Vaccines and immunotherapies for the prevention of infectious diseases having cutaneous manifestations

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Although the development of antimicrobial drugs has advanced rapidly in the past several years, such agents act against only certain groups of microbes and are associated with increasing rates of resistance. These limitations of treatment force physicians to continue to rely on prevention, which is more effective and cost-effective than therapy. From the use of the smallpox vaccine by Jenner in the 1700s to the current concerns about biologic warfare, the technology for vaccine development has seen numerous advances. The currently available vaccines for viral illnesses include Dryvax for smallpox; the combination measles, mumps, and rubella vaccine; inactivated vaccine for hepatitis A; plasma-derived vaccine for hepatitis B; and the live attenuated Oka strain vaccine for varicella zoster. Vaccines available against bacterial illnesses include those for anthrax, Haemophilus influenzae, and Neisseria meningitidis. Currently in development for both prophylactic and therapeutic purposes are vaccines for HIV, herpes simplex virus, and human papillomavirus. Other vaccines being investigated for prevention are those for cytomegalovirus, respiratory syncytial virus, parainfluenza virus, hepatitis C, and dengue fever, among many others. Fungal and protozoan diseases are also subjects of vaccine research. Among immunoglobulins approved for prophylactic and therapeutic use are those against cytomegalovirus, hepatitis A and B, measles, rabies, and tetanus. With this progress, it is hoped that effective vaccines soon will be developed for many more infectious diseases with cutaneous manifestations. (J Am Acad Dermatol 2004;50:495-528.)

Learning objective: At the completion of this learning activity, participants should be familiar with the current and experimental vaccines and immunotherapies for infectious diseases with cutaneous manifestations.
not met, the eradication is estimated to be complete by 2005. A number of other vaccines have led to notable decreases in infections and complications. For instance, after years of widespread measles vaccination, a record low of 37 measles cases was reported in the United States in 2002. Vaccines for Haemophilus influenzae and Neisseria meningitidis have greatly decreased the incidence of these diseases. These currently available vaccines also provide a basic framework of knowledge and experience with which other vaccines can be developed.

Unfortunately, the progress in finding new vaccines cannot be rapid enough. The incidence of many viral infections, such as HIV, HSV, and human papillomaviruses (HPVs), is increasing despite the currently available antiviral agents. The development of prospective vaccines is generally measured in decades (Table I), marked by small increments of advancement before the final product becomes available. Numerous different vaccine candidates often are developed and evaluated over many years before a single effective vaccine is licensed.

### Table I. Timeline of vaccine development*

<table>
<thead>
<tr>
<th>Year</th>
<th>Vaccine</th>
</tr>
</thead>
<tbody>
<tr>
<td>1796</td>
<td>Smallpox</td>
</tr>
<tr>
<td>1800</td>
<td>Anthrax</td>
</tr>
<tr>
<td>1885</td>
<td>Rabies</td>
</tr>
<tr>
<td>1900</td>
<td>Influenza</td>
</tr>
<tr>
<td>1945</td>
<td>Yellow fever</td>
</tr>
<tr>
<td>1963</td>
<td>IPV, OPV (Poliomyelitis)</td>
</tr>
<tr>
<td>1963</td>
<td>Measles</td>
</tr>
<tr>
<td>1967</td>
<td>Mumps</td>
</tr>
<tr>
<td>1969</td>
<td>Rubella</td>
</tr>
<tr>
<td>1975</td>
<td>Neisseria meningitidis</td>
</tr>
<tr>
<td>1980</td>
<td>Adenovirus</td>
</tr>
<tr>
<td>1980</td>
<td>Rabies (IMOVAX: first FDA-approved rabies vaccine)</td>
</tr>
<tr>
<td>1981</td>
<td>Hepatitis B</td>
</tr>
<tr>
<td>1987</td>
<td>Haemophilus influenzae</td>
</tr>
<tr>
<td>1992</td>
<td>Japanese encephalitis</td>
</tr>
<tr>
<td>1992</td>
<td>DTaP (diphtheria-tetanus-acellular pertussis)</td>
</tr>
<tr>
<td>1995</td>
<td>Hepatitis A, Varicella</td>
</tr>
<tr>
<td>1998</td>
<td>Lyme disease†</td>
</tr>
<tr>
<td>1998</td>
<td>Rotavirus†</td>
</tr>
<tr>
<td>2000</td>
<td>Streptococcus pneumoniae</td>
</tr>
<tr>
<td>2000</td>
<td>Dryvax (smallpox)</td>
</tr>
<tr>
<td>2002</td>
<td>Pediarix (DTaP-HepB-IPV)</td>
</tr>
</tbody>
</table>

*Dates for smallpox, anthrax, and rabies vaccines are of the first published results of vaccine usage. Remaining dates are those of approval of vaccines by the Food and Drug Administration (FDA).
†No longer available.

### CURRENTLY LICENSED VACCINES

#### Viruses

**Smallpox.** In 1796 Edward Jenner first demonstrated that inoculation of cowpox virus into human skin could lead to protection from subsequent smallpox infection. He named the inoculation substance vaccine, based on the Latin word vacca, meaning cow. The most recent vaccines used for smallpox vaccination were derived from a species similar to cowpox, called vaccinia virus. Vaccinia virus is a member of the orthopox virus family, which includes variola virus, the virus that causes smallpox, and other viruses such as cowpox and monkeypox. Several strains of the live attenuated virus vaccine were employed in the eradication of disease. The smallpox vaccine has been the prototype of success of a viral vaccine. Prior to immunization, smallpox infection relentlessly killed hundreds of millions of persons. The complete eradication of this disease could easily be considered the greatest success story in medical history.

Vaccine production ended 2 decades ago after the eradication of smallpox, and approximately 60 million vaccine doses remain worldwide. Because of recent concerns over potential biologic warfare in the future, the threat of smallpox may not have been
completely eliminated with eradication of the disease. Therefore, renewed interest in the production of smallpox vaccines has developed.

The destruction of the 2 remaining smallpox virus reserves, in Atlanta and Moscow, has been a source of ongoing debate. Opponents of destruction contend that the virus stocks would be helpful for future research, such as smallpox pathogenesis and the production of new antiviral agents. Proponents argue that the virus genome has already been cloned and sequenced and is unnecessary for such research. The World Health Assembly in May 1999 resolved to postpone the previously arranged smallpox destruction scheduled for June 30 of that year.

All US smallpox vaccine formulations contain the New York City Board of Health vaccinia strain, which is less reactogenic than other strains. The U.S. National Pharmaceutical Stockpile contains 4 formulations of smallpox vaccine: 2 previously manufactured calf-lymph-derived vaccines, Dryvax (Wyeth Laboratories, Marietta, Pa) and Aventis Pasteur vaccine (Swiftwater, Pa); and two newly developed vaccines from Acambis/Baxter Pharmaceuticals (Cambridge, Mass), ACAM1000 and ACAM2000 (Centers for Disease Control [CDC] Drug Services, unpublished data, 2002). ACAM1000 is grown in human embryonic lung cell culture (MRC-5), and ACAM2000 is grown in African green monkey kidney cells (Vero cells).

Dryvax is licensed and is currently being used for immunization against smallpox in public health and health-care response teams and laboratory workers who are involved with research activities that involve vaccinia virus. This vaccine will be used to fulfill the recommendations of the national Advisory Committee on Immunization Practices. An emergency vaccination strategy has been developed in the event of confirmed cases of smallpox. Priority will be given to those with early diagnosis, all who had been in contact with the patient since onset of fever, all household members of the contacts, and health-care workers, public health personnel, first responders, and other personnel who will be assisting with outbreak control measures and emergency response activities.

Dryvax is freeze-dried and reconstituted before use with a diluent that contains 50% glycerin and 0.25% phenol. Contraindications to use of this vaccine in the absence of smallpox include allergies to polymyxin B sulfate, streptomycin sulfate, chlorotetracycline hydrochloride, and neomycin sulfate. In the case of contact with persons with smallpox or in the presence of smallpox, the vaccine should be administered along with antihistamines or glucocorticoids.

The CDC is holding the other 3 vaccines in reserve. Studies are currently under way to determine the reactogenicity of the 2 newer cell culture vaccines. The tissue culture cell vaccines are being developed in hopes of supplanting the calf-lymph vaccine if a more extensive vaccination program is needed.

All 4 vaccines are administered by means of inoculation into the superficial layers of the skin, which allow the virus to grow and induce an immunologic response that protects the host against smallpox. The vaccines elicit antibody and cell-mediated immunity. A successful vaccination, defined as an antibody titer of 1:10 or greater, occurs in more than 95% of persons within 1-2 weeks of immunization. The protective duration of the vaccine occurs for 3-5 years and begins fading after 5 years, with minimal protection after 20 years. In persons who have undergone revaccination with smallpox, residual immunity may last 30 years or more. Individuals undergoing postexposure vaccination should receive the smallpox vaccination within 3 days of exposure to prevent the natural history of smallpox. Some epidemiologic evidence suggests that vaccination up to 4-7 days after exposure also may alter the severity of the disease and provide protection from death.

Adverse reactions of the smallpox vaccine are similar to those caused by other vaccines and include local skin reactions, fever, erythema multiforme, and anaphylaxis. There recently have been 30 cases of cardiac-related events, such as myocarditis, pericarditis, myocardial infarction, and angina during the civilian smallpox vaccination program.

Other adverse effects specific to smallpox vaccination include eczema vaccinatum, fetal vaccinia, generalized vaccinia, inadvertent inoculation, ocular vaccinia (Fig 1), progressive vaccinia, and postvaccinal encephalopathy and encephalomyelitis. These specific complications of smallpox vaccina-
tion can be treated with vaccinia immunoglobulin (VIG), cidofovir, and ophthalmic antiviral agents, although none of these therapies have undergone clinical trials.\textsuperscript{22} VIG is considered first-line treatment because of worldwide historical experience with it in the treatment of vaccinia-related adverse events. However, supplies of VIG are limited. Although cidofovir has never been used to treat vaccinia infections in human beings, the first use of cidofovir in 3 HIV-positive men with recurrent molluscum contagiosum virus cleared the lesions.\textsuperscript{23} These were the first documented cases in which cidofovir was used to treat poxvirus infections in human beings.

Smallpox vaccination is contraindicated for persons with any of the following conditions or who have had close contact with any of the following conditions: atopic dermatitis or active acute or chronic exfoliative skin conditions that disrupt the epidermis, Darier disease, pregnancy or a desire to become pregnant within the 28 days after vaccination, and compromised immune systems.\textsuperscript{22} Other contraindications that apply only to potential vaccine candidates but do not include their close contacts are moderate to severe intercurrent illness, smallpox vaccine-component allergies, age <18 years, breastfeeding, and the use of topical ocular steroid medications.\textsuperscript{22}

**Measles, mumps, and rubella.** Vaccination for these 3 classic childhood diseases typically is administered as a combination measles-mumps-rubella (MMR) vaccine (Tables II and III). Live virus vaccines for all 3 viruses were introduced in the 1960s, and after widespread implementation in the United States, the number of cases of these infections reported annually has declined by more than 98%.\textsuperscript{21} This result is largely due to the recommendation that all states require a 2-dose MMR vaccine in children before they enter school. The use of 2-dose MMR vaccine is to induce immunity in that small percentage of persons with failure to develop an immunologic response to one or more components of the first dose. The most recent recommendations by the CDC are that vaccination with the first MMR dose be administered at 12-15 months and the second dose at 4-6 years of age.\textsuperscript{25} Each MMR vaccine contains 0.3 mg of human albumin, 25 \(\mu\)g of neomycin, 14.5 mg of sorbitol, and 14.5 mg of hydrolyzed gelatin (Merck, manufacturer’s package insert). Because these vaccines consist of live attenuated viruses, they should not be administered to pregnant women or those planning to become pregnant within the following 3 months. The theoretical risk of congenital rubella syndrome after immunization has been the primary concern. However, a study of 321 women who had received the rubella vaccine 3 months before or after conception revealed no congenital malformations compatible with congenital rubella infection.\textsuperscript{26} Immunization is also contraindicated in immunosuppressed patients, although it can be administered to individuals with asymptomatic HIV infection as well as to persons with mild immunosuppression. In healthy individuals, minor illnesses with or without fever are not a contraindication to vaccination. Patients with a history of anaphylactic hypersensitivity to neomycin should not receive the MMR vaccine. The vaccine can be administered to patients with an allergy to eggs, since the risk of severe anaphylactic reactions is exceedingly low.\textsuperscript{25} It is recommended that these patients be observed for 90 minutes after immunization.\textsuperscript{27}

**Measles.** Measles virus has been noted to be the most infectious disease of humankind in terms of the minimal number of virions necessary to produce infection.\textsuperscript{28} An estimated 75% of susceptible family contacts who are exposed to a case of measles develop the disease.\textsuperscript{29} Because human beings are the only reservoir for measles virus, global eradication is technically feasible. The World Health Organization, the CDC, and the Pan American Health Organization convened in 1996 and adopted the goal of global eradication by 2005-2010.\textsuperscript{30} Owing to universal childhood immunization in the United States, measles is no longer considered an indigenous disease in this country.\textsuperscript{31} The reported incidence of measles has decreased by >99% since the measles vaccine became available. In 2000 there were 86 reported measles cases, of which 26 were internationally imported. Of the remaining 60 indigenous cases, 9 were definitely due to imported virus, 18 were linked to imported virus, and 33 were of unknown source.\textsuperscript{32} In 2001 there were 116 reported cases of measles,\textsuperscript{33} but in 2002 only 37 measles cases were reported in the United States.\textsuperscript{33} The increase of 35% from 2000 to 2001 should remind us that we need to guard against complacency. Lack of compliance with routine MMR vaccination in the past led to a resurgence of measles infection in the United States between 1989 and 1991, with some deaths reported.\textsuperscript{34} Moreover, more than 1 million children die of measles each year in third-world countries.\textsuperscript{34}

In 1963 the initial measles vaccine, which was a live attenuated vaccine, was licensed. In 1968 the current measles vaccine was licensed. It uses the Enders-Edmonston virus strain, a further attenuated version of the live preparations previously available, resulting in fewer adverse reactions in recipients. It is produced by culturing the Moraten virus strain in chick embryo cells. Measles vaccination produces a mild or unapparent infection that is noncommuni-
Both humoral and cellular immune responses develop as a result. After receiving 2 doses of vaccine, 95% to 99% of recipients develop serologic evidence of immunity to measles. Immunity is thought to be lifelong, similar to that acquired after infection with the wild-type virus. However rare, measles infection has been reported in patients with previously documented postimmunization seroconversion.

Adverse effects after measles vaccination typically are mild. Five percent to 15% of recipients develop a fever of at least 103°F for 1 to 2 days, generally 5 to 12 days after immunization. These persons are largely asymptomatic, but some may develop a transient viral exanthem. An associated encephalitis or encephalopathy rarely has been reported after immunization and occurs in fewer than 1 in 1 million vaccinees. There have been early concerns about the association of measles vaccination and subacute sclerosing panencephalitis (SSPE), since this complication may occur with natural infection. A small number of reports have described the occurrence of SSPE in persons with a history of vaccination but no known history of infection. More recent evidence indicates that at least some of those cases had unrecognized natural measles infection prior to vaccination, and the SSPE was directly related

<table>
<thead>
<tr>
<th>Vaccine</th>
<th>Target population</th>
<th>Route</th>
<th>Dosage</th>
<th>Comments</th>
</tr>
</thead>
<tbody>
<tr>
<td>Adenovirus</td>
<td>Military personnel; military personnel, and those involved in producing quantities of cultures or those with high potential for aerosol exposure</td>
<td>Oral</td>
<td>1 dose</td>
<td></td>
</tr>
<tr>
<td>Anthrax</td>
<td></td>
<td>SC</td>
<td>3 doses: 0, 2, and 4 wk; boosters at 6, 12, and 18 mo</td>
<td>Current recommendation is for annual boosters.</td>
</tr>
<tr>
<td>DT (diphtheria-tetanus)</td>
<td>All adults</td>
<td>IM</td>
<td>1 dose booster every 10 y; for adults, the primary series is 3 doses; the first 2 doses given at least 4 wk apart and the third dose, 6-12 mo after the second</td>
<td>Give 1 dose if patient had received the primary series and the last vaccination was ≥10 y ago.</td>
</tr>
<tr>
<td>Hepatitis A*</td>
<td>Persons with behavioral, medical, or occupational indications</td>
<td>IM</td>
<td>2 doses: 0 and 6-12 mo</td>
<td>Other indications are travel to or work in countries that have high incidence of hepatitis A.</td>
</tr>
<tr>
<td>Hepatitis B*</td>
<td>Persons with behavioral, medical, or occupational indications</td>
<td>IM</td>
<td>3 doses: 0, 1-2, and 4-6 mo</td>
<td>Other target populations are inmates of correctional facilities, home contacts and sex partners of persons with chronic hepatitis B viral (HBV) infection, and travelers to countries with high prevalence of chronic HBV infection.</td>
</tr>
<tr>
<td>Influenza</td>
<td>Persons over 50 y; those with medical or occupational indications, and home contacts of persons with indications</td>
<td>IM</td>
<td>1 dose annually</td>
<td>October or November is the optimal time, but December to March is acceptable. Cont'd on page 500</td>
</tr>
</tbody>
</table>

HDCV, Human diploid cell vaccine; ID, intradermal; IM, intramuscular; PCEC, purified chick embryo cell culture vaccine; SC, subcutaneous.

*A combination vaccine (Twinrix), now available, contains Havrix (Hepatitis A) and Engerix-B (Hepatitis B). It thus reduces the number of vaccinations from 5 to 3.
to the infection.\textsuperscript{25} Widespread measles immunization has nearly eliminated SSPE in the United States, and the live measles vaccine does not increase the risk for this complication.\textsuperscript{25}

**Mumps.** Since the introduction of live mumps vaccine in 1967 and its recommended use in 1977, the incidence of mumps has decreased steadily by more than 99%. The live attenuated mumps vaccine (Jeryl-Lynn strain) is prepared in chick embryo cell culture. Immunization produces a mild subclinical infection that is noncommunicable. Early clinical studies have shown that 97% of children and 93% of adults develop serologic evidence of immunity after vaccination.\textsuperscript{46-48} Outbreak-based studies, however, have reported lower efficacy rates, ranging from 75% to 95% protection from infection.\textsuperscript{49-52} Although the duration of immunity in vaccine recipients is not completely known, serologic and epidemiologic evidence suggests that immunity persists for at least 30 years.\textsuperscript{53-56}

Adverse reactions generally are mild and uncommon after mumps vaccination. Low-grade fever, mild parotitis, and a viral exanthem have been reported. Serious reactions, such as adverse neurologic effects, are extremely rare and have not been causally associated with the mumps vaccine.\textsuperscript{57}

**Rubella.** Two different live attenuated rubella vaccine strains, HPV-77 and Cendehill, were initially

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**Table II. Cont’d**

<table>
<thead>
<tr>
<th>Vaccine</th>
<th>Target population</th>
<th>Route</th>
<th>Dosage</th>
<th>Comments</th>
</tr>
</thead>
<tbody>
<tr>
<td>Japanese encephalitis</td>
<td>Persons traveling to Asia Consider for persons 15-70 years old who are exposed to areas of moderate to high risk</td>
<td>SC</td>
<td>3 doses: 0, 7, and 30 d 3 doses: 0, 1, 12 mo</td>
<td>Doses 1 and 3 to be given several wk before tick season.</td>
</tr>
<tr>
<td>Lyme disease*</td>
<td>Adults born in or after 1957 should receive at least 1 dose of MMR vaccine unless they have acceptable evidence of immunity or a medical contraindication.</td>
<td>IM</td>
<td>1 dose if measles, mumps, or rubella vaccination history is unreliable; if second dose is recommended, administer no sooner than 4 wk after first dose.</td>
<td>Second dose of MMR vaccine is recommended for those who work in health care facilities, plan to travel internationally, were recently exposed to measles, were previously vaccinated with killed measles vaccine, were vaccinated with an unknown vaccine between 1963 and 1967, or are students in post secondary educational institutions.</td>
</tr>
<tr>
<td>MMR (measles-mumps-rubella)</td>
<td>Adult</td>
<td>SC</td>
<td>1 dose if measles, mumps, or rubella vaccination history is unreliable; if second dose is recommended, administer no sooner than 4 wk after first dose.</td>
<td>Another indication is travel to countries in which disease is epidemic.</td>
</tr>
<tr>
<td><em>Neisseria meningitidis</em></td>
<td>Consider vaccination for persons with medical indications (anatomic or functional asplenia, terminal complement component deficiencies) and college students who live in dormitories.</td>
<td>SC</td>
<td>1 dose; revaccination at 3-5 years may be indicated for persons at high risk for infection.</td>
<td>Another indication is travel to countries in which disease is epidemic.</td>
</tr>
<tr>
<td>Poliomyelitis</td>
<td>Not recommended for persons &gt;18 y old.</td>
<td>IM or SC</td>
<td>1 dose; revaccination at 3-5 years may be indicated for persons at high risk for infection.</td>
<td>Those who never received or completed the pediatric series do not need to be vaccinated unless they travel to areas where wild-type virus exposure may occur.</td>
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</table>

*Lyman disease vaccine no longer available.
developed and licensed in the United States in 1969. They were replaced in 1979 by the RA 27/3 (rubella abortus 27, explant 3) vaccine, which is grown in human diploid fibroblast cell culture. This vaccine produces nasal antibodies, as well as higher and more persistent antibody titers, that better mimic the immune protection developed after natural infection.\(^5^8,^5^9\) Vaccination induces an antibody response in more than 97% of recipients.\(^4^7,^6^0\) Immunity in vaccine recipients is thought to be lifelong and has been shown to persist for at least 16 years.\(^6^1,^6^2\) In addition, this vaccine was associated with fewer adverse events compared with the previous rubella vaccines.

Adverse effects after rubella vaccination typically are mild. Five percent to 15% of vaccinated children develop fever, lymphadenopathy, or a viral exanthem, usually 5 to 12 days after vaccination.\(^5^7,^6^3\) Arthralgias and arthritis are frequent complications in adult vaccinees, particularly women, and may develop in 25% to 40% of this population.\(^6^4,^6^6\) But occurrences in children are rare (0.5%).\(^6^4\) These joint symptoms typically begin within the first 3 weeks after vaccination and remit within 11 days.\(^2^6\) The

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**Table II. Cont’d**

<table>
<thead>
<tr>
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<th>Target population</th>
<th>Route</th>
<th>Dosage</th>
<th>Comments</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rabies</td>
<td>Persons at risk of rabies exposure or those recently exposed.</td>
<td>IM</td>
<td>Preexposure: 3 doses at 0, 7, and 21 or 28 d; HDCD: Imovax</td>
<td>Previously vaccinated persons require only 2 doses after rabies exposure at 0 and 3 d.</td>
</tr>
<tr>
<td>RA 27/3 (rubella abortus 27, explant 3) vaccine</td>
<td></td>
<td>IM</td>
<td>Postexposure: 5 doses at 0, 3, 7, 14, and 28 d; HDCD: Imovax Rabies ID</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>IM</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>IM</td>
<td>Rabies Vaccine Absorbed</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>IM</td>
<td>PCEC: PabAvert</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>ID</td>
<td>HDCD: Imovax Rabies ID</td>
<td></td>
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<td></td>
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<td></td>
<td></td>
</tr>
<tr>
<td>Smallpox</td>
<td>Those with early diagnosis, all who had been in contact with the patient since onset of fever, all household members of the contacts, smallpox public health and healthcare response teams, and laboratory workers who are involved with vaccinia virus</td>
<td>SC</td>
<td>1 dose; if there is no evidence of a local vaccine reaction, can repeat vaccination after 7 d.</td>
<td>Postexposure vaccination; should receive the smallpox vaccination within 3 d.</td>
</tr>
<tr>
<td>Streptococcus pneumoniae</td>
<td>Persons &gt; 65 years old and those with chronic cardiovascular, hepatic, pulmonary, or renal diseases, diabetes mellitus, anatomic or functional asplenia, or immunosuppression</td>
<td>IM or SC</td>
<td>1 dose</td>
<td>Other populations are Alaskan natives, certain American Indian populations, and residents of nursing homes and other long-term care facilities.</td>
</tr>
<tr>
<td>Varicella zoster</td>
<td>Susceptible adults; recommended for those who do not have reliable clinical history of varicella infection or serologic evidence of varicella zoster virus infection</td>
<td>SC</td>
<td>2 doses: 0, and 4-8 wk</td>
<td></td>
</tr>
<tr>
<td>Yellow fever</td>
<td>Persons traveling to endemic countries (parts of Africa, South America, and southeast Asia)</td>
<td>SC</td>
<td>1 dose</td>
<td>Booster is given every 10 years for recertification for travel into endemic countries.</td>
</tr>
</tbody>
</table>
knees and the fingers are most frequently involved, but any joint may be affected.63

**Hepatitis A virus.** Both inactivated and attenuated forms of hepatitis A vaccines have been developed and studied. However, the inactivated vaccine is the only type licensed and available in the United States (Havrix and Vaqta). These vaccines are propagated in human diploid fibroblast culture and inactivated by formalin. Immunization generally involves 2 doses given 6-12 months apart in adults and children 2 years and older. Studies of both available inactivated vaccines show excellent as well as comparable immunogenicity and efficacy rates. Overall, 97% to 99% of recipients develop protective levels of

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<tr>
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<th>Comments</th>
</tr>
</thead>
<tbody>
<tr>
<td>DTaP (diphtheria, tetanus, acellular pertussis)</td>
<td>Infants and toddlers</td>
<td>IM</td>
<td>4 doses: 2, 4, 6, and 15-18 mo</td>
<td>The fourth dose of DTaP may be given as early as age 12 mo if 6 mo have elapsed since the third dose, and the child is unlikely to return at age 15-18 mo.</td>
</tr>
<tr>
<td>DT (diphtheria-tetanus)</td>
<td>Children aged 11-12 y</td>
<td>IM</td>
<td>Recommended if at least 5 y have elapsed since the last dose of diphtheria and tetanus toxoid-containing vaccine.</td>
<td>Recommended routine DT boosters every 10 y</td>
</tr>
<tr>
<td>Hepatitis A*</td>
<td>Children</td>
<td>IM</td>
<td>2 doses: Havrix, ≥2 y: 0 and 6-12 mo; Vaqta, 2-17 y: 0 and 6-18 mo; &gt;17 y: 0 and 6 mo</td>
<td>Minimum interval between doses is 6 mo.</td>
</tr>
<tr>
<td>Hepatitis B*</td>
<td>Children</td>
<td>IM</td>
<td>3 doses: infants, birth to 2 mo, 1-4 mo, and 6-18 mo; children and adolescents; 0, 2, and 4 months</td>
<td>Infants with HBsAg-positive mothers receive first dose within 12 h of birth, second dose at 1 mo, and third dose at 6 mo.</td>
</tr>
<tr>
<td>Hib (Haemophilus influenzae type b)</td>
<td>Infant and toddlers</td>
<td>IM</td>
<td>4 doses: 2, 4, 6, and 12-15 mo</td>
<td>If PRP-OMP (Pedvax HIB or ComVax [Merck]) is given at ages 2 and 4 mo, the 6-mo dose is not required.</td>
</tr>
<tr>
<td>Influenza</td>
<td>Children older than 6 mo who have chronic cardiovascular, pulmonary, or renal diseases, chronic metabolic diseases, hemoglobinopathies, immunosuppression, and those receiving long-term aspirin therapy (risk of Reye syndrome)</td>
<td>IM</td>
<td>6 mo-8 y, 1 or 2 doses of split virus only, at least 1 mo apart; 9-12 y, 1 dose of split virus only; &gt;12 yrs, 1 dose of whole or split virus</td>
<td>October and November for those more susceptible to flu complications, and November and December for all others</td>
</tr>
<tr>
<td>MMR (measles, mumps, rubella)</td>
<td>Children</td>
<td>SC</td>
<td>2 doses: 12-15 mo and 4-6 y</td>
<td>Second dose may be administered during any visit, provided at least 4 wk have elapsed since the first dose; second dose should be given by 11 to 12 y</td>
</tr>
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</table>

IM, Intramuscular; HBsAg, hepatitis B surface antigen; IPV, inactivated polio vaccine; OPV, oral polio vaccine; SC, subcutaneous.

*A combination vaccine (Twinrix), now available, contains Havrix (Hepatitis A) and Engerix-B (Hepatitis B). It thus reduces the number of vaccinations from 5 to 3.

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**Table III. Vaccines: recommendations for immunizations in children**
antibodies 1 month after the first dose, and 99% to 100% of recipients are protected 1 month after the second dose.67-72 When studied in placebo-controlled clinical trials in Thailand (which has high rates of hepatitis A), 2 doses of the inactivated vaccine were 94% effective in protecting against hepatitis A infection.73 A similar study in New York children showed 100% efficacy after a single dose of vaccine.74

Because of limited long-term data about this vaccine, the duration of immunity has yet to be determined. In one study of a 3-dose series in adults, detectable antibodies were documented in all subjects 4 years after immunization.75 According to kinetic models of antibody concentration decline, protective levels of hepatitis A antibodies can be expected to persist for 20 years76 and perhaps up to 30 years.77 A separate mathematical evaluation of long-term immunity after a primary dose and booster dose for hepatitis A has calculated that protective antibody levels should persist for 24 to 47 years.78 It is unknown whether vaccine-induced immunity will persist beyond the loss of detectable antibody levels, as occurs with hepatitis B immunization, but this phenomenon has been suggested to occur.78

The hepatitis A vaccine is recommended for persons at least 2 years of age living in or traveling to areas of high endemicity for hepatitis A. It is also recommended for persons with chronic liver disease due to causes other than hepatitis A, persons engaging in high-risk sexual activity, residents of a community experiencing an outbreak of hepatitis A, and users of illicit injectable drugs. In addition, the hepatitis A vaccine currently is recommended for routine pediatric use in some states and regions.

Adverse effects with hepatitis A vaccination generally are mild, and no serious side effects have been attributed to the vaccine in clinical trials.73 Other than soreness at the injection site, the most common adverse effects include headache (14%) and malaise (7%) in adults and feeding problems (8%) and headache (4%) in children.

**Hepatitis B virus.** Prior to the development of the hepatitis B vaccine, an estimated 200,000-300,000 persons worldwide were being infected with the hepatitis B virus (HBV) annually.79 Immunization for hepatitis B became a reality in 1981 when the plasma-derived vaccine was licensed in the United States. This vaccine was highly effective in inducing immunity but was associated with several drawbacks. The supply of suitable carrier plasma necessary to make the vaccine was not sufficient for large-scale production. Also, despite the chemical treatment of plasma products for safety, there was some concern about the risk, albeit small,

<table>
<thead>
<tr>
<th>Table III. Cont’d</th>
<th>Vaccine</th>
<th>Target population</th>
<th>Route</th>
<th>Dosage</th>
<th>Comments</th>
</tr>
</thead>
<tbody>
<tr>
<td>Poliomyelitis</td>
<td>Children</td>
<td>SC</td>
<td>Sequential series, 4 doses: 2 mo, IPV; 4 mo, IPV. 6-18 mo, OPV; 4-6 y, OPV.</td>
<td>Regimens with all IPV or all OPV are given in the same time frame; all IPV doses are indicated for immunosuppressed patients or contacts; all OPV dosing is accepted in certain circumstances only.</td>
<td></td>
</tr>
<tr>
<td>Rotavirus</td>
<td>Infants</td>
<td>Oral</td>
<td>3 doses: 2, 4, and 6 mo</td>
<td>Vaccine is currently suspended for investigation regarding the association with intussusception.</td>
<td></td>
</tr>
<tr>
<td>Streptococcus pneumoniae</td>
<td>All children aged 2-23 mo, and recommended for certain children aged 24-59 mo</td>
<td>SC or IM</td>
<td>4 doses: 2, 4, 6, and 12-15 mo</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Varicella zoster</td>
<td>Children</td>
<td>SC</td>
<td>1 dose at ages 12 mo to 13 y; 2 doses (4-8 wk apart) in susceptible persons ≥13 y</td>
<td>Do not give to children younger than 12 mo.</td>
<td></td>
</tr>
</tbody>
</table>

*IM,* Intramuscular; *HBsAg,* hepatitis B surface antigen; *IPV,* inactivated polio vaccine; *OPV,* oral polio vaccine; *SC,* subcutaneous.

*A combination vaccine (Twinrix), now available, contains Havrix (Hepatitis A) and Engerix-B (Hepatitis B). It thus reduces the number of vaccinations from 5 to 3.
of HIV transmission. Both of these issues were addressed in 1986, when the yeast recombinant hepatitis B vaccine was licensed. This particular vaccine has been a major breakthrough for the field of medicine. It was the first licensed recombinant viral vaccine prototype, as well as the first effective viral vaccine for a sexually transmitted disease. This vaccine is produced by means of recombinant DNA technology, which involves insertion of the gene for hepatitis B surface antigen (HBsAg) into the yeast *Saccharomyces cerevisiae* (baker’s yeast). Clinical studies in high-risk homosexual men demonstrated three-dose vaccine efficacy of 82% to 95% in preventing acute hepatitis B. Overall, approximately 5% of immunocompetent adults fail to develop significant antibody titers after hepatitis B vaccination. Nearly 99% of children respond to vaccination, while only 50% to 70% of those over age 60 acquire immunity. Variables associated with a lower likelihood of seroconversion include immunosuppression, renal failure, premature reactivation with low birth weight, age older than 40 years, obesity, and smoking. Because of the decreased rates of seroconversion in specific populations, additional research is focused on methods of increasing immunogenicity to hepatitis B vaccines. Alternative delivery systems, such as adeno-viruses and vaccinia vectors, are under evaluation. Clinical trials are currently investigating the addition of adjuvants to the current recombinant vaccine in an effort to increase the host immune response. Several different types of vaccines also in development include DNA vaccines and Pre-S vaccines, which incorporate the surface proteins of the hepatitis B virus.

The duration of immunity afforded by vaccination merits further long-term studies, but according to present data, long-term efficacy is expected. Antibody levels decline rapidly in the first year after vaccination and then level off to a slow pace of decline. The loss of detectable antibodies to hepatitis B years after vaccination does not necessarily indicate a lack of immunity. The majority of persons are protected by immunologic memory in B lymphocytes, which mount an anamnestic response to natural infection. Rare cases of hepatitis B infection in previously vaccinated patients have been described. These patients generally have subclinical disease, and none has developed chronic infection or serious complications.

The regimen for immunization includes 3 doses, given at months 0, 1, and 6. Hepatitis B vaccination is recommended for adults at risk (ie, persons living in or traveling to areas of high endemicity of hepatitis B, health care 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, and police and fire department personnel who render first aid) and all children aged 0-18 years. Because of the current widespread use in children, a thimerosal-free vaccine was recently approved by the FDA. Thimerosal is a preservative that contains mercury, which has prompted the limitation of its use in children. In persons in whom vaccine-induced protection is less complete, such as in hemodialysis patients, the need for a booster dose should be assessed by means of annual antibody testing.

Adverse effects after hepatitis B vaccination are generally mild and well tolerated. Most commonly reported is fatigue (15%), followed by headache (9%) and fever (1%-9%). 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. Several isolated reports since that time have described the rare occurrence of adverse effects such as thrombocytopenic purpura, vasculitis, rheumatoid arthritis, lichen planus, and lichenoid reaction. However, it appears that these conditions do not occur at a higher rate than in the unvaccinated population. 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.

On May 11, 2001, the FDA licensed a new combination vaccine that protects people at least 18 years of age against hepatitis A virus and hepatitis B virus. This vaccine, Twinrix, combines 2 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 combination reduces the number of injections from 5 to 3. This vaccine is given at the same schedule that is used for the hepatitis B vaccine, administered at months 0, 1, and 6. The adverse effects of this vaccine are similar in type and frequency to those of the monovalent hepatitis A and hepatitis B vaccines. The data from 11 clinical trials indicate that 99.9% of vaccinees developed seroconversion for antibodies against hepatitis A virus and 98.5% antibodies against HBsAg, with persistence up to 4 years (GlaxoSmithKline Biologicals, unpublished data, 2001).

In 2002, the FDA approved DTaP-HepB-IPV (Pediarix, SmithKline Beecham Biologicals, Rixensart, Belgium), a combined diphtheria and tetanus toxoids and acellular pertussis adsorbed (DTaP), hepatitis B (HepB) (recombinant), and inactivated poliovirus vaccine (IPV). After 3 doses of DTaP-HepB-IPV, the immunologic responses were similar...
to those following 3 doses of separately administered vaccines for diphtheria, tetanus, 3 pertussis antigens, hepatitis B, and poliovirus types 1, 2, and 3. However, there are no immunologic data regarding simultaneous administration of DTaP-HepB-IPV and both Haemophilus influenzae type b (Hib) conjugate vaccine and pneumococcal conjugate vaccine. The rates of most solicited local and systemic adverse events, with the exception of fever, after administration of DTaP-HepB-IPV were similar to rates that followed separate administration of US-licensed vaccines. Furthermore, administration of DTaP-HepB-IPV together with Hib vaccine was associated with higher rates of fever in comparison with separately administered vaccines.

The FDA has approved Pediarix for use in infants aged 2, 4, and 6 months. The Advisory Committee on Immunization Practices recommends that for infants who are born to women who are HBsAg-positive or whose HBsAg status is unknown, a birth dose of single-antigen vaccine is preferred, but the birth dose can then be followed by 3 doses of Pediarix at ages 2, 4, and 6 months. The recommended period between doses is 6-8 weeks, preferably 8 weeks, and the third dose of Pediarix should be given at least 16 weeks after the first dose and at least 8 weeks after the second dose but not before age 6 months. It should not be given to any infant aged <6 weeks or any person aged ≥7 years.

**Varicella-zoster virus** (Fig 2). Prior to the widespread availability of varicella vaccine, yearly US figures for varicella disease included approximately 4 million cases, 11,000 hospitalizations, and 100 deaths. The currently available varicella vaccine in the United States is a live attenuated Oka strain vaccine approved in 1995. The vaccine is very safe and effective. Clinical trials began more than 20 years earlier in Japan, after the vaccine was developed by means of attenuation of virus isolated from the vesicular fluid of a healthy boy (with the surname Oka) who had natural varicella infection. These initial studies showed a 90% seroconversion rate 4 weeks after vaccination with few clinical reactions. Follow-up studies showed that the vaccine protected against chickenpox for at least 17 to 19 years, and all of the subjects had persistent antibodies and delayed-type skin reactions to the varicella-zoster antigen. In the United States, a double-blind, placebo-controlled study of the Oka vaccine in 914 children revealed an efficacy of 100% at 9 months. After a 7-year follow-up, 95% of the vaccinated subjects remained free of clinical disease with chickenpox. In comparison with the disease rates of unvaccinated children in the United States, it appears that the Oka vaccine reduces the rate of varicella in children participating in the clinical trials by 65% to 90%.

Additional studies in the United States have shown that the Oka vaccine induces humoral and cell-mediated immunity in healthy children, both of which have been shown to persist for at least 8 years. Delayed-type hypersensitivity skin reactions to varicella-zoster virus antigens have also been shown to occur for at least 10 years after vaccination. Case series have also shown that vaccinated persons have less severe varicella (<50 lesions, no fever, and shorter duration of illness) than those unvaccinated. Recent data indicate that the varicella vaccine effectiveness was >95% for preventing disease and 100% for preventing moderate or severe disease in susceptible contacts when given within 36 hours of exposure.

Studies of adolescents and adults have shown that 2 doses administered 4-8 weeks apart were necessary to produce seroconversion rates and antibody responses similar to those obtained in healthy children. Vaccination is recommended for susceptible adults, particularly those in high-risk situations (eg, health-care personnel). The vaccine is recommended for all children who have no history of chickenpox and is required for all who attend school in most states. Clinical studies of the use of vaccination in immunosuppressed children and adolescents, particularly in those with acute lymphocytic leukemia, have been performed. Results indicated that vaccination is safe for those who are at least 1 year away from induction chemotherapy if the current chemotherapy is halted around the time of vaccination and the patient’s lymphocyte counts are ≥700/mm³. The immune response in these persons is lower than that of healthy recipients, thus requiring the administration of 2 doses separated by 3 months. Transmission of varicella from these vaccinees may occur if a vaccine-associated rash develops, although the risk of transmission is about one fourth that of natural varicella (20%-25% vs 87%).

Another study of hematopoietic-cell transplant recipients, without a history of zoster, showed that zoster developed in only 13% of those who had...
received the inactivated varicella vaccine but in 30% of those who were not vaccinated. These data suggest that the varicella vaccine may boost immunity and prevent zoster.

Waning cellular immunity is strongly correlated with the development of herpes zoster. Levin et al found that the varicella-zoster virus-responding T-cell frequency was still significantly improved over initial baseline measurements, as well as expected measurements for this age cohort. In this vaccinated population, the frequency of herpes zoster was within the range of expected incidents for this age cohort. However, in all of the cases of herpes zoster in the study, the number of lesions was small, the associated pain was minimal, and postherpetic neuralgia did not occur. These findings suggest that vaccination in the elderly may be able to attenuate the course of herpes zoster.

Multiple studies have reported a modified varicella-like syndrome in some vaccinated children after exposure to the natural wild-type varicella virus. The average yearly rate of modified varicella-like syndrome varies from 0 to 2.72% among children who have been vaccinated with the U.S.-licensed Oka strain vaccine. These children typically develop a milder form of disease with fewer than 50 lesions. Most children do not have associated fever, and only 50% of them develop vesicular lesions. None of the cases has been associated with systemic or serious disease. It has been noted that more complete and long-lasting protection from varicella is associated with a stronger antibody response to vaccination.

It is known that immunization with the Oka strain vaccine can lead to latent infection. In children, the incidence of primary varicella is between 18 and 77 per 1 million person years of follow-up. Herpes zoster can develop later, either from this vaccine-type virus or from natural wild-type varicella-zoster virus. There have been several reports of mild herpes zoster in previously healthy children who had received the varicella vaccine. The incidence is less than that seen in children with prior chickenpox, such that vaccinated children may perhaps have a decreased risk for herpes zoster.

The Oka vaccine should be given as a single dose to children 12 months to 12 years of age. Persons over the age of 13 should receive 2 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 for some.

The Oka vaccine is generally well tolerated. In both adults and children, the most common side effect is mild tenderness, erythema, or induration at the injection site (19.3% to 24.4%). Fever occurred in 10.2% to 14.7% of clinical trial subjects, and a generalized varicella-like rash developed in 3.8% to 5.5%. A localized varicella-like rash at the injection site may also occur. The likelihood of transmission of the vaccine virus from a healthy vaccinee is low but may be more likely if a rash develops after vaccination, especially in those who are immunocompromised hosts. One case of transmission from a vaccinated child to a susceptible mother has been reported in the United States, but it is suspected that the child may have been concurrently infected with natural wild-type varicella. Persons who undergo vaccination should avoid close association with susceptible high-risk individuals for up to 6 weeks, if possible. This vaccination is also contraindicated in pregnant women or in any woman planning to become pregnant within 3 months, since vaccination involves use of a live attenuated virus and natural varicella is known to cause fetal harm.

Bacteria

Anthrax. Interest in Bacillus anthracis was raised after the deliberate contamination of mail with spores after the September 11, 2001, attacks on the United States. This aerobic or facultative anaerobic gram-positive rod forms highly resistant endospores under extreme conditions. The spores cause human disease by means of direct contact, ingestion, or inhalation. Cutaneous anthrax occurs when a break in the skin allows spores to enter. The incubation time is usually 2-3 days but can occur as soon as 12 hours or as late as 2 weeks after exposure. Initially, a papule forms, and within 24 hours, a ring of vesicles follows. These lesions ulcerate and become black, necrotic, and edematous. Mortality is about 20% in untreated cases.

The toxins of B anthracis come from 2 genes, pX01 and pX02. The gene pX01 encodes protective antigen (PA), lethal factor, and edema factor, which make up the anthrax toxin. The latter 2 com-
bined with the PA to form lethal toxin and edema toxin. Edema toxin causes unregulated adenylate cyclase activity, which leads to production of unphysiologically high concentrations of cyclic adenosine monophosphate.\textsuperscript{156} This event causes massive edema and organ failure. Plasmid pX02 encodes the poly-y-linked D-glutamic acid (PGA) capsule, which prevents the antiphagocytic activity of macrophages.\textsuperscript{157} Strains of \textit{B. anthracis} that lack pX02 are avirulent.

As early as 1881, Pasteur demonstrated the protective efficacy of the first anthrax vaccine when he injected sheep with heat-attenuated \textit{B. anthracis}. In the 1930s, widespread vaccination with attenuated strains such as the Sterne strain significantly decreased the incidence of anthrax in domesticated animals in industrialized countries. In the United States, the licensed human vaccine (anthrax vaccine adsorbed [AVA], newly renamed BioThrax) is a culture supernatant of a toxigenic, nonencapsulated \textit{B. anthracis} strain, V770-NP1-R, derived from the Sterne strain.\textsuperscript{158} A similar culture supernatant-derived human vaccine (PI 1511/0058) is produced in the United Kingdom.\textsuperscript{159}

A 1950s study of wool sorters immunized with a vaccine similar to AVA, coupled with long experience with AVA and the United Kingdom vaccine, have shown that a critical level of serum antibodies to the PA confers immunity to anthrax.\textsuperscript{160,161} After regular immunizations the previous year, staff of the Government Wool Disinfection Station in Liverpool were free of the disease with a total protective efficacy of 92.5\%.\textsuperscript{162} Laboratory animals and cattle also are protected by AVA from both cutaneous and inhalational \textit{B. anthracis} challenges.\textsuperscript{163,164}

The CDC recommends anthrax vaccinations for persons engaged in the production of quantities of or concentrations of \textit{B. anthracis} cultures, those engaged in activities with a high potential for aerosol exposure, persons in the military, and other select populations for which a calculable risk can be assessed.\textsuperscript{165} Preexposure vaccinations are not recommended for emergency first responders, medical practitioners, and private citizens.

Although efficacious and safe,\textsuperscript{166} AVA has multiple limitations. First, standardization of AVA is based on the protection of guinea pigs challenged intracutaneously with \textit{B. anthracis} spores.\textsuperscript{164} PA is not measured in the vaccine, and there is no standardized assay of PA antibodies in animals or humans vaccinated with AVA. Second, the schedule of AVA administration (subcutaneous injections at 0, 2, and 4 weeks and boosters at 6, 12, and 18 months with recommended annual boosters to maintain immunity) is likely suboptimal. This schedule was designed for rapid induction of immunity,\textsuperscript{167} but a recent study showed that increasing the interval between the first 2 injections enhances the level of AVA-induced antibodies to PA.\textsuperscript{168} Furthermore, a schedule of injections at 6, 12, and 18 months is not well defined. The long-term protective efficacy in human beings is unknown. In animal studies, the protective efficacy may last up to 2 years after 2 inoculations.\textsuperscript{169,170} Finally, AVA contains other cellular elements that contribute to the relatively high rate of local and systemic adverse reactions.\textsuperscript{166} The most common adverse effects included injection-site hypersensitivity, edema, pain, headache, arthralgia, asthenia, and pruritus. In 20% of recipients, mild cutaneous reactions included erythema, edema, and induration. In \(<1\% of recipients, systemic reactions included fever, chills, nausea, and body aches.\textsuperscript{160}

Spores and PGA have also been studied as possible targets for immunization. PA antibodies have been found to have unexpected sporicidal properties in vivo. PA antibodies both enhance phagocytosis of spores by macrophages and suppress germination.\textsuperscript{171} Active immunization of guinea pigs and mice with formalin-inactivated spores also confers immunity to infection.\textsuperscript{172,173}

Encapsulated \textit{B. anthracis} strains are virulent in mice, regardless of whether they produce toxin.\textsuperscript{174} PGA is the major virulence factor in mice, and vaccines based on PA show reduced efficacy in those animals.\textsuperscript{172} It may be easier to demonstrate a role for anti-PA antibodies in protective immunity with the use of mice. However, there is no definitive test of whether anticapsular antibodies contribute to human immunity to \textit{B. anthracis}. Furthermore, it will be difficult to extrapolate conclusions about capsular antibodies from mouse anthrax infection to human anthrax infection, given that the contributions of PGA and PA to pathogenesis differ substantially between mice and humans.

Live vaccines are potential candidates for administration as an oral, single dose. They consist of organisms in which the gene encoding the vaccine has been introduced. After delivery, the microorganism either colonizes the mucosal surface or causes a limited infection during which the vaccine candidate is expressed and presented to the immune system. Two live vector systems have been described for PA.\textsuperscript{173,175} The main disadvantage of live vaccines is the need to culture and store the organism prior to use.

By contrast, DNA vaccines are extremely simple and cost-effective. They can be defined broadly as plasmid DNA expression vectors that result in expression of an antigen in situ leading to the induction of antigen-specific immunity.\textsuperscript{176} DNA-based
vaccines have multiple advantages over conventional vaccines. Unlike subunit vaccines, which require extensive purification, DNA is relatively cheap to produce. The vaccines are heat-stable and are amenable to genetic manipulation. DNA vaccines based on PA have been constructed and have been shown to be capable of inducing PA immune responses and of protecting mice against lethal toxin.178

In the short term, improved anthrax vaccines will focus on PA. The National Institute of Allergy and Infectious Diseases has an accelerated program for vaccine development to make 25 million doses of a recombinant PA vaccine available within 2 years.179

**Lyme disease** (Fig 3). Lyme disease is the most commonly diagnosed vector-borne disease in the United States. Since 1982 the annual incidence of Lyme disease has increased more than 25-fold.180 Between 1993 and 1997, an average of 12,451 new cases were reported each year to the CDC. Lyme disease is a tick-borne zoonosis caused by an infection with the spirochete *Borrelia burgdorferi*.

*B. burgdorferi* has several outer surface proteins. In naturally acquired infections, animals and humans mount an immune response against the spirochete’s flagellin (41 kd) and the 23-kd outer-surface protein (OspC).181 However, in culture, the dominant outer-surface protein expressed is OspA, a 31-kd lipoprotein.182,183 Experiments have shown that OspA is expressed abundantly by *B. burgdorferi* in the midgut of infected ticks.185 When the tick attaches itself to a host, the spirochetes undergo a substantial antigenic change during their migration to the tick’s salivary gland, where OspA expression is decreased and OspC expression is increased.184,185 This temperature-sensitive ability is likely an acquired protective mechanism that enables *B. burgdorferi* to become established in warm-blooded animals. OspA is uniform across different isolates in the United States, but OspC is highly variable among different regions. An immune response directed against OspC after Lyme disease is unlikely to protect against a heterologous infection. However, cross-strain protection would be expected with an immune response directed against the spirochete’s OspA in the tick gut.186

In 1990, a mouse model of Lyme disease was developed. Shortly afterward, a recombinant OspA (rOspA) vaccine was shown to be protective against syringe-inoculated infection.187,188 Both passive immunization and active immunization were shown to be protective. The rOspA vaccine was then shown to be protective against naturally occurring tick-transmitted infection.189-191 The effectiveness of rOspA vaccine initially was surprising because organisms isolated from infected animals do not express OspA until naturally occurring late infections. It was discovered that the vaccine acts primarily in the tick midgut rather than in the vaccinated host.192 As a feeding tick ingests a blood meal, the ingested blood has circulating anti-OspA antibodies that destroy *B. burgdorferi* in the midgut of the tick. This finding is significant because it suggests that a high level of circulating antibodies has to be maintained in the host to protect against infection at the time of a bite by an infected tick.

The rOspA vaccine was also shown to be effective in protecting dogs and rhesus monkeys against Lyme disease.193,194 On the basis of these promising results, human immunogenicity, efficacy, and safety trials were performed to evaluate recombinant, lipitated OspA vaccine (LYMErix) of *B burgdorferi sensu stricto*. The rOspA protein is expressed in *Escherichia coli* and purified. LYMErix was found to be immunogenic and safe in phases I and II clinical trials.195,196 In a randomized, controlled clinical phase III trial,197 10,936 people were recruited from 31 sites in Lyme-endemic areas. Each subject received 3 doses of vaccine (30 μg) or placebo on a 0-, 1-, and 12-month schedule and timed so that each subject received the initial vaccination several weeks before *B burgdorferi* transmission season. These subjects were followed for clinical manifestations of Lyme disease or adverse events for 20 months.

Vaccine efficacy in protecting against symptomatic infection (ie, the presence of erythema migrans or objective neurologic, musculoskeletal, or cardiovascular manifestations) was 49% after 2 doses and 76% after 3 doses. The efficacy in protecting against asymptomatic infection (no recognized symptoms) was 83% after 2 doses and 100% after 3 doses. All subjects had laboratory-confirmed infection by means of cultural isolation, polymerase chain reaction positivity, or seroconversion. A subset of subjects had serologic testing for immunogenicity to correlate to protection. Protective immunity

Fig 3. Erythema migrans of the inguinal region, a symptom of Lyme disease.
was achieved after 2 doses, but antibody levels declined rapidly, resulting in protective levels for less than a full year. With 3 doses, a significant anamnestic response developed with maintenance of protective antibody levels throughout the following exposure season.

The most common adverse event was soreness at the injection site (24.1% of vaccine recipients versus 7.6% of placebo recipients). Other side effects included arthralgia, myalgias, flulike symptoms, and fever and chills, but all were uncommon (less than 3.2%). The vaccine was found to be safe in subjects with a history of Lyme disease. However, there was a greater incidence of musculoskeletal symptoms within the first 30 days after vaccination in subjects previously afflicted with Lyme disease than in subjects without Lyme disease (20% versus 13%).\(^{198}\) After the first 30 days since vaccination, there was no significant difference between vaccine and placebo recipients with a history of Lyme disease (33% versus 35%).\(^{197}\)

The Pasteur Merieux Connaught vaccine, Imu-Lyme, was studied in a similar phase III trial involving 10,305 adult subjects.\(^{199}\) The immunogen is also recombinant OspA lipoprotein, but unlike the LYMErix vaccine, it does not have an aluminum adjuvant. Subjects received 3 doses of 30 \(\mu\)g of vaccine or placebo on a 0-, 1-, and 12-month schedule. At 12 months, 7,515 subjects received booster doses. The primary end point was the total number of new clinical cases of Lyme disease, confirmed by means of serology. The efficacy of vaccine was 68% after 2 doses of vaccine in the first year of study and 92% in those who received the third dose of vaccine in the second year. Local injection site reactions were more common in the vaccine group during the first week; otherwise, there was no difference between the vaccine and placebo groups in the occurrence of late adverse reactions.

An analysis of the cost per quality-adjusted life-year gains with vaccination showed use of the Lyme vaccine to be economically attractive only in persons whose seasonal risk for Lyme disease exceeds 1%.\(^{200}\) In areas with a seasonal attack rate of 0.5%, the cost per quality-adjusted life-year gained was $145,200. Areas with annual attack rates of 2.5% to 10% have been reported,\(^{201}\) but large endemic areas, such as those in which the 2 large Lyme disease vaccine trials were done, had annual attack rates of about 1%. In 1998, the LYMErix vaccine was approved by the FDA for use in persons aged 15 to 70 years on a 0-, 1-, and 12-month schedule. The CDC recommends use of the Lyme disease vaccine in persons aged 15-70 years who reside, work, or play in areas of high or moderate risk and in travelers to areas of high or moderate risk with prolonged and frequent exposures to infected ticks.

Several specific issues surround the safety of Lyme vaccines.\(^{202}\) The first is a theoretical concern that OspA-based vaccine could induce inflammatory arthritis in genetically susceptible persons through an autoimmune mechanism. The second issue is the question of cross-strain protection; will the current vaccine protect against all strains of \(B\) burgdorferi in the United States? The third is concern that booster doses will be required to maintain adequate circulating antibody levels, but the need for and safety of booster doses is not known. The fourth issue is whether the vaccine might alter the clinical course of infection if antibody levels fall below the level needed for complete protection. Another concern is whether alternative vaccine schedules might allow the development of protective immunity in less than 1 year. The final concern is the lack of information about the safety and immunogenicity of the vaccine in children.

Although the vaccine appeared safe and effective, it was taken off the market in early 2003 because of a lack of general use in the medical community.

**Haemophilus influenzae.** The early 1980s saw the introduction of the first vaccine for Hib, which consisted of polyribosyl ribitol phosphate (PRP), the capsular polysaccharide of Hib. A large field trial performed in Finland showed that antibody response to plain PRP vaccine correlated with prevention of invasive Hib disease.\(^{203}\) One month after vaccination, an antibody concentration of 1 g/mL in serum was associated with long-term protection from disease.\(^{204}\)

In December 1987, the first Hib conjugate vaccine, PRP-D (PRP conjugated to diphtheria toxoid; ProHIBIT, Aventis Pasteur) was licensed in the United States. The PRP-D vaccine was found to prevent Hib disease in Finnish infants immunized during the first 6 months of life.\(^{205}\)\(^{206}\) However, a study of native Alaskan infants immunized at 2, 4, and 6 months showed no significant protection,\(^{207}\) and the FDA rejected the application to license PRP-D for use in infants.\(^{208}\) In 1987 the FDA approved PRP-D for use only in children older than 18 months.\(^{209}\)

Two subsequent Hib conjugate vaccines were licensed in the United States for use with infants beginning at 2 months of age: HibOC (Hib [PRP] oligosaccharides conjugated to CRM,\(^{197}\) a cross-reacting, mutant, nontoxic diphtheria toxin; HibTITER, Wyeth Laboratories) and PRP-OMP (PRP conjugated to an outer-membrane protein complex; PedvaxHib, Merck Sharp and Dohme).\(^{210}\)\(^{211}\) Three randomized,
placebo-controlled trials conducted in infants immunized beginning at 2 months of age showed the efficacy of these vaccines. Further, these 2 vaccines were found to be significantly more immunogenic in infants than PRP-D.211,215,216

In 1993, the FDA approved 2 more Hib conjugate vaccines, PRP-T (PRP conjugated to tetanus toxoid) vaccine (ActHIB, Aventis Pasteur) and a combined diphtheria and tetanus toxoids and whole-cell pertussis vaccine (DTP) and Haemophilus b conjugate vaccine (Tetramune, Lederle-Praxis Biologicals). Two studies showed that PRP-T has an immunogenicity profile similar to that of HbOC vaccine in infants immunized at 2, 4, and 6 months of age. The DTP-HbOC vaccine was also found to be safe and more immunogenic than separate vaccination with DTP and HbOC.220,221

Before Hib conjugate vaccines became available, Hib was the principal cause of bacterial meningitis and a major cause of other serious invasive diseases among children aged <5 years in the United States. In the prevaccine era, the incidence of Hib invasive disease was 100 per 100,000 among children aged <5 years. In 1991 all infants at age 2 months were recommended to receive Hib conjugate vaccines, and by 1996 the incidence of Hib invasive disease among children aged <5 years had declined by more than 99%. During 1998-2000, a total of 824 Hib invasive disease cases was reported among children younger than 5 years, and rates were 1.4 per 100,000 children in 1998 and 1999 and 1.6 in 2000. The Hib conjugate vaccines have been equally efficacious in the rest of the world, as well.226,227

Neisseria meningitidis (Fig 4). The important determinant of virulence of Neisseria meningitidis is the polysaccharide capsule. Mutants without capsular expression are nonpathogenic, since they are killed by complement. There are licensed polysaccharide vaccines against serogroups A, C, Y, and W135, but there currently is none against serogroup B.

Thirty years ago, the first successful capsular polysaccharide vaccines against groups A and C were developed for epidemics of meningitis among U.S. military recruits. Studies performed in the United States, Africa, and Europe showed that these vaccines were safe and induced bactericidal antibodies in older children and adults. However, most polysaccharide vaccines are poor immunogens in infants and do not elicit immunologic memory in people of any age. Group A polysaccharide is immunogenic in children <2 years of age, but protective immunity after 1 dose lasts a short duration, and even after repeated injections, booster responses are low. Furthermore, repeated vaccination with group C polysaccharide vaccine causes immune hyporesponsiveness, which may cause difficulties for patients who need long-term protection.

As with Hib vaccines, meningococcal conjugate vaccines were developed and found to induce long-term protection and to be safe and immunogenic in young infants. In November 1999, meningococcal group C conjugate vaccine entered routine immunization in the United Kingdom. Infants received 3 doses of vaccine at 2, 3, and 4 months of age as their routine primary immunizations. Also, a catch-up campaign between November 1999 and December 2000 was targeted at all children of >4 months and <18 years of age. Prospective nationwide surveillance showed that confirmed cases of invasive group C disease fell overall by 85%, in both the primary immunized and the catch-up populations. However, a concern about mass immunization with meningococcal group C conjugate vaccine is capsular group replacement by strains not contained in the vaccine. Capsule switching between B and C strains has been documented. However, careful and extensive surveillance in the meningococcal group C conjugate vaccine study in the United Kingdom showed no evidence of capsular group replacement.

Multiple clinical trials have shown that bivalent A plus C polysaccharide conjugate vaccines are well tolerated and immunogenic in infants, toddlers, and adults. The currently available formulation in the United States is the tetravalent A, C, Y, W-135 vaccine (Menomune-A, C, Y, W-135; Aventis Pasteur). It is safe and has an efficacy of 85%, and it could prevent more than 60% of cases in college students. The current recommendation for the tetravalent vaccine is selective immunization based on risk factors for meningococcal disease, instead of universal immunization in the pediatric population.
INVESTIGATIONAL VACCINES

Many of the vaccines currently under investigation more than likely will expand the focus of immunization. All the vaccines available up to the end of the 20th century have been used solely to prevent disease. However, several new candidate vaccines are being developed and evaluated in the treatment of already acquired infections. Table IV lists the predominant diseases for which candidate vaccines are presently under investigation.

Viruses

HIV. As the AIDS epidemic persists and spreads unabated in much of the world, the search for an effective HIV vaccine is becoming critical. In 1997 President Clinton challenged scientists to develop an effective HIV vaccine by the year 2007. Since clinical trials first began in 1987, at least 34 different HIV candidate vaccines have begun phase I trials, and a handful of these have progressed to phase II trials.254 Seventy-four additional HIV vaccine candidates are reported to be in research and development or preclinical testing in animals, and this number probably has increased.254 The predominant types of HIV vaccines under investigation are listed in Table V. Thus far, only one AIDS vaccine had advanced to phase III trials, the VaxGen AIDS vaccine AIDSVax, which was evaluated in the United States and in Thailand. Earlier research suggests that this recombinant gp120 vaccine stimulates antibody production but may not induce cellular immunity. HIV research has shown that the induction of cytotoxic T lymphocytes may be an important correlate for protective efficacy of HIV vaccines.255 However, the recombinant gp120 HIV vaccine failed. In the 3-year study, of 5,009 volunteers at 59 trial sites in the United States, Canada, the Netherlands, and Puerto Rico, 5.7% of those who were vaccinated developed HIV compared with 5.8% of those given placebo.256

Recombinant subunit HIV vaccines are genetically engineered from HIV surface envelope proteins, such as gp120 or gp160. Because they do not contain live virus or DNA, there is no risk of causing infection. A therapeutic trial was carried out with gp160 subunit immunization every 3 months for 3 years in HIV-positive persons, in addition to antiretroviral therapy.257 Results demonstrated a modest effect on CD4 counts but no clinical benefit. These results were consistent with similar, earlier studies.258,259

Recombinant live-virus vector vaccines involve the use of virus carriers that are genetically engineered to express particular HIV genes. The first candidate to be tested was a vaccinia vector with the insertion of HIV gp160 gene. The vaccine alone induced little antibody.254 However, when it was used as a primer and boosted with the recombinant gp160 vaccine, results showed strong induction of cellular immunity and antibody responses.260 Phase I trials are under way for a recombinant vaccinia HIV primer followed by boosting with a recombinant gp120 vaccine.254

Because of concerns over shedding of the vaccinia virus and possible disseminated disease in immunosuppressed persons, more attention has been focused on canarypox and adenovirus vectors, which can infect humans but cannot replicate. Replication in humans continues long enough to produce the necessary HIV proteins before abortion of the cycle.261 Early results have shown that recombinant canarypox vector vaccines can induce humoral and cellular immune responses, including cytotoxic lymphocytes.262 The greatest interest for these vaccine candidates lies in the prime and boost approach. The canarypox vaccine primer induces a

Table IV. Infectious diseases with cutaneous manifestations for which vaccines are under development

<table>
<thead>
<tr>
<th>Prophylactic</th>
<th>Therapeutic</th>
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<tbody>
<tr>
<td>HIV</td>
<td>HIV</td>
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<tr>
<td>HSV</td>
<td>HSV</td>
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<tr>
<td>HPV</td>
<td>HPV</td>
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<tr>
<td>Cytomegalovirus</td>
<td>Varicella-zoster virus</td>
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<tr>
<td>Respiratory syncytial virus</td>
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<tr>
<td>Parainfluenza virus</td>
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<tr>
<td>Hepatitis C</td>
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<tr>
<td>Dengue fever</td>
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<tr>
<td>Epstein-Barr virus</td>
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<tr>
<td>Anthrax</td>
<td></td>
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<tr>
<td>Lyme disease</td>
<td></td>
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<tr>
<td>Neisseria meningitidis serotype B</td>
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<tr>
<td>Nontypeable Haemophilus influenzae</td>
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<tr>
<td>Rocky Mountain spotted fever</td>
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<tr>
<td>Staphylococcus aureus</td>
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<tr>
<td>Staphylococcus epidermidis</td>
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<tr>
<td>Streptococcus pyogenes</td>
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<td>Pseudomonas aeruginosa</td>
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<tr>
<td>Francisella tularensis</td>
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<tr>
<td>Brucella melitensis</td>
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<tr>
<td>Leptospira interrogans</td>
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<tr>
<td>Candida species</td>
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<tr>
<td>Aspergillus species</td>
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<tr>
<td>Cryptococcus neoformans</td>
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<tr>
<td>Coccioides immitis</td>
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<tr>
<td>Blastomyces dermatitidis</td>
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<tr>
<td>Histoplasma capsulatum</td>
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<tr>
<td>Leishmania</td>
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</tbody>
</table>
Table V. Predominant types of HIV vaccines under investigation

| DNA plasmid: gp120, gp160, GagPol, Pol, techniques such as multispliclads, interleukin-2 adjuvants, genetic adjuvants, conventional adjuvants, DNA priming, microspheres, and in vivo electroporation |
| Epitopes: CTL or HTL |
| Live attenuated virus: HIV, simian-human immunodeficiency virus |
| Peptides: synthetic V3-peptides, Tat, lipopeptides |
| Recombinant live vector: adeno-associated viruses, replication-defective adenovirus serotype 5 vector, alphaviruses, canarypox and other avian and mammalian poxviruses, herpes simplex virus type-1, picornaviruses, rhabdoviruses, vaccinia, modified vaccinia virus Ankara vector, vesicular stomatitis virus, bacterial vectors |
| Recombinant subunit: rgp120, rgp160, Env |
| Retroviral vectors: recombinant murine retrovirus |
| RNA optimized constructs: pGag, pEnv |
| Self-replicating RNA: replicon particles based on Venezuelan equine encephalitis virus |
| Virus-like particles (VLP): p170/p24:Ty VLP |
| Whole inactivated virus: envelope depleted whole inactivated HIV-1 |

CTL, Cytotoxic T lymphocyte; HTL, helper T lymphocyte.

strong cellular immunity, followed by a recombinant subunit vaccine that boosts the antibody response. The combination of both vaccines induces a stronger immune response than either one alone. Recent results from a phase II trial showed that 93% of subjects who received the combination of vaccines developed neutralizing antibodies. Also, almost one-third of the recipients developed a cytotoxic lymphocyte response. Additional studies have investigated canarypox vectors expressing gp160 or gp120/gag/pol HIV-1 antigens given along with recombinant gp160 or gp120 subunit vaccines. In a comparison of data from different trials of several candidate canarypox vector HIV vaccines, more than half of the recipients developed durable, HIV-specific cytotoxic T-lymphocyte responses. Researchers suggest that a broader recombinant vector vaccine would probably increase the percentage of responders.

A trial in Uganda of the effect of a canarypox vector vaccine alone commenced in February 1999. The vaccine, called Alvac vCP205, contains 3 HIV genes in a weakened version of canarypox virus. The particular genes come from clade B viruses, which are the predominant subtype of HIV found in the United States and Europe. However, the majority of HIV infections that occur in Uganda are due to clades A and D. This study will first evaluate the cross-reactivity among these viral sub-units and compare the immune responses in recipients.

Use of the vesicular stomatitis virus as a vector to express HIV protein has also shown promise. One study involved vaccination of rhesus monkeys with live attenuated vesicular stomatitis virus expressing Env and Gag, and the animals were challenged with simian-human immunodeficiency virus. Two years after the challenge, none of the animals has developed AIDS and none have detectable virus. Another study involved evaluation of immune responses of animals vaccinated either intranasally or intramuscularly with the vesicular stomatitis virus vector expressing Env and Gag. After a challenge with simian and human immunodeficiency virus, the animals vaccinated intranasally had a slight loss of CD4 T lymphocytes. However, the animals vaccinated intramuscularly had a significant decrease in CD4 T lymphocytes, which returned to 70% of baseline. The virus load was undetectable in both groups.

DNA vaccines are another promising prospect for HIV immunization. With this approach, purified DNA that encodes for particular immunogenic antigens is injected. These antigens are presented to the host immune system in their native form and are processed in a way similar to that for a natural viral infection. Therapeutic immunization with a plasmid/gp160 and gag+pol DNA vaccine in HIV-positive chimpanzees revealed a significant decrease in viral load and a boost in the immune response. Studies in seronegative primates demonstrated the induction of neutralizing antibodies and cytotoxic T-lymphocyte responses, but the vaccine did not protect against infection. A phase I clinical trial of 2 DNA vaccine candidates is currently in progress.

Several other approaches to HIV vaccine development are under investigation. Live attenuated virus vaccines are known to generate a broad and durable immune response, but they have not been tested in humans owing to potential safety concerns with live HIV virus. Whole-inactivated vaccines are generally thought to be safer than live attenuated ones. However, inactivation of the virus often leads to a vaccine that is less potent or immunogenic. Studies of whole-killed virus vaccines in chimpanzees thus far have not been able to demonstrate protection from HIV infection. In addition, there is concern that inadvertently incomplete inactivation could lead to HIV infection of vaccine recipients. Virus-like particles (VLP) are a safer option, since they consist of a noninfectious HIV “look-alike” that does not contain the HIV genome. One such candidate, known as p17/p24:TYP, has reached the stage...
of clinical trials. Early results have shown that this vaccine leads to low levels of HIV binding antibodies and T-cell memory responses but induces very little cytotoxic T-lymphocyte activity.\textsuperscript{254} Other VLP candidates are under development. Among the many important controversies that exist in HIV vaccine development is whether neutralizing antibodies as typically measured are relevant to clinical protection.

**HSV** (Fig 5). The search for a vaccine for herpes simplex virus (HSV 1 and 2) spans 9 decades. In the 1920s, untreated vesicular fluid from herpes lesions was injected into patients in an attempt to induce immunity.\textsuperscript{254} This method, to say the least, did not withstand the test of time. Inactivated whole virus vaccines were developed in the 1930s and were made from HSV-infected animal tissue, such as rabbit brain.\textsuperscript{271} Despite the many advances made with inactivated virus vaccines through the years, none of the candidates proved to be sufficiently immunogenic. With increasing technology, several different approaches for HSV vaccines are currently in development and under evaluation.

Two separate recombinant subunit vaccines have been investigated in phase III trials. One such candidate developed by Chiron contained HSV-2 surface glycoproteins B and D and the adjuvant MF59. The development of this vaccine was halted prematurely because results demonstrated overall lack of efficacy for both preventive and therapeutic use.\textsuperscript{272,273} A second recombinant vaccine was developed by SmithKline Beecham and contains the glycoprotein D and the adjuvant monophosphoryl lipid A immunostimulant.\textsuperscript{274} Results of clinical trials with this candidate indicate that it has a clinical efficacy of 73% in protecting women who are serologically negative for both HSV-1 and HSV-2 from acquiring HSV-2 disease.\textsuperscript{275} Another approach combines the safety profile of a killed vaccine with the immunogenic potential of a live virus vaccine.\textsuperscript{276} The disabled infectious single-cycle vaccine lacks the glycoprotein H gene necessary for virus entry into cells. After a single replication cycle, the virus is unable to spread to surrounding cells and thus remains non-infectious. Studies in guinea pigs showed encouraging results for both preventive and therapeutic treatment.\textsuperscript{276,277} Although phase I studies showed the candidate to be safe and well tolerated, phase II trials showed that the vaccine failed as therapy. Trials are also planned to evaluate the efficacy in preventing infection in seronegative partners in discordant couples.\textsuperscript{254}

DNA vaccines are also in development for HSV immunization. Animal studies that involve inoculations of plasmid DNA carrying the desired viral genes have shown promising results for the prevention of infection.\textsuperscript{278,279} These vaccines are able to express only 1 or 2 viral antigens but can induce cell-mediated immunity without the need for potent adjuvants. One such candidate that encodes glycoprotein D2 is currently in phase I clinical trials, and several others are in preclinical development.

Live attenuated HSV vaccines have been rather difficult to develop, as viruses that are the safest and most attenuated tend to lack immunogenicity. Research in the past has shown that stable attenuation of HSV was not achieved after passage in cell culture. After immunization, the vaccine strain would then have the potential to revert to its virulent state and cause disease. A genetically engineered HSV mutant vaccine was found to be safe and effective in animal studies\textsuperscript{280} but in humans was overly attenuated and lacked sufficient immunogenicity.\textsuperscript{281} New genetically engineered strains are currently under development.

**HPV** (Fig 6). Certain types of HPV are associated with the development of cervical cancer. Thus the search for a prophylactic or therapeutic HPV vaccine has been an important endeavor. Although more than 30 types of HPV are known to be sexually transmissible, the major types associated with malignancy (16, 18, 31, 33, 45, 52, 58) and condylomata (6 and 11) are relatively few in number, allowing for more focused strategies for immunization against these specific types. Vaccine development has been
hampered in the past because of the inability to culture HPV. However, an in vitro culture system for HPV has recently been developed, furthering the prospect for advances in this field.254

VLP are produced by means of recombinant DNA technology and are designed to self-assemble into conformations that resemble natural HPV. These vaccines contain no viral DNA and thus carry no risk of infection or oncogenic exposure. VLP have been designed for all of the major HPV subtypes, and clinical trials are currently under way for HPV-11 L1 VLP,282 HPV-6 L1 VLP,283 and HPV-16 L1 VLP.284 A recent double-blind, placebo-controlled study showed that persons who were HPV-16 negative and received the HPV-16 vaccine had a significantly reduced incidence of both HPV-16 infections and related cervical intraepithelial neoplasia at a median follow-up of 17 months.285

Fusion protein vaccines are currently under evaluation for the immunotherapy of cervical cancer and genital warts. TA-HPV is a live recombinant vaccinia virus that has been engineered to express the E6 and E7 protein genes for HPV 16 and 18 as a treatment for cervical cancer.254 This method also involves utilization of the viral vector approach, with vaccinia as a vehicle. Viral vector vaccines can be polyvalent and have the potential to produce immunity similar to that induced by live attenuated vaccines. A phase I-II clinical trial of TA-HPV286 has shown encouraging results, and further studies are under way. TA-GW is a recombinant fusion protein vaccine consisting of HPV-6 L2 and E7 proteins and is under investigation for the treatment of genital warts. A phase Ia clinical trial showed the vaccine to be immunogenic, with encouraging clinical responses.287 A third protein vaccine, TA-CIN, is in preclinical development for the treatment of cervical dysplasia.254

Peptide-based vaccines have been shown to be protective against HPV-induced tumors in mice, although the T-cell repertoires in mice and humans differ. Two early-stage human clinical trials are under way, one involving HLA-A*0201 binding HPV16-E7 peptides, to assess the possible therapeutic implications these vaccines may offer.157 Other investigational approaches to HPV immunization include DNA vaccines,288 bacterial vectors,289-291 and dendritic cells pulsed with HPV epitopes.292

In a study, the cost-effectiveness of vaccinating adolescent girls who are at high risk of HPV infection was evaluated.293 Relative to current practice, a vaccine with a 75% probability of immunity against HPV infection resulted in a life-expectancy gain of 2.8 days or 4.0 quality-adjusted days at a cost of $246 (incremental cost-effectiveness of $22,755 per quality-adjusted life year). If all 12-year-old girls in the United States (approximately at 1,988,600) were vaccinated, more than 224,255 cases of HPV, 3,317 cases of cervical cancer, and 1,340 deaths from cervical cancer would be prevented.

Fungi

The idea of an antifungal vaccine has been investigated for more than 40 years.294 Development of an effective fungal vaccine is difficult because fungi, like other eukaryotic organisms, are orders of magnitude more complex than bacteria or viruses.295 The main obstacle of vaccine development is the isolation of the smallest possible immunogenic substance from multiple proteins and carbohydrates that safely confers lasting immunity against infection. A second major challenge is the lack of scientific knowledge about natural fungal immunity and the relative contributions of the innate, acquired humoral and cellular systems.296,297 Currently, there is no FDA-approved fungal vaccine for clinical use.

Attenuated low-virulence strains of Candida spp have been used in experimental animal models with some success.298-300 Other preparations, such as inactivated whole cells of the fungus, secretory and cell surface–located molecules, and major cytoplasmic and cell wall enzymes, have been used with varying levels of success.301-304

The challenge in developing a candida vaccine is to purify antigens associated with invasive forms, not colonized forms. In one report, heat-killed wild-type Candida albicans was combined with a novel adjuvant from a Vibrio cholerae enterotoxin to augment the immune response.305 The vaccine was given intranasally to mice, and there was a response of induced cellular and humoral immunity by antibody titers and a footpad delayed hypersensitivity in response to cutaneous antigen inoculation. There was a 90% survival rate among vaccinated mice, compared with 0 among the control animals, when they were challenged with a moderate inoculum of live, virulent organisms.
In another trial, researchers examined the immunogenicity of a specific candida antigen, mannan, which is a carbohydrate associated with the cell wall during adhesion to host macrophages. Poorly immunogenic by itself, when encapsulated within a liposome, it is effective. It has a short shelf life and requires multiple dosing. The same researchers conjugated mannan to bovine albumin and with 2 doses of vaccine, and survival improved from 0 at 20 days in controls to 80% at 65 days in vaccinated animals. The vaccine also showed transferable protective immunity to naive animals. The same researchers conjugated mannan to bovine albumin and with 2 doses of vaccine, and survival improved from 0 at 20 days in controls to 80% at 65 days in vaccinated animals. The vaccine also showed transferable protective immunity to naive animals.

A conjugate vaccine of cryptococcal capsular glucuronoxylomannan covalently linked to tetanus toxoid confers 70%-80% protection in mice by stimulating both cellular and humoral immunity. Passive immunization with monoclonal antibodies against Cryptococcus neoformans has been successfully employed in a murine model of disseminated disease.

A new strategy is the use of vaccines to neutralize fungal virulence factors. Fungal phospholipases have been found to be important mediators of fungal infection. β-Blockers, which have antiphospholipase activity, have been used successfully in combination with fluconazole to increase survival in mice with disseminated candidiasis.

Protozoa

Leishmaniasis (Fig 7). The World Health Organization considers leishmaniasis to be one of the most significant parasitic diseases, with about 350 million people at risk of contracting it. The best option in controlling leishmaniasis is considered to be vaccination. Numerous antigens have been tested with variable success against cutaneous leishmaniasis in in vitro and mouse models, but there currently are no commercially available effective vaccines. The general opinion is that a vaccine against leishmaniasis is feasible. Vaccines have the additional advantage of avoiding the problems of drug resistance, which has been an increasing problem in the control of leishmaniasis.

Since cured individuals are protected from further disease, vaccine development mainly has been focused on cutaneous leishmaniasis. The development of a Leishmania vaccine has been hampered by several factors, including the antigenic diversity, the complex life cycle, the use of experimental animal models not representative of human disease, and the lack of sufficient financial support to develop a vaccine for the population of third-world nations.

Recently, the UNDP/World Bank/WHO Special Programme for Research and Training in Tropical Diseases has identified several promising vaccine candidates, including those based on recombinant antigens and whole-cell vaccines. These approaches have shown promise in preclinical studies and are being further evaluated in human trials.

### Table VI. Immunoglobulins: indications for administration

<table>
<thead>
<tr>
<th>Infectious Disease</th>
<th>Approved Indication</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cytomegalovirus</td>
<td>Cytomegalovirus prophylaxis in seronegative renal transplant recipients of a kidney from a cytomegalovirus-positive donor</td>
</tr>
<tr>
<td>Hepatitis A</td>
<td>Within 2 weeks of exposure in susceptible persons</td>
</tr>
<tr>
<td>Hepatitis B</td>
<td>Hepatitis B exposure in susceptible persons, first dose as soon as possible and the second within a month</td>
</tr>
<tr>
<td>Measles</td>
<td>Within 6 days of exposure in susceptible persons</td>
</tr>
<tr>
<td>Rabies</td>
<td>Rabies exposure in previously unvaccinated persons</td>
</tr>
<tr>
<td>Respiratory syncytial virus</td>
<td>Prophylaxis in high-risk infants (e.g., those with bronchopulmonary dysplasia, prematurity, or chronic lung disease)</td>
</tr>
<tr>
<td>Tetanus</td>
<td>Single dose after exposure; some recommend an injection around the site of infection</td>
</tr>
<tr>
<td>Vaccinia</td>
<td>For complications of vaccinia vaccination</td>
</tr>
<tr>
<td>Varicella</td>
<td>Within 96 hours of exposure for susceptible persons exposed to varicella who have a high risk for complications (e.g., immunocompromised patients and neonates)</td>
</tr>
<tr>
<td>Other uses for intravenous immunoglobulin</td>
<td>Primary immunodeficiencies (ataxia telangiectasia, agammaglobulinemia, common variable immunodeficiency, severe combined immunodeficiency, etc.), Guillain-Barré syndrome with ascending paralysis, immune thrombocytopenic purpura, bone marrow transplantation, Kawasaki disease, chronic B-cell lymphocytic leukemia, and in children with AIDS who are hypogammaglobulinemic or have had 2 or more serious bacterial infections, parvovirus B19 infection, failure to make specific antibodies after immunization, or enterovirus infection</td>
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Diseases has organized a comparative study of several leading recombinant antigens to test candidate antigens as potential vaccine candidates. The study involved use of recombinant antigens from Leishmania major, L braziliensis, and L mexicana expressed in Escherichia coli, either as a single recombinant protein or as a mixture. The trial consisted of testing the antigens for human peripheral blood mononuclear cell proliferation and conducting challenge experiments in immunized mice.

Experiments of peripheral blood mononuclear cell stimulation showed that most antigens induced some interferon-γ production and proliferative responses. This finding suggests that the selected antigens have some degree of immunogenicity in humans. However, none of the antigens induced significant protection in the mouse challenge experiments. Because of these conflicting results, it is difficult to draw any definitive conclusions. The usefulness of these antigens as a human vaccine still remains unknown.

DNA vaccination may be a new approach where conventional vaccines have failed. In DNA vaccination, plasmid DNA containing the gene that encodes the vaccine candidate is introduced into the tissue through intramuscular injection or particle bombardment. The DNA is taken up by cells and translocated to the nucleus, where it is transcribed into RNA and then translated in the cytoplasm. Although the uptake and expression in DNA are extremely low, the antigen is expressed in enough quantity to induce a specific and potent immune response and to confer future protection.

The effectiveness of DNA vaccines against infectious diseases has been demonstrated in several animal models, including a murine model for leishmaniasis in which a GP63-based DNA vaccine was studied. Vaccination with DNA encoding Leishmania homologue of receptors for activated C kinase (LACK) conferred protection to mice against infection with L major. Although the amino acid sequence of the LACK antigen was highly conserved, the efficacy of this vaccine antigen against strains other than L major could not be shown. DNA vaccination with a L donovani LACK DNA vaccine induced a strong parasite-specific T cell immune response (interferon-γ but not interleukin-4 production) and primed for an in vivo T-cell response to inoculated parasites in one study. However, it did not induce protection against cutaneous or systemic L donovani challenge in mice.

DNA vaccination holds enormous promise for the future of vaccine development against infectious agents, especially in developing countries. Such vaccines are attractive because they ensure correct folding of the antigen, generate appropriate immune responses, are simple to produce, and do not require adjuvants, all of which may make them stable and affordable. DNA vaccines are also most likely to confer long-term cellular immunity. Before DNA vaccines can be studied in clinical trials, it is important that safety issues, such as the induction of autoimmune disease and the integration of the vaccine DNA in the human genome, be appropriately addressed.

Traditional molecular biologic methods work on one gene at a time. Because of the limited output of such methods, the whole picture of gene function is difficult to obtain. With the recently developed microarray technology, DNA molecules representing many genes are placed in thousands of discrete spots on a microscope slide. This technique allows scientists to look at many genes at once and to determine which ones are expressed in a particular cell type.

In 1994 the Leishmania Genome Network was launched in Rio de Janeiro, Brazil, and was aimed at sequencing the genome of the reference strain Leishmania major Friedlin. It is expected that microarray technology, together with the Leishmania genome project, will allow the identification of many new drug and vaccine targets. From initial studies, more than 100 unknown genes were identified and are now being tested as new vaccine candidates.

**IMMUNOGLOBULINS**

Table VI lists the current immunoglobulins that are used for infectious diseases. Immunoglobulins are proteins made by B lymphocytes and plasma cells as part of the humoral portion of the immune system. Commercial sterile preparations are made from pooled human plasma of several thousand donors and consist of purified immunoglobulin G along with small amounts of other globulins. These
preparations were first used to treat immune deficiency diseases in 1952, with the discovery of Bruton’s agammaglobulinemia. Early preparations were associated with frequent side effects when they were given intravenously and thus required frequent and painful intramuscular administration. In 1981 an improved preparation of immunoglobulin was licensed for intravenous use (IVIG). FDA-approved indications include the following 6 conditions: primary immunodeficiencies, immune-mediated thrombocytopenia, Kawasaki disease, recent bone marrow transplantation (in persons at least 20 years of age), chronic B-cell lymphocytic leukemia, and pediatric HIV-1 infection. IVIG is also used in clinical practice for numerous other conditions, such as multiple sclerosis. Intravenous preparations are manufactured by several different companies, and owing to differences in the production process and donor populations, the products available may vary considerably.

The intramuscular form of immunoglobulin is still available and is approved for the prophylaxis of hepatitis A and measles. Several immunoglobulin preparations with high titers to individual viruses are also available for the prophylaxis of specific viral infections. These hyperimmune globulins are available for rabies, varicella, cytomegalovirus, respiratory syncytial virus, and hepatitis B.

IVIG has also been shown to be of potential benefit for HIV-infected children. This population frequently suffers from bacterial infections with common encapsulated bacteria, whereas infected adults more frequently develop opportunistic infections. Several small studies with IVIG have shown decreased bacterial infections and sepsis, as well as improved survival in HIV-positive children. Another study of HIV-infected children who were undergoing treatment with zidovudine showed a benefit with the use of IVIG, but only in those subjects not receiving trimethoprim-sulfamethoxazole as antibiotic prophylaxis. The exact role of IVIG in this situation remains unclear.

Intramuscular immunoglobulin can be given for the prevention of measles in susceptible individuals (those with no previous infection or immunization). Its use is indicated for exposed persons with an increased risk of complications from disease, such as immunocompromised patients or children less than 1 year old. This treatment should be given within 6 days of exposure. The second indication for intramuscular immunoglobulin is the prophylaxis of hepatitis A. The protective effect is of most value if given prior to or immediately after exposure, and at least within 2 weeks of exposure. If given within several days of exposure, the immunoglobulin prevents infection in 80% to 95% of patients. This treatment was also indicated for travelers who plan to stay in areas with poor sanitation, but the hepatitis A vaccine is now the preferred method for hepatitis A prevention in travelers.

Hepatitis B immune globulin is given with hepatitis B vaccine in certain situations as part of the recommended postexposure prophylaxis regimen. The following susceptible persons who were exposed to hepatitis B virus should receive hepatitis B hyperimmune globulin in addition to immunization: persons with acute exposure to HBsAg-positive blood, infants with perinatal exposure to HBsAg-positive mothers, persons with sexual exposure to HBsAg-positive partners, and infants with an HBsAg-positive primary caregiver. Hepatitis hyperimmune globulin has been evaluated for the prophylactic treatment of HBsAg-positive liver transplant recipients. High-dose, long-term treatment has led to increased survival and decreased serologic recurrence in a number of studies. However, maintenance treatment is required for the prevention of recurrence, and long-term treatment is expensive.

Varicella-zoster immune globulin should be administered to susceptible persons exposed to varicella virus who have an increased risk for complications (i.e., immunocompromised persons). The postexposure prophylaxis should be administered as soon as possible and no later than 96 hours after exposure. The protection lasts at least 3 weeks after the injection.

Adverse reactions to any of the immunoglobulin preparations are rare. The incidence of systemic side effects is generally less than 5%, and these reactions are typically mild and self-limited. Fever, chills, headache, backache, nausea, vomiting, chest tightness, myalgias, and dyspnea have all been reported. With intravenous preparations, slowing of the infusion rate can be of benefit in alleviating the side effects. Hydrocortisone and antihistamines are also useful. Anaphylactic reactions may also occur in immunoglobulin A–deficient patients receiving IVIG, but this complication is rare. From 1985 to 1998, acute renal failure has been described in 120 IVIG recipients worldwide. Acute renal failure appears most closely associated with IVIG preparations with high sucrose content. The majority of affected patients developed renal failure within 7 days of IVIG administration, and 40% required dialysis owing to the degree of failure. Although this complication remains infrequent, it is associated with significant morbidity and mortality. Other uncommon adverse effects include encephalopathy and thromboembolism.
Because these biologic products are derived from human plasma, viral contamination poses a potential, though small, risk. In 1994 the association of 2 different IVIG preparations with hepatitis C contamination led to numerous cases of acute hepatitis C infection (more than 100 cases in the United States). The manufacturers added a solvent-detergent treatment for viral inactivation, and users of the products no longer are considered to be at risk for hepatitis C.542 All current IVIG preparations come from donors who are screened for hepatitis B and C, HIV, and elevated levels of liver enzymes. Also, the majority of manufacturers include a viral inactivation step in the production process. No cases of HIV transmission have been reported with IVIG.

CONCLUSION

It is clear that vaccination is one of the greatest achievements in the history of mankind. Immunization should be used in conjunction with public health measures such as sanitation, blood product screening, safe sex techniques, vector control, dietary education, and exercise promotion. Although immunization is considered widely as a major success, there are the public health challenges of implementing vaccine programs and allaying unfounded fears of the public about immunization side effects. Vaccine-preventable infectious diseases still remain major causes of morbidity and mortality in the world. Many children in the world still die from measles, tetanus, and pertussis, and influenza-related pneumonia and sepsis are among the top 10 causes of death in the United States and in the world.544,545 Despite the data that show efficacy of vaccinations, there is a small movement of antivaccination activists who may be partly contributing to the lower rates of adult immunization.546,547

The implementation of routine immunizations not only has a significant impact on the overall incidence of disease but also markedly decreases the direct and indirect costs associated with health care. For instance, a 1994 study of the cost-effectiveness of a varicella vaccination program in the United States showed savings of $384 million dollars per year.548 The cost reduction with varicella is mostly due to a decrease in time lost from work by caregivers, although this savings is significant. Vaccines for more serious diseases that often require hospitalization, such as respiratory syncytial virus in infants, will likely result in a more beneficial cost-effective profile. Historically, the cost savings of the eradication of smallpox, a disease that killed millions of people, approaches infinity when the millions more that would have been affected are considered. With recent current events, the use of weapons of mass destruction, specifically smallpox and anthrax, is now a realistic and potential biologic threat. A smallpox vaccination program has now been initiated by the United States in preparation for the possibility of terrorist attacks and in protecting persons in the front lines of a biologic attack. These individuals include military personnel, medical professionals, and emergency personnel and response teams who would be first on the scene in a smallpox emergency. A similar situation exists for poliomyelitis, which is expected to be eradicated worldwide in the near future. The cost savings for an HIV vaccine would also be phenomenal, considering the long-term treatment and numerous complications involved with this chronic infection.

Immunization has successfully led to the reduction in incidence of numerous diseases. Careful development and clinical evaluation have provided safe and effective vaccines with few adverse effects. Many reported adverse reactions after vaccination may be coincidental and have no proven direct relationship with the vaccine in question. Although serious side effects may rarely occur from vaccines, a much greater risk for morbidity and mortality results from the failure to become immunized. One vaccine, however, was recently removed from the market owing to safety issues. Rotashield was a live, oral, tetravalent rotavirus vaccine that was associated with several cases of intussusception and is considered to be causal.549 Most associations between vaccines and adverse events are not, however, shown to be causal. For example, the MMR vaccine was reported recently not to have a causal relationship to autism.550-552 Likewise, a causal relationship between the hepatitis B vaccine and a variety of autoimmune diseases has been disproved. This vaccine does not increase the risk of multiple sclerosis553 nor does it cause a relapse of preexisting multiple sclerosis.554 A claim that the rising rates of diabetes mellitus in children may be due to increased exposure to vaccination has been rigorously refuted.555,556 Nevertheless, suspected relationships between vaccines and adverse events need to be reported to the Vaccine Adverse Event Reporting System (800-822-7967) so that the 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 advances include genetically engineered vectors and VLP, among many others. 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 such as hepatitis B virus and Norwalk virus. This form of vaccination would offer a convenient, painless, and inexpensive approach to widespread control of disease and thus would be accessible to developing countries.

It is anticipated that the future will bring safe and effective vaccines for a variety of infectious diseases. Although few vaccines are available for the therapy of infectious diseases, our concept of vaccines is now being expanded with ongoing clinical trials of therapeutic vaccines.

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