

Vaccines and Vaccination

Vaccination is a highly efficient way of preventing viral diseases and remains one of the most effective healthcare measures introduced into medical practice. Indeed the control of so many diseases by vaccination is probably the single most outstanding achievement of medical science over the past century after the introduction of clean water. The eradication of smallpox is rightly hailed as a major achievement of modern medicine. Poliomyelitis is likely to be consigned to history also within the next few years. Morbidity and mortality due to measles virus have declined precipitately over the past decades, despite unwarranted public concerns in some countries resulting in a temporary drop in vaccination uptake and the re-emergence of local epidemics. Other vaccines should make a greater impact than is currently the case but there is frequently a lack of political will to implement effective and sustained vaccination programs.

Newer technologies are expected to bring improvements to existing vaccines, both in terms of delivery and efficacy, for example, the development of multi-seasonal influenza vaccines. Despite considerable effort, however, control of HIV and hepatitis C by vaccination remains out of reach for the foreseeable future, due to a number of scientific and logistic obstacles (see Chapter 23: Retroviruses and Chapter 36: Flaviviruses). Considerably more optimism surrounds the development of a quadrivalent dengue vaccine, one of the most important unmet medical needs worldwide.

Edward Jenner introduced vaccination in 1798 to protect people against smallpox using fluid from cowpox pustules. Indeed, the term vaccine is derived from the Latin word for cow, *vacca*. Nearly a century later, the concept was shown to have wider applications by Louis Pasteur, including his use of an experimental killed rabies “vaccine” to protect a boy bitten by a rabid dog. In the 1930s Max Theiler developed the 17D yellow fever vaccine, arguably one of the most significant advances in the control of an arthropod-transmitted disease (Box 11.1).

With the advent of cell culture techniques in the 1950s, a second era of vaccine development began, and many attenuated virus and inactivated virus vaccines were developed. Notably, Albert Sabin and Jonas Salk independently developed live and killed poliovirus vaccines respectively—“oral” (OPV) and “inactivated” (IPV). These

illustrate two traditional strategies for the development of viral vaccines, either by attenuating virulent virus sufficiently to abrogate its disease potential while retaining immunogenicity (live attenuated vaccines), or by chemical inactivation of virus infectivity without destroying those antigens necessary to elicit a protective immune response. Each has distinct advantages and disadvantages as to efficacy, duration of immunity, delivery, cost, and safety.

Today, the field of vaccinology has entered a new era through the application of advanced technologies in a myriad of different ways. Some have produced significant new vaccines, most notably against hepatitis B and chimeric vaccines against Japanese encephalitis virus (JEV). Although other candidate vaccines produced by recombinant DNA technology are yet to realize their full potential, it is clear that these will provide major advances in developing vaccination against contemporary diseases such as dengue and Zika viruses. Despite such developments, our understanding of the mechanisms by which vaccines prevent disease is still incomplete in many instances.

VACCINE DESIGN AND PRODUCTION

The rational design of any vaccine takes account of efficacy, safety, and practical usage, including cost. Vaccine efficacy is most often measured by the prevention of clinical disease rather than an absolute prevention of infection, the latter sometimes referred to as “sterile immunity.” The long-term duration of immunity is important, especially among communities where exposure is likely to occur throughout life. The number of doses required and the requirement for a cold chain become critical issues in regions where healthcare facilities are either minimal or difficult to access (Box 11.2).

Considerable attention is paid to the safety of vaccines. New products undergo rigorous safety testing prior to licensure. Although care often needs to be exercised among vaccine recipients known to have natural or acquired immunodeficiency or be pregnant, heightened concerns by the public as to the safety of individual vaccines are largely unfounded.

Vaccine design also requires knowledge of the virus, its antigens, and to what extent phenotypic and antigenic

BOX 11.1 Edward Jenner and Vaccination Against Smallpox

Edward Jenner (1749–1823), an English country physician and naturalist, is credited as the pioneer of vaccination. As with many physicians of his day, he practiced variolation whereby small quantities of smallpox virus were deliberately scratched into the skin of people in order to stimulate protection against naturally acquired disease. However, Jenner was intrigued by country lore that those who contracted cowpox from milking and cows appeared protected from smallpox. In 1798 one such milkmaid, Sarah Nelmes, consulted Jenner as to a rash that had developed on a hand through handling a cow (named Blossom!) with evidence of cowpox on her udders. Jenner realized this was an opportunity to test the protective effect of cowpox for someone yet to catch smallpox. Thus he chose the eight-year-old son of his gardener, James Phipps, to test his hypothesis. Not only was Jenner able to transmit cowpox from Sarah Nelmes to James Phipps, he then found James was now protected from smallpox by subjecting the boy to variolation. To Jenner's relief, James was fully protected. Although there are records of others who had considered previously the use of cowpox in humans, the challenging of James Phipps with smallpox to demonstrate immunity is the seminal contribution Jenner made, almost a century before our understanding as to the nature of infectious agents and the early days of immunology.



Portrait of Edward Jenner by an unknown artist, Wellcome Library, London.

BOX 11.2 The Objective of Vaccination

The object of vaccination is to stimulate immunological memory, thereby protecting the recipient against future disease. Any subsequent infection with wild-type virus is likely to be subclinical as a result. Vaccination should ideally also totally prevent infection in the recipient after future exposure, and prevent transmission of virus to contacts. At a population level, this will increase herd immunity and reduce the circulation of virus in the community.

TABLE 11.1 The Characteristics of an Ideal Vaccine

High efficacy in target populations
Few or no adverse reactions
Manufacture is inexpensive and easy to translate into developing countries
Safe in immunocompromised individuals and pregnant women
Easy and inexpensive to deliver
Stable during transport and storage
Induces life-long immunity
Stimulates both humoral and cell-mediated immune responses
Reproducible manufacturing process
Delivery informed by adequate and detailed epidemiological knowledge of the disease and its outcomes

variation influences the quality of protective immunity. Of these factors, antigenic drift, for example, among influenza viruses, or the presence of multiple variants as is the case with human immunodeficiency virus (HIV), presents real challenges (Table 11.1).

Attenuated Virus Vaccines

Attenuated virus vaccines have dramatically reduced the incidence of several important diseases and the spread of wild-type virus. The vaccine virus replicates in the recipient and in so doing, amplifies the amount of antigen available for presentation to the host's immune system. There are important benefits in this, since the replication of the vaccine virus mimics that of wild-type virus, the host immune response resembling what occurs after natural infection. This is not the case with either inactivated or subunit vaccines (see below). Most attenuated vaccines are injected either subcutaneously or intramuscularly, but some are delivered orally, and a few intranasally (Table 11.2, Figs. 11.1–11.3).

Attenuated virus vaccines are derived from several sources. The first vaccine, introduced by Jenner in 1798 for the control of human smallpox, utilized cowpox virus, a

TABLE 11.2 Methods for Production of Viral Vaccines**Live-Virus Vaccines**

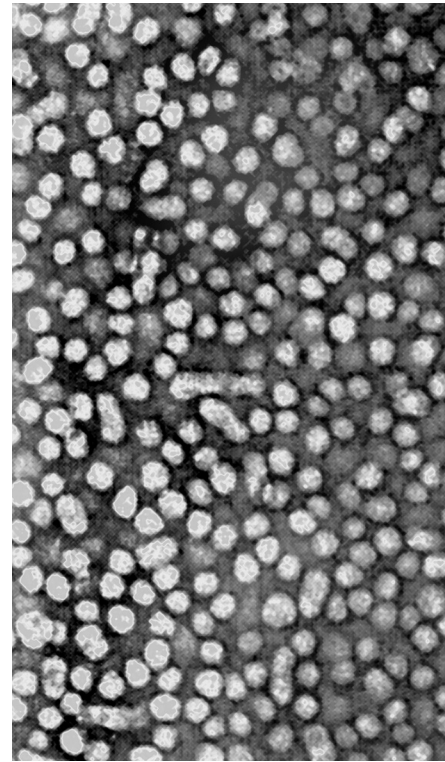
Related virus from a heterologous host species (e.g., cowpox)
 Naturally occurring attenuated virus strain
 Serial passage in a heterologous host animal
 Serial passage in cultured cells
 Selection of cold-adapted mutants

Non-replicating Native Antigen Vaccines

Vaccines produced from inactivated whole virions
 Vaccines produced from native virion subunits
 Vaccines produced from purified native virion proteins

Vaccines Produced by Recombinant-DNA and Other Technologies

Attenuation of viruses using recombinant DNA technology for gene deletion or site-directed mutagenesis
 Expression of viral proteins in eukaryotic (yeast, mammalian, insect) or bacterial cells by recombinant DNA technology
In vitro expression of viral proteins that self-assemble into virus-like particles
 The expression of viral antigens by viral or bacterial vectors delivered *in vivo*
 Use of reassortants or of viral chimeras (viruses with the replicative machinery of one virus and the protective antigens of another)
 Chemical synthesis of viral peptides
 The production and administration of anti-idiotypic antibodies
 DNA immunogens

**FIGURE 11.1** Hepatitis B surface antigen (HBsAg) purified from human plasma was introduced as a human vaccine in the late 1970s, but has subsequently been replaced in most countries by HBsAg particles produced by recombinant DNA technology.

natural pathogen of cattle. This virus produced only a mild lesion in humans, but it conferred protection against the severe disease because it is antigenically related to smallpox virus. The derivation of vaccinia virus subsequently used for over 150 years to eradicate smallpox is unknown, but is thought to have arisen by cross-contamination with other poxviruses during the early decades of the 19th century.

Second, serial passage in a heterologous host was a classic means of empirically attenuating viruses for use as vaccines before the advent of cell culture. For example, in the 1930s yellow fever virus was adapted to grow in mouse brain and in embryonated hens' eggs: the 17D yellow fever vaccine virus was developed by such empirical serial passage of virulent Asibi strain virus, first in mice by intracerebral inoculation, then in mouse embryo cells, and finally in chick embryo cell culture. Attenuation was laboriously assessed at every few passages by inoculation of experimental animals. At the point when the virus became sufficiently attenuated it was formulated into the 17D yellow fever vaccine that remains in extensive use today. The 17D yellow fever vaccine also offers attractive opportunities as a vector for vaccines against other flavivirus diseases, such as and dengue, diseases for which new or improved vaccines are urgently needed. These chimera

vaccine products generated by heterologous recombination are likely to offer much needed products in the next few years.

Third, the majority of attenuated virus vaccines in present use were derived empirically by serial passage in cultured cells. Adaptation of virus to more vigorous growth in cultured cells is fortuitously accompanied by progressive loss of virulence for the natural host. Loss of virulence may be demonstrated initially in a convenient laboratory model, often a mouse, before being confirmed by clinical trials. Because of the practical requirement that the vaccine must not be so attenuated that it fails to replicate satisfactorily *in vivo*, it is sometimes necessary to compromise by using a strain that may induce mild clinical signs among a few recipients.

It has been shown that for most viruses, a number of different genes contribute to virulence and tropism and do so in several different ways (see, e.g., Chapter 7: Pathogenesis of Virus Infections). Vaccine candidate viruses accumulate numerous point mutations in one or more viral genes during multiple passages in cultured cells, leading cumulatively to attenuation. In recent years, sequencing of virus genomes has brought considerable understanding about the basis of attenuation and has allowed for better predictions as to vaccine efficacy and safety.

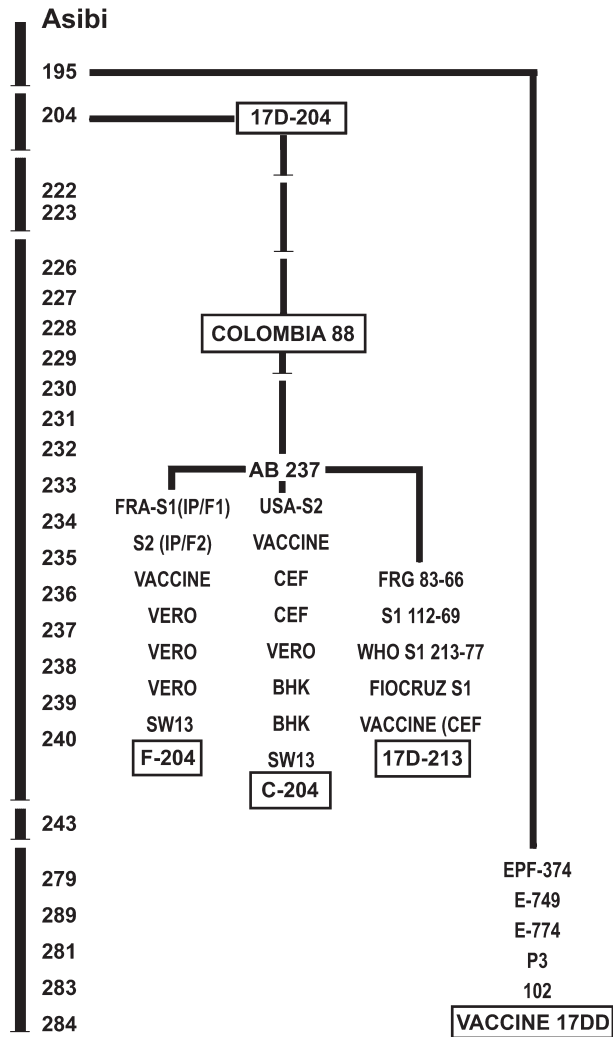


FIGURE 11.2 Passage history of the yellow fever 17D vaccine. The original Asibi monkey isolate was subcultured in embryonic mouse tissue and minced whole chicken embryo, yielding the parent 17D strain at passage 180 and the 17D-204 strain at passage 204. Further subcultures were carried out in embryonated chicken eggs, different cell lines and chick embryo fibroblasts to produce experimental vaccine batches. *Adapted from Bonaldo, M.C., et al., 2000. Mem. Inst. Oswaldo. Cruz. 95 (Suppl. 1), 215–223.*

The observation that temperature-sensitive (*ts*) mutants (mutants unable to replicate satisfactorily at temperatures greater than normal body temperature) generally display reduced virulence, suggested that such mutants might make satisfactory live vaccines. Unfortunately, candidate vaccines containing one or more *ts* mutations have displayed a disturbing tendency to revert toward virulence as a result of replication within recipients. Attention accordingly has moved to cold-adapted mutants, derived by adaptation of virus to grow at suboptimal temperatures. In general, these mutants have proven to be more stable. One rationale for the use of vaccines containing a cold-adapted mutant virus is that they may be suitable for intranasal administration—the

mutant virus replicating at the lower temperature of the nasal cavity (about 33°C), but not at the temperature of the more susceptible lower respiratory tract. In 1997, after extensive clinical trials, cold-adapted influenza vaccines containing mutations in almost every gene were licensed for use in the United States.

Inactivated Virus Vaccines

Inactivated virus vaccines are usually made by exposure of virulent virus to chemical or physical agents, for example, formalin or β -propiolactone, in order to destroy infectivity while retaining immunogenicity. Initially, virus for this purpose was often obtained from infected animal sources, for example, mouse brain, but infected cell cultures provide cleaner starting material.

The need to use large amounts of antigen to elicit an adequate antibody response is a major disadvantage. Generally with such vaccines, the primary vaccination course comprises two or three injections; further “booster” doses may be required at intervals to maintain protective immunity. The chemical or physical treatment used to eliminate infectivity of inactivated virus vaccines may be damaging enough to modify immunogenicity, especially of antigens needed to elicit cell-mediated immune responses. The result is an immune response shorter in duration, narrower in spectrum for viral antigens, weaker cell-mediated and mucosal immune responses, and possibly less effective in preventing viral entry. The most commonly used inactivating agent, formalin, is known to induce irreversible changes in many viral antigens; its continued use derives from the conservative attitude of regulatory agencies and vaccine manufacturers and a paucity of research in this area. The use of β -propiolactone in the manufacture of some human rabies vaccines has advantages in that proteins are not damaged and the inactivating agent is completely hydrolyzed within hours to non-toxic products.

Non-ionic detergents such as the polyoxyethylene ethers are used in the case of enveloped viruses to solubilize virions and release glycoprotein peplomers and other envelope proteins. Differential centrifugation or ultrafiltration is used to semi-purify the solubilized glycoproteins prior to formulation for use as so-called “split” vaccines. Some commonly used influenza vaccines are produced by this procedure.

Vaccines Produced by Alternative Methods

Hepatitis B vaccines were the first to prevent a human cancer of viral etiology, namely hepatocellular carcinoma. The first HBV vaccines consisted of purified hepatitis B surface antigen (HBsAg) particles isolated from the blood of chronically infected carriers of the virus. The concentrated products were treated with two or three inactivating regimens to ensure that no infectious hepatitis

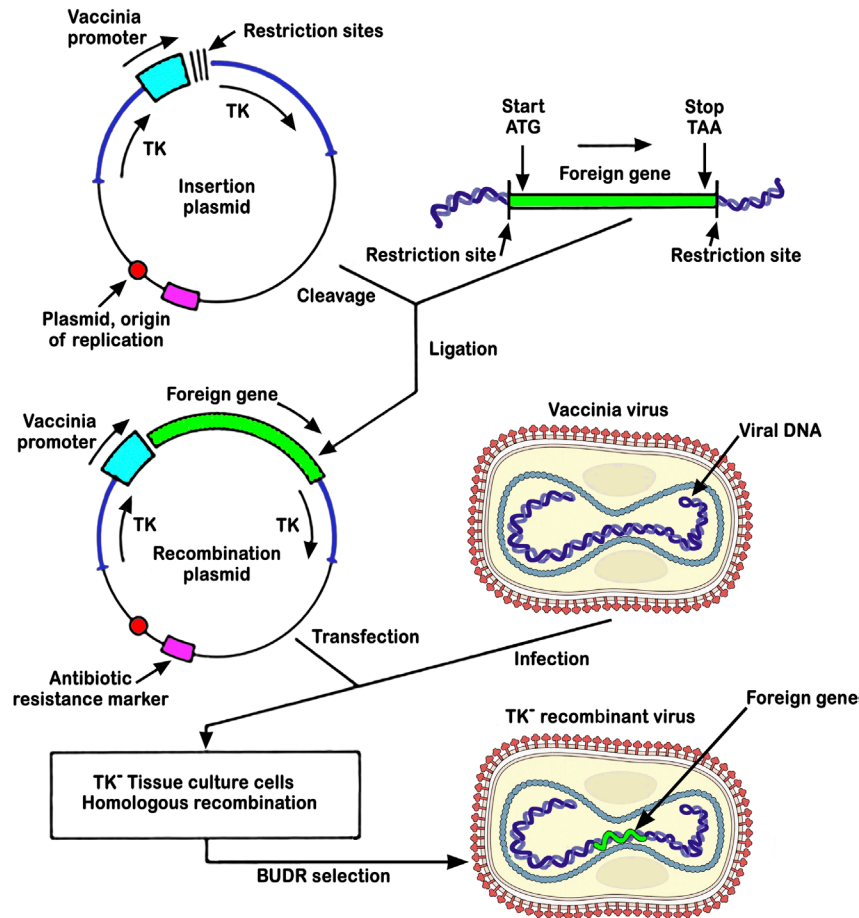


FIGURE 11.3 Method of constructing a vaccinia virus vector carrying a selected gene from another virus. This approach has been used to develop several successful veterinary vaccines, but as yet no human vaccine has been licensed. It has also been extended by using other viruses as vectors (e.g., adenoviruses, paramyxoviruses, parvoviruses), and is used for gene expression in virus research and for delivery of many kinds of therapeutic genes. As shown in the figure, a vaccinia promoter is inserted into the plasmid just upstream of the intended insertion site, vaccinia virus DNA sequences (e.g., from an interrupted thymidine kinase gene) are ligated upstream and downstream of the site, and the foreign gene is inserted. Tissue culture cells are infected with vaccinia virus and transfected with the insertion plasmid, and the vaccinia TK sequences direct homologous recombination insertion into the vaccinia virus genome. Stable recombinant viruses are then selected; in this example, the recombinant virus has an interrupted thymidine kinase gene and can be selected for by use of 5-bromodeoxyuridine in the culture medium. *TK*, thymidine kinase gene of vaccinia virus; *BudR*, bromodeoxyuridine. Courtesy of B. Moss and J. Esposito, with permission.

B virus remained in the final product. The initial doubt that impurities may cause unwanted autoimmune reactions proved unfounded. More recently, these vaccines have been largely but not completely replaced by HBsAg expressed in yeast cells using recombinant DNA technology.

A raft of new technologies is being explored commercially. These are directed toward solving problems of low yield or purity, or by exploiting unrelated carrier organisms to improve routes of delivery, for example, by delivery of relevant immunogens to lymphoid tissue in the gut. However, up till now most of the vaccines in widespread human use are based on well-established, conventional technologies (Table 11.2).

The problem of back mutation (that is, a mutation by which the vaccine virus regains virulence) may be largely

circumvented by completely deleting those genes that are essential for virulence. The large DNA viruses, in particular, carry a significant number of genes that are required for replication *in vivo* but not for replication in cultured cells. Genetic manipulation may be used to construct deletion mutants that are attenuated and stable over many passages. Experimental herpesvirus vaccines have been constructed using this strategy; one such vaccine, a pseudorabies vaccine for swine with a deletion of the whole thymidine kinase (TK) gene of the virus, is in wide use by veterinary practitioners in many countries. This vaccine is the model for herpes simplex virus vaccines currently under development for human use.

Site-directed mutagenesis permits further variations in this strategy—not only are large deletions easy to identify,

construct and test, but prescribed nucleotide substitutions and insertions are now feasible. As more becomes known about particular genes determining viral virulence and immunogenicity, these genes can be modified with the aim of improving the balance between immunogenicity and virulence.

Expression of Viral Proteins in Recombinant Systems

Recombinant-DNA technology provides a means of producing large and consistent amounts of viral proteins, that are easy to purify and can be readily formulated into vaccines. Once the critical viral protein conferring protection has been identified, the gene (or, in the case of an RNA virus, a cDNA copy of the gene) may be cloned into one of a wide choice of expression plasmids and expressed in any number of cell systems. If the immunogenic viral protein of interest is glycosylated, eukaryotic expression systems must be used to ensure the expressed protein is also glycosylated and secreted in its native conformation.

Useful eukaryotic expression systems include yeast cells (*Saccharomyces cerevisiae*), insect cells (*Spodoptera frugiperda*), and various mammalian cells deemed free of adventitious agents, for example, MRC-5 cells. Yeast offers the advantage that there is extensive experience with scale-up for industrial production; the first vaccine produced by expression of a cloned gene, hepatitis B vaccine, was produced in yeast. Insect cells offer the advantage of simple technology derived from the silk industry: moth cell cultures (or whole caterpillars) may be made to express very large amounts of viral proteins through infection with baculoviruses carrying the gene(s) of the virus of interest. The promoter for the gene encoding the baculovirus polyhedrin protein is so effective that, when the heterologous gene is inserted within the baculovirus polyhedrin gene, the viral protein may comprise up to half of the mass of the infected moth cells or caterpillars. However, mammalian cells offer the advantage over cells from lower eukaryotes in that they are more likely to possess the machinery for correct post-translational processing of viral proteins, including glycosylation and secretion.

Virus-Like Particles

Capsid proteins may assemble into virus-like particles (VLPs) when the genes coding for the production and cleavage of capsid proteins of certain non-enveloped icosahedral viruses are cloned into plasmids and co-expressed. These particles may be used as a vaccine. Examples of viruses for which VLPs have been made and shown to be immunogenic include papillomaviruses, picornaviruses, caliciviruses, rotaviruses, and reoviruses. VLPs may be equated to so-called naturally occurring “empty

virus particles” in that VLPs are totally devoid of nucleic acid and therefore non-infectious. The VLPs are similar to inactivated whole virus vaccines, but avoid the need for the potentially epitope-damaging step of chemical inactivation. The first human VLP-based vaccine in wide commercial use was the vaccine against human papillomavirus, which was introduced in many countries around 2007.

Use of Live Vectors and Reassortants

Recombinant DNA technology allows the expression of viral epitopes on the surface of non-pathogenic bacteria, which can be directly administered to non-immune individuals. The general approach is to insert the DNA encoding a protective viral antigen into a region of the genome of a bacterium or one of its plasmids that encodes a prominent surface protein. Provided that the added viral protein structures do not seriously interfere with the transport, packaging, stability, or function of the bacterial protein, the bacterium can multiply and present the viral epitope to the immune system of the recipient. Many consider bacteria that multiply naturally in the intestinal lumen as ideal expression vectors for presenting protective antigens of virulent enteric viruses to the gut-associated lymphoid tissue (GALT). Vaccines under development employ attenuated strains of *Escherichia coli*, *Salmonella* spp. and *Mycobacterium* spp. for immunization against enteric organisms and/or for the preferential stimulation of mucosal immunity.

Recombinant DNA techniques allow the introduction of heterologous genes coding for protective antigens of another virus into the genome of an avirulent virus acting as a vector: the foreign gene is then expressed in cells susceptible to the avirulent carrier. Cells in which the vector virus replicates *in vivo* will express the heterologous protein, with the recipient mounting both a humoral and a cell-mediated immune response to the relevant viral antigen. Viral vectors are also used to deliver into target cells genes of interest for gene therapy, cancer therapy, degenerative disease therapy, etc.

Although vectors such as adenovirus have shown considerable promise in the laboratory, an adenovirus-vectored HIV vaccine proved unsuccessful early on in clinical trials. However, success may be in part dependent upon the particular heterologous antigen being expressed, as a similar experimental vaccine against Ebola virus has shown total protection in non-human primates and has been deployed in an attempt to control the 2014 epidemic of Ebola virus in West Africa.

Another widely used vector is vaccinia virus. Vaccination of experimental animals with these recombinant vectored vaccines has, in nearly every case, produced a good antibody response. For example, vaccinia-virus vectored rabies vaccines have been shown to protect foxes and raccoons when incorporated into orally administered baits. Since the large vaccinia genome can accommodate at least

a dozen foreign genes and still be packaged satisfactorily within the virion, it would theoretically be possible to construct a vector capable of protecting against several different viral diseases. Fowlpox virus and canarypox viruses have been tested as substitutes for vaccinia virus: these viruses do not undergo a complete infection cycle in mammals, including humans, but can undergo an aborted infection cycle sufficient to allow the expression of any inserted heterologous gene, with the result that antibodies to the heterologous gene product(s) are produced. Research suggests that adenoviruses, herpesviruses, and parvoviruses also have value as vectors, and indeed may have certain advantages in terms of long-term antigen expression.

Chimeras and Reassortants

Virus strains with proven, useful vaccine properties (e.g., safety, high yield) may be altered to substitute relevant antigen genes or gene segments from wild-type pathogenic strains. The value of such viral chimeras was well established with influenza viruses even before the advent of recombinant DNA technology. Reassortant influenza viruses were produced by co-cultivation of a vaccine virus with a new isolate to encourage genome segment exchange. Viruses with the desired growth properties of the vaccine virus but with the immunogenic properties of the recent isolate were selected, cloned, and used in inactivated vaccines. This approach was adapted for the H5N1 avian influenza virus that was causing severe illness and deaths in Hong Kong in 1995. However, the H5N1 virus grows poorly in both cultured cells and embryonated hens' eggs—because it is so rapidly lethal in these systems. In this case, the virus that was used for vaccine production was a reassortant virus containing most of the genes of the high-yielding existing PR8 vaccine strain, plus the hemagglutinin and neuraminidase genes of the H5N1 virus. Furthermore, the multiple basic amino acids in the HA cleavage site that are associated with virulence in H5 avian viruses were changed to a sequence characteristic of low-virulence avian strains.

Synthetic Peptides

Techniques have been developed for locating protective epitopes on viral proteins and chemically synthesizing peptides corresponding to these epitopes (Table 11.2). Such synthetic peptides have been shown to elicit neutralizing antibodies, but in general this approach has been disappointing. One limitation is that most epitopes eliciting humoral immunity are conformational, that is, are not composed of linear arrays of contiguous amino acids, but rather are assembled from amino acids separated in the primary sequence and brought into close apposition by the folding of one or more polypeptides. An effective antigenic stimulus requires that the three-dimensional shape of an

epitope present in either the native protein molecule or virus particle is maintained in a vaccine. Since short synthetic peptides lack any tertiary or quaternary conformation, most antibodies raised against these molecules are incapable of binding to whole virions. When neutralizing antibody is present after injection of a peptide, the titer is often orders of magnitude lower than that induced by inactivated whole virus vaccines or purified intact proteins.

In contrast, epitopes recognized by T lymphocytes are mostly composed of short linear peptides. Some of these epitopes are conserved between strains of virus and therefore elicit a broad T cell response. Thus attention moved toward the construction of artificial heteropolymers of T cell epitopes and B cell epitopes, in some cases also coupled to peptides facilitating fusion with cell membranes to enhance uptake. Such constructs prime T cells to respond more vigorously when either boosted with an inactivated whole-virus vaccine or when challenged by infection.

DNA Vaccination

Perhaps the most revolutionary new approach to vaccination stems from the discovery that naked viral DNA can be used as an immunogen. In the early 1990s, it was observed that a plasmid construct expressing β -galactosidase gene after inoculation into mouse skeletal muscle expressed the enzyme for up to 60 days post inoculation. In a few short years, this approach that would previously have been ridiculed by grant-giving bodies became an area of great promise, and DNA vaccines were extensively explored. Experimental DNA vaccines exist for a wide range of applications including viral, bacterial, and parasitic infectious agents, as well as for anti-tumor and anti-allergic therapies. Experimentally, the methodology can also be applied to the screening of protective antigens.

It was shown for many viruses that whole viral DNA, RNA, or cDNA of viral RNA when transfected into cells could undergo a full cycle viral replication. In principle, the technique is relatively simple; most notably, it eliminates the step of using an infectious viral or bacterial vector, or the need to prepare large amounts of antigenic protein. Recombinant plasmids are constructed to contain genes encoding viral antigens. The DNA insert in the plasmid, upon injection, is transcribed and the mRNA is translated. Antigen-presenting cells of bone marrow origin infiltrate the site and take up the antigen, from where it is transported to a regional lymph node. Direct transfection of antigen-presenting cells, particularly Langerhans cells of the skin, with the inoculated DNA also occurs at the site of injection. The expressed protein elicits an immune response similar to that during an infection. Both humoral and cellular immune responses can be measured. Most importantly, for a growing number of viruses the immune response following injection has been shown to confer protection.

An improvement in the delivery of DNA vaccines has been achieved by coating the plasmid DNA onto microparticles, commonly 1 to 3 μm diameter gold particles, and injecting an aerosol of particles using a gas-driven gun-like apparatus (the *gene gun*); less than 1 μg of DNA delivered subcutaneously by this means is often immunogenic. As with other recombinant vaccines, the incorporation of genes encoding immunostimulatory proteins including IL-2, IL-12, and GM-CSF adds to the immunogenicity of the viral DNA.

Advantages of DNA vaccines include purity, physiochemical stability, simplicity and relatively low cost of production, distribution, and delivery. Multiple antigens can be included in a single plasmid, new antigens can be added easily, and antigens are expressed in a native conformation resembling that on the virion, thereby facilitating both processing and presentation to the immune system. Repeated injection may be given without interference and T cell immunity as well as antibody responses are elicited. One interesting aspect of DNA immunization is that immunity can develop in the presence of maternal antibodies. However, for reasons not fully understood, the efficacy of DNA vaccines demonstrated in mice is not always reproduced in non-human primates or humans.

Methods for Enhancing Immunogenicity

The immunogenicity of inactivated vaccines, and especially that of purified protein vaccines and synthetic peptides, usually needs to be enhanced; this may be achieved by mixing the antigen with an *adjuvant*, by incorporating the antigen in *liposomes*, or incorporating the antigen in immunostimulating complexes (ISCOMs). Adjuvants are selected for the property of recruiting additional signaling pathways involved in the activation of the innate immune system and antigen-presenting cells, as well as promoting the persistence of the immunizing antigen through the establishment of an immunogenic depot.

Adjuvants

Adjuvants are substances that, when mixed with vaccine immunogens, potentiate the immune response resulting in the need for either a lesser quantity of antigen or fewer doses, or both. Considerable variation exists between adjuvants as to chemical composition and mode of action. Mechanisms of action include:

1. prolongation of release of antigen;
2. activation of macrophages, leading to secretion of lymphokines and attraction of lymphocytes; and
3. mitogenicity for lymphocytes.

Until recently alum was the only adjuvant licensed for use in humans, and has been widely used for many years.

However, alum is not particularly effective, as cell-mediated immunity is often poorly stimulated. Mineral oil (e.g., Freund's adjuvants) and other adjuvants are used in animals but generally these are far too reactogenic to be acceptable for human use. There has been a clear requirement for better adjuvants for some considerable time, preferably chemically defined and of known mode of action.

Antibody responses can be improved by combining alum with an additional immunostimulatory compound, for example, a modified synthetic form of monophosphoryl lipid A (MPL) that is a ligand for Toll-like receptor 4 (TLR-4) on the surface of antigen-presenting cells. Such formulations also stimulate IFN- γ to produce additional primed CD4⁺ helper T cells. Cervarix, a VLP preparation of human papilloma virus, is so formulated and is the first vaccine to contain an additional TLR-4 ligand approved for human use. An alternative is MF59, a squalene oil-in-water emulsion successfully used to adjuvant seasonal influenza vaccines leading to greater cross-reactive antibodies with higher avidity.

Liposomes and ISCOMs

Liposomes are artificial spherical vesicles composed of a lipid bilayer, into which viral proteins can be incorporated. When purified viral envelope proteins are included, the resulting "virosomes" (or "immunosomes") resemble somewhat the original envelope of the virion. This enables not only a reconstitution of virus envelope-like structures lacking nucleic acid and other viral components, but also allows the incorporation of non-pyrogenic lipids with the properties of adjuvants.

A spherical cage-like structure 40 nm in diameter is formed when viral envelope glycoproteins or synthetic peptides are mixed with cholesterol plus a glycoside known as Quil A. These ISCOMs (immunostimulating complexes) display significantly enhanced immunogenicity. A combined influenza/hepatitis A liposome vaccine is in limited use.

VACCINE SAFETY, EFFICACY, AND PRACTICAL USAGE

The practical advantages and limitations of different vaccines can be illustrated by comparing the properties of live, non-replicating and DNA vaccines (Table 11.3). Attenuated virus vaccines delivered orally or intranasally depend critically for efficacy on vaccine replication in the intestinal or respiratory tract respectively. Interference can occur between the vaccine and other enteric or respiratory viruses incidentally co-infecting the person at the time of vaccination—a common situation among infants, particularly in poor socio-economic conditions. Interference can occur between different live viruses contained in vaccines delivered via the natural route, for example, between the three virus components of the Sabin oral polio vaccine, if

TABLE 11.3 Advantages and Limitations of Attenuated, Inactivated, and DNA Vaccines

Property	Attenuated Virus Vaccine	Inactivated Virus Vaccine	DNA Vaccine
Route of administration	Injection, inhalation, or oral	Injection	Injection, gene gun
Amount of virus in vaccine dose	Low	High	Nil
Number of doses	Single, generally	Multiple	Single, or in prime-boost regimen
Need for adjuvant	No	Yes	No
Duration of immunity	Many years	More limited, for example, several years	Not known
Antibody response	IgG, IgA (if via mucosal route)	IgG	IgG
Cell-mediated response	Good	Generally modest	Good
Heat lability	Yes, for most viruses	No	No
Interference by prior antibody	Yes	Usually no	Apparently no
Side-effects	Occasional, local, or systemic	Occasional, local	Uncertain
Use in pregnant women	Usually not advised	Yes	Yes
Reversion to virulence	Rare	No	No
Cost	Low	High	Low
Potential hazards	Contaminating viruses	Vaccine-enhanced disease	Not yet known

respective concentrations are not appropriately balanced. It is for this reason that the Sabin oral polio vaccine is routinely administered on three occasions, separated by at least two months. Interference is not a problem with systemically administered polyvalent vaccines, for example, the live attenuated measles/mumps/rubella vaccine.

IgA is the most important class of immunoglobulin relevant to the prevention of infection of mucosal surfaces, such as those of the intestinal, respiratory, genitourinary, and ocular epithelia. One of the advantages of an oral attenuated virus vaccine, for example, the Sabin polio vaccine, is that replication of the attenuated virus in the intestinal tract leads to prolonged synthesis of local IgA antibodies. By preventing infection, circulation of virus in the community is suppressed and the prospect of eradication of the virus from the local population has become possible.

The duration of immunity after vaccination with live attenuated vaccines is usually many years or decades, particularly against those viruses spreading via the blood to reach target organs. Re-infections, if they occur, are usually asymptomatic and short-lived, and lead to a boost in immunity. This solid immunity is attributable to systemic antibody of the IgG class successfully neutralizing the infectivity of any incoming virus. In contrast, protection afforded by mucosal IgA antibodies tends to be much shorter in duration, for example, with respiratory and enteric viruses where pathogenicity is largely restricted to the site of body entry. As each new vaccine is introduced, its duration of protection needs to be documented by long-term field studies.

There are particular difficulties in producing vaccines against viruses known to establish persistent infections, such as herpesviruses and retroviruses; a vaccine must be effective in inducing sterilizing immunity if it is to prevent not only the primary disease, but also the establishment of lifelong latency. As already stated, attenuated virus vaccines are generally found to be much more effective in eliciting cell-mediated immunity than inactivated viruses. Further, cell-mediated immunity is known to be the most effective arm of the immune system in modulating, if not eliminating, latent/persistent infections.

Attenuated vaccines are susceptible to inactivation by high ambient temperatures, a particular problem in the tropics. Formidable problems can be encountered in maintaining the “cold chain” from manufacturer to the point of administration in remote, often hot, rural areas. To some extent the problem has been alleviated by the addition of stabilizing agents to some vaccines (e.g., polio vaccine), selection of vaccine strains that are inherently more heat stable, and by preparing freeze-dried products for reconstitution immediately before administration (e.g., vaccinia). Portable refrigerators for use in vehicles and temporary field laboratories have proven invaluable in recent years to overcome these difficulties.

FACTORS AFFECTING VACCINE SAFETY

To be accepted, a vaccine must be safe as well as efficacious. Concerns about safety and unwarranted effects of vaccines



FIGURE 11.4 The Cow-Pock—or—the Wonderful Effects of the New Inoculation!—Publications of the Anti-Vaccine Society Print (color engraving) published June 12, 1802 by H. Humphrey, St. James's Street. In this cartoon, the British satirist James Gillray caricatured a scene at the Smallpox and Inoculation Hospital at St. Pancras, showing cowpox vaccine being administered to frightened young women, and cows emerging from different parts of people's bodies. The cartoon was inspired by the controversy over inoculating against the dreaded disease, smallpox. Opponents of vaccination had depicted cases of vaccinees developing bovine features and this is picked up and exaggerated by Gillray. Although the central figure is often assumed to be Edward Jenner, circumstantial evidence suggests this may not be so. Although the director of the Smallpox Hospital William Woodville had originally supported Jenner, he and his colleague George Pearson, were in dispute with Jenner by the time the caricature was published. *From Wikimedia Commons.*

have been expressed ever since the earliest use of cowpox as a vaccine (Fig. 11.4). Licensing authorities are extremely vigilant and insist on rigorous safety tests since residual live virulent virus in certain batches of inactivated vaccines caused a number of tragedies in pioneering days. Live attenuated vaccines present a different set of challenges that must be met before any particular product is released onto the market; these are discussed below. Vaccines are made under a broad set of guidelines, termed Good Manufacturing Practices (GMP). After successfully completing phase III studies, the release of a new vaccine is accompanied by firm data on the recorded side effects and the frequency and severity of each.

Some excellent human viral vaccines in routine use, such as rubella and measles vaccines, can produce some symptoms—in effect, a very mild case of the disease—in a minority of recipients. Attempts to attenuate virulence further by additional passages in cultured cells have been accompanied by a decline in the capacity of the virus to replicate in humans, with a corresponding loss of

immunogenicity. Such trivial side effects as do occur with current human viral vaccines are of limited consequence and do not prove to be a significant disincentive to immunization, provided that parents and recipients are adequately informed in advance.

Spread of a live vaccine to contacts is not usually a problem if it occurs, but is not a preferred outcome. Some vaccine strains may revert toward virulence during replication in the recipient, or in those close contacts to which the vaccine virus has spread. Most vaccine viruses are incapable of such spread, but in those that do there may be an accumulation of back mutations that gradually leads to a restoration of virulence. The principal example of this phenomenon is the reversion to virulence of Sabin poliovirus type 3 oral vaccine, which can lead to paralytic disease in one of up to 3 million vaccine recipients.

The 17D yellow fever vaccine is widely regarded as one of the safest vaccines yet a problem arose when, during World War II, 17D yellow fever vaccine was administered to US servicemen along with human immune serum. Among

approximately 2.5 million troops vaccinated, 28,600 cases of icteric hepatitis occurred, leading to 62 deaths. Careful epidemiological studies led to the conclusion that some of the lots of immune serum were contaminated with a hepatitis virus now known to be hepatitis B virus. When the use of immune serum along with the yellow fever vaccine was discontinued, the problem ceased.

Since vaccine viruses are grown in cells derived from humans or animals, there is always a possibility that a vaccine will be contaminated with another virus, derived from those cells or from the medium (especially the serum) in which those cells are cultured. For example, primary monkey kidney cell cultures, once widely used for the manufacture of polio vaccines, have, at one time or another, yielded over 75 simian viruses, some of which are considered pathogenic for man. Some early batches of inactivated poliovirus vaccine were contaminated with SV40 virus but there has been no evidence of any adverse consequences among recipients. This danger has led to a move away from primary cell cultures and toward well-characterized continuous cell lines that can be subjected to comprehensive screening for endogenous agents before being certified as safe, for example, MRC-5 cells.

Another important source of viral contaminants is bovine fetal calf serum, universally used in cell cultures; all batches must be screened for contamination with bovine viruses. Likewise, porcine viruses have been contaminants of crude preparations of trypsin prepared from swine pancreases, which is commonly used in the preparation of cell cultures. The risk of contaminating viruses is obviously greatest with live-virus vaccines, but may also occur with inactivated whole-virus vaccines, since some viruses are more resistant to inactivation than others.

Vaccination During Pregnancy

Ideally women planning a pregnancy should be up to date with immunizations at least one month before conception. These should include as a minimum influenza, measles, mumps, rubella, and chickenpox in addition to diphtheria, whooping cough, and tetanus. As high fever is linked to a slight increase in risk of fetal defects and possibly miscarriage, the possibility of vaccine-associated febrile reactions means that immunization is generally avoided during pregnancy, particularly with live vaccines. For this reason, and for the theoretical possibility of infection of the fetus, it is recommended that rubella vaccination should not be undertaken within 28 days of the intention to become pregnant, or during pregnancy, although in the limited instances where this has happened inadvertently, no harmful consequences to the fetus have been seen.

However, where a particular virus disease represents a significant risk to both a pregnant woman and the unborn child, then the benefits of immunization are usually considered as outweighing the risk of vaccine reactions.

Influenza, for example, can pose serious risks during pregnancy and thus vaccination with the standard inactivated vaccine is recommended at any time.

Vaccination of Immunocompromised Persons

Most estimates of the efficiency of immunization among immunocompromised recipients are based upon the measurement of antibodies. The titer of the antibody response may be reduced or even absent, but this is not necessarily a direct measure of protection. Limited evidence suggests that inactivated vaccines have the same safety profile in immunocompromised individuals as in those fully immunocompetent. Live attenuated vaccines are not generally recommended, although there may be exceptions, and prolonged excretion of vaccine virus by immunocompromised vaccinees is well described. Nevertheless, MMR and varicella vaccines have been successfully administered to children infected with HIV and displaying only mild or moderate immunodeficiency. Any decision to administer a vaccine has to be considered in the context of risk and benefit, for example, likely exposure to a vaccine-preventable disease and the risk of developing adverse reactions to the vaccine. In the case of immunocompetent patients facing the prospect of immunosuppressive medication, live vaccines should be given at least four weeks, and inactivated vaccines two weeks, before the start of medication.

An important precautionary measure is the immunization of household and close contacts of the immunocompromised individual in order to reduce their risk of exposure to vaccine virus undergoing reversion. Care needs to be exercised, however, in giving live attenuated vaccines, for example, rotavirus and oral poliovirus vaccines may be shed by vaccinated contacts.

PASSIVE PROTECTION

As an adjunct to the above strategies for stimulating the body to induce *active* immunity, short-term *passive* protection can be achieved by the intramuscular or intravenous administration of preformed antibody. The available preparations and policies for their use are described in Chapter 14: Control, Prevention, and Eradication.

VACCINATION POLICY AND PROGRAMS

The major human viral vaccines in widespread usage are set out in [Table 11.4](#). Many are recommended to be given routinely to all infants or older individuals (see [Table 14.2](#)). Others are used against viruses of restricted geographical distribution or for individuals at particular risk, such as Japanese encephalitis, yellow fever, tick-borne encephalitis, rabies, and hepatitis A. There are many more vaccines in various stages of development or clinical trial, for example,

TABLE 11.4 Vaccines in Use for Routine Immunization

Bacterial	Viral—Live	Viral—Non-replicating
BCG	Polio (Sabin, OPV)	Polio (Salk, IPV)
Diphtheria (toxoid)	Measles	HBV–recombinant
Tetanus (toxoid)	Mumps	Human papillomavirus
Pertussis (acellular)	Rubella	Japanese encephalitis
Hemophilus influenzae b	Rotavirus	Tick-borne encephalitis
Pneumococcal polysaccharide	Yellow fever	Hepatitis A
Typhoid	Varicella-zoster	Influenza
Cholera	Vaccinia	Rabies
Meningococcal C conjugate		

vaccines against HIV, HCV, several herpesviruses, and several respiratory viruses. The subjects of vaccination policy and eradication of diseases by vaccination are discussed in Chapter 14: Control, Prevention, and Eradication, together with a typical schedule for the immunization of infants against common viral diseases (Table 14.2). Detailed discussion of current immunization practices and/or vaccine development prospects for individual viral diseases is provided in the relevant chapters of Part II.

Recently Introduced or Under-Utilized Viral Vaccines

Cervical Cancer Due to Human Papillomavirus (HPV) Infection

This is the second most common cancer in women, accounting for 13% of all female cancers in developing countries. There are an estimated 500,000 new cases each year. Virtually all cervical cancer cases are linked to persistent genital human papillomavirus (HPV) infection, the most common viral infection of the female reproductive tract. Hence the target group for immunization is adolescent females, although some programs now also include males. Available HPV vaccines consist of VLPs made up of the viral L1 capsid protein. These are highly efficacious in preventing infection with, and cancer due to, HPV types 16 and 18, which together are responsible for approximately 70% of cervical cancer cases worldwide. One product is also highly efficacious in preventing genital warts caused by types 6 and 11 (see Chapter 19: Papillomaviruses).

Rotaviruses

Rotaviruses are the most common cause of severe diarrheal disease in infants and young children across the developing world, accounting for around 530,000 deaths annually, with more than 85% of these deaths occurring in low-income countries in Africa and Asia: over 2 million infants are hospitalized each year suffering from dehydration, adding a further burden to often very limited healthcare resources. Over a third of diarrhea cases among hospitalized children less than five years of age have been estimated as due to rotavirus infection. Children between six months and two years of age are the most susceptible to severe gastroenteritis.

Vaccination is an important measure in the control of rotavirus diarrheal disease, along with improvements in hygiene and sanitation, oral rehydration, and zinc supplementation. Two vaccine products became available in the mid-2000s, both consisting of living attenuated strains and both compatible with national childhood vaccination schedules and the recommendations of the WHO Expanded Program on Immunization (EPI). Rotavirus vaccination is strongly recommended for developing countries where rotavirus mortality is important, and presently an increasing number of the more developed countries are adding rotavirus vaccine to their childhood vaccination schedules to prevent common non-fatal diarrheal disease. An early vaccine was withdrawn because of an increased risk of intussusception, but this concern has not been seen with current vaccines. One vaccine, RV5, is administered orally in a 3-dose series, with doses administered at ages two, four, and six months; a second vaccine, RV1, is administered orally in a 2-dose series, with doses administered at ages two and four months.

Japanese Encephalitis Virus

Japanese encephalitis virus is the main cause of encephalitis in many Asian countries. Transmission is difficult to halt, given the virus exists in a transmission cycle between mosquitoes, pigs, and/or aquatic birds. Humans become infected incidentally when bitten by an infected mosquito and the disease is usually prevalent both in rural and periurban areas (see Chapter 36: Flaviviruses). As the disease has spread over the past half-century as agricultural development and intensive rice cultivation have been expanded in many Asian countries, the need to employ effective vaccination policies has become ever greater. Indeed the disease is most likely under-reported, with annual mortality estimated at 10 to 15,000 deaths. Recent estimates put the number of clinical cases at 60,000, a third of whom develop long-term neurological impairment.

Vaccines have been available for many years, although price and inconvenient vaccination schedules together with a lack of governmental awareness as to the extent

of infection have hampered immunization programs. For many years vaccines were prepared as purified, inactivated products made from either the Nakayama or Beijing strains of virus grown in mouse brain tissue. An alternative, a live attenuated SA 14-14-2 vaccine produced in China, has been extensively used both in China and in other Asian countries.

More recent cell-derived, inactivated vaccines have come onto the market, one designed to protect travelers from outside of endemic areas and the second for childhood immunization in Japan. In addition, a live recombinant product based on the yellow fever vaccine has been introduced in some Japanese encephalitis virus-endemic countries.

Therapeutic Immunization—A Goal for the Future?

Some attention is now directed to the possibility of inducing the body to clear established persistent infections (HIV, HCV, HBV) or even virus-related cancers (cervical carcinoma associated with HPV) by using a vaccine strategy to boost immunity. A number of possible endpoints could be adopted for success—a lowering of viral load, suppression of viral replication, total elimination of virus, or just slowing disease progression. These attempts, which have had limited but tantalizing results, would of course be helped by better understanding of how these viruses avoid immune elimination.

Commercial Aspects of Vaccine Manufacture

Vaccine development and manufacture are not as financially attractive to the private sector as are antiviral drugs. The development costs, including the need to carry out large-scale phase III trials to ensure safety, are considerable, and many of the populations in greatest need of new vaccines are in parts of the world where health expenditure per person is extremely low. Vaccines are used at most several times only during the life of the individual, so revenues are limited, and there is detailed community scrutiny for rare adverse events

TABLE 11.5 Commercial Attractiveness of Vaccines Compared to Antiviral Drugs

Vaccines	Antivirals
Used 1 to 3 times only, then maybe an occasional booster	Dosing is repeated, e.g., several times every day, sometimes for months
Used <i>before</i> the need arises, similar to insurance	Used <i>after</i> the subject has acquired the disease or high risk
Community scrutinizes rare adverse events critically, often does not appreciate risk	Community accepts risk/benefit ratio
Suspicion of authorities, big pharmaceutical companies	Similar

that may lead to legal ramifications. Thus, the enormous global benefit of vaccination against common serious infections is offset by the reticence of some manufacturers to take the necessary commercial risks. Efforts by government and public–private partnerships such as the vaccine alliance GAVI and the Bill and Melinda Gates Foundation, are helping to address this difficulty (Table 11.5).

FURTHER READING

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