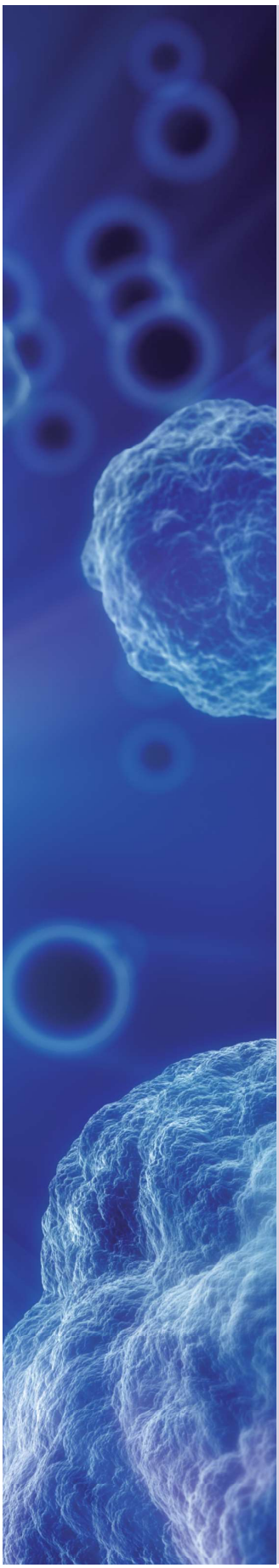


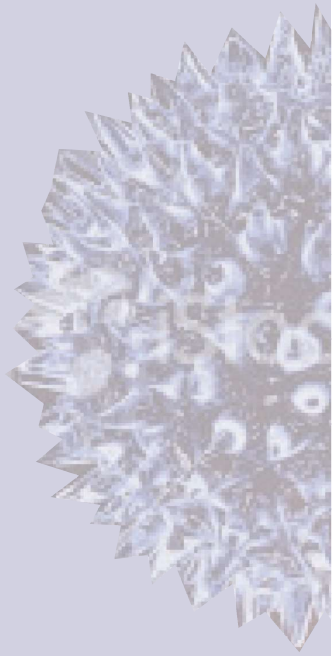
CHAPTER 13

Vaccines

Key Topics

Just to Recap …

The mechanisms by which we resist the onslaught of microbes have been discussed. These mechanisms include humoral, cellular and innate immunity. One of the great triumphs of medicine has been the ability to harness these mechanisms through vaccination to protect against a host of infectious diseases.



Introduction

The control of infection is approached from several directions. Improvements in public health—water supply, sewage systems, education in personal hygiene—prevent the spread of cholera and many other diseases. Antibiotics have had a major impact on bacterial diseases. Another strategy is to give the immune response a helping hand. This can be achieved by administering individual components of the immune response, such as defensins or antibodies, by using immunopotentiating agents such as cytokines, or more commonly by exposing the immune system to an antigen in order to stimulate the acquired immune response to generate memory—a procedure referred to as vaccination (see Milestone 13.1). Vaccines have traditionally been aimed at generating responses against infectious agents, but increasingly they are also being explored in areas such as malignancy.

Roitt's Essential Immunology, Twelfth Edition. Peter J. Delves, Seamus J. Martin, Dennis R. Burton, Ivan M. Roitt.

Milestone 13.1—Vaccination

The notion that survivors of serious infectious disease seldom contract that infection again has been embedded in folklore for centuries. In an account of the terrible plague that afflicted Athens, Thucydides noted that, in the main, those nursing the sick were individuals who had already been infected and yet recovered from the plague. Deliberate attempts to ward off infections by inducing a minor form of the disease in otherwise healthy subjects were common in China in the Middle Ages. There, they developed the practice of inhaling a powder made from **smallpox** scabs as protection against any future infection. The Indians inoculated the scab material into small skin wounds, and this practice of **variolation** (Latin *varus*, a pustular facial disease) was introduced into Turkey where the inhabitants were determined to prevent the ravages of smallpox epidemics interfering with the lucrative sale of their gorgeous daughters to the harems of the wealthy.

The writer Voltaire, in 1773, tells us that the credit for spreading the practice of variolation to western Europe should be attributed to Lady Wortley Montague, a remarkably enterprising woman who was the wife of the English Ambassador to Constantinople in the time of George I. With little scruple, she inoculated her daughter with smallpox in the face of the protestations of her chaplain who felt that it could only succeed with infidels, not Christians. All went well however and the practice was taken up in England despite the hazardous nature of the procedure that had a case fatality of 0.5–2%. These dreadful risks were taken because, at that time, as Voltaire recorded "…three score persons in every hundred have the smallpox. Of these three score, twenty die of it in the most favorable season of life, and as many more wear the disagreeable remains of it on their faces so long as they live."

Edward Jenner (1749–1823) (Figure M13.1.1), a country physician in Gloucestershire, suggested to one of his patients that she might have smallpox, but she assured him that his diagnosis was impossible as she had already contracted cowpox through her chores as a milkmaid (folklore again!). This led Jenner to the series of experiments in which he showed that prior inoculation with cowpox, which was nonvirulent (i.e. nonpathogenic) in the human, protected against subsequent challenge with smallpox (cf. p. 351). His ideas initially met with violent opposition but were eventually accepted and he achieved world fame; learned societies everywhere elected him to membership, although it is intriguing to note that the College of Physicians in London required him to pass an examination in classics and the Royal Society honored him with a Fellowship on the basis of his work on the nesting behavior of the cuckoo. In the end he inoculated thousands of people in the shed in the garden of his house in Berkeley, Gloucestershire, which now functions as a museum and venue for small symposia (rather fun to visit if you get the chance).

The next seminal development in vaccines came through the research of Louis Pasteur who had developed the germ theory of disease. A culture of chicken cholera bacillus, which had accidently been left on a bench during the warm summer months, lost much of its ability to cause disease; nonetheless, birds that had been inoculated with this old culture were resistant to fresh virulent cultures of the bacillus. This **attenuation** of **virulent** organisms was reproduced by Pasteur for anthrax and rabies using abnormal culture and passage conditions. Recognizing the relevance of Jenner's research for his own experiments, Pasteur called his treatment **vaccination**, a term that has stood the test of time.

Figure M13.1.1. Edward Jenner among patients in the Smallpox and Inoculation Hospital at St Pancras, London.

Etching after J. Gillray, 1802. (Kindly supplied by the Wellcome Centre Medical Photographic Library, London.)

Passively acquired immunity

Passively administered antibody

Temporary protection against infection and clearance of toxins can be achieved by giving antibody isolated from the plasma of an individual having a high antibody titer to the pathogen or a hyperimmunized animal (Table 13.1 and Figure 13.1). Prior to the introduction of antibiotics, horse serum containing anti-tetanus or anti-diphtheria toxins was extensively employed prophylactically, but nowadays it is used less commonly because of the complication of serum sickness (a type III hypersensitivity) and immediate (type I) hypersensitivity developing in response to the foreign protein. Furthermore, as the acquired antibodies are utilized by combination with antigen or are catabolized in the normal way, this protection is lost. The use of passive immunization is currently largely restricted to anti-venoms, in which an immediate therapeutic effect is required for a usually rare event such as a snake bite, and in prophylaxis for certain viral infections including cytomegalovirus (CMV) and rabies. However, with the emergence of antibiotic-resistant strains of bacteria, and concerns about possible bioterrorism, there is a renewed interest in passive immunization against infectious

Table 13.1. Examples of passive therapy against infection and toxins.

Condition	Source of antibody	Use
Tetanus infection	Human polyclonal	Antitoxin. Management of tetanus-prone wounds in patients where immunization is incomplete or uncertain
Botulism	Horse polyclonal	Antitoxin. Post-exposure prophylaxis of botulism
Snake bites (various)	Horse polyclonal	Antivenom. Treatment following venomous snake bite
Spider bites (various)	Horse polyclonal, rabbit polyclonal	Antivenom. Treatment following venomous spider bite
Paralysis tick bite	Dog polyclonal	Antivenom. Treatment following bite from paralysis tick
Stonefish sting	Horse polyclonal	Antivenom. Treatment following stonefish sting
Jellyfish sting	Sheep polyclonal	Antivenom. Treatment following venomous jellyfish sting
Hepatitis B infection	Human polyclonal	Antiviral. Prevention of infection in laboratory and other personnel accidentally inoculated with hepatitis B virus, and in infants of mothers infected during pregnancy or who are high-risk carriers
Rabies infection	Human polyclonal/monoclonal	Antiviral. Following bite from a possibly infected animal
Varicella-zoster virus infection	Human polyclonal	Antiviral. Seronegative individuals at increased risk of severe varicella (chickenpox)
Cytomegalovirus infection	Human polyclonal	Antiviral. Prophylaxis in immunosuppressed patients
Respiratory syncytial virus infection	Humanized mouse IgG1 monoclonal	Antiviral. Prevention of serious lower respiratory tract disease in high-risk children and infants

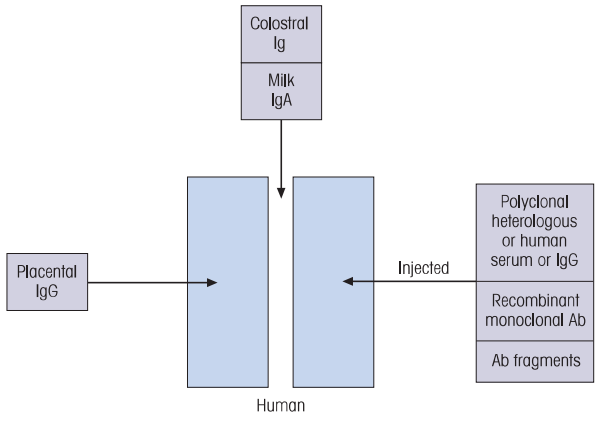


Figure 13.1. Passive immunization produced by: transplacental passage of IgG from mother to fetus, acquisition of IgA from mother's colostrum and milk by the infant, and injection of polyclonal antibodies, recombinant monoclonal antibodies, or antibody (Ab) fragments (Fab or scFv).

agents. Increasingly, it is likely that polyclonal antibody preparations will be replaced by human monoclonal antibodies or combinations of such antibodies. For instance, a humanized mouse monoclonal antibody (Synagis®, MedImmune) is in use to prevent disease due to respiratory syncytial virus (RSV) in babies and young infants. A cocktail of two human monoclonal antibodies against rabies virus is being developed for use as a post-exposure prophylactic following a bite or a scratch from a rabid animal such as a dog or a bat. In this case, there is a window of opportunity for intervention as the rabies virus needs to gain access to the CNS to cause disease and circulating antibody can prevent this. Passive antibody in rabies treatment is augmented with vaccination.

Maternally acquired antibody

In the first few months of life, while the baby's own lymphoid system is slowly getting under way, protection is afforded to the fetus by maternally derived IgG antibodies acquired by placental transfer and to the neonate by intestinal absorption of colostral immunoglobulins (Figure 13.1). The major immunoglobulin in milk is secretory IgA (SIgA) and this is not absorbed by the baby but remains in the intestine to protect the mucosal surfaces. In this respect it is quite striking that the SIgA antibodies are directed against bacterial and viral antigens often present in the intestine, and it is presumed that IgA-producing cells, responding to gut antigens, migrate and colonize breast tissue (as part of the mucosal immune system; see pp. 324–5), where the antibodies they produce appear in the milk. The case for mucosal vaccination of future mothers against selected infections is strong. It should also be noted that it has been argued that one of the most important functions of antibody is in the maternally acquired role. The hypothesis is that maternal antibody attenuates many infections allowing cellular immunity to mature under controlled conditions.

Intravenous immunoglobulin (IVIg)

Intravenous immunoglobulin (IVIg) is a preparation of IgG obtained by large-scale fractionation of plasma pooled from thousands of healthy blood donors. The preparations are given to individuals with immunodeficiencies associated with reduced or absent circulating antibody. IVIg is also of value in the treatment of a number of infection-associated conditions such as streptococcal toxic shock syndrome. IVIg also has efficacy in the treatment of several autoimmune and inflammatory diseases such as idiopathic thrombocytopenic purpura, chronic inflammatory demyelinating polyneuropathy and Guillain–Barré syndrome. The mechanism of action in these non-immunodeficient patients remains unclear, although recent evidence suggests IVIg probably modulates immune activity through sialic acids on the Ig molecule.

Adoptive transfer of cytotoxic T-cells

This is a labor-intensive operation and will be restricted to autologous cells or instances in which the donor shares an MHC class I allele. Adoptive transfer of autologous cytotoxic T-lymphocytes has been shown to be effective in enhancing EBV-specific immune responses and in reducing the viral load in patients with post-transplant lymphoproliferative disease.

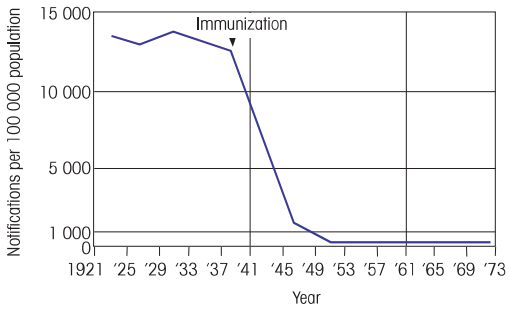


Figure 13.2. Notification of diphtheria in England and Wales per 100 000 population showing dramatic fall after immunization.

(Reproduced from Dick G. (1978) *Immunisation*. Update Books; with kind permission of the author and publishers.)

Principles of vaccination

Herd immunity

In the case of tetanus, active immunization is of benefit to the individual but not to the community as it will not eliminate the organism that is found in the feces of domestic animals and persists in the soil as highly resistant spores. Where a disease depends on human transmission, immunity in just a proportion of the population can help the whole community if it leads to a fall in the reproduction rate (i.e. the number of further cases produced by each infected individual) to less than one; under these circumstances the disease will die out: witness, for example, the disappearance of diphtheria from communities in which around 75% of the children have been immunized (Figure 13.2). But this figure must be maintained; there is no room for complacency. In contrast, focal outbreaks of measles have occurred in communities that object to immunization on religious grounds, raising an important point for parents in general. Each individual must compare any perceived disadvantage associated with vaccination in relation to the increased risk of disease in their unprotected child.

How vaccines work

Vaccines are effective because of adaptive immunity and immune memory. Antibody memory exists in two compartments. First as pre-existing antibody in the blood and tissues ready to attack the pathogen without cellular triggering—this is probably the most powerful first line of defense against exposure to many pathogens. This antibody can be maintained at relatively high levels for many years, probably produced by

long-lived plasma cells in the bone marrow, although this is not universally accepted. In a sense, the most crucial part of antibody "memory" might be equated with the long life of these plasma cells. However, the second form of the antibody memory component, memory B-cells, might also be crucial for vaccine-mediated protection in some cases. In this case, contact with pathogen stimulates B-cells to proliferate and differentiate to produce copious amounts of antibody. Equally, the contact of memory B-cells with pathogen might be important to boost plasma-cell numbers and serum-antibody concentrations for the next encounter with the pathogen. T-cell memory also exists in two compartments. Effector memory T-cells are found in peripheral tissues where they can respond immediately to pathogen-infected cell contact with effector activities. Central memory T-cells are found mainly in lymph nodes where they can respond to pathogen contact with expansion and differentiation to effectors. T-cell memory consists of both $CD8^+$ and $CD4^+$ T-cell responses. Clearly T cells responses are most pertinent to virus, parasite and intracellular bacterial infections.

The best correlate of protection for many current vaccines is antibody and it is likely that, in these cases, antibody is the most important mechanism of vaccine-induced resistance to disease. This is consistent with the notion that T-cells are the largest contribution to viral immunity during primary infection and antibodies during secondary infection (Figure 13.3). However, it is important to note that the mechanisms of vaccine protection may vary widely between different pathogens, different individuals, different doses of pathogen to which the individual is exposed and different routes of exposure.

In addition to an ability to engender effective immunity, a number of mundane but nonetheless crucial conditions must be satisfied for a vaccine to be considered successful (Table 13.2). The antigens must be readily available, and the preparation should be stable, cheap and certainly, safe, bearing in mind that the recipients are most often healthy children. Clearly, the first contact with antigen during vaccination should not be injurious and the maneuver is to avoid the pathogenic effects of infection, while maintaining protective immunogens.

The primary approaches to the generation of existing vaccines are shown in Figure 13.4. and these are now considered in turn.

Killed organisms as vaccines

The simplest way to destroy the ability of microbes to cause disease, yet maintain their antigenic constitution, is to prevent their replication by killing in an appropriate manner. Parasitic worms and, to a lesser extent, protozoa are extremely difficult to grow up in bulk to manufacture killed vaccines. This problem does not arise for many bacteria and viruses and, in these cases, the inactivated microorganisms have provided a number of safe antigens for immunization. Examples are influenza, cholera and inactivated poliomyelitis (Salk) vaccines (Figure 13.5). Care has to be taken to ensure that important protective antigens are not destroyed in the inactivation process.

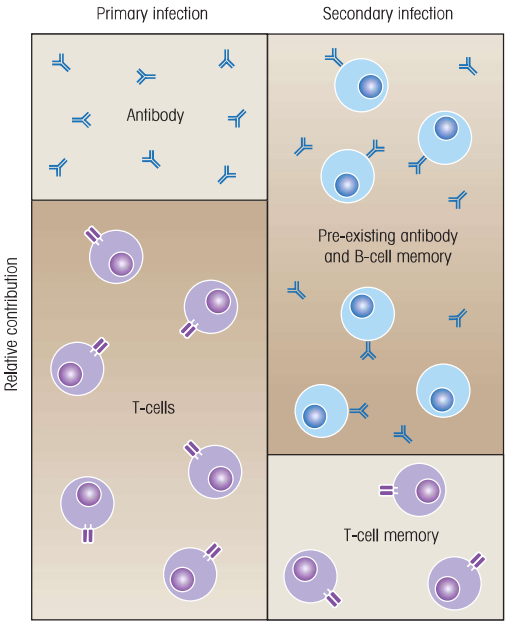


Figure 13.3. A schematic view of the relative contributions of humoral and cellular immunity during primary or secondary viral infection.

During primary viral infection, antiviral T-cell responses are critical for reducing viral replication in addition to contributing to the development of an effective antibody response. Primary T-cell-dependent antibody responses are mounted during the course of infection and take time to undergo immunoglobulin class-switching and somatic hypermutation to possibly provide assistance to virus specific T-cells in resolving the infection. Following recovery from primary infection (or vaccination), persisting virus-specific antibody represents the first line of defense against secondary infection. If secondary infection does occur, then circulating antibodies and presumably memory B-cells that proliferate and differentiate into antibody secreting cells will reduce virus dissemination and allow time for the development of an antiviral T-cell response. Memory B-cells are highly efficient at presenting specific antigen and therefore may also be involved with more rapid and efficient presentation to T-cells as well. Pre-existing T-cell memory will also play a role in protection against secondary infection. However, even if T-cell memory has declined or is lost, the long-term maintenance of antiviral antibody responses will suppress virus replication until a new virus-specific T-cell response is mounted from the naive repertoire. (Adapted from Amanna I.J. & Slifka M.K. (2009) *Antiviral Research* **84**, 119–130.)

Table 13.2. Factors required for a successful vaccine.

Factor	Requirements
Effectiveness	Must evoke protective levels of immunity: at the appropriate site of relevant nature (Ab, Tc, Th1, Th2) of adequate duration
Availability	Readily cultured in bulk or accessible source of subunit
Stability	Stable under extreme climatic conditions, preferably not requiring refrigeration
Cheapness	What is cheap in the West may be expensive in developing countries but the Bill and Melinda Gates Foundation and governments help
Safety	Eliminate any pathogenicity

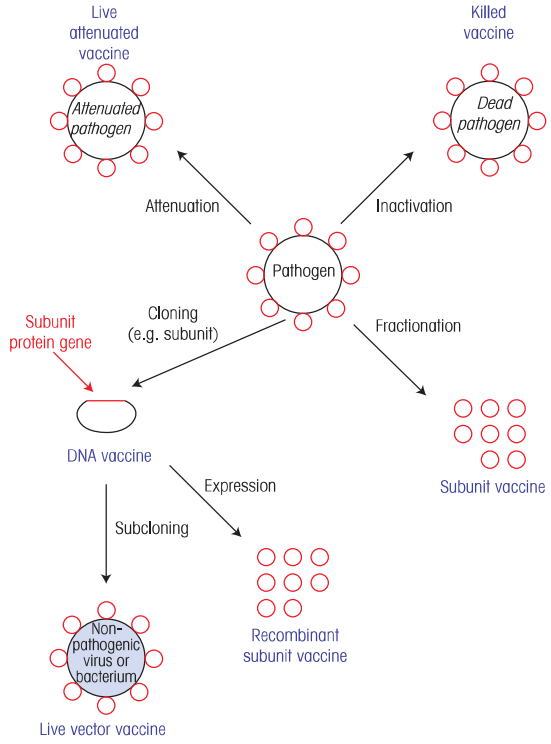


Figure 13.4. Classical vaccine approaches.

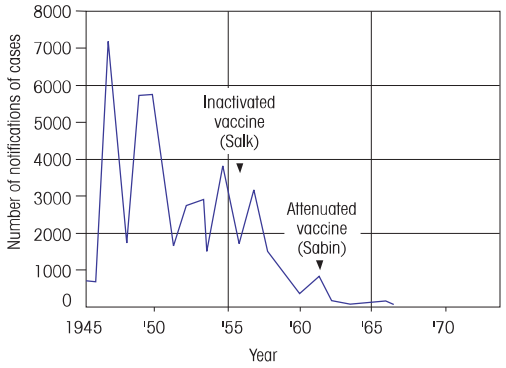


Figure 13.5. Notifications of paralytic poliomyelitis in England and Wales showing the beneficial effects of community immunization with killed and live vaccines.

(Reproduced from Dick G. (1978) *Immunisation*. Update Books; with kind permission of the author and publishers.)

Live attenuated organisms have many advantages as vaccines

The objective of attenuation is to produce a modified organism that mimics the natural behavior of the original microbe without causing significant disease. In many instances the immunity conferred by killed vaccines, even when given with adjuvant (see below), is often inferior to that resulting from infection with live organisms. This must be partly because the replication of the living microbes confronts the host with a **larger and more sustained dose of antigen** and that, with budding viruses, infected cells are required for the establishment of good **cytotoxic T-cell memory**. Another significant advantage of using live organisms is that the immune **response takes place largely at the site of the natural infection**. This is well illustrated by the nasopharyngeal IgA response to immunization with polio vaccine. In contrast with the ineffectiveness of parenteral injection of killed vaccine, intranasal administration evoked a good local antibody response; however, whereas this declined over a period of 2 months or so, per oral immunization with *live attenuated* virus established a persistently high IgA antibody level (Figure 13.6).

There is in fact a strong upsurge of interest in strategies for mucosal immunization. Remember, the mucosal immune system involves mucous membranes covering the aerodigestive

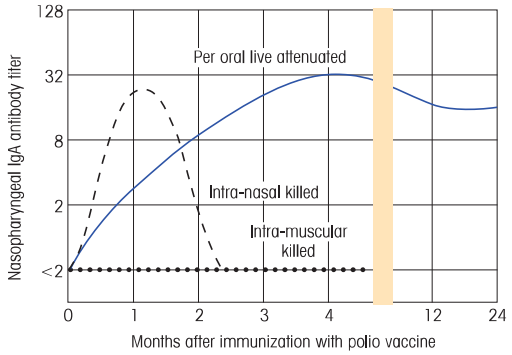


Figure 13.6. Local IgA response to polio vaccine.

Local secretory antibody synthesis is confined to the specific anatomical sites that have been directly stimulated by contact with antigen. (Data from Ogra P.L. *et al.* (1975). In Notkins A.L. (ed.) *Viral Immunology and Immunopathology*. Academic Press, New York, p. 67.)

and urogenital tracts as well as the conjunctiva, the ear and the ducts of all exocrine glands whose protection includes SIgA antibodies. Resident T-cells in these tissues produce large amounts of transforming growth factor-β (TGFβ), and the interleukins IL-10 and IL-4, which promote the B-cell switch to IgA, and note also that human intestinal epithelial cells themselves are major sources of TGFβ and IL-10.

Classical methods of attenuation

The objective of attenuation, that of producing an organism that causes only a very mild form of the natural disease, can be equally well attained if one can identify heterologous strains that are virulent for another species, but avirulent in humans. The best example of this was Jenner's seminal demonstration that cowpox would protect against smallpox. Subsequently, a truly remarkable global effort by the World Health Organization (WHO), combining extensive vaccination and selective epidemiological control methods, **completely eradicated smallpox as a human disease**—a wonderful achievement. Thus, although 300 million people are estimated to have died of smallpox in the twentieth century, no-one has died of the virus since 1978. Emboldened by this success, the WHO embarked upon a program to eradicate polio using attenuated polio vaccine to block transmission of the virus and, despite setbacks such as a temporary halt in vaccination in northern Nigeria following unfounded rumours regarding the safety of the vaccine, it is hoped that this goal will be achieved in the not too distant future. One can even follow the progress of this campaign on http://www.polioeradication.org.

Attenuation itself was originally achieved by empirical modification of the conditions under which an organism grows. Pasteur first achieved the production of live but non-virulent forms of chicken cholera bacillus and anthrax (cf. Milestone 13.1) by such artifices as culture at higher temperatures and under anerobic conditions, and was able to confer immunity by infection with the attenuated organisms. A virulent strain of *Mycobacterium tuberculosis* became attenuated by chance in 1908 when Calmette and Guérin at the Institut Pasteur, Lille, France added bile to the culture medium in an attempt to achieve dispersed growth. After 13 years of culture in bile-containing medium, the strain remained attenuated and was used successfully to vaccinate children against tuberculosis. The same organism, bCG (bacille Calmette–Guérin), is widely used today in many countries for the immunization of infants and of tuberculin-negative children and adolescents. However, its efficacy varies widely from, for example, protection in 80% of vaccinated individuals in the UK, to a total lack of efficacy in Southern India. This variability is not fully understood, but is thought to be due to a number of factors including local differences in the antigenic composition of the vaccine and in the environmental mycobacterial strains, and differences in MHC alleles and other genetic factors in the various human populations. Attenuation by cold adaptation has been applied to influenza and other respiratory viruses; the organism can grow at the lower temperatures (32–34°C) of the upper respiratory tract, but fails to produce clinical disease because of its inability to replicate in the lower respiratory tract (37°C). An intranasal vaccine containing cold-adapted attenuated influenza virus strains was licensed for use in the USA in 2003.

Attenuation by recombinant DNA technology

It must be said that many of the classical methods of attenuation are somewhat empirical and the outcome is difficult to control or predict. With knowledge of the genetic makeup of these microorganisms, we can apply the molecular biologist's delicate scalpel to deliberately target the alterations that are needed for successful attenuation. Thus genetic recombination is being used to develop various attenuated strains of viruses, such as influenza, with not only a lower virulence for humans but also an increased multiplication rate in eggs (enabling newly endemic strains of influenza to be adapted for rapid vaccine production). Not surprisingly, strains of HIV-1, with vicious deletions of the regulatory genes, are being investigated as protective vaccines. The potential is clearly quite enormous.

The **tropism** of attenuated organisms for **the site** at which **natural infection** occurs is likely to be exploited dramatically in the near future to establish gut immunity to typhoid and cholera using attenuated forms of *Salmonella typhi* and *Vibrio cholerae* in which the virulence genes have been identified and modified by genetic engineering.

Microbial vectors as vaccines

An ingenious trick is to use a nonpathogenic virus as a Trojan horse for genes encoding proteins of a pathogen. Incorporation of such "foreign" genes into attenuated recombinant viral vectors, such as fowlpox and canarypox virus and the modified

vaccinia Ankara (MVA) strain virus that infect mammalian hosts, but are unable to replicate effectively, provides a powerful vaccination strategy with many benefits. The genes may be derived from organisms that are difficult to grow or inherently dangerous, and the constructs themselves are replication deficient, non-integrating, stable and relatively easy to prepare. The proteins encoded by these genes are appropriately expressed *in vivo* with respect to glycosylation and secretion, and are processed for MHC presentation by the infected cells, thus effectively endowing the host with both humoral and cell-mediated immunity.

A wide variety of genes have been expressed in vaccinia virus vectors, and it has been demonstrated that the products of genes coding for viral envelope proteins, such as influenza virus hemagglutinin, vesicular stomatitis virus glycoprotein, HIV-1 gp120 and herpes simplex virus glycoprotein D, could be correctly processed. Hepatitis B surface antigen (HBsAg) was secreted from recombinant vaccinia virus-infected cells as the characteristic 22 nm particles (Figure 13.7). It is an impressive approach and chimpanzees have been protected against the clinical effects of hepatitis B virus, while mice that were inoculated with recombinant influenza hemagglutinin generated cytotoxic T-cells and were protected against influenza infection.

Attention has also been paid to bCG as a vehicle for antigens required to evoke CD4-mediated T-cell immunity. The organism is avirulent, has a low frequency of serious complications, can be administered any time after birth, has strong adjuvant properties and gives long-lasting cell-mediated immunity after a single injection.

The ability of *Salmonella* to elicit **mucosal responses by oral immunization** has been exploited in the design of vectors that allow the expression of any protein antigen linked to *E. coli* enterotoxin, a powerful mucosal immunostimulant. There is an attractive possibility that the oral route of vaccination may be applicable not only for the establishment of gut mucosal immunity but also for providing systemic protection. For example, *Salmonella typhimurium* not only invades the mucosal lining of the gut, but also infects cells of the mononuclear phagocyte system throughout the body, thereby stimulating the production of humoral and secretory antibodies as well as $CD4^+$ and $CD8^+$ T cell-mediated immunity. As attenuated *Salmonella* can be made to express proteins from *Shigella*, cholera, malaria sporozoites and so on, it is entirely feasible to consider these as potential oral vaccines. *Salmonella* may also carry "foreign genes" within separate DNA plasmids and, after phagocytosis by antigen-presenting cells, the plasmids can be released from the phagosome into the cytosol if the plasmid bears a recombinant listeriolysin gene or the bacterium is a mutant whose cell walls disintegrate within the phagosome; the plasmid then moves to the nucleus where it is transcribed to produce the desired antigen. Quite strikingly, these attenuated organisms are very effective when inhaled and can elicit substantive mucosal and systemic immune responses comparable with those obtained by the parenteral route.

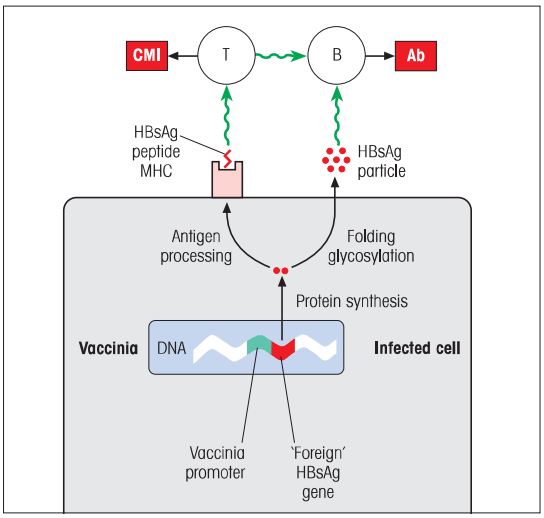


Figure 13.7. Hepatitis B surface antigen (HBsAg) vaccine using an attenuated vaccinia virus carrier.

The HBsAg protein is synthesized by the machinery of the host cell: some is secreted to form the HBsAg 22 nm particle that stimulates antibody (Ab) production, and some follows the antigen processing pathway to stimulate cell-mediated immunity (CMI) and T-helper activity.

Constraints on the use of attenuated vaccines

Attenuated vaccines for poliomyelitis (Sabin), measles, mumps, rubella, varicella-zoster and yellow fever have gained general acceptance. However, with live viral vaccines there is a possibility that the nucleic acid might be incorporated into the host's genome or that there may be reversion to a virulent form. Reversion is less likely if the attenuated strains contain several mutations. Another disadvantage of attenuated strains is the difficulty and expense of maintaining appropriate cold-storage facilities, especially in out-of-the-way places. In diseases such as viral hepatitis, AIDS and cancer, the dangers associated with live vaccines are daunting. With most vaccines there is a very small, but still real, risk of developing complications and it cannot be emphasized too often that this **risk must be balanced against the expected chance of contracting the disease with its own complications**. Where this is minimal, some may prefer to avoid general vaccination and to rely upon a crash course backed up if necessary by passive immunization in the localities around isolated outbreaks of infectious disease.

It is important to recognize those children with immunodeficiency before injection of live organisms; a child with impaired T-cell reactivity can become overwhelmed by BCG and die. It is also inadvisable to give live vaccines to patients being treated with steroids, immunosuppressive drugs or

radiotherapy or who have malignant conditions such as lymphoma and leukemia; pregnant mothers must also be included here because of the vulnerability of the fetus.

Use in a veterinary context

For veterinary use, of course, there is a little less concern about minor side-effects and excellent results have been obtained using existing vaccinia strains with rinderpest in cattle and rabies in foxes, for example. In the latter case, a recombinant vaccinia virus vaccine expressing the rabies surface glycoprotein was distributed with bait from the air and immunized approximately 80% of the foxes in that area. No cases of rabies were subsequently seen, but epidemiological considerations indicate that, with the higher fox density that this leads to, the higher the percentage that have to be made immune; thus, either one has to increase the efficacy of the vaccine, or culling of the animals must continue—an interesting consequence of interference with ecosystems. Less complicated is the use of such immunization to control local outbreaks of rabies in rare mammalian species, such as the African wild dog, which are threatened with extinction by the virus in certain game reserves.

Subunit vaccines

A whole pathogen usually contains many antigens that are not concerned in the protective response of the host but may give rise to problems by suppressing the response to protective antigens or by provoking hypersensitivity, as we saw in the last chapter. Vaccination with the isolated protective antigens may avoid these complications, and identification of these antigens then opens up the possibility of producing them synthetically in circumstances in which bulk growth of the organism is impractical or isolation of the individual components too expensive.

The use of purified components as bacterial vaccines

Bacterial exotoxins such as those produced by diphtheria and tetanus bacilli have long been used as immunogens. First, they must of course be detoxified and this may be achieved by formaldehyde treatment when this does not destroy the major immunogenic determinants (Figure 13.8). Immunization with the **toxoid** will, therefore, provoke the formation of protective antibodies, which neutralize the toxin by stereochemically blocking the active site, and encourage removal by phagocytic cells. The toxoid is generally given after adsorption to aluminum hydroxide, which acts as an adjuvant and produces higher antibody titers. In addition to their use as vaccines to generate a protective antibody response against tetanus and diphtheria, the toxoids are often conjugated to other proteins, peptides or polysaccharides to provide helper T-cell epitopes for these antigens. Nontoxic variants of the toxins themselves, such as the CRM197 variant of diphtheria toxin, can also be used to provide helper T-cell epitopes for antigens such as the *Haemophilus influenzae* type b (Hib) polysaccharide.

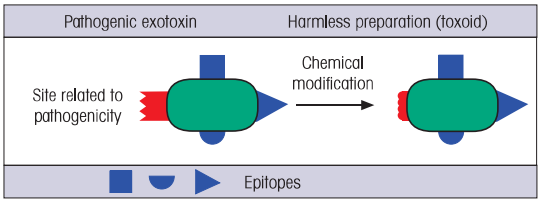


Figure 13.8. Modification of toxin to harmless toxoid without losing many of the antigenic determinants.

Thus, antibodies to the toxoid will react well with the original toxin.

The emphasis now is to move towards gene cloning of individual proteins once they have been identified immunologically and biochemically. In general, a protein subunit used in a vaccine should contain a sufficient number of T-cell epitopes to avoid HLA-related unresponsiveness within the immunized population. In order to maintain a pool of memory B-cells over a reasonable period of time, persistence of antigen on the follicular dendritic cells (DCs) in a form resistant to proteolytic degradation with retention of the native three-dimensional configuration is needed.

A viral subunit vaccine: hepatitis B virus (HBV)

In 1965, Baruch Blumberg first described an antigen in the blood of Australian aborigines associated with hepatitis. This "Australia antigen" was subsequently shown to be a particle formed from the surface antigen of hepatitis B virus. Initially antigen particles were isolated from the plasma of HBV carriers and inactivated and used as a vaccine. Later, the particles were prepared in yeast. The HBV subunit vaccine was a milestone in vaccinology as it was the first manufactured using recombinant DNA technology. One very interesting facet of this vaccine is that it was originally used for small at-risk groups exposed to blood products such as doctors and nurses. Later, it became very widely used including in the developing world. As HBV is associated with hepatic cancer and more than 300 million people are infected worldwide, the HBV vaccine represents the first to prevent cancer on a large scale.

Carbohydrate vaccines

The dense surface distribution of characteristic glycan structures on diverse pathogens and on malignant cells makes carbohydrates attractive antibody-based vaccine targets (Figure 13.9). However, the nature of glycans presents some severe problems in terms of the induction of protective antibodies. First, glycans tend to be poorly immunogenic. They should be coupled to a carrier protein to provide a source of $CD4^+$ T-cell help. Second, anti-glycan antibodies typically have low affinities relative to anti-protein antibodies. They rely heavily on avidity effects to achieve binding at physiological concentrations. Third,

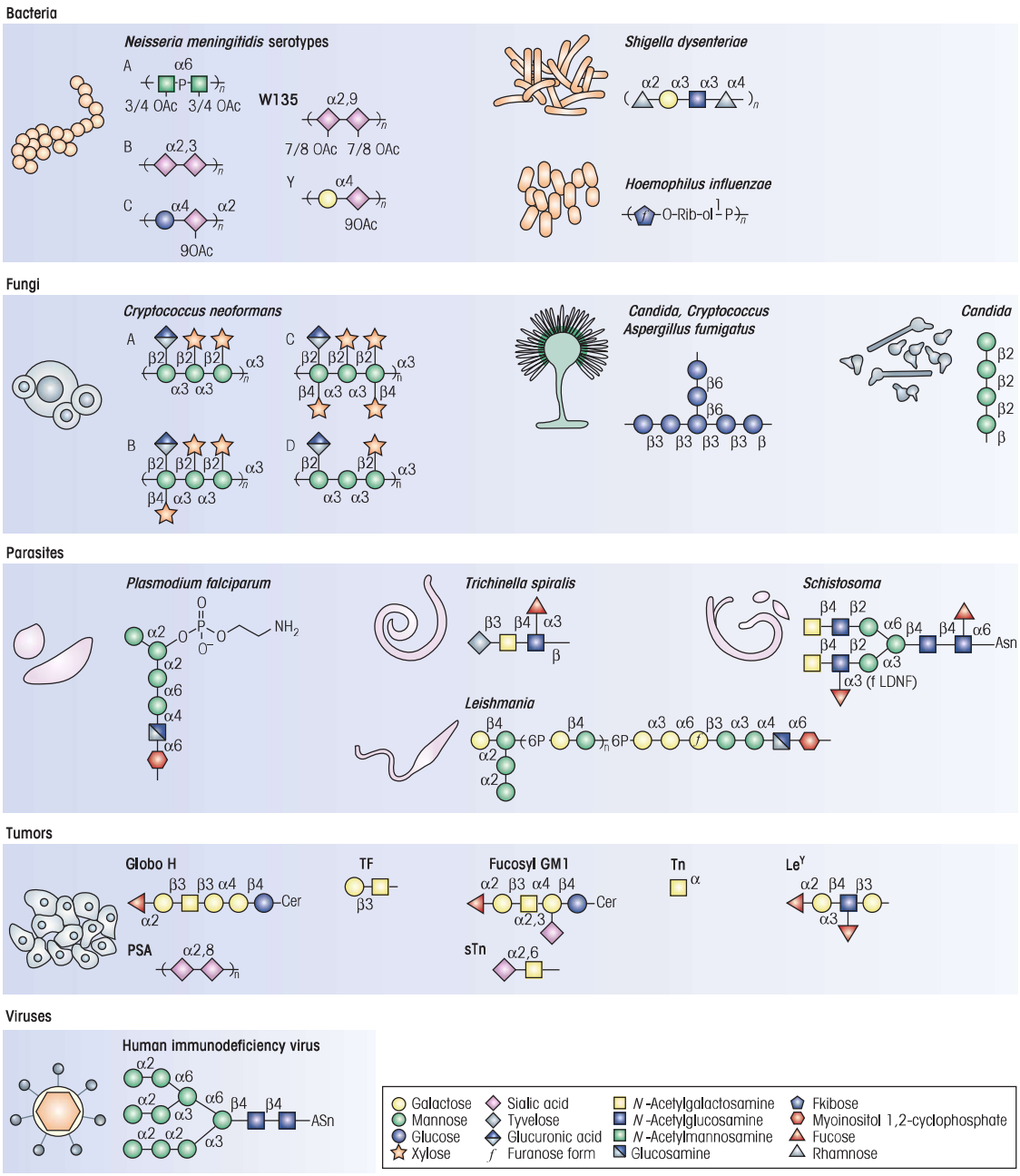


Figure 13.9. A diverse array of disease-causing agents and glycan antigens is targeted by existing and developmental carbohydrate vaccines.

Bacteria: capsular polysaccharide repeats associated with particular species (and serotypes). Fungi: common glucuronoxylomannan (GXM) motifs for serotypes A–D (*Cryptococcus*); β-glucan (*Candida*, *Cryptococcus* and *Aspergillus*); β-mannan (*Candida*). Parasites: synthetic glycosylphosphatidylinositol motif (*Plasmodium falciparum*); common tyvelose-containing antigen (*Trichinella*); LacdiNAc (LDN) and fucosylated LDN (LDNF) (*Schistosoma*); common lipophosphoglycan (*Leishmania*). Tumors: common glycan antigens associated with glycolipids (globohexaosylceramide (Globo H), fucosyl GM1, Lewis Y (Le^Y)) and glycoproteins (Thomsen–Friedenreich (TF), Le^Y, 2–6-α-*N*-acetylgalactosamine (Tn), sialyl Tn and polysialic acid (PSA)) found on various malignant tissues. Viruses: high mannose $GlcNAc_2Man_9$ (HIV). Mannose residues may be 6-*O*-acetylated on GXM motifs. (From Astronomo R.D. & Burton D.R. (2010) *Nature Reviews Drug Discovery* **9**, 308–324).

glycans are typically heterogeneous on target pathogens or cells and therefore the efficacy of any specific anti-glycan response is diluted. Nevertheless, glycoconjugates are increasingly being designed (Figure 13.10) as vaccine candidates. Licensed carbohydrate vaccines include those against *Haemophilus influenzae* type b (Hib), *Neisseria meningitidis*, *Salmonella typhi* and *Streptococcus pneumoniae*.

DNA vaccines

Teams working with J. Wolff and P. Felgner experimented with a new strategy for gene therapy that involved binding the negatively charged DNA to cationic lipids, which would themselves attach to the negatively charged surface of living cells and then presumably gain entry. The surprise was that controls injected with DNA without the lipids actually showed an *even higher uptake of DNA* and expression of the protein it encoded, so giving rise to the whole new technology of **DNA vaccination or genetic immunization**. As Wolff put it: "We tried it again and it worked. By the fourth or fifth time we knew we were onto something big. Even now I get a chill down my spine when I see it working." It was quickly appreciated that the injected DNA functions as a source of immunogen *in situ* and can induce strong immune responses, particularly cellular immune responses. The DNA used in this procedure is sometimes referred to as **naked DNA** to reflect the fact that the nucleic acid is stripped bare of its associated proteins.

The transcription unit composed of the cDNA gene with a poly A terminator is stitched in place in a DNA plasmid with a promoter such as that from cytomegalovirus and a CpG bacterial sequence as an adjuvant. It is usually injected into muscle where it can give prolonged expression of protein. The pivotal cell is the dendritic antigen-presenting cell that may be transfected directly, could endocytose soluble antigen secreted by the muscle cells into the interstitial spaces of the muscle, and could take up cells that have been killed or injured by the vaccine. The CpG immunostimulatory sequences engage Toll-like receptor 9 (TLR9) and thereby provoke the synthesis of IFNα and β, IL-12 and IL-18, which promote the formation of T helper (Th)1 cells; this in turn generates good cell-mediated immunity, helps the B-cell synthesis of certain antibody classes (e.g. IgG2a in the mouse) and induces good cytotoxic T-cell responses, presumably reflecting the cytosolic expression of the protein and its processing in the MHC class I pathway. Let's look at an example. It will be recalled that frequent point mutations (drift; p. 331) in the gene encoding influenza surface hemagglutinin give rise to substantial antigenic variation, whereas the major internal proteins, which elicit T-cell-mediated immunity responses, have been relatively conserved. On this line of reasoning, nucleoprotein DNA should give broad protection against other influenza strains and indeed it does (Figure 13.11). A combination of DNAs encoding the hemagglutinin (included only for statutory reasons) and nucleoprotein genes gave nonhuman primates and ferrets good protection against infection, and protected ferrets against challenge with an antigenically distinct epidemic human virus strain more effectively than the contemporary clinically licensed vaccine. Vaccination can also be achieved by coating the plasmids onto minute gold particles or cationic poly (lactide co-glycolide) (PLG) microparticles and shooting them into skin epidermal cells by the high-pressure "biolistic" helium gun, a technique that uses between 10- and 100-fold less plasmid DNA than the muscle injection.

To date, straightforward DNA vaccination has not been as successful in humans or nonhuman primates as in mice. Nevertheless the many potential advantages of the approach, including its simplicity and ease of quality control for example, mean that many efforts on improving DNA vaccination in humans are being explored. One is a "prime–boost" protocol. The persistent but low level of expression of the protein antigen by DNA vaccines establishes a pool of relatively high affinity memory B-cells that can readily be revealed by boosting with protein antigen (Figure 13.12). This has given rise to a prime–boost strategy in which these memory cells are expanded by boosting with a non-replicating viral vector, such as fowlpox virus or Ankara strain-modified vaccinia virus, bearing a gene encoding the antigen. Mice immunized in this fashion with influenza virus hemagglutinin produced satisfyingly high levels of IgG2a antibody and were protected against challenge with live virus. Remarkably, up to 30% of circulating CD8 T-cells were specific for the immunizing epitope as shown by MHC class I tetramer binding (cf. p. 176). A similar strategy with *Plasmodium berghei* produced high levels of peptide-specific CD8 T cells secreting IFNγ which protected against challenge by sporozoites. An ongoing HIV vaccine trial uses a DNA prime followed by an adenoviral boost, with both prime and boost encoding a number of HIV proteins.

Newer approaches to vaccine development

Conventional vaccines, which have been enormously successful against a range of pathogens, can be described as following a "simple mimicry" strategy going back to the work of Jenner and Pasteur. The essential strategy is to use attenuated or killed pathogens, with the occasional use of purified or recombinant subunits. These vaccines mostly target pathogens that have very little antigenic diversity and appear to be largely dependent on antibody-based protection. The conventional approach has tended to find much less success for a range of other pathogens, notably those showing considerable antigenic diversity or for which T-cell immunity may be of greater protective import (Figure 13.13). For example consider HIV. A live attenuated vaccine protects monkeys against challenge with the same strain of SIV (simian immunodeficiency virus, the monkey equivalent of HIV) but is far less effective against other strains of SIV. For a human vaccine against HIV to be effective clearly it should protect against the majority of circulating global viral strains. Killed and subunit vaccines against HIV/SIV tend to be ineffective because of the enormous variability and instability of the

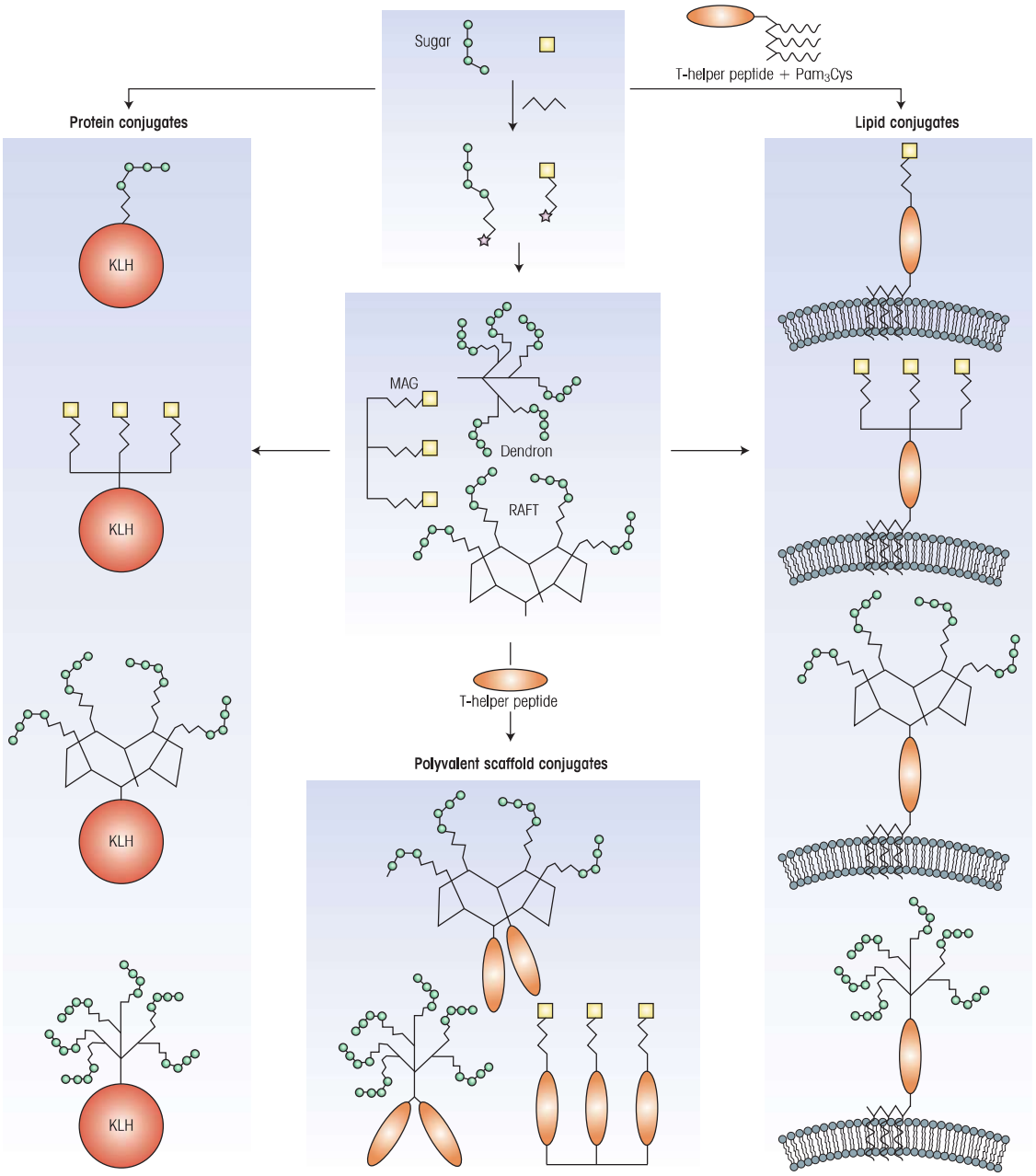


Figure 13.10. Schematic representation of glycoconjugate immunogen design.

Starting from activated glycans (star denotes activated group) from natural or synthetic sources, the production of three categories of glycoconjugate immunogens is shown: protein conjugates, lipid conjugates and polyvalent scaffold conjugates. The requirement for both polyvalent display and helper T-cell epitopes, crucial for achieving strong, long-lasting and class-switched antibody responses, are satisfied in each category. For protein conjugates, activated glycans are covalently attached to immunogenic protein carriers—for example, keyhole limpet hemocyanin (KLH)—which provide helper T-cell epitopes and enable polyvalent display. Lipid conjugates, made by covalent linkage of activated glycans to helper T-cell peptides attached to lipid moieties, allow polyvalency through formulation into lipid membranes. In addition, activated glycans may first be conjugated to synthetic polyvalent scaffolds—for example, dendron, multiple antigen glycopeptide (mAG) and regioselectively addressable functionalized template (RAFT)—which may then be used to make protein and lipid conjugates. Alternatively, polyvalent scaffold conjugates may be made through addition of helper T-cell peptides alone. Adjuvants (see later) are usually included in the final glycoconjugate vaccine formulations (for example, alum or QS-21). Note that tripalmitoyl-*S*-glyceryl-cysteinylserine (Pam_3Cys) has adjuvant properties. (From Astronomo R.D. & Burton D.R. (2010) *Nature Reviews Drug Discovery* **9**, 308–324.)

surface envelope proteins (see also chapter 14). Another highly problematical disease for vaccine development is tuberculosis; immunity to this intracellular pathogen is likely to involve T-cell rather than antibody-based protective activities.

In recent years, vaccine development has increasingly turned to the tools of modern molecular biology. For bacterial vaccines, the rise of genomics has been crucial. At least one complete sequence is now available for all of the major human pathogens. This has facilitated the development of "reverse vaccinology," championed by Rino Rappuoli and colleagues. The essential strategy identifies the complete repertoire of bacterial surface antigens, investigates the ability of antigens to elicit immunity in animal models and then designs a combination of antigens to be used in the vaccine. This elegant approach is illustrated in Figure 13.14 for the successful development of a vaccine to serogroup B *Neisseria meningitidis* (MenB), which is the most common cause of meningococcal disease in the developed world and had defined conventional vaccine approaches for decades.

Highly variable viruses such as HIV and hepatitis C virus (HCV) also provide severe vaccine development problems. Here one of the approaches being adopted can be described as reverse engineering or structural vaccinology. Thus broadly neutralizing antibodies capable of acting against a wide spectrum of global isolates as required by a vaccine have been described in natural infection and are being studied in terms of their interaction with surface envelope proteins. The notion is that the molecular information gained can be used to modify envelope proteins or to design novel immunogens that can be used as vaccines to elicit broadly neutralizing antibodies. This same concept might provide a universal influenza vaccine that would protect against most or all subtypes and stains of flu and obviate the need for annual immunizations. Immunodominance is one of the great problems in developing vaccines to highly variable pathogens i.e. the pathogen has evolved so that the strongest immune responses tend to be elicited to the most variable regions of the pathogen. A host of strategies are now being explored to try and focus B-cell and T-cell responses on to the most conserved epitopes.

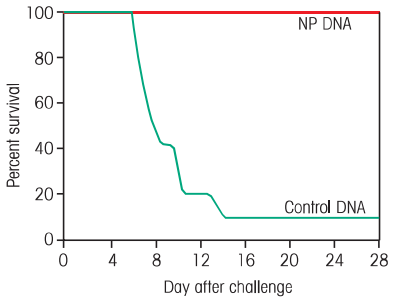


Figure 13.11. Protection from cross-strain influenza challenge after vaccination with nucleoprotein DNA.

Mice were immunized three times at 3-week intervals with 200 mg of nucleoprotein (NP) or vector (control) DNA and lethally challenged with a heterologous influenza strain 3 weeks after the last immunization. Survival of mice given NP DNA was significantly higher than in mice receiving vector ($p = 0.0005$). (Data kindly provided by Dr Margaret A. Liu and colleagues (1993) *DNA and Cell Biology* **12**, 777–783, and reproduced by permission of Mary Ann Liebert Inc.)

Current vaccines

The established vaccines in current use and the schedules for their administration are set out in Tables 13.3 and 13.4. Regional differences in immunization schedules reflect not only different degrees of perceived risk of infection but also other local considerations. Children under 2 years of age make inadequate responses to the T-independent *H. influenzae* capsular polysaccharide, so they are now routinely immunized with the antigen conjugated with tetanus toxoid or the

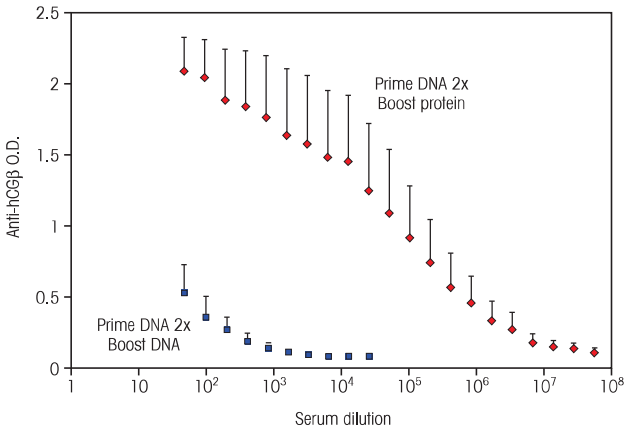


Figure 13.12. Induction of memory cells by DNA vaccine and boost of antibody production with the protein immunogen, human chorionic gonadotropin β-chain (hCG β).

Groups of five (C57BL/6 × BALB/c)F1 mice each received 50 mg of the hCG β DNA plasmid at weeks 0 and 2; one group received a further injection of the plasmid, while the other was boosted with 5 mg of the hCG β protein antigen in RIBI (cf. p. 365) adjuvant. Dilutions of serum were tested for antibodies to hCG β by indirect ELISA. Mean titers + SE are shown. (Data from Laylor R. *et al.* (1999) *Clinical and Experimental Immunology* **117**, 106.)

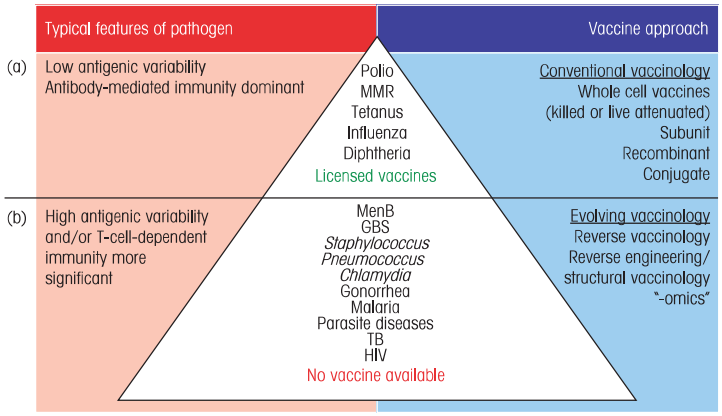


Figure 13.13. Schematic view of conventional vaccinology and evolving vaccinology in the post-genome era.

(a) Most licensed vaccines target pathogens that have low antigenic variability and pathogens for which protection depends on antibody-mediated immunity. These vaccines have typically been developed using conventional vaccinology. (b) Several pathogens are shown for which no vaccine is available, due to either their high antigenic variability and/or the need to induce T-cell-dependent immunity to elicit protection. New approaches are being applied to vaccine development for these pathogens in the post-genome era. Vaccines/diseases shown in the figure are selected examples of each category and are not a complete list. TB, *Mycobacterium tuberculosis*; MMR, mumps, measles, rubella; MenB, meningitis B; GBS, group B *Streptococcus*. (Figure with permission from Rinuado C.D., Telford J.L., Rappuoli R. & Seib K.L. (2009) *Journal of Clinical Investigation* **9**, 2515–2525 and modified.)

Table 13.3. Current licensed vaccines for use in USA and/or Europe.

Vaccine	Antigenic component	Use
Bacterial infections (+ viral in some combinations)		
Anthrax	Alum adsorbed protective antigen (PA) from *Bacillus anthracis*	Individuals who handle infected animals or animal products. Laboratory staff working with *B. anthracis*
BCG	Bacillus Calmette-Guérin live attenuated strain of *Mycobacterium bovis*	Children and adolescents in geographical regions where the vaccine has been shown to be effective, including UK. Not routinely used in the USA
Cholera	Inactivated Vibrio cholerae together with recombinant B-subunit of the cholera toxin	Drinkable oral vaccine for travelers to endemic or epidemic areas
Diphtheria, tetanus, pertussis, poliomyelitis, hepatitis B	Alum-adsorbed diphtheria toxoid, tetanus toxoid, acellular pertussis, inactivated poliomyelitis virus and recombinant hepatitis B virus surface antigen	Routine immunization of children
Diphtheria, tetanus, pertussis, poliomyelitis, *Haemophilus influenzae* type b	Another pentavalent combination vaccine, including *Haemophilus influenzae* type b capsular polysaccharides conjugated to tetanus toxoid or to the CRM197 nontoxic variant of diphtheria toxin	Routine immunization of children

Table 13.3. (*Continued*)

Vaccine	Antigenic component	Use
Meningococcal group C	Four serotypes of meningococcus polysaccharide conjugated to diphtheria toxoid	Routine immunization of children in UK. As nearly all cases of childhood meningococcal disease in the UK are caused by groups B and C, the vaccine used for routine immunization contains only group C. A vaccine against meningococcal groups A, C, W-135 and Y is also available
Pneumococcal	Polysaccharide from either each of the 23 or from each of seven capsular types of pneumococcus, conjugated to diphtheria toxoid and adsorbed onto alum	Routine immunization of children (USA). Individuals at risk of pneumococcal infection, e.g. elderly, persons who have undergone splenectomy or with various chronic diseases (UK)
Typhoid fever	Vi polysaccharide antigen of *Salmonella typhi*	Travelers to countries with poor sanitation, laboratory workers handling specimens from suspected cases
Viral infections		
Hepatitis A	Alum-adsorbed inactivated hepatitis A virus	At risk individuals, e.g. laboratory staff working with the virus, patients with hemophilia, travelers to high-risk areas
Hepatitis B	Alum-adsorbed recombinant hepatitis B virussurface antigen (HBsAg)	Routine immunization of children (USA). Individuals at high risk of contracting hepatitis B (UK)
Influenza (inactivated)	Inactivated trivalent WHO recommended strains of influenza virus	Routine immunization of infants (USA). Individuals at high risk of complications from contracting influenza virus (UK)
Influenza (live attenuated)	Attenuated trivalent WHO recommended strains of influenza virus	Individuals aged 5–49 at high risk of complications from contracting influenza virus
Japanese encephalitis virus	Inactivated Japanese encephalitis virus	Individuals at risk of contracting Japanese encephalitis virus
Measles, Mumps and Rubella (MMR)	Live attenuated measles, mumps and rubella viruses	Routine immunization of children
Papillomavirus	Virus-like particles	Prophylaxis against human papillomavirus (HPV) infections including prevention of cervical cancer
Polio (Inactivated, Salk)	Inactivated poliovirus types 1, 2 & 3	Routine immunization of children. Protects against polio paralysis but does not prevent spread of wild polio virus (for which the oral polio vaccine [Sabin] containing live attenuated types 1, 2 & 3 virus is used)
Rabies	Inactivated rabies virus	At risk individuals
Rotavirus	Live attenuated virus	Oral, to prevent rotavirus-associated diarrhea and dehydration in infants
Tick-borne encephalitis	Inactivated tick-borne encephalitis virus	At risk individuals, e.g. working, walking or camping in infected areas
Varicella–zoster	Live attenuated varicella–zoster virus	Seronegative healthy children over 1 year old who come into close contact with individuals at high risk of severe varicella infections. Seronegative healthcare workers who come into direct contact with patients. A concentrated attenuated virus vaccine is used to prevent shingles in the elderly
Yellow fever	Live attenuated yellow fever virus	Those traveling or living in areas where infection is endemic, and laboratory staff who handle the virus or clinical samples from suspected cases

Vaccines separately containing the individual components of the polyvalent vaccines are also licensed.

Figure 13.14. MenB vaccine development.

Preclinical development was based on a reverse vaccinology approach, in which the genome sequence of the virulent meningitis B (MenB) strain MC58 was used to identify open reading frames (ORFs) predicted to encode proteins that were surface exposed (i.e. secreted [S] or located in the outer membrane [OM]), which were then expressed in *E. coli*, purified, and used to immunize mice. Antibodies generated in mice were then used to confirm surface exposure of the vaccine candidate by FACS and to identify proteins that induced bactericidal activity. This screening process resulted in identification of several novel vaccine candidates, including GNA 1870 (which is fHBP), GNA 1994 (which is NadA), GNA2132, GNA 1030, and GNA2091. The formulation for the comprehensive MenB vaccine consists of four components: fHBPGNA2091 and GNA2132–GNA1030 fusion proteins, NadA, and OMV from the New Zealand MeNZB vaccine strain. Clinical development using this formulation has shown in phase I and II trials that the vaccine is well tolerated and immunogenic. The vaccine induced bactericidal activity using human complement (hSBA) with titers greater than 1:4, which indicates the generation of antibodies able to kill the bacteria at a level that correlates with protection against the bacteria, in more than 90% of infants after the fourth dose. This vaccine entered phase III clinical trials in 2008. P, periplasm; IM, inner membrane; C, cytoplasm. (Figure with permission from Rinuado C.D., Telford J.L., Rappuoli R. & Seib K.L. (2009) *Journal of Clinical Investigation* **9**, 2515–2525.)

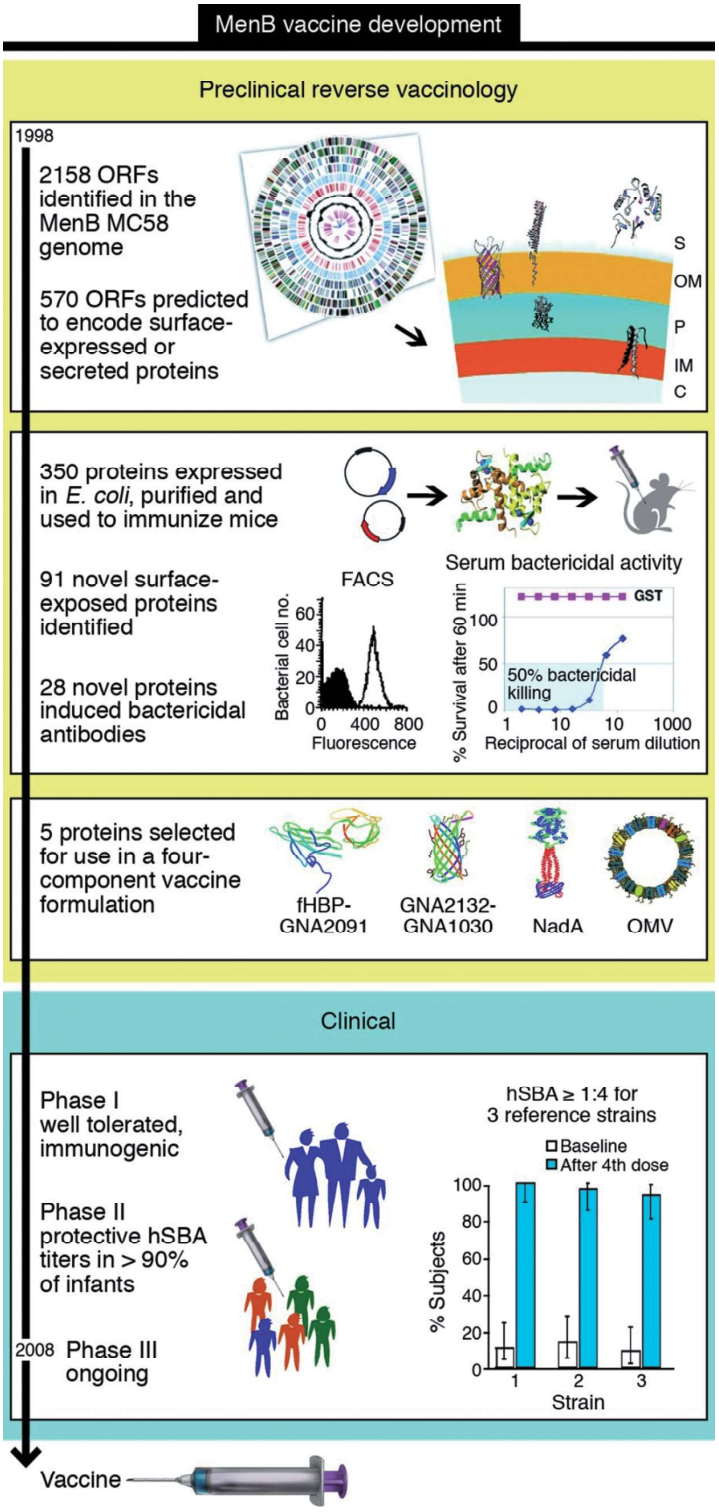


Table 13.4. Centers for Disease Control and Prevention (CDC)— recommended immunizations schedule for persons aged 0–6 years in the USA, 2008. Ranges are shown when there is flexibility in the immunization schedule e.g. for HepB, the first immunization is given at birth, the second at 1–2 months and the 3rd at 6–18 months. (http://www.cdc.gov/vaccines/recs/schedules/default.htm)

Age ► / Vaccine ▼	Birth	1 month	2 months	4 months	6 months	12 months	15 months	18 months	19–23 months	2–3 years	4–6 years
Hepatitis B	Hep B	Hep B			Hep B						
Rotavirus			Rota	Rota	Rota						
Diphtheria, tetanus, pertussis			DTaP	DTaP	DTaP		DTaP				DTaP
Haemophilus influenzae type b			Hib	Hib	*Hib*	Hib					
Pneumococcal			PCV	PCV	PCV	PCV				PPV	
Inactivated poliovirus			IPV	IPV	IPV						IPV
Influenza					Influenza (Yearly)						
Measles, mumps, rubella						MMR					MMR
Varicella						Varicella					Varicella
Hepatitis A						HepA (2 doses)				HepA Series	
Meningococcal										MCV4	

Range of recommended ages

Certain high-risk groups

CRM197 nontoxic variant of diphtheria toxin. The considerable morbidity and mortality associated with hepatitis B infection, its complex epidemiology and the difficulty in identifying high-risk individuals have led to routine vaccination in the USA from the time of birth. In the UK, BCG vaccination is routinely given. However, this is not the case in the USA, where the fact that vaccination leads to individuals becoming positive to the Mantoux skin test, thus resulting in an inability to use this test as a means of excluding tuberculosis during the investigation of suspected infection, is seen as too much of a disadvantage. Due to the constant antigenic drift and occasional antigen shift that occurs with the influenza virus, a new vaccine has to be produced each year for each hemisphere.

Vaccines under development

As with outer pharmaceutical agents, the development of vaccines comprises several stages. Successful pre-clinical studies in animal models are followed by phase I clinical trials in volunteers to initially evaluate safety and the immune response. If all goes well, phase II trials are then carried out in a small number of individuals to gain an indication of efficacy. If the phase II trial is successful, and the company and regulatory authorities decide to proceed, this is followed by a much larger (phase III) study to fully establish efficacy and safety, after which regulatory approval for distribution is given. Phase IV clinical trials finally establish efficacy and safety in large numbers of people. This whole process may take up to 20 years and cost in excess of $500 m.

There are many vaccines currently under development for diseases in which there is at present no vaccine available or where the vaccines that are available are left wanting (Table 13.5). **Tuberculosis** is a good example of the latter situation. The bacille Calmette–Guérin (bCG) vaccine has been in use for over 80 years but is only efficacious in protecting children and adolescents against disseminated and meningeal TB, and then only in some areas of the world, and is largely ineffective against pulmonary TB, which is the commonest form of the disease in adults. Indeed, TB remains a truly major problem in developing countries, and cases have also increased dramatically in Western countries. The alarmingly heightened susceptibility to TB in individuals with HIV/AIDS has led to TB in up to half of HIV-infected individuals, and worldwide multidrug-resistant strains are appearing. This has led to an urgent search for improved vaccine candidates.

Vaccines against parasitic diseases have proved particularly difficult to develop: malaria

A major advance in malaria control has been the finding that the impregnation of bed nets with the insecticide pyrethroid reduces *Plasmodium falciparum* deaths by 40%. However, with the emergence of drug-resistant strains of malaria parasites and reports of increasing mosquito resistance to insecticides, vaccines must be developed. The goal seems achievable as, although children are very susceptible, adults resident in highly endemic areas acquire a protective but non-sterilizing immunity possibly mediated by antibodies.

Malaria is a complex mosquito-borne parasitic disease (Figure 13.15). Traditionally, vaccines have targeted a single stage of the infectious cycle. These include the sporozoite,

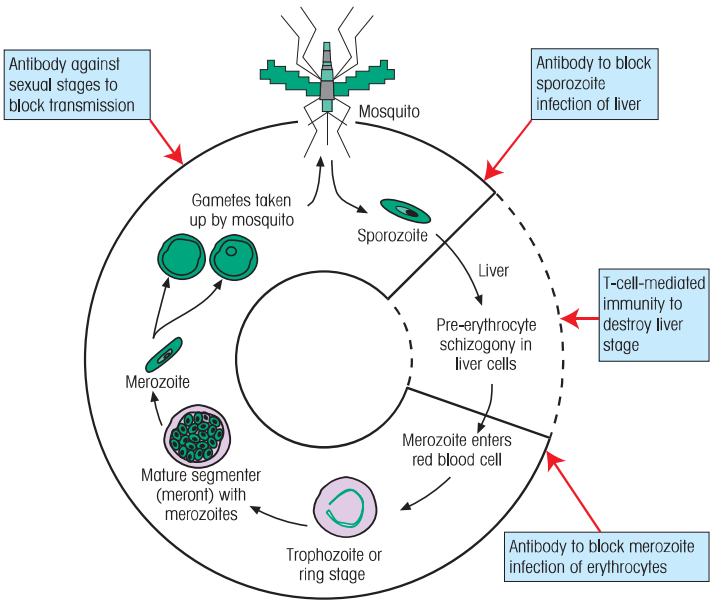


Figure 13.15. Vaccine targeting of the malaria life cycle.

Some of the most investigated potential stages of the cycle to be targeted by vaccine strategies are illustrated.

Table 13.5. Adjuvants in development for human vaccines. From Reed S.G. *et al.* (2008) *Trends in Immunology* **30**, 23–32.

Adjuvants in development for human vaccines		
Adjuvants	**Formulation**	**In pre-clinical or clinical trials**
Montanides	Water-in-oil emulsions	Malaria (Phase I), HIV, cancer (Phase I/II)
Saponins (QS-21)	Aqueous	Cancer (Phase II), herpes (Phase I), HIV (Phase I)
SAF	Oil-in-water emulsion conteining squalene, Tween™ 80, Pluronic™ L121	HIV (Phase I – Chiron)
AS03	Oil-in-water emulsion conteining α-tocopherol, squalene, Tween™ 80	Pandemic flu (GSK)
MTP-PtdEtn	Oil-in-water emulsion	HSV
Exotoxins	*P. aeruginosa* E. coli heat-labile enterotoxin LT	*P. aeruginosa*, cystic fibrosis (AERUGEN – Crucell/Berna) ETEC (Phase II – Iornai Corp.)
ISCOMS	Phospholipids, cholesterol, QS-21	Influenza, HSV, HIV, HBV, malaria, cancer
TLR ligands		
MPL®-SE	Oil-in-water emulsion	*Leishmania* (Phase I/II – IDRI)
Synthetic Lipid A	Oil-in-water emulsion	Various indications (Avanti/IDRI)
MPL®-AF	Aqueous	Allergy (ATL); cancer (Biomira)
AS01	Liposomal	HIV (Phase I), malaria (ASO1, Phase III, GSK) cancer (Phase II/III, Biomira/MerckKGaA)
AS02	Oil-in-water emulsion containing MPL® and QS-21	HPV (Cervarix), HIV, tuberculosis, malaria (Phase III), herpes (GSK)
AS04	Alum + aqueous MPL®	HPV, HAV (GSK)
AS15	AS01 + CpG	Cancer therapy (GSK)
RC529	Aqueous	HBV, pneumovax
TLR-9	n/a	Cancer (ProMune – Coley/Pfizer)
(CpG)		HCV (ACTILON Coley)
TLR-9 ISS series	n/a	HIV, HBV, HSV, anthrax (VaxImmune Coley/GSK/Chiron) HBV (HEPLISAV, Phase III – Dynavax) Cancer (Phase II, Dynavax)
TLR-9 IMO series	n/a	Cancer (IMOxine, Phase I, Hybridon Inc.)
(YpG, CpR motif)	n/a	Cancer (IMO-2055, Phase II, Idera Pharm.) HIV (Remune, Phase I, Idera/IMNR)
TLR-9 agonist (MIDGE®)	n/a	Cancer (Phase I, Mologen AG)
TLR-7/8 (Imiquimod)	n/a	Melanoma (3M Pharmaceutical) HIV (prelinical), leishmaniasis
TLR-7/8 (Resiquimod)	n/a	HSV, HCV (Phase II – 3M Pharmaceuticals)

Abbreviations: ETEC, enterotoxigenic *Escherichia coli*; HBV, hepatitis B virus; HCV, hepatitis C virus; HPV, human papillomavirus; HSV, herpes simplex virus.

which is the form with which the host is first infected after a mosquito bite, the liver stage of infection, the blood stage in which red blood cells become infected and the transmission stage in which gametes are taken up by the mosquito to complete the cycle. One of the problems faced by vaccine developers is the very considerate sequence variation apparent in malarial proteins.

In 2008, a candidate vaccine—RTS,S/AS01E (GlaxoSmithKline, GSK)—was reported to have reduced the incidence of clinical malaria cases in trial of about 800 children aged 5–17 months in Kenya and Tanzania in a single malarial season by about a half. Thus there was only one episode of severe malaria in the vaccine group but eight episodes amongst seven children in the placebo group. This moderate success was hailed as a significant milestone along the road to an effective malaria vaccine as children in the age group of 0–5 years in sub-Saharan Africa are precisely the demographic most at risk from severe disease due to the parasite. The vaccine stimulates immune responses to the circumsporozoite protein (CSP) of *Plasmodium falciparum* in the liver stage of infection. The vaccine induces anti-CSP antibodies but no correlation was found between anti-CSP levels and protection from disease. The "AS01E" designator refers to the antigenic formulation used in the vaccine. The next stage in vaccine development is a phase III trial involving up to 16 000 African children across 7 countries. The trial was initiated in May 2009, the data will be made available to regulatory authorities in 2012 and the vaccine available for targeted use among children aged 5–17 months as early as 2013.

Despite the modest success of RTS,S/AS01E there is a strong argument made that the most effective vaccine is likely to target many antigens at different stages of the life-cycle of the parasite. For example, it has been noted that $CD8^+$ T-cells can provide sterile protection against liver-stage malaria parasites in mice. However, the number of antigen-specific $CD8^+$ T cells is very high suggesting that reliance on this mechanism alone might be unwise. Encouraging data has emerged on combining immune responses to liver and blood stages of the parasite, particularly using viral vectors that can induce both effective antibody and T-cell responses. In addition, data in humans and mice suggests that antibodies blocking transmission can be beneficial.

One of the most promising opportunities for malaria vaccine research relates to definition of the complete malaria genome that should help identify more vaccine targets as described above for "reverse vaccinology." Whole organism based malarial vaccines e.g. irradiated sporozoites are an alternative to recombinant vaccines that are being investigated.

Finally, it should be noted that, as with a number of viral infections, it is possible that T-cell-mediated immunity may contribute to malarial pathology. Infiltrating leukocytes have been observed in the brains of patients who have died of cerebral malaria and resistance to malarial disease has been correlated with a deficit in T-cell function in certain instances.

Vaccines for protection against bioterrorism

Biological warfare has a long and dark history. One early example occurred in 1346 when a group of Tartars catapulted plague-infected bodies and heads over the city walls of the Black Sea town of Kaffa in an attempt to re-capture the town from the Genovese. In 1763, the British were fighting Delaware Indians and, as a supposed gesture of "goodwill," donated smallpox-infected blankets to the Indians resulting in the death of many of the native tribes. Throughout the last century many countries throughout the world had biological weapons programs. Particularly alarming for citizens of the USA were the anthrax cases that occurred in late 2001 following exposure to mail items deliberately contaminated with anthrax spores and sent to news media offices in New York City and Boca Raton, Florida, and to two US Senators in Washington, DC. Aside from the anthrax (*Bacillus anthracis*), smallpox (variola) and plague (*Yersinia pestis*) mentioned above, many other infectious agents can potentially be used for bioterrorism including *Clostridium botulinum* toxin (botulism), *Francisella tularensis* (tularemia) and the Ebola, Marburg, Lassa and South American hemorrhagic fever viruses. Efforts are, therefore, underway to develop vaccines against these diseases in those cases in which effective vaccines are not currently available. Considerable success has been noted at the Vaccine Research Center of the National Institutes of Health in Bethesda, Maryland, USA in developing a vaccine that protects monkeys against lethal challenge with Ebola virus. The vaccine uses non-replicating adenovirus as a vector for the introduction of Ebola virus genes and expression of Ebola virus proteins in the animals prior to exposure to virus. Following the eradication of smallpox, routine vaccination against smallpox was discontinued. Concern that this agent could be used as a biological weapon has led to calls for the re-introduction of routine vaccination against smallpox. Currently only a small number of laboratory researchers, key healthcare workers and military personnel are vaccinated because it is felt that routine vaccination of the entire population would inevitably lead to a small number of vaccine-related deaths, a scenario accepted when a disease is endemic but not for a currently "extinct" disease. However, the vaccine is being stockpiled just in case. Incidentally, vaccination against smallpox would also protect against the related monkeypox virus.

Immunization against cancer

The realization that several different types of human cancer are closely associated with infectious agents suggests that vaccination against agents such as human papillomaviruses (cervical cancer), Epstein–Barr virus (Burkitt's and other lymphomas, nasopharyngeal carcinoma), *Helicobacter pylori* (stomach cancer), hepatitis B virus (liver cancer), HTLV-1 (adult T-cell leukemia) and human herpesvirus 8 (Kaposi's sarcoma) should lead to a substantial reduction in the

incidence of such tumors. Cancer vaccines have also been developed against a number of tumor-associated antigens including carcinoembryonic antigen (colorectal cancer), immunoglobulin idiotypes (B-cell lymphoma), MAGE (melanoma), and so on. Results to date have been somewhat less than spectacular using tumor-associated self antigens but there is hope that strategies such as targeted activation of dendritic cells will lead to improved response rates.

Other applications for vaccines

A vaccine based on the human chorionic gonadotropin hormone, which is made by the preimplantation blastocyst and is essential for the establishment of early pregnancy, has undergone clinical trials as an immunological contraceptive. Vaccines are also being developed for the treatment of allergies and autoimmune diseases. These are generally aimed at re-setting the Th1/Th2 balance, activating regulatory T-cells, or re-establishing tolerance by clonal deletion or anergy. A vaccine for the treatment of addiction to tobacco consists of nicotine coupled to a bacteriophage Qb protein, which assembles into a complex of 180 protein monomers to form virus-like particles (VLPs). The vaccine has shown possible efficacy in a clinical trial in which there was a correlation between the levels of antibody induced against nicotine and continuous abstinence from smoking in some individuals. An anti-cocaine vaccine consisting of a derivate of cocaine conjugated to recombinant cholera toxin B with alum adjuvant is also undergoing clinical trials.

Adjuvants

For practical and economic reasons, prophylactic immunization should involve the minimum number of injections and the least amount of antigen. We have referred to the undoubted advantages of replicating attenuated organisms in this respect, but nonliving organisms, and especially purified products, frequently require an adjuvant that, by definition, is a substance incorporated into or injected simultaneously with antigen that potentiates the immune response (Latin *adjuvare*—to help).

Two types of action have been described for adjuvants; **immunostimulation** and **antigen delivery**. Immunostimulation results from the action of molecules to directly enhance immune responses. Immunostimulants include Toll-like receptor (TLR) agonists, cytokines and bacterial exotoxins. The explosion of understanding in innate immunity in the last decade has greatly increased the potential for the rational design of immunostimulants. The activation of DCs is particularly important here as this leads to increased antigen uptake, migration to lymph nodes and priming of $CD4^+$ T-cell help for B- and T-cell responses. Antigen delivery vehicles serve to optimally present antigens to the immune system by, at least in part, preventing dispersal of antigen and promoting slow release of antigen ("depot effects"). Such vehicles can deliver not only antigen but also immunostimulants more effectively. Examples include mineral salts such as alum, emulsions such as Freund's adjuvant, liposomes and immune-stimulating complexes or ISCOMs. In reality many adjuvants combine to varying degrees the properties of immunostimulation and antigen delivery.

As stated above, conventional live attenuated vaccines typically do not require adjuvants, although responses sometimes can be enhanced by adjuvantation e.g. hepatitis A vaccination. However, the immunogenicity of proteins is typically relatively poor and the use of adjuvants is required. This is particularly the case if the protein is presented as a soluble monomeric form such as HIV gp120 as opposed to in a multimeric repeating particulate form like HBV surface antigen. The most widely used adjuvants in humans are based on gels formed by aluminium salts and are referred to collectively as **"alum" adjuvants**. Antigens are adsorbed on the aluminium particles and the appropriate adjuvant formulation selected based on immunogenicity. The activity of alum is ascribed to depot effects and immunostimulatory effects based on particle formation and induction of inflammation. Alum is used in several licensed vaccines, including hepatitis A, human papillomavirus (HPV), diphtheria–pertussis–tetanus (DPT), *Haemophilus influenze* b and inactivated polio.

Emulsions have been much used in vaccine research and are beginning to appear in human use. The classical adjuvant is Freund's, which is a water-in-oil emulsion. The complete form consists of a water-in-paraffin-oil emulsion plus inactivated mycobacteria; the incomplete form lacks the mycobacteria. The lifelong persistence of oil in the tissues and the occasional production of sterile abscesses mean this adjuvant (incomplete form, the complete form is even less suitable) is not used in human vaccines. The montanides are similar to incomplete Freund's but are biodegradable and have been used in HIV, malaria and cancer vaccine trials. Ribi, a commonly used formulation in experimental work, is a water-in-oil emulsion incorporating monophosphoryl lipid A (MPL) and mycobacterial trehalose dimycolate (TDM). MLA is a derivative of one of the most potent stimulators of antigen-presenting cells, namely lipid A from Gram-negative bacterial lipopolysaccharide (LPS). However, lipid A has many side effects although its derivative, MLA, is far less toxic. MF59 (Chiron—now Novartis) is an oil-in-water emulsion that has been safely used in millions of doses in an influenza vaccine in Europe. It effectively stimulates antibody and $CD4^+$ T-cell responses but not $CD8^+$ T-cell responses in humans and nonhuman primates. AS02 (GlaxoSmithKline) is an oil-in-water emulsion to which two immunostimulants, 3D-MPL and QS21 have been added. 3D-MPL is a derivative of MPL and QS21 is a saponin, originally derived from tree bark, that stimulates both antibody and cell-mediated immunity. Therefore AS02 is seen as a potentially powerful adjuvant for vaccines in which antibody and T-cell-mediated immunity may be important such as HIV or in which T-cell-mediated immunity is likely to be key such as TB.

Particulate antigens elicit much better immune responses than soluble proteins. ISCOMS or immune stimulating complexes take advantage of this by trapping antigens in cage-like structures with saponins. ISCOMATRIX (CSL) refines this

basic concept. Synthetic oligonucleotides (deoxyribonucleotides) containing unmethylated CpG motifs (CpG ODN) are powerful immunostimulants acting through interaction with TLR9. Different families of CpG ODN can preferentially stimulate different cells—B-cells, NK cells, DCs, $CD8^+$ T-cells—involved in immune responses. Liposomes, virosomes and virus-like particles have the ability to present antigens in a multimeric form and can stimulate enhanced immune responses.

A number of pathogens gain entry to the body via mucosal surfaces and the induction of immune responses at these surfaces can be crucial in providing the best protection against disease. Many of the adjuvants described above can be used as mucosal adjuvants. However, there are also a number of molecules that are particularly effective as mucosal adjuvants, most notably cholera toxin (CT) and *E. coli* heat-stable enterotoxin (LT). Modified forms of the toxins and their subunits can powerfully stimulate mucosal responses through mechanisms that are not well understood.

Table 13.5 summarizes some of the adjuvants under development for use in human vaccines.

SUMMARY

Passively acquired immunity

- Temporary protection against infection or clearance of toxins can be achieved with passively administered antibody preparations. Antisera from hyperimmunized animals and from immune humans are classically used in passive protection but increasingly human monoclonal antibodies are becoming available.
- Maternal antibody provides crucial protection to the newborn as its immune system matures.

Principles of vaccination

- Vaccines are effective because of humoral and cellular immune memory. Probably antibodies induced by vaccination are crucial in protecting against most bacteria and many viruses and parasites.
- Herd immunity is important in reducing disease incidence when transmission occurs between humans.

Killed organisms as vaccines

- Killed bacteria and viruses have been widely used as effective vaccines.

Live attenuated organisms

- The advantages include the larger antigen dose typically provided by a replicating organism, the tendency to elicit better cellular immunity and the generation of an immune response at the site of the natural infection.
- Nonpathogenic vectors such as adenovirus, attenuated fowlpox and modified vaccinia Ankara virus can serve as Trojan horses for genes from pathogenic organisms that are difficult to attenuate.
- BCG is a good vehicle for antigens requiring CD4 T-cell immunity and salmonella constructs may give oral and systemic immunity. Intranasal immunization is gaining popularity.
- The risk with live attenuated organisms is reversion to the virulent form and danger to immunocompromised individuals.

Subunit vaccines

- Whole organisms have a multiplicity of antigens, some of which are not protective, may induce hypersensitivity or might even be immunosuppressive.
- It makes particular sense in these cases to use purified components or those made recombinantly.
- Toxoids, inactivated toxins, are effective as vaccines in preventing illness due to some bacterial agents.
- The hepatitis B surface antigen particle is a classic example of an effective subunit viral vaccine.
- Many successful bacterial vaccines target glycans on the surface of the organism using glycoconjugate preparations.
- DNA encoding the proteins from a pathogen can be injected directly into muscle injected directly into muscle to generate the proteins *in situ* and produce immune responses. The advantages are stability, ease of production and cheapness. The method has not been as effective in humans as in mice but newer developments such as a DNA prime with a protein or vector boost are promising.

Newer approaches to vaccines

- The rise of genomics has been crucial in allowing a rational approach to the identification of many more bacterial vaccine targets. "Reverse vaccinology" has been successfully applied to the development of a MenB vaccine.
- Highly variable pathogens such as HIV and HCV present particular problems to vaccine design in that they require the elicitation of broadly protective immune responses. Here molecular approaches are being adopted to describe how broadly neutralizing antibodies interact with their targets and use the information to rationally design vaccine candidates.

Current vaccines

- Children in both the USA and UK are routinely immunized with diphtheria and tetanus toxoids and acellular pertussis (DTP triple vaccine), attenuated