vaccines are genetically engineered from HIV surface envelope proteins, gp160 and gp120. These vaccines do not contain live virus or DNA. Therapeutic studies using the gp160 subunit, administered every 3 months for 3 years, demonstrate modest effects on CD4 counts but no clinical benefit in HIV positive persons taking antiretroviral therapy.[52,305,308]

Virus vectors are genetically engineered to express certain HIV genes. The vaccinia virus was the first recombinant live-virus vector to be tested. The HIV gp160 gene was inserted into the vaccina virus genome. This vaccine alone did not stimulate much antibody production.[52] However, this vaccine followed by a boosted recombinant gp160 vaccine induces a strong cellular immunity and antibody response.[308] It remains to be determined if this candidate vaccine will be able to prevent infection in seronegative individuals. The safety and immunogenicity of combinations of recombinant subtype E and B HIV type 1 envelope gp120 vaccines has been tested.[306] No serious adverse events were found to be related to the vaccination and a $>95\%$ lymphoproliferative response and a 100% binding antibody was found in all vaccine recipients.[306]

Other potential viral vectors include the canarypox and adenovirus. Unlike the vaccinia virus, the canarypox and adenovirus infect humans but do not replicate. This is important since the vaccinia virus can be shed and possibly disseminate in immunosuppressed hosts. The canarypox and adenovirus produce the necessary HIV proteins (gp160 or gp120), then terminate any further replication.[309]

The canarypox vector is able to induce humoral and cellular immune responses.[309] In February of 1999, a trial commenced in Uganda using the canarypox vector vaccine, ALVAC cCP205. This vaccine contains three HIV genes from clade B viruses. Clade B virus is the predominant subtype of HIV found in the U.S. and Europe. In Uganda, the most common subtypes of HIV are clades A and D. This study will evaluate the cross-reactivity among these viral subunits and compare the immune response in recipients.

The canarypox vaccine's greatest potential lies in the prime and boost approach. An initial canarypox vaccine primer is able to induce a strong cellular immunity. This primer is then followed by a recombinant subunit vaccine which boosts the antibody response.[310,311] The combination of primer followed by a boost induces a stronger immune response than either one alone.[312,313] The results of a phase II trial showed that 93% of subjects who received the combination of vaccines developed neutralizing antibodies and one-third developed a HIV-specific cytotoxic T-lymphocyte response.[314] A broader recombinant vector vaccine would likely increase the percentage of responders.[315]

DNA (or nucleic acid) vaccines are another potential prospect for HIV immunization. Purified DNA that encodes for particular immunogenic antigens is injected in vaccine recipients. This antigen is presented to the host immune system in its native form and is processed similar to that for a natural viral infection.[316] Therapeutic immunization with a plasmid gp160 and gag + pol DNA vaccine in HIV-positive chimpanzees have produced a significant decrease in viral load and a boost in the immune response.[317] In seronegative primates, studies show an induction of neutralizing antibodies and cytotoxic T-lymphocyte response. However, the vaccine does not protect against HIV infection.[52] Two DNA vaccine candidates are currently in phase I trials.[52]

Other potential prospects for HIV vaccine development are under investigation. In animal models, live-attenuated HIV vaccines are able to generate a broad and durable immune response. Due to potential safety concerns with live HIV virus, this vaccine has not been tested in humans.[52] Whole-inactivated vaccines have been used in chimpanzees, but inactivation of the virus often leads to a vaccine that is less potent or immunogenic without providing protection against HIV infection.[52] There is a risk that incomplete inactivation could lead to HIV infection of vaccine recipients. Virus-like particles (VLPs) have also been attempted. They provide a safer option since they consist of a non-infectious HIV 'look-alike' that does not contain the HIV genome. Early results have shown that a VLP candidate, known as p17/p24:TY, leads to low levels of HIV binding antibodies and T-cell memory responses, but induces very little cytotoxic T-lymphocyte activity.[52] Other VLP candidates are under research and development, and results of clinical trials should be available in the near future.

Other vaccines

Vaccines for several other viral diseases are currently in the early stages of development. At least four different types of hepatitis C vaccines are in preclinical development. However, research for these candidate vaccines is hampered by the lack of reproducible tissue culture or a convenient small animal model for testing and the defining of what constitutes a protective immune response and what role antibodies to hepatitis C virus serve.[52] Early studies in chimpanzees with several hepatitis C vaccines are currently underway.

Three different Epstein–Barr virus vaccine types are reported to be in phase I studies, including a glycoprotein subunit (gp350) vaccine, a vaccinia recombinant vaccine expressing gp350, and peptide induction of cutaneous T lymphocytes.[52] It is unknown if the specific antigenic components of these vaccines are sufficient to prevent infection.

At least 14 different vaccines are under development for dengue virus. While most are in preclinical stages, a live attenuated monovalent vaccine is in phase III clinical trials and a combined quadrivalent vaccine is in phase I trials. The live-attenuated vaccines have shown promise in the prevention of infection.[52]

An Ebola vaccine has recently been developed. This experimental vaccine has been shown to successfully protect non-human primates against Ebola disease with the generation of Ebola-specific CD8 T cell and antibody responses. More than 6 months, however, were required to complete immunizations in the non-human primates tested.[318]

Potential West Nile virus (WNV) vaccines include formalin-inactivated, naked DNA, and live attenuated recombinant viruses. Of these, the molecular live-attenuated (WN/DEN4) and the live, attenuated recombinant vaccine (ChimeriVax) appear to be the most promising WNV vaccine candidates. The molecular live-attenuated WNV vaccines are genetically engineered to bear the membrane precursor and envelope protein genes of WNV on a backbone of dengue 4 virus (DEN4). In animal studies, the WN/DEN 4 induced a moderate-to-high titer of neutralizing antibodies and prevented viremia in monkeys challenged with WNV.[319] The

ChimeriVax replaces genes encoding the premembrane (prM) and envelope (E) protein of yellow fever 17D vaccine with the envelope genes of the WNV. The resulting virion replicates in the host like the yellow fever virus but allows for immunity specifically against the WNV.[320-322] In animal studies, this vaccine induced high humoral antibody responses.[320-322] Human clinical trials are underway for both of these WNV vaccines.

The severe acute respiratory syndrome (SARS) accelerated vaccine initiative (SAVI) has developed and is testing three potential SARS vaccines in animals.[323-325] In animal models (i.e., mice) these vaccines induce broad immunity to virus specific structural SARS-associated coronavirus antigens such as the spike protein S1 fragment, membrane protein and nucelocapsid protein. The results of these studies should be available this year and possibly for testing in China, if the vaccine works, as early as 2005. An adenoviral delivery of codon-optimised SARS-CoV strain antigens has been shown to induce virus-specific broad immunity in rhesus monkeys.[325] Other potential SARS vaccines utilize inactivated virus and DNA that encodes for an outer surface protein on the SARS virus.

Conclusion

Immunization has successfully reduced the incidence of numerous diseases. Careful development and clinical evaluation have provided safe and effective vaccines with few adverse effects. Many reported adverse reactions following vaccination might be coincidental and have no proven direct relationship with the vaccine in question. Although serious side effects may occur from vaccines, a much greater risk for morbidity and mortality results from the failure to become immunized. One vaccine was recently removed from the market due to safety issues. Rotashield was a live, oral tetravalent, rotavirus vaccine that was associated with several cases of intussusception and considered to be causal.[326] Most associations between vaccines and adverse events are not demonstrated to be causal. For example, the measles mumps rubella (MMR) vaccine was reported not to have a causal relationship to autism.[77-79] Nevertheless, suspected relationships between vaccines and adverse events need to be reported to the Vaccine Adverse Event Reporting System (1-800-822-7967) so that an excellent safety record of vaccines can be maintained.

The technology of vaccine development has progressed dramatically in the last decade. While more conventional methods have consisted of whole-killed or live attenuated viruses, more recent advancements include genetically engineered vectors and virus-like particles. Anticipated vaccine developments in the future show exciting promise in several areas, such as immunization with plants. Potatoes, tomatoes and bananas are currently undergoing genetic engineering to express immunizing antigens against infections.[327,328] This form of vaccination would offer a convenient, painless and inexpensive approach to widespread control of disease and would thus be accessible to developing countries. It is anticipated that the future will bring safe and effective vaccines for a variety of viral diseases while constantly and continually expanding our concept of vaccines with ongoing experimental and clinical trials of therapeutic vaccines.

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