12 Vaccines: Ancient Medicines to Modern Therapeutics

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INTRODUCTION

A BRIEF HISTORY OF VACCINE DEVELOPMENT

The concept that individuals who survive an infectious disease do not get infected a second time is as old as humankind. Thucydides recorded that in the Peloponnesian War (431 BC) survivors of the plague took care of the sick believing they would not get the disease again. We now know that this is due to our immune systems which recognize invading materials as foreign and organizes a defense against them. This will be discussed in the following chapter although it has to be admitted that not all of the details are clearly defined even today. Pliny the Elder reported in his encyclopedia of natural science that the Romans explored the use of the livers from dogs who had died from rabies in order to prevent the disease in man but this effort remains an antique curiosity.

Although two centuries have gone by since the investigations by Jenner in the 1790s into *Vaccinia* that began the modern phase of vaccine development, vaccination as such can be traced back much earlier.

Around 1716 Lady Mary Pierrepont, wife of the then British Ambassador to the Turkish Porte or Court in Istanbul, Sir Edward Wortley Montagu, and herself scarred from an earlier attack of smallpox, reported that Turkish village women exposed healthy individuals to scabs and pustules obtained from patients who manifested mild cases of the disease. Two points need to be made from this observation, namely that the disease can vary in intensity from patient to patient and that immunity can be transferred. Since she had also lost a brother to the disease she reported this observation in letters home and, when she eventually arrived back in London, she introduced the practice to society and had her own children successfully vaccinated.

It might be worth pointing out here that the Turkish practice probably originated in China centuries prior to this observation. Effectively what these ancients had discovered was the variability of the intensity of the infection (*virulence*), a key issue in the development of a safe vaccine.

Toward the end of the 18th century Edward Jenner, a country doctor, and coincidentally already vaccinated against smallpox by the then fashionable process introduced by Lady Pierrepont, had noted that there was a legend amongst country people that milk maids suffered a mild form of smallpox called cowpox that was nonfatal and did not leave the debilitating scars associated with smallpox itself. To his eternal credit Jenner decided to investigate this phenomenon and discovered that the legend was true. Cowpox protected against the more virulent smallpox and could be used as a vaccine. At the time the British medical establishment mocked these
findings, but doctors across Europe followed through and confirmed that cowpox
made an effective and safe vaccine to protect patients at risk from smallpox. Jenner
is now regarded as the “Father of Vaccination.” Later Louis Pasteur developed and
tested clinically an attenuated form of rabies that was less virulent but cured the
disease as opposed to only protecting against subsequent infection. In 1901 von
Behring received the first Nobel Prize in Medicine for his discovery of what would
come to be known as **antibodies**.

Although **Vaccinia** was eliminated from the international scene as recently as
two decades ago, terrorism fears have generated renewed interest in the large-scale
protection of an unprotected population against the disease, which has occasionally
reappeared as a result of laboratory accidents and, in some cases, of deliberate
dissemination of the virus. This is one example of the primary need for protection
against the disease and for suitable vaccine delivery systems.

**BCG—The Only Tuberculosis Vaccine**

The vaccine known as BCG, or Bacille Calmette-Guérin, is officially defined as a
living culture of an attenuated form of **Mycobacterium bovis**, an organism similar
to **Mycobacterium tuberculosis** which is the leading cause of tuberculosis in humans.
The vaccine was developed at the turn of the 20th century as part of an attempt to
halt the spread of tuberculosis in European industrial cities. This was, and remains,
a significant disease that caused a great deal of human misery but, at that time, it
was also becoming a significant factor in slowing the economic revolution then being
experienced by the Europeans.

Little was known about the cause of the disease up to the last quarter of the
19th century but tuberculosis had become known as the “White Plague” and was a
major cause of premature death in the working classes. However, it was by no means
confined to the poor and there was little that could be done to stop the spread of the
disease except to isolate the patients. Treatment consisted of providing good nutrition
and sometimes heroic surgical procedures like collapsing or removing lungs. The
clinics or sanitaria were often located in isolated areas such as the tops of mountains.
To get a good idea of what conditions were like at that time, the novel *The Magic
Mountain* (1924) by Thomas Mann provides an excellent description of the contem-
porary treatment.

The causative organism was isolated by Robert Koch in 1882 and named **M.
tuberculosis** although, at the time, this observation proved controversial. Koch went
on to claim that a sterile filtrate of a growing virulent strain of the tuberculosis
organism could act as a vaccine against the disease. Unfortunately this claim proved
to be invalid, although the filtrate (“Old Tuberculin”) became a valuable diagnostic
agent, producing a marked dermal reaction in a patient who had tuberculosis anti-
obodies even if clinical signs of the disease were not present.

Spread mainly as an airborne droplet infection, the disease remains prevalent in
overcrowded communities, such as those found in industrial cities of the time. Today
the disease has often become associated with AIDS. In France the disease was
endemic among the working classes of the city of Lille. In 1894 the physician Albert
Calmette was sent from the Institut Pasteur in Paris to set up a branch laboratory
devoted to studying the disease in the community and, if possible develop a vaccine against it. Calmette was mainly interested in the social impact of the disease and spent much of his time organizing clinics and obtaining relief for the factory workers affected by the disease.

He was joined in 1897 by Camille Guérin, a microbiologist. In 1908 they discovered accidentally that the addition of a bile extract to a growing culture of tuberculosis isolates allowed the hydrophobic cells to be dispersed more evenly, thus allowing reproducible samples to be taken more readily from the growing culture. Using an extremely virulent bovine strain of tuberculosis (the original culture has been lost so the designation may not be correct) they started what turned out to be a long process of progressively affecting the virulence of the organism by passage. The organism is very slow growing so this process involved growing a culture for 3 weeks and placing a sample in a fresh sterile broth which was then grown for another 3 weeks and so on. It took a total of 13 years and 231 transplants for the organism to lose its virulence, initially to a series of different animals such as mice, rats, and guinea pigs. Eventually the investigators became convinced that it was safe to test in humans. It is interesting to note that virulence of this organism has never been restored. The immunological protection, although it has never been lost, has been affected sometimes by cultural conditions. Another interesting observation about this work is that the vaccine was initially administered orally and was proven effective by this route. The dermal scarification procedure now used was only introduced into medicine much later.

Like all other crowded industrial manufacturing centers, Chicago was badly affected by tuberculosis, especially among the poorest members of the community. There was a small laboratory in Cook County Hospital (the local hospital in Chicago set up in 1835 for the poor community that still serves this function) under the direction of Dr. Frederick Tice who was studying the disease in the 1930s. The main efforts were devoted to controlling the spread of the disease by setting up and maintaining a sanitarium south of Chicago. However, Dr. Tice sent a young doctor, Sol Roy Rosenthal, to study at the Institut Pasteur from 1933 to 1934; in particular, to study the new and controversial BCG vaccine as part of his doctoral studies. Dr. Rosenthal returned and brought back with him samples of the Pasteur BCG vaccine given to him by Guérin for further study. This was part of an enlightened policy adopted by the Institut Pasteur to allow the attenuated organism to be both freely and readily available to all countries.

Scientifically this may have had some unforeseen effects in the fact that each national laboratory began to grow the vaccine under different cultural conditions from those laid down by the originators. The net result was that a number of different BCG vaccines, each identified by its country of origin, began to appear and, with hindsight, these different cultural procedures resulted in vaccines with different potencies.

Dr. Rosenthal was aware of this and, in fact, by 1950 had developed his own BCG vaccine which he claimed was more potent than most of the other brands then available. To show his gratitude he named the improved vaccine after his mentor and it is still known as the Tice strain BCG. In all fairness, in many tests undertaken since that time the Tice vaccine has usually been demonstrated to be superior to
most of the other sources of vaccine, with the possible exception of the original Pasteur strain. In the United States the use of BCG vaccination in the community proved to be different from almost every other country. Rosenthal encountered an entrenched medical opinion, especially with members of the U.S. Public Health Service who assumed a defeatist attitude toward BCG. The medical establishment had decided by the late 1930s, based on some incorrect or otherwise unconvincing evidence, that BCG was unreliable and too dangerous to be used on the American public. Rosenthal spent most of his working life trying to convince them otherwise. He organized clinical trials of the vaccine on a large scale on the south side of Chicago, then a major industrial hub associated with meat processing. These trials are interesting because he managed to organize follow-up examination of the patients, in some cases for as long as 25 years, and, in the process, made some interesting parallel discoveries. He may have been among the first to report in 1936 that BCG was a potent stimulant of the reticuloendothelial system, finding that neoplasms could be suppressed by BCG. For example, an unexpected observation from the tuberculosis trials in Chicago involving over 1500 patients was that diseases such as leukemia and soft tissue tumors were significantly suppressed among the patients treated with BCG, quite apart from a reduction in the development of active tuberculosis.

The significance of this observation has only been appreciated more recently. Rosenthal wrote a book in 1957 called *BCG Vaccination Against Tuberculosis*. The second edition of 1980, substantially rewritten from the first, was called *BCG Vaccine: Tuberculosis–Cancer*, and this simple change graphically illustrates the change in emphasis in the use of BCG. By 1960 the menace of tuberculosis was perceived as being overcome by the use of antibacterials such as streptomycin and isoniazide, and the need for a tuberculosis vaccine was generally considered to be less relevant to the needs of the time.

However, since then there has been a major resurgence of drug resistant-tuberculosis, associated with the AIDS pandemic, and this has caused increasing concern over the past decade. BCG vaccination of medical and nursing personnel involved in these areas has now become an accepted precaution since there is no other approved vaccine available and any acellular vaccine, for example, will probably not receive regulatory approval for at least another decade, if at all.

The Tice substrain of BCG is still the only licensed source of the vaccine in the United States, although manufacturing is now carried out in North Carolina. The fresh liquid form of the vaccine, with a shelf life of only 10 days, was later improved by freeze drying the product, extending the shelf life to 18 months.

**REQUIREMENTS OF AN IDEAL VACCINE FOR TODAY**

Before discussing the way in which the immune system functions and how vaccines can work, it might be a useful exercise for the student to consider what an ideal vaccine should consist of. For example, most vaccines today are administered parenterally and this can sometimes be a painful or distressing experience, especially for young children.
At the 1990 International Task Force for Vaccine Development meeting in New York, a children’s vaccine initiative (CVI) was set up with the aim of developing an ideal vaccine. The following criteria were established although the list may not be totally inclusive.

1. A vaccine should only require a single dose.
2. A vaccine should be given early in life.
3. The route of administration should be nonparenteral.
4. Vaccines should be combined in order to reduce the number of visits to a doctor or medical center.
5. Vaccines should be heat stable and retain activity during transport and storage, especially in tropical climates.
6. Vaccines need to be developed against diseases with high mortality rates, such as AIDS, pneumonic plague, acute respiratory infections, diarrhea, and parasitic diseases such as malaria.
7. And, above all, the cost must be low throughout the world.

The ultimate vaccines in the future will be required to fit these criteria and it is not difficult to see that the cost may be the biggest problem.

In the 15 years since these criteria were promulgated by declaration, it will be evident that most vaccines are still administered parenterally with the exception of polio and typhoid vaccines. In many ways this can be attributed to the physicochemical characteristics of vaccine antigens themselves, which are large molecules susceptible to proteolytic degradation, denaturation, and rapid clearance from plasma. Some combination vaccines are available which reduce the number of injections. However, the MMR (measles, mumps, and rubella) combination vaccine has gained an unsafe image in the popular press, mainly due to a reputed link with autism in some children that as yet remains unproven scientifically. In some quarters the autism was associated with the use of thiomersalate as a mercurial preservative in multidose injections but, again, this supposition remains unproven.

The controversy has reappeared with the introduction of a five component children’s vaccine containing diphtheria, polio, measles, mumps, and rubella; although this should be much more convenient and contains a killed polio instead of an attenuated virus which is known to occasionally revert to the active form, albeit in single numbers per million injections. In this case the children’s vaccine should be safer and more convenient.

Throughout this chapter the reader should bear in mind the possibility that modern biotechnology, the subject of the book as a whole, is making progress toward the ideal criteria outlined above.

**TYPES OF MODERN VACCINES**

Modern vaccines can be classified into groups according to whether the organism is alive or dead or whether the vaccine is prepared from naturally occurring fragments or synthetically derived components (Figure 12.1).
Attenuated live vaccines are less common than they were a century ago, examples being the original Pasteur rabies vaccine and BCG, but the recent approval of a nasal influenza vaccine, FluMist® (MedImmune Vaccines, Inc., Gaithersburg, MD) shows that the approach remains valid.

Other examples include polio, mumps, and rubella vaccines. The attenuated polio vaccine can be given orally and is known to occasionally revert to an active form which does represent a slight but measurable issue with this vaccine.

The attenuated mumps, measles, and rubella viruses are usually administered in one combined vaccine, MMR. The three viruses are grown separately, lyophilized with various cryoprotectants such as sorbitol and amino acids or hydrolyzed gelatin, and combined in a final pack, usually with neomycin as a preservative. The combined vaccine has to be kept refrigerated prior to use.

Some viruses and bacteria have been explored as genetically engineered organisms to express appropriate peptides or proteins or even specific epitopes to raise the protective immunity of the entire organism against aggressive pathogens.

Killed inactivated vaccines

To some extent the simplest vaccines are those in which the bacterial or viral pathogens has been killed by chemicals or heat so that they themselves cannot cause disease but can confer protection against invasion. This has been used, for example, for the combined diphtheria-tetanus-pertussis vaccine. In this case there were concerns about the presence of entire cells that could cause complications other than the needed protection. Recently acellular systems have been introduced which may
be more effective and safer. This concept of using only the fragments of an invading organism that cause the problems is attractive because very often side effects due to other components of the intact organism are avoided. Such components may be proteins or fragments of protein, carbohydrates, lipids, or even DNA fragments, but while they are often effective, their immunological potential may well be weak. This emphasizes the need for adjuvants to increase their effectiveness and this will be dealt with in a later section.

**Conjugate Vaccines**

Conjugate vaccines combine toxoids with polysaccharides and attempt to train the immune system to recognize the polysaccharides as being foreign. Young children cannot react to pathogens covered with polysaccharide and Hib conjugates have lowered the infection rate in children from 1:60 to 1:100,000, a worthwhile achievement.

Combination vaccines are more convenient in use and are exploited in the familiar DPT and MMR vaccines. However, there is always the possibility of autoimmune reactions and an increased risk of side effects. As noted, these issues have been obscured legally by claims that autism in children is caused by such combinations or the use of thiomersalate as a mercurial preservative. Scientifically it is probably safe to say that these side effects have not been demonstrated convincingly but these issues have caused difficulties for the manufacturers.

DNA vaccines are being explored but it has proved to be difficult to deliver the naked DNA to cellular sites where the appropriate antigen will be produced. Gold particles coated with DNA plasmids have been evaluated. Naked DNA is wasteful in some senses because relatively large quantities are required for even small quantities of translation and adjuvants are required. In addition there is no protection against environmental DNAase enzymes. However, DNA and the appropriate protein antigen may have more potential.

**Subunit Vaccines**

In the early 20th century it was recognized that some components of a microbacterial cell were more important than others for protection and thus came the concept of subunit vaccines. When combined with the discovery that bacterial toxins could be inactivated with formaldehyde, the result was the introduction of a diphtheria subunit vaccine in 1923 and a tetanus subunit vaccine in 1927.

Modern subunit vaccines contain one or more selected antigenic subunits that have been found to provide protection against a particular pathogen. They are better defined from a physicochemical aspect and have fewer side effects than vaccines, which contain intact cells, whether inactivated or attenuated. Current subunit antigens include viral and bacterial proteins as well as bacterial capsular polysaccharides. Toxins from *Corynebacterium diphtheriae* or *Clostridium tetani* are water soluble proteins, which effectively constitute the respective vaccine antigens. However, they are treated with formaldehyde to eliminate or reduce the associated toxicity to
form toxoids. It is also necessary to preserve the epitopes responsible for antibody formation and the process is usually a balance between the two requirements.

Toxoids generally have a poor immunogenicity and they are usually adsorbed onto aluminum salt suspensions which act as adjuvants.

Genetic manipulation of *B. pertussis* has resulted in acellular toxoids which are claimed to be devoid of toxicity, and acellular vaccines have been introduced in the United States and Japan for the treatment of older children. Trials have demonstrated that the efficacy of acellular vaccines is comparable to whole-cell vaccines but have virtually no side effects.

The genetic engineering of hepatitis B subunit vaccine in yeast cells has resulted in a subunit vaccine replacing the conventional whole cell vaccines obtained from the plasma of infected humans. The main advantages of recombinant DNA (rDNA) vaccines when compared with human plasma products is that they offer higher yields, are of more consistent quality, and are safer, thereby being easier to produce and cheaper.

**EMERGING VACCINE TYPES**

**Protein Vaccines**

The majority of pathogenic antigens in nature are proteins with specific biological functions. For example, the human immunodeficiency virus (HIV) glycoprotein (gp) 120 binds onto the surface receptor cluster designation 4, CD4, on leukocytes to facilitate entry of the virus into the cell. The antigen 85 protein complex of *M. tuberculosis* is needed for the synthesis of factors that mediate bacterial cell wall integrity and immunomodulation. In vaccine manufacture pathogen proteins are either purified from the pathogen itself or are synthesized by recombinant methods.

However, in practice, it is generally found that protective immunity is rarely provided by just a single component from the pathogen, multiple components being needed as in the DPT vaccine. For regulatory reasons it is usually better to explore single proteins or protein complexes. Unfortunately, with a few exceptions such as the *Bacillus anthracis* protective antigen (PA) and the urease enzyme from *Helicobacter pylori*, most single protein antigens tend to have weak immunoprotective activity and require the use of adjuvants or immunostimulators (see later). In addition, without these additional materials protein antigens tend to induce a type 2 immune response, which may not always provide adequate protection, especially in the case of intracellular pathogens.

**DNA Vaccines**

Outside the remit of this present chapter, it might be noted that a significant number of DNA sequences have had no identifiable function and were initially labeled as “junk.” However, gradually some functions for this so-called junk DNA have been identified, especially those of a regulatory nature. This has resulted in a greater interest in the possibility of providing DNA sequences that synthesize antigenic proteins.
The issue was one of getting the DNA into a cell and this gave rise to the use of viral delivery systems and bacterial plasmid DNA (pDNA) vectors. pDNA vectors are useful since they are much safer to manufacture on a large scale, inexpensive, and easily customized—apart from being safer for the patient. However, the downside is that these systems are poorly immunogenic although, as noted above, this might be improved by the addition of an appropriate adjuvant.

In the body, expressed proteins are processed through the major histocompatibility class I systems which generally results in a type I immune response and this is usually effective against intracellular pathogens. In the case of the transgenic product eliciting a type II response the expressed protein is taken up by phagocytes and antigen presenting cells.

One of the main safety aspects of using a pDNA vaccine is that, unlike the viral delivery systems, these cannot replicate and infect the host. In addition, it does not combine with the host genome which could otherwise lead to a potential cancerous reaction. Trials of so-called naked DNA suggested that a reduced cellular residence time may have been responsible for a poor protective response but this may also have been due to other physical and biological barriers.

Loss of vaccine by any first-pass effects could be minimized by avoiding the intravenous route and careful selection of mucosal routes would be of benefit. Indeed, unlike small molecular weight drugs, vaccines are not administered through the IV route and alternatives are required.

Retention times within the cell may be increased through the use of nuclear retention signal peptides and this with other approaches are certain to be tried in the immediate future.

Lipid and Carbohydrate Antigen Vaccines

As will be discussed in a later section, adaptive immune responses are mediated through the major histocompatibility complex (MHC) class I or II antigen presentation pathways. However, some MHC-independent antigen presentation pathways have been identified for pathogenic lipids and carbohydrates. Antigens presenting leukocytes express the CD1 molecules in a structure similar to that identified in the MHC class I, except that there are two deep cavities that create a hydrophobic environment suitable for lipids, glycolipids, or lipoproteins. Two groups of CD1 molecules elicit the production of type I cytokines such as interferon (through the group 1 CD1 pathway) that can recognize mycobacterial lipid/glycolipid antigens which lyse infected dendritic cells and secrete bactericidal cytokines. Since group 2 CD1 molecules have been detected in the human gastrointestinal epithelial cells, this has some implications for the viability of oral vaccines.

Recombinant Live Carriers

The main invasive pathogens are bacteria and viruses and recombinant live carriers using bacterial and viruses have been described. Viral carriers rely on the established and efficient methods for invading and infecting eukaryotic cells and their in vivo replicative process improves the induction of type I and type II immune responses.
These are discussed elsewhere but include a recombinant vaccinia virus that expresses rabies virus glycoproteins used for oral vaccination.

**THE IMMUNE SYSTEM AND MECHANISMS OF ACTION**

Although some aspects of the human immunological system remain obscure and beyond direct explanation, much of this very subtle protective mechanism has been elucidated and used.

Many diseases in humans and the animal kingdom are caused by invading pathogenic microorganisms that proliferate and disturb the normal physiological functions of the intact body. There has been a tendency for the medical community to label microorganisms as pathogenic or nonpathogenic, but the sad truth of the matter is that almost all bacteria and viruses in the wrong place at the wrong time will manifest some type of disease. It is also true that some organisms are much more aggressive than others so that only a small number of anthrax cells, as an example, introduced into an animal or human will often result in various forms of the disease. This concept of aggressiveness or pathogenicity is therefore easily understood. Some microorganisms vary in their effect, as noted above, but here there may be two causes for this: (a) the organism itself losing the ability to affect the body, and (b) the body being able to resist the invasion by some mechanism.

The story is told of Mithradates, the 1st century BC king of Pontus, who was so afraid of being poisoned that he immunized himself by taking increasing doses of known poisons in order to develop a natural resistance to them. It is this concept that underlies vaccines and their mode of action. The body is “vaccinated” against a disease causing microorganism so that, after a first infection it is able to resist any subsequent invasions. Here the first invasion occurs in the form of a highly controlled infection in the form of a vaccine which may consist of an attenuated bacterium or one that is modified in some way to produce some symptoms of the disease but, in the process, stimulates the body to defend itself.

This is the concept. The questions remain as to how and why this happens, a subject that has puzzled scientists since Jenner’s time. The subject has become known as immunology and in recent years following the elucidation of the gene coding and the way proteins are synthesized in the body has taken up a significant degree of current scientific effort.

Considering a theoretical pathogen consisting of a bacterial cell attached to some surface such as a blood cell, on its own it is unlikely that anything will happen. However, the pathogens may grow and start to release material from their cells (toxins) which interfere with the normal physiological functioning of the body—the “disease.” These secretions, the bacterial toxins, are often countered by the body producing antitoxins which adsorb or neutralize the toxic components. If there are few pathogenic cells this will often be sufficient to protect the body but the system also has “memory” so that if the same type of invasion occurred again a successful defense could be readily mounted. The pathogen may then make some slight changes in the design of the toxins so that the body now has to reinvent itself and come up with
new antitoxin or antibody molecules in order to renew the defense. The process is ongoing and we are all subject to the vagaries of bacterial combat throughout our lives.

**Antigens**

The materials capable of stimulating the lymphoid tissues to produce antibodies are termed **antigens** and comprise bacterial and viruses as well as some smaller molecular entities. However, the response is not to the intact organisms but rather to some specific parts which have characteristic three-dimensional structures, the **epitopes**, and this sensitivity to structure is a characteristic feature of the immune response. Once an animal is in contact with an epitope the response can be in the circulatory or **humoral** system or directly as a cell-mediated response, but it is exquisitely sensitive to the specific antigen and rarely to any other.

Antigens themselves consist of two classes, the first being substances which are capable of generating an immune response on their own—**immunogens**—and smaller molecules that can react weakly with antibodies, so-called **haptens**. However, if haptens are attached to larger molecular moieties they begin to function as immunogens.

**Antibodies**

Antibodies remove antigens by binding directly to the three-dimensional epitope. Cell-mediated responses are against sites within antigens. The main effectors cells are the T-lymphocytes which have T-cell receptors capable of binding antigens when presented to them. The T-cell receptor is able to recognize both the antigen fragment and the structure to which it is bound, effectively being able to recognize small pieces of foreign molecules bound to the host-cell surface molecules. There appears to be a molecular weight cut-off for immunogenic foreign entities of greater than approximately 5 kDa. Haptens with molecular weights below this limit usually need to be attached to larger molecular weight entities or form complexes with tissue proteins acting as carriers, which would then have the overall required properties.

Epitopes on invading organisms cause the formation of corresponding antibodies, often more than one epitope being present on the organism and each responsible for its own antibody. The overall and characteristic epitope shape can be formed from a single segment of the antigenic molecular moiety or it can be formed in three dimensions by folding of the molecule in its native environment. The interacting section of the host antibody or T-cell receptor is called the **paratope** and forms part of the terminal groups of the corresponding **immunoglobulin**.

**Immunoglobulins**

Antibodies or immunoglobulins (these terms are interchangeable) are **glycoproteins** which bind specifically to the antigens that induced their formation and each one is formed as a unique response to that particular antigen. In the body they are present in body fluids and certain types of cell. Serum, the fluid that separates when blood is allowed to clot, contains no cells but it does contain the immunoglobulins which can be separated electrophoretically. They have different molecular weights and
charge, and there are five basic classes or isotypes: IgG, IgA, IgM, IgD, and IgE. Of these, IgG is the most common by weight and has a molecular weight of around 160 kDa. There is a pentameric variant, IgM, which has a molecular weight of around 970 kDa; and the rest have molecular weights of 160-188 kDa and are present in varying amounts in normal human serum.

Structurally antibody molecules are similar in that they have the same basic structure consisting of two light chains and two heavy chains lying together in the shape of a Y. The chains are help together by disulfide bridges and some noncovalent interactions. Along the molecules some areas are conserved and others are totally unique. Different carbohydrates are attached to the main molecule which determines some of the subsequent properties of the antibody. The most common antibody in serum is IgG but it has at least four subclasses in humans. It is the major antibody of the secondary immune system and is found in both serum and tissue fluids. With all immunogens the antigen-recognizing paratope is contained in the so-called Fab end of the molecule and, as noted, the remainder of the molecule is the effector portion. Because the reaction between the epitope and its corresponding paratope involves for the most part short-range noncovalent forces (in three-dimensions) the two must have close proximity and therefore close fitting structures in order to interact. This heightens the specificity of the reaction. (For a more detailed description, see Figure 4.1 and Chapter 4.)

**T-CELL RECEPTORS**

The T-lymphocytes carry a number of glycoproteins on their surfaces that are involved in antigen recognition. These molecules or receptors are responsible for the recognition of the specific major histocompatibility complex (MHC) and antigen complexes and will be different for every T-cell so there are mechanisms in place for diversity.

**MAJOR HISTOCOMPATIBILITY COMPLEX (MHC)**

The major histocompatibility complex (MHC) is part of the system that codes for molecules important in immune recognition, including graft rejection. MHC class I and II molecules present antigen fragments to T-lymphocytes. For example, class I molecules bind viral proteins and present them to the CD8+ T cells. Exogenous antigens such as proteins taken into the cell by endocytosis are processed within the cell and presented to CD4+ cells.

There are a number of MHC class I and class II polymorphic molecules, all with substantially the same basic structure. The MHC class I molecule is a dimer consisting of a glycosylated transmembrane peptide of molecular weight 45 kDa covalently linked to a 12 kDa peptide; it is found on the surface of most nucleated cells within the body. It is believed that the polypeptide backbones fold in such as way as to form a platform of \( \beta \)-pleated sheet structures to support a peptide binding cleft in which the antigen fragments are held and presented to the T cells.

The class II molecules are similar but are held together by noncovalent links. These are less widely distributed, being mainly found on the surface of some cells.
Pharmaceutical Biotechnology

of the immune system such as β-lymphocytes, macrophages, monocytes, and activated T-lymphocytes. MHC antigens are essential for recognition by T-lymphocytes since they will only recognize epitopes when presented in the cleft of the MHC.

Naive CD4 T-cells need to be activated by MHC class II antigen and then start to secrete a wide range of cytokines including interleukins (IL) 2, 3, 4, 5, and 10 as well as interferon (IFN)-γ (Figure 12.2). These cells are then converted to Th1 (inflammatory T-cells) which secrete IL-2, IFN-γ, and tumor necrosis factor (TNF). Alternatively they can be converted to the Th2 cells which secrete IL-4, 5, 6, and 10. The Th1 pathway results in a powerful stimulation of macrophages to kill phagocytosed microorganisms and encourages other macrophages, lymphocytes, and neutrophils to come to the site of activation or invasion. The Th2 cytokines, on the other hand, activate B-cells that can differentiate into antibody-secreting cells. In some disease states the two Th1 and Th2 subsets can get out of balance. It has been suggested that autoimmune diseases, including rheumatoid arthritis, are due to an excess of Th1 activity with the associated cytokines inducing an inflammatory response.

Antigen MHC class I complexes have a different function and present their antigen to CD8+ cytolytic T cells, which produce an appropriate group of effector molecules leading to apoptosis of target cells.

**FIGURE 12.2** Cytokines and cells activating Th1 and Th2 cells.

**TYPES OF IMMUNE DEFENSE MECHANISMS**

It will be evident from the above historical background that empirically it was appreciated that immunity to a disease could be both active and passive. Immunity was shown to be specific and nonspecific with two main responses, humoral (body fluids) and cellular.

**Passive immunity** only lasts a short while and occurs when the mother passes protective agents such as immunoglobulins to the child in, for example, breast milk. Tetanus immunization is another example since it only lasts 10 years. The Rhesus response where the first child immunizes the mother against other children is also well known. Passive immunity also has the potential to produce undesirable immune responses such as allergic reactions or anaphylactic shock.
Active immunity includes an efficient set of mechanisms to deal with infection and these are highly adaptable to situations as they arise.

Systemic immunity protects the blood and organs and interior tissues of the body. Antibodies and specialized cells circulate throughout the body looking to destroy foreign cells or tissues. A recognition pattern is remembered for subsequent invasions by the same organisms.

Innate or nonspecific immunity has probably developed during evolution and includes anatomical barriers such as the skin, cilia in the lungs, and bronchial tubes, as well as the presence of specific components in specialized tissues that combat invasion.

Adaptive or specific immunity is generally slower to respond but recognizes nonself molecules or invaders and is capable of destroying them if they have particular molecules on their surface.

Mucosal immunity represents the first line of defense against invasion of viruses and bacteria by utilizing immunoglobulins and other materials as antibodies. This will be discussed later.

Other materials that assist in resisting invasion include:

Fibronectin (Fn) is ubiquitous throughout the body and serves a number of functions, including coating bacterial and foreign particles with apoprotein which promotes recognition and destruction by circulating cells of the immune system.

Lysozyme is an enzyme that occurs in tears and serum and breaks down bacterial cell walls.

Interferon is capable of inhibiting viral replication processes and activates cells that kill pathogens.

Tumor necrosis factor (TNF)α is capable of suppressing viral replication and also activates phagocytes.

Transferrin and lactoferrin deprive organisms of the trace quantities of iron needed for metabolism.

**THE MUCOSAL SYSTEM**

In recent years it has become evident that many pathogens invade the body through the mucosal system, which in itself appears to provide a first line of defense against invasion. All mucosal surfaces are accessible to pathogens, which are effectively living particles, but if these can access the body through this route then it follows that particulate vaccine carriers could also enter by the same means. This site is an attractive alternative to parenteral administration of vaccines because it is relatively large in surface area (some 400 m² as opposed to 2 m² for the external skin), although not all of it is directly accessible. It includes the nasal passages, eye lids and surrounding tissues, mouth and lungs, the whole of the gastrointestinal tract, and the vagina. These surfaces are of interest for direct drug delivery since particulate systems can be delivered to specific targets and in many cases made to adhere to the mucosa.
The net effect is that mucosal administration of a vaccine is an attractive and more effective alternative to parenteral administration which, in any case, tends to only provide systemic protection.

ASSOCIATED LYMPHOID TISSUES

The common mucosal immune system (CMIS) is now well established as a separate component of the host’s immune apparatus, quite distinct from and independent of the systemic immune system described above. Moreover, if an immune response is induced at one site in the mucosal system this generally leads to responses at distal mucosal sites of the CMIS, presenting a potentially large advantage. It should be noted that there are approximately $6 \times 10^{10}$ antibody producing cells in mucosal tissues and $2.5 \times 10^{10}$ lymphocytes in the entire lymphatic system.

Needless to say, there are issues associated with mucosal vaccine delivery, including in some places a harsh environment. One example would be the low pH of the stomach and upper part of the gastrointestinal (GI) tract and the widespread availability of proteolytic enzymes, especially along the whole of the GI tract. There is the potential to develop tolerance to materials delivered orally although the oral route is considered to be the safest of all. The nasal route, on the other hand, is currently under investigation in a number of laboratories and appears to be safe, although there is some evidence of antigen transfer to neuronal tissues through the olfactory bulb in mice.

MUCOSAL-ASSOCIATED LYMPHOID TISSUES

Mucosal-associated lymphoid tissues (MALT) differ in various sites as follows.

Nasal-associated lymphoid tissues (NALT) consist of lymphoid follicles with overlying ciliated epithelium to sweep the mucus along the site, mucus goblet cells producing the mucus and numerous membranous or microfolded cells, the M cells (Figure 12.3 and Figure 12.4). These organized cellular structures are found at the entrance to the nasopharyngeal duct in the mouse but have also been described in the human proximal nasal passage. Nasal administration demonstrates higher permeability

![FIGURE 12.3 Follicle-associated epithelium (FAE).](image-url)
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than other mucosal sites and offers an alternative site for vaccine delivery. The nasal cavity has a large and readily accessible mucosal surface. There are fewer challenges to absorption and a milder environment than that experienced while traveling down the GI tract. In addition, any material passing through the nasal wall is distributed systemically without first passing through the liver, avoiding the first pass effects associated with most drug absorption pathways. Nevertheless, the exact mechanisms for drug or vaccine absorption through the nasal mucosal wall remain at present obscure, especially in humans.

Gut associated lymphoid tissues (GALT) contain the so-called Peyer’s patches which are organized lymphoid follicles with overlying M cells and are considered to be the main entry point for particulate matter during passage down the GI tract (Figure 12.3 and Figure 12.4).

Bronchus associated lymphoid tissues (BALT).

Rectal associated lymphoid tissue (RALT).

The mucosal system has been shown to form direct protective mechanisms for the body as a whole against pathogenic invasion. These include the secretion and movement of mucus along the tissue concerned (“trafficking”), the secretion of stomach acids, enzymatic degradation, peristaltic movements, and the presence of tight junctions. However, it might also be argued that the main function of these is in the digestion of food, especially from the gut, but food is often absorbed as small particles or, in the case of lipids, droplets. If this process could be mimicked by vaccine delivery systems, this would be a clear advantage. However, the mucosal system also has its own immunoprotective action irrelevant to food processing.

Mucosal immunization prevents pathogens from infiltrating or infecting the body whereas systemic immunization resolves an infection after the invasion has occurred, thereby suppressing the disease. There is also clear evidence that both systemic and mucosal immunity is induced by mucosal immunization. As noted earlier, there is also evidence that immunization at a single site in the body can result in protection of the entire mucosal system so there must be some form of communication between sites; and this justifies the term common mucosal immune system.

Mucosal immunity is divided into two main components, the inductive phase and the effector phase. In the inductive phase antigen is presented which results in

**FIGURE 12.4** Movement of antigen through the M cell.
lymphocytes being primed and moved from their inductive site to the lymph nodes and the circulating blood. This may represent the means by which the distant mucosal components communicate and allows the lymphocytes to reach the effector phase sites. Here the secretory immunity, sigA, is induced by the production of appropriate antibodies. In the intraepithelial CD8+ T-cell mediated immunity and the CD4+ T-cytokine production occurs in the lamina propria cells of the intestine.

M cells are potentially important targets for vaccine delivery and are found in all inductive mucosal sites. Some pathogens are capable of entering the body through these cells whose primary function is the sampling of luminal antigens and moving antigenic material to underlying lymphoid tissues, transcytosis. They are characterized by their lack of brush border microvilli and there is no mucus secreted although, in the GI tract, for example, there is slow movement of mucus across their surface. They are also capable of efficiently endocytosing adherent micromolecules and particles, dead or alive.

**VACCINE ADJUVANTS**

Although there are a number of advantages associated with the use of subunit vaccines (e.g., highly purified peptides, proteins or DNA) as vaccines (e.g., specificity), one feature they all have in common is that they are generally poorly immunogenic. The more traditional vaccines contain many other components, some of which elicit additional T-cell assistance or function as adjuvants. An adjuvant is a substance that acts as an immunostimulator, one example being the bacterial DNA in a whole cell vaccine. The overall result is a more robust immune response than that provided by the antigen alone.

Adjuvants improve the antigenic response by a number of mechanisms, such as

- Increasing the immunogenicity of weak antigens
- Enhancing the speed and duration of the immune response
- Modulating the antibody activity
- Stimulating the cellular mediated immunity
- Promoting the induction of mucosal immunity
- Enhancing the immune response in immunologically immature individuals
- Reducing the dose of an antigen required for a response
- Increasing safety and reducing production costs

A wide variety of materials have been explored for their adjuvant activity, although not all are equally effective or nontoxic, especially in humans. Alum and other aluminum salts were first recognized in 1926 and remain the most effective agents licensed for human use by the FDA, although some French products also use calcium phosphate. However, in recent years it has become evident that new and improved vaccine adjuvants are needed.

Although widely approved and effective as adjuvants, alum and other aluminum salts do have some issues since they require relatively large quantities of antigen,
which requires repeated dosing, are nonbioadhesive, cannot elicit cell-mediated immunity, and require constant refrigeration. In addition, alums are not effective by the mucosal route as adjuvants and there are concerns relating to the production of IgG when alum is used. These are becoming more relevant as vaccines for use in tropical underdeveloped nations become of increasing concern. There is now a movement toward heat-stable, single-dose vaccines and this may be achieved by using microparticle formulated vaccines.

Until very recently the development of adjuvants has remained substantially a trial-and-error process. This probably accounts for the wide variety of materials described as being suitable for the purpose.

Bacterial DNA, long a component of the earlier whole cell vaccines, has been shown to have an immunostimulatory effect on immune cells and is a potent inducer of cytokines such as IL-1, IL-6, and IL-12. Monophosphoryl A, a component of mycobacterial cell walls, reacts with receptors on antigen producing cells and generates a Th1 response due to the production of IL-2 and IFN-γ.

Perhaps one of the most potent immunostimulants is the Freund's Complete Adjuvant (FCA). This consists of a water-in-oil emulsion of killed mycobacteria in mineral oil. The exact mode of action is uncertain although it appears to be connected with the way in which the mycobacterial cells are presented to the surrounding tissues when injected. The reaction is rapid and devastating to the point where it cannot be used in humans and there is a move to stop using it in animals. There is an Incomplete Freund's Adjuvant (IFA), which is an emulsion without the mycobacteria and the reaction is less severe. In addition, MF59 is a squalene oil-in-water emulsion without additional immunostimulatory materials and has proved to be a potent adjuvant currently under testing. Some components of mycobacterial cells may also have immunostimulatory action. Synthetic and semisynthetic derivatives have been tested, including muramyl di- and tripeptides, MDP and MTP. MDP, –acetyl-muramyl-L-alanyl-D-isoglutamine, is a small glycopeptide which appears to represent the smallest structure essential for mycobacterial adjuvanticity. However, synthetic MDP and some other analogues have the ability to enhance nonspecific resistance against diverse microbial infections and are capable of conferring resistance against a wide variety of pathogens, including influenza, herpes simplex, vaccinia, and Sendai virus. A purified monophosphoryl A in an emulsion has been evaluated clinically although it does not appear to have progressed to the market place.

Some plant materials have been evaluated, including the soap-like saponins from Quilaja saponaria and the highly purified Quil A extract obtained from saponins which induce the production of cytokines. In common with all saponins these materials have hemolytic activity that limits their direct use in humans although it is used in veterinary medicine. However, combination of Quil A with cholesterol, phospholipids, and antigens forms a human-compatible adjuvant called immunostimulatory complexes (ISCOMs). ISCOMs have an interesting range of adjuvant activities resulting in an increased Th1 response but have the advantage that they are active orally. More recently other purified Quil A extracts have been prepared and shown to be less toxic.
The most potent mucosal adjuvants have been shown to be the toxins derived from *Vibrio cholerae* or *Escherichia coli*, which should not be surprising since these organisms invade the body through the GI tract. Obviously too toxic for human use because they are the source of cholera or diarrhoea, heat labile enterotoxins have been tested in mice and shown to be potent adjuvants for orally or nasally administered influenza vaccine. The potency of heat-labile enterotoxin mutants may also be enhanced by formulation into bioadhesive particulate delivery systems, and this is an area under current exploration.

**MODERN MICROPARTICULATE VACCINE VEHICLES**

A wide variety of particulate systems have been explored as drug delivery systems and are now being evaluated for the delivery of vaccines or vaccine components. Examples of particles are latex emulsions, carbon particles, liposomes, and polystyrene and poly(lactide-co-glucoside) particles. As noted earlier, antigens or antigenic epitopes attached to a particulate carrier are more likely to bring about a successful immunological reaction and some, such as chitosan particles, can act as adjuvants in their own right. Liposomes have been discussed in an earlier chapter and will not be reviewed here.

Natural polymers such as gelatin or albumin have been used as particulate drug delivery systems, although they are of uncertain purity and certainly have the potential for immunogenicity.

**BIODEGRADABLE POLYMERS**

Synthetic polymers, especially polyesters of lactic and glycolic acids, have been used since the 1960s as resorbable sutures and microparticulate drug delivery systems. Extensive studies of these materials and other similar biologically acceptable polymers have demonstrated that they can degrade by random cleavage in the particle matrix or simply hydrolyse at the particle surface, producing organic acids at the interface. This hydrolysis means that the surrounding environment will be acidic and this can sometimes have implications for the stability of any incorporated drug substance. Other than this caveat, polylactide (PL) and polylactide-co-glucoside (PLGA) polymers have become accepted as safe and effective as controlled-release vehicles.

Low molecular weight PLA and PLGA can be produced by direct catalysis of mixtures of lactic or glycolic acid using antimony trioxide as catalyst; higher molecular weight entities being produced using antimony, tin, or lead catalysts. The composition of the polymer and the molecular weight are controlled by selection of appropriate molar ratios of the two primary acids and polymerization conditions. Factors such as crystallinity, polydispersity geometry, and polymer structure are all controlled during the manufacturing process. Breakdown in the body occurs by hydrolysis, with high molecular weight material reverting to polymers of lower molecular weight. It is generally accepted that enzymic degradation is hardly involved in the breakdown, the main process being by simple hydrolytic breakdown. However, polymers with a high lactic acid content are more stable to hydrolytic attack than...
those with intermediate ratios of lactic:glycolic acids. Hydrolysis is also likely to be slower through regions of crystallinity in the matrix. A 50:50 lactide:glycolide co-polymer degrades the most rapidly because it is unlikely to contain crystalline blocks of either of the constituent polymers. The glucoside component is thought to encourage the penetration of water into the solid matrix, thereby forming water channels and causing the surface hydrolysis over a wider area. As the molecular weight falls the relative numbers of the end groups increase so that what was initially a hydrophobic block becomes progressively more hydrophillic. This is seen with the higher molecular weight polymers which exhibit two distinct release phases of water uptake, separated by a short lag period. Moreover, in an alkaline medium or solutions of high ionic strength the hydrolysis rate is accelerated. Effectively the hydrolytic process is autocatalytic and the interior of microparticles has been observed to degrade faster than the interface.

The release of incorporated proteins from polymer matrices has been described as a three-stage process, a lag phase followed by a burst and then a steady state. The lag phase is due to water penetrating into the matrix but the burst effect is likely to be due to material at or near the matrix interface which is readily released as soon as it is wetted. After that the release profile settles down into a steady state until most of the drug has been released. These three phases are not always clearly separated, depending on the composition of the matrix and the amount of drug incorporated, but it should be possible to design a system that will manifest a zero order–release pattern over a reasonable period of time ideal for vaccine administration. If antigens are delivered to the mucosal system incorporated into small particles the main enzymatic barrier to absorption in the form of the exo- and endopeptidases in the lumen and the mucosal cellular membranes can be minimized, allowing both a mucosal and systemic immune response to be obtained. Antigen uptake is promoted or antigen can be directly delivered to the lymph nodes, although this will not necessarily result in the induction of an immune response. A full immunological response requires the antigen-presenting cell (APC) in the presence of other immunostimulants and cytokines. Coincidentally, perhaps, microparticles have the same approximate dimensions as invading pathogenic microorganisms with which the immune system has evolved to combat. Typically a vaccination requires one initial dose followed by two or more booster doses and some authors have considered the possibility of developing controlled release formulations which will mimic this effect but work by using a single dose (Alpar et al. 2000).

Vaccine antigens have been encapsulated inside polymeric particles and shown to stimulate production of antigen-specific serum antibody responses as well as mucosal IgA. Since bioadhesive microparticles can be produced this is proving to be an attractive avenue for the exploration of intranasal and other mucosal vaccines.

**LIPID PARTICLES AS ADJUVANTS AND DELIVERY SYSTEMS**

Liposomes (see Chapter 9) have been used to deliver vaccines and have been observed to have immunostimulant activity. When administered orally liposomes with encapsulated antigens have been claimed to provide protection from the gastric proteolytic enzymes. Liposomes also have potential as mucosal delivery systems...
since not only are they immunogenic in their own right but physical association of the antigen with the liposomal structure is not a requirement for intranasal immunostimulation. Simple mixtures of liposomes with other agents such as chitosan have potential for nasal delivery and have facilitated enhanced responses to vaccines administered orally.

Cochleate systems consisting of calcium-precipitated protein–phospholipid complexes are stable solid sheets that roll up into a spiral with no internal aqueous space, and the calcium ions bridge adjacent sheets. Oral administration of vaccines in cochlear delivery systems has been shown to induce strong long-lasting circulation and mucosal antibody responses with long-term immunologocal memory to influenza glycoproteins.

Virosomes are viral glycoproteins encapsulated in lipid vesicles, which have been shown to be effective as experimental vaccines delivered by both mucosal and systemic routes. Viruses and their surface glycoproteins have a high affinity for receptors on mucosal surfaces, especially along the respiratory tract.

**CHITOSANS**

Chitin is almost as common in nature as cellulose and is a main structural element of Crustacea, molluscs, and insects. Because it has limited solubility in industrial solvents, it has limited use; but when deacetylated under alkaline conditions it is converted to chitosan. Chitosan has terminal free amino groups distributed along its molecular chain (Figure 12.5), giving it a higher chemical and biochemical reactivity and thereby allowing it to be applied in a number of areas, including cosmetics and drug delivery. While inexpensive and readily available commercially, this material is also claimed to be nontoxic, biodegradable, and biocompatible. One other advantage, less widely recognized, is that it is a mucoadhesive and it also appears to act as an immunoadjuvant which, in the context of vaccine delivery, might provide a considerable advantage. In addition, the adjuvanticity of chitosan can be enhanced by the addition of secondary adjuvants so, overall, this material is seen to be very promising.

Commercially chitosans can have molecular weights varying from 4 to 2000 kDa and vary in the degree of deacetylation from 66% to 95%. Because of the free amino groups chitosan behaves as a weak base, with a pKa of 6.2–7.0 and is insoluble in water or organic solvents. It is a polyamine and therefore dissolves in hydrochloric acid.

![FIGURE 12.5 Chitosan structure. (From Leone et al. 2004.)](image)
acid and various organic acids including acetic, oxalic, and lactic acids, thereby forming salts. Chitosan salts are soluble in water, the solubility depending on the type of acid involved. For example, sebacic, phosphoric, and sulfuric salts are all less soluble and this provides a means of making dispersed insoluble particles of chitosan. In one method the chitosan powder is dissolved in a dilute acetic acid solution and poured into a solution of sodium sulfate, forming a fine dispersion of chitosan microparticles that can be collected and dried.

The stability of unmodified chitosan particles in an aqueous environment may be questionable and some authors have made covalent cross-linked chitosan microparticles by taking advantage of the formation of Schiff bases with the free amino groups using a reactive aldehyde such as glutaraldehyde. These cross-linked particles may be less soluble in water and they are more stable physically but need to be loaded with any drug only after the remaining glutaraldehyde is thoroughly washed out and neutralized with sodium metabisulfite.

THE FUTURE OF VACCINES AND VACCINATION

It is evident that currently vaccine research is a vigorous and developing topic but a number of goals remain elusive. For example, an ideal vaccine should elicit the required immunological response against specified pathogens, whether it requires a specialized delivery system or adjuvants. This entails comparison of the requirements for an ideal vaccine, with progress to date.

In addition it is becoming clear that vaccines should be heat stable since many are required in tropical countries where the cold supply chain used for many current vaccines is not available. Any newly developed vaccine must be completely safe since it should not cause disease or manifest side effects. This consideration is difficult to achieve in many cases because the side effects may not always be evident at first and may only show up after thousands of patients have been treated, often as an idiosyncratic reaction in just a few individuals. This issue is also experienced when developing small molecule drugs for the market place.

An advantage would be obtained if protection could be achieved using only a single dose that would be effective for the rest of the patients life. In some instances a controlled-release formulation has been tested and pulsatile systems that could mimic the administration of a booster dose may also have the desired effect.

Finally, the vaccine must be inexpensive and this is by no means an easy requirement. To illustrate the point, if a vaccine costs $25 to produce, pack, and deliver to the patient, how can this be acceptable in a country where the amount of money available per patient is only $250 per annum? Of course, money is saved on the subsequent savings in healthcare costs throughout the remainder of the patient’s life. The rest of the debate is limited to a discussion of the value of a life, but in human terms this cannot be measured.

An interesting issue has surfaced at the time of writing (October 2004) when the supply of influenza vaccine in both the United States and United Kingdom became severely limited owing to a failure in good manufacturing practices within
one organization. This resulted in the closure of the plant involved but, more to the point, a loss of about 50% of available doses for the winter season. Since there are only two plants worldwide making this vaccine, this has affected affluent members of society who would otherwise not consider themselves affected by the economic constraints that affect developing nations. The media has probably contributed to the hysterical discussion of which members of society should or should not receive the vaccine, also promoted by some politicians as the vaccine that could make the difference between life or death. The more responsible media has suggested that there may be insufficient economic return and too high a commercial risk for many pharmaceutical manufacturers to wish to get involved in vaccine production in general so that failures of this magnitude are almost inevitable. This entire issue will not go away and it is certain the discussion will continue in the foreseeable future.

On the basis of current research it may be possible to speculate about future directions for vaccine development. For example, controlled-release drug systems are well recognized and have appeared in clinical practice. It seems reasonable to ask if the same technology could be applied to vaccines. What would be ideal is a vaccine that only required a single dose which incorporated that booster dose so often necessary for complete effectiveness. If this in turn was combined with heat stability to overcome the problem of maintaining an effective cold chain for distribution in tropical countries we would be well on the way to providing an ideal product.

The probability is that acellular and subcellular vaccines will represent the future because they are generally much safer although, without appropriate adjuvants, they may be less effective. Viral shells, without their DNA should not be able to grow in vivo but should be capable of triggering an immune response in the form of the production of antibodies and memory cells. Isolated bacterial flagellae may also be capable of the same response. Acellular vaccines against Haemophilus influenzae B (Hib) bacterial flagellae have been cultivated and tested. An anthrax vaccine uses the protective antigen of anthrax, and there have been a number of attempts to develop a vaccine against the GP 120 surface protein of the HIV virus.

Recombinant DNA vaccines offer alternatives as subunit vaccines and organisms can be engineered to produce antigens or even epitopes. A hepatitis B vaccine has been engineered using yeast as the host cell. Adverse reactions are rare to subunit vaccines, making them safer for use in immunocompromised patients.

A recent development has been the use of genetically modified plants to produce vaccine components. Examples include common foodstuffs such as tomatoes, bananas, potatoes, and corn. The prospects for oral vaccines in bananas especially would appear to be promising since this is a staple food in many tropical countries. Vaccine epitopes have also been produced in the milk of goats, sheep, and cows although these may be difficult to purify, process, and formulate.

Bacterial toxins such as diphtheria and tetanus can damage host cells but the isolated toxins can also be immunogenic. However, the induced response may not always be very strong and booster shots are required every 10 years. Adjuvants could improve the response and both diphtheria and tetanus toxoids are more effective when combined with pertussis subunit vaccines, the DPT combination at present used clinically.
SOME FUTURE VACCINE OPPORTUNITIES

There appear to be a myriad of opportunities for vaccines against, for example, cancers, allergies, hepatitis, tuberculosis, and HIV. Vaccines to treat drug addicts, including tobacco products, nicotine, and cocaine are all within the realm of possibility.

There is a perceived need to develop effective vaccines against bovine spongiform encephalopathy (BSE) and the human form, new variant Creutzfeldt-Jacob (nvCJ) disease. Organ transplant vaccines would obviously be beneficial and tetanus toxoid has been shown to lower cholesterol in animals. Tumor-specific antigens have also been explored and heat stress proteins have been evaluated as adjuvants in this case.

Attention has recently become focused on epidermal administration of vaccines, either as polymer rods inserted subdermally or by particulate systems fired into the skin using the high pressure jets developed by companies such as PowderJect Pharmaceuticals (Oxford, UK). The epidermis contains immune cells and less vaccine may be required to achieve a response which also reduces costs.

RISK-BENEFIT RATIOS

The increased use of vaccines has drawn attention to ethical issues associated with safety and the risk–benefit ratio in some cases has come under scrutiny. Vaccines are usually given to patients who are otherwise healthy and have a lower tolerance for risk. Adverse reactions are either quite common (>>10%) or very rare (<<0.0001%). The question of justifiable risk in a healthy population then becomes problematic and difficult to identify.

To identify a justified risk in a healthy population requires clinical trials and if the risk is very low inordinate numbers of patients are required to identify issues. These become extremely expensive in practice and this is one reason why vaccines are subject to postlicensing and postmarketing surveillance for safety.

Animal models are rarely valuable in predicting human responses. Animals are required to be immunized twice monthly and monitored for at least 6 months. Some reassurance might be provided if there were no deaths or obvious and unusual effects. However, postmortem examination is required to ensure that no macroscopic or microscopic changes have been produced in any of the internal organs.

Once the animal studies are completed human studies can commence, moving through phase I (up to 50 naïve patients to establish a sufficient immune response); phase II (several hundred patients in several locations) to phase III in which expanded studies on as many as thousands of patients. The trials require to be randomized and closely controlled by being blinded to avoid bias in interpretation. The patients all have to provide informed consent and strict adherence to good clinical practices in accordance with ethical principles is required to ensure risk-benefit considerations justify the risks associated with all trials of this type. The safety of the patients is an overall requirement and trials must be supervised by the appropriate independent ethics committee or the local Institutional Review Board.

It should be noted that, despite all these precautions, some individuals have become impatient with the system and have tried to take shortcuts, with inevitable severe repercussions when things go wrong. The system is there to protect the patients
and the complexity of these issues certainly accounts for the high cost of commercially available vaccines (and drugs in general).

REFERENCES AND FURTHER READING


