CHAPTER 1

Vaccines: Past, Present and Future

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A. Bacterial Vaccines: Past, Present and Future

I. Past

The earliest bacterial vaccines were live strains derived from virulent cultures isolated from cases of natural infection. These were attenuated by empirical methods based on sub-culture under adverse conditions. Sometimes this involved exposure to unusually high temperatures or culture on media that contained substances inhibitory to wild-type strains. Occasionally attempts were made to select strains by passage in unnatural host species, but generally this approach was less successful than for viral attenuation. Examples of vaccines produced by these processes include the fowl cholera (*Pasteurella multocida*) and anthrax vaccines developed by PASTEUR (1880, and PASTEUR et al. 1881) and the bacille Calmette-Guérin (BCG) tuberculosis vaccine developed by CALMETTE et al. (1928). The Pasteur anthrax vaccine strains were selected by growth at high temperature (42°C) and were notoriously unstable and difficult to administer consistently.

The reasons for this did not become apparent until quite recently when the genetic basis of the pathogenicity of *Bacillus anthracis* was established. It now seems probable that the Pasteur anthrax vaccine consisted of organisms from which the plasmid pXO 1 encoding the toxin complex had been deleted. Such a strain would still synthesize the other major virulence determinant, the poly-D-glutamic acid capsule encoded by the pXO2 plasmid, but would not stimulate a protective response as this material is not antigenic. It is assumed that the protective activity resulted from the presence of small numbers of virulent organisms, hence the difficulty of achieving both safe and effective batches. This problem was not resolved until the development of the Sterne strain of *B. anthracis* from which the pXO2 capsule plasmid has been deleted but in the toxin-encoding pXO1 plasmid is retained. This strain was also selected by empirical methods, but the use of effective and reliable in vivo assays assured attenuation and efficacy.

The BCG strain was selected by repeated sub-culture in the presence of ox bile of a mycobacterial strain isolated from bovine milk (CALMETTE et al. 1921). The basis of attenuation of this strain is still not understood, nor is the basis of its immunizing activity. Furthermore, numerous variants have arisen during

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the course of repeated serial sub-culture, and there is evidence that these differ in protective activity. This has resulted in problems in assuring consistency between vaccines produced by different manufacturers and even by the same manufacturer over an extended time interval. The problem is exacerbated by the absence, even today, of a potency assay which predicts immunizing activity in humans.

Many other live attenuated bacterial vaccines were developed against a variety of diseases including cholera, dysentery, paratyphoid, plague, typhoid and tularaemia. Most proved inconsistent in safety, efficacy or both. In general, because of these problems the development of bacterial vaccines tended to move away from live attenuated strains. Two other main approaches were followed, the use of killed virulent bacteria and the production of detoxified toxins (anatoxins or toxoids).

The development of killed vaccines required methods that would ensure loss of viability but not destroy protective antigens. Usually mild heat treatment was used, but in some cases this needed to be supplemented with chemical treatment. The inactivation procedures were all developed empirically and still tend to vary in detail between manufacturers even for similar types of product. Nevertheless, this approach was successful in producing effective vaccines against a number of diseases. Currently available bacterial vaccines prepared from killed organisms include cholera, pertussis, plague and typhoid whole-cell vaccines. Attempts were also made to apply this approach to many other organisms including gonococci, meningococci, pneumococci and mycobacteria but without success. The reasons for these failures were not understood at the time because of lack of basic knowledge of bacterial structure and the immunology of infection. They included inability to stimulate protection and also unacceptable toxicity in the case of vaccines prepared from gram negative cocci. The lack of efficacy resulted from the wide diversity of antigenic types, the modulation of expression of antigens during growth in vivo and the need in the case of certain organisms such as mycobacteria, to stimulate cell-mediated immunity to relevant antigens. None of these factors was identified until many years later, and this lack of knowledge impeded the development of vaccines against many infectious agents.

Even for bacteria that were amenable to the killed cell approach, variations in expression of protective factors by different strains and by the same strains grown under different cultural conditions caused inconsistencies between manufacturers and even between different batches produced by the same manufacturer. This emerged as a particular problem with killed pertussis and typhoid vaccines. In the case of the former this was not resolved until an effective potency test was developed. In the case of typhoid vaccines no satisfactory potency test predictive of performance in humans has yet been developed and the production of whole-cell vaccines followed a very uneven course. After the initial successes reported by Wright and colleagues, subsequent studies indicated poor efficacy. Eventually it was established that the inactivation procedure should preserve the Vi antigen intact (and probably

coincidentally many protein antigens). This was achieved by inactivation with cold ethanol or acetone. Interestingly, although acetone-inactivated preparations showed the highest efficacy in field trials (YUGOSLAVIA TYPHOID COMMISSION 1964), this type of preparation was not developed commercially, most manufacturers preferring to retain heat-phenolized preparations. However, these are now being superseded by purified sub-unit or live attenuated strains (KLUGMAN et al. 1987; LEVINE et al. 1990).

Because of their ill-defined composition killed whole-cell vaccines can cause difficulties. Nevertheless, some are still in use and provide protection against important infectious diseases. Possibly the best example is killed whole-cell pertussis vaccine. The results of recent clinical trials in Germany, Senegal and Sweden have confirmed that such vaccines, when subject to effective quality control, are capable of achieving levels of efficacy which match or exceed those of even the most effective sub-unit preparations (OLIN et al. 1997).

Somewhat better defined are the bacterial toxoid vaccines. These represented the first successful attempt to develop vaccines targetted against defined bacterial products and in particular against the factors primarily responsible for inducing the disease process. Once it was recognized that the pathological effects of certain infections could be reproduced by administration of cell-free extracts of the organisms or their culture fluids, attempts were made to render these non-toxic while retaining immunizing capacity. This did not prove entirely straightforward and the selection of reliable inactivating agents was difficult. Neutralization with antitoxin was used to produce a toxin-antitoxin complex for immunization. This approach was later combined with early toxoided preparations to protect against reversion. Although preparations of this type remained in production for many years, they required very careful preparation with precise control of the proportions of toxin and antitoxin used.

Many problems resulted from dissociation or incomplete neutralization of the toxin and the use of chemical inactivating agents was favoured. Early attempts to produce a diphtheria vaccine used iodine to inactivate the toxin. It proved difficult to achieve consistent results with this and frequent problems were encountered with inadequate detoxification or reversion to toxicity. Conversely, excessive treatment led to a degraded and non-protective product. The introduction of formaldehyde for detoxification represented a major advance and made possible the large-scale production of effective vaccines against diphtheria, tetanus and other toxigenic clostridial diseases (RAMON 1923). However, the interaction of formaldehyde with proteins is complex, and careful control of the process is essential. Nevertheless, formol toxoids are still favoured for the production of diphtheria and clostridial vaccines, including tetanus. Formaldehyde has also been adopted for the detoxification of pertussis toxin used in some current sub-unit vaccines.

Toxoids of this type are fairly easy and relatively inexpensive to manufacture. As diphtheria and tetanus vaccines produced by this means have proved

safe and very effective, there has been considerable reluctance on the part of manufacturers to apply more sophisticated approaches, such as genetic detoxification, to these vaccines even though this has been technically feasible for some considerable time.

II. Present

The types of bacterial vaccine currently in use, together with some examples are listed in Table 1. Of these the live attenuated, killed whole-cell and toxoid categories were developed many years ago and include preparations with a well-established track record. BCG vaccine has had a rather controversial history in relation to efficacy against post-primary tuberculosis but persists as no more effective alternative has yet been introduced.

Table 1. Currently available bacterial vaccines

Vaccine type	Reactogenicity	Efficacy
Killed whole cell		
Cholera	Moderate-high	Low (<50%)
Pertussis	Moderate-high	Up to 95%
Plague	High	Uncertain, short term
Q fever	Moderate	Approx. 80%
Typhoid	High	Up to 75%
Live attenuated		
BCG (tuberculosis)	Low	Variable (0%–75%)
Cholera CVD103HgR	Low	Still in trials
Tularaemia LVS	Low	Uncertain
Typhoid Ty21a	Low	Variable (20%–70%)
Purified sub-unit		
Anthrax	Moderate-high	Uncertain
Acellular pertusssis	Low	70%–85%
Meningococcal group B OMV	Low	Approx. 55%[c]
Toxoid		
Botulism (1–5 component)	Moderate	Probably high
Diphtheria	Low-moderate	>90%
Tetanus	Low-moderate	>95%
Polysaccharide		
Meningococcal A, C, AC, $ACW_{135}Y$	Low	Up to 90%, short term[a]
Pneumococcal 23-component	Low	56%–67%[a]
Typhoid Vi	Low	Up to 70%
Polysaccharide-protein conjugate		
Haemophilus influenzae b (various compositions in use)	Low	>95%
Meningococcal C	Low	In trials[b]
Meningococcal AC	Low	In trials[b]
Pneumococcal 7-valent	Low	In trials[b]

[a] Not suitable for use in recipients aged under 2 years.
[b] Preliminary results predict high efficacy based on immunogenicity data.
[c] In clinical trials.

More recently the emphasis has been on the development of vaccines of more defined composition and properties. This has included the elaboration of vaccines based on purified sub-units and production of attenuated strains containing predetermined genetic modifications. An approach has also been made to improvement of the immunogenicity of natural antigens by chemical modification. This has led to the development of the polysaccharide-protein conjugate vaccines.

1. Purified Sub-unit Vaccines

a) Polysaccharides

These are prepared from the capsular polysaccharides of encapsulated bacterial strains. While many pathogenic bacterial species synthesize capsular polysaccharides, these have been exploited for vaccine production in only a few instances. These include the A, C, W and Y serogroup antigens of *Neisseria meningitidis*, the capsular antigens of a variety of *Streptococcus pneumoniae* serotypes, the capsular antigen of *Haemophilus influenzae* type b (Hib) and the Vi antigen of *Salmonella typhi*. These polysaccharides can be produced to a high level of purity and are essentially free of toxic or reactogenic components. However, to possess immunogenicity they must be of a minimum molecular size as well as retaining their original repeating sub-unit composition. Such vaccines have been used successfully in certain situations but have certain limitations. In particular, they behave as T cell independent antigens and are inefficient in stimulating isotype and sub-class switching, tend to evoke antibodies of low avidity and do not stimulate a memory response. Of even greater importance is their failure to evoke responses in the very young (AUSTRIAN 1981). This has proved a particular disadvantage in the case of Hib, meningococcal and pneumococcal polysaccharides where much of the burden of disease caused by the corresponding organisms falls on infants. This problem has stimulated the search for ways of making these antigens more effective. This has been achieved by conjugating them to proteins to produce semi-synthetic glycoconjugates (SCHNEERSON et al. 1989).

b) Protein-Polysaccharide Conjugate Vaccines

These are produced from purified bacterial polysaccharides or oligosaccharide sub-units by chemical bonding to a protein carrier which acts as a source of T_H epitopes (MOREAU 1996). Either of two main conjugation strategies may be adopted: (a) direct conjugation of activated polysaccharide to an activated protein, (b) indirect conjugation of activated polysaccharide to a linker molecule followed by conjugation to an activated protein which itself may or may not be attached to a linker molecule. Option (a) is to be preferred when possible as the use of linker molecules may induce a substantial response to protein-linker neoepitopes at the expense of the saccharide. With successful construction polysaccharide-protein conjugates can induce excellent T cell dependent responses to carbohydrate antigens. They have been outstandingly successful in

the case of Hib conjugates SCHNEERSON et al. (1980) and pre-clinical animal studies and clinical trial data suggest that similar achievements are possible with meningococcal group C and multivalent pneumococcal conjugates. Many other conjugates have been prepared and demonstrated experimentally to stimulate antibodies against a variety of bacteria including *Bacteroides*, *Escherichia coli*, *Klebsiella*, *Pseudomonas*, *Salmonella* and *Streptococcus* group B. Vaccines can also be prepared against surface polysaccharides of non-encapsulated bacteria, for example, *Brucella*, *Shigella*, by conjugating detoxified lipopolysaccharide to a carrier protein (TAYLOR et al. 1993).

The number of carrier proteins used to date has been limited. Manufacturers have favoured established vaccine components such as diphtheria or tetanus toxoids or the non-toxic diphtheria mutant protein CRM 197 as carriers in the belief that this would more easily satisfy regulatory requirements. However, this is already beginning to present problems of potential overload of these components in complex combination vaccines. Increased reactogenicity and epitope suppression or enhancement are possible complications of excessive exposure to individual antigens. This has led to the search for additional carrier proteins (ROBBINS et al. 1996).

2. Purified Protein Sub-units

These target individual protective antigens. This is relatively straightforward in the case of toxigenic infections such as diphtheria or tetanus but the identification of candidate antigens has proved more elusive for infections where the mechanism of pathogenesis is more complex, for example, *N. meningitidis* serogroup B. For *Bordetella pertussis* various combinations have been used in formulating acellular vaccines. All include detoxified pertussis toxin in some form but with or without the addition of other components including filamentous haemagglutinin, pertactin and fimbriae (fim 2 and 3). Clinical trial data suggest that efficacy is improved with increasing number of components, but that the method of detoxification can also influence the results (OLIN et al. 1997).

Protein components for vaccines can be produced by purification from the natural strain or by rDNA technology in a heterologous host. The latter system can circumvent some purification problems but rDNA proteins may show post-translational modifications distinct from those seen in the natural product. Some complex proteins may also be difficult to express and assemble in heterologous systems, an example being pertussis toxin. The genetically detoxified protein must be expressed in a *B. pertussis* strain from which the wild-type gene has been deleted (PIZZA et al. 1989).

The rDNA approach may be the only practicable way of producing antigens from organisms that are not amenable to in vitro culture, for example, *Treponema pallidum* and *Mycobacterium leprae*.

An increasing number of vaccines are being developed from recombinant proteins, for example, new anthrax, plague, *Helicobacter* and Lyme disease

vaccines, but with the exceptions of isolated examples of meningococcal group B and acellular pertussis vaccines none is in routine production.

3. Genetically Modified Strains

Current policy is to develop live attenuated strains containing defined mutations. An early attempt to do this produced the Ty 21a live typhoid vaccine. This strain was thought to contain a mutation in the galactose epimerase gene as the attenuating modification. However, this has been demonstrated not to be the case. The Ty 21a strain was produced by mutagenesis with nitrosoguanidine and contains numerous mutations, many of which may contribute to attenuation.

It is now the practice to introduce specific mutations in identified genes by site-directed mutagenesis. These usually involve key metabolic or "housekeeping "genes such as those responsible for the shikimic acid pathway (Aro mutants) or pho or cya mutants (HOISETH and STOCKER 1981). Another approach is to produce deletions or modifications to genes encoding specific virulence determinants, for example, the CVD103HgR mutant of *Vibrio cholerae*. In this the cholera toxin, zonula occludens toxin and haemolysin genes have been deleted or rendered inoperative. This strain and aro mutants of *Salmonella typhi* have reached marketing authorization application stage in some countries. Many other genetically modified strains are currently undergoing preclinical evaluation.

The application of such organisms as delivery systems for a variety of protective antigens has attracted considerable interest. Modified *Salmonella* and BCG strains have been specifically targetted although naturally non-pathogenic bacteria such as *Streptococcus lactis* have also been studied (CHATFIELD et al. 1993). Although some success has been obtained with these systems in experimental studies, none has so far achieved routine application. Similarly, many other non-living delivery systems and new adjuvants are currently under investigation (POWELL and NEWMAN 1995). These are more appropriately considered in terms of future developments.

III. Future

Approaches to vaccine design that are under examination include: synthetic peptides, anti-idiotypes, nucleic acids, live recombinants, multivalent combinations, slow-release/single-dose presentations, and oral/mucosal delivery systems.

1. Synthetic Peptides

Linear peptides reproducing part of the primary sequence of bacterial antigens have met with little success in achieving protection against bacterial diseases. They tend to stimulate responses which are too narrow in terms of HLA restriction. Circular and complex peptides have shown rather more

promising results in experimental studies, for example, in stimulating responses to the meningococcal group B por A variable regions. They are also more likely to stimulate adequate T and B lymphocyte responses (FITZMAURICE et al. 1996). However, potentially the most useful is the mimeotope approach. This uses non-linear arrays of peptide sequence to simulate the three-dimensional structure and charge distribution of epitopes which may be of protein or non-protein composition. Modification of the structure allows "fine tuning" to reduce or increase cross reactivity and avidity. This approach could be extremely useful for simulating non-protein antigens that may be impossible to purify or to produce by recombinant methods.

2. Anti-idiotypes

These are natural mimeotopes that use monoclonal antibodies to the combining site of antibody to the native antigen. Again this approach is potentially most useful for non-protein antigens such as polysaccharides. There are many problems associated with the use of such materials as vaccines, such as assuring freedom from extraneous agents, and none intended for human use has so far advanced beyond the experimental stage. The refinement of recombinant antibody production methods may overcome these problems.

3. Nucleic Acids

Vaccines may be prepared by construction of plasmids containing the cloned gene for an antigen together with the sequences necessary for transcription and expression within eukaryotic cells. These, when suitably presented, may trigger protective immune responses against a variety of antigens. This approach has given encouraging results with a number of bacterial pathogens. However, there are many regulatory and safety issues to be addressed with this type of preparation. Nucleic acid vaccines will probably prove most useful for those infections which are not easily amenable to prevention by other methods. An example is tuberculosis, against which DNA vaccines are hitherto the only preparations to have stimulated protection approaching that given by BCG (HUYGEN et al. 1996). They may also provide a means of constructing compatible multivalent vaccines which can be delivered by the mucosal route.

4. Live Recombinants

These may consist of individual pathogens attenuated by defined mutations, for example, AroAC$^-$ mutants of *S. typhi* or more complex constructs expressing protective antigens for multiple pathogens. Thus in the future it may be possible to design a "universal" enteric vaccine which expresses the antigens of *Campylobacter*, *Entamoeba*, *Giardia*, *S. typhi*, *Shigella*, and *V. cholerae* in a single preparation. Hitherto the development of such constructs has been impeded by interference between some of the components, for example, the long O chains of *Salmonella* have masked the shorter O chains of *V. cholerae*.

However, these problems may be overcome by targetting different antigens or by using a different presenting system.

5. Multivalent Combinations

The proliferation of new vaccines and the difficulty of integrating them into acceptable immunization schedules has stimulated the development of complex combinations, for example, a "universal paediatric vaccine" containing acellular pertussis, diphtheria, tetanus, Hib, hepatitis B, and inactivated poliomyelitis in a single formulation. This has not proved straightforward as some incompatibilities have emerged, such as interference between acellular pertussis and Hib components. The future trend may be to target syndromes, for example, "meningitis vaccines" containing Hib, meningococcal and pneumococcal antigens, "enteric vaccines" and "sexually transmitted disease vaccines" each covering the major diseases in these groups. It is also likely that combinations will be formulated with delivery systems appropriate to the route of entry of the pathogen.

6. Slow-Release/Single-Dose Vaccines

Related to the problems addressed by multivalent vaccines is the concept of single-dose preparations. For much of the world population access to medical facilities is limited, and the implementation of complex multi-dose immunization schedules impracticable. This has directed efforts towards vaccines which will produce lasting immunity after a single dose. These have been based on systems which either release antigens slowly over a prolonged period, or which produce pulsed release to simulate multiple doses. In practice, sustained release has been more easily achieved. Current developments favour biodegradable microspheres (Gander and Merkle 1998), but future preparations are likely to use more sophisticated vehicles, particularly those that achieve high-grade responses when given by the oral route. Single dose preparations will also be designed to provide simultaneous immunization against multiple pathogens.

7. Oral/Mucosal Delivery Systems

The ideal vaccine would stimulate lasting immunity against a variety of pathogens without adverse side effects when given as a single dose by the oral route. Such an objective has yet to be achieved but may not be totally unattainable. Investigations are proceeding along the lines of live vectors and non-living delivery systems. The ideal will incorporate multiple antigens and single-dose schedules. Systems employing antigens encapsulated in microparticles or nanopartcles have shown promising results when delivered by oral, respiratory or parenteral routes (Gander and Merkle 1998). Live delivery systems, although offering some advantages, also have limitations and these may prove crucial.

The cloning and expression of genes encoding bacterial antigens in plants offers the prospect of delivering vaccines in foods. There are many unresolved issues over dosage and regulation of such products, and their greatest future advantage may lie in the bulk production of vaccine components without the need for elaborate and expensive fermentation facilities.

B. Viral Vaccines

I. Past

Vaccines are among the most effective medical intervention against viral diseases. Partly this is because specific anti-viral antibiotics are rare as a result of the intimate relationship between the cell and the virus, and partly because it is clear infection with a particular pathogen often gives long-lived protection if the patient survives. Small pox is usually regarded as the first human disease for which vaccination was developed as a deliberate intervention in a healthy subject to prevent subsequent infection. Jenner not only vaccinated James Phipps with cowpox virus in 1796, he also challenged him with live smallpox afterwards. While this was somewhat dubious ethically, it is generally considered to have set the scene for the eventual eradication of smallpox from the world. Much of the vaccine used was primitive by modern standard, much of it grown on the scarified flanks of cattle with poor control of the seed virus or contamination. Adverse reactions were common; fever and swollen lymph glands were inevitable in first time vaccinees and disseminated vaccinia and encephalitis occurred at frequencies which would be entirely unacceptable nowadays. Nonetheless the use of the vaccine eradicated the disease from the globe. It is probable that the selection of more appropriate strains and better production methods could have limited the adverse reactions seen. Current attempts to use the smallpox vaccine as a vector for other antigens are handicapped by its undesirable side effects, but it can be argued that it was the most successful vaccine so far, in that it no longer needs to be used.

While Jenner used an existing virus of animals to vaccinate humans, Pasteur is credited with the first scientific development of a vaccine. The material which was developed as a post-exposure treatment consisted of the central nervous system of rabbits infected with human rabies, a procedure thought to attenuate the virulence of the virus for humans. Similar types of killed vaccine, usually phenol-treated extracts, are still in use in many parts of the world, despite their known hazards, which include the induction of immune encephalitis because of the contamination with the donor animal CNS. The potency is also questionable in many cases. Slightly better versions included rabies virus grown in duck eggs, which while not causing encephalitis did not induce much protection either as the virus titre was so low. Many of the problems associated with the early types of vaccine were to do with poorly defined production conditions, and this is the main area of improvement. Certainly the mechanism by which the vaccines against viral diseases in

current use confer protection rather than cause disease is still very poorly understood.

II. Present

In the context of viral vaccines the present may be taken to have started in 1937 when Theiler developed the 17D strain of yellow fever vaccine (Barrett 1997). The strain was developed from a wild-type isolate, Asibi, by repeated passage in mouse embryo, chick embryo and minced chick embryo devoid of nervous tissue. After 176 passages the virus was attenuated and is among the safest and most effective vaccines in use today, despite the fact that it has been passaged to different degrees in different laboratories, so that different vaccines are by no means identical. The vaccine is still grown in embryonated hens-eggs, which also form the production substrate for the second vaccine of the modern era, that against influenza. Influenza vaccines, however, are killed. In the early days all influenza vaccines consisted of whole inactivated virus particles. These preparations were associated with adverse reactions, such as mild fever, and it was found that splitting the virus with detergent and solvent reduced the reactions seen. In recent times the reaction rate has been reduced still further by the use of subunit vaccines which consist of just the two outer glycoproteins the haemagglutinin and neuraminidase. While an immune response to these two proteins confers protection, they are not as immunogenic as the whole virus preparations. A reduced immunogenicity of subunit vaccines seems to be a general feature of vaccines against viral diseases.

Polio vaccines were developed in the 1950s by Salk and Sabin (Minor 1997a,b). Salk devised a method for very careful treatment of a polio virus preparation with dilute formalin such that the infectivity was destroyed while the antigenic structure of the virus, which is somewhat fragile, was retained. There were immediate difficulties with the inactivation process, leading to the Cutter incident, in which a number of recipients contracted poliomyelitis from the vaccine. However the production process was modified very rapidly, and current vaccines are based on much the same process. They have proved both safe and effective in the course of over forty years" use. The live attenuated strains developed by Sabin were the product of a painstakingly careful consideration of the pathogenesis of poliomyelitis.

Polio virus normally infects the human gut without ill effects or detectable symptoms. Occasionally there is a viraemic phase when sites away from the gut become infected, and even more rarely the central nervous system, specifically the motor neurons of the spinal cord become infected and destroyed, leading to paralytic poliomyelitis. It was reasoned by Sabin that the viruses growing in the different locations are different in character, and that the type of virus required as a vaccine is one which is able to grow in the human gut but not the CNS. The strains in current use, and which are likely to eliminate the wild-type polio virus from the world in the near future have these properties. While they

adapt as they replicate in the recipient and very rarely are able to change sufficiently to cause paralytic disease, their great effectiveness indicates the important of an understanding of pathogenesis in vaccine development if serious vaccine associated disease is to be avoided.

Measles is a common childhood infection which in developed countries is regarded as unpleasant but largely trivial. In developing countries, however, it is a major cause of death in childhood, with an estimated 1.4 million deaths occurring annually. The vaccines used generally derive from the Edmonston strain isolated by Enders in the United States in the 1950s. They are live attenuated vaccine strains given by intramuscular injection, and if correctly used, are highly effective. However, they do not work in the presence of maternal antibody, so that the recommended age of first vaccination is 12–15 months in developed countries and 9 months in developing areas. The vaccine strains are stability attenuated, and while they may cause a low-grade fever some 7–10 days post-immunization, the incidence of serious reactions is low. Understanding of their mode of action is poor, and this handicaps efforts to produce vaccines which can be safely and effectively given at an earlier age, as is discussed below.

Mumps is a childhood infection which is also widely regarded as trivial, although in the absence of vaccination strategies it is the most common cause of hospitalization due to meningitis. The vaccines against mumps are in general effective but virologically poorly defined (MINOR 1997a,b). The Urabe strain was implicated in aseptic meningitis in recipients but is fairly well characterized. Two other commonly used live vaccine strains are Rubini, which by its passage history should be derived from a wild-type isolate from a Swiss Italian child but is in fact almost certainly derived from the Enders strain, a commonly used laboratory virus, and the Jeryl Lynn strain, which is a mixture of two distinct strains, both probably originating from America.

One of the few viral vaccines in current use which is a product of a modern molecular virology is the hepatitis B vaccine. Early vaccines were derived from the plasma of chronic carriers of hepatitis B, who produce an excess of the viral surface antigen, which can be purified in the form of immunogenic particles, and after appropriate treatments to remove any infectious virus is extremely effective. Plasma-derived vaccines of this type are in use in certain countries at present, but other products are manufactured by expression of the gene encoding the surface antigen in yeast. This results in the formation of similar highly immunogenic particles without any conceivable risk of infection. Production depended on the identification of the surface antigen as the protective immunogen, the identification of the viral gene encoding it and its subsequent expression in a suitable host cell. Bacterial expression was not satisfactory. Hepatitis B vaccines therefore afford one of the few examples of a viral vaccine produced by recombinant technology as well as one of the few where a subunit vaccine is entirely effective. Shortly after the licensure of the hepatitis B vaccine a vaccine against hepatitis A was developed, hepatitis A is

caused by a virus similar to polio virus, and the vaccine is a formalin-inactivated preparation similar in nature to inactivated polio vaccine. The well-tested empirical methods of development of vaccines against viral diseases are therefore still effective.

III. Future

In the near future a number of classical type viral vaccines are likely to be used, including a rotavirus vaccine consisting of a simian rotavirus strain and other strains derived from it. This is essentially the same approach as that used by Jenner in 1796, of taking a non-human virus and using it in human recipients. While the classical approach has been very successful in a large number of important instances, there have been a number of notable insuperable problems.

No vaccine has been developed against the common cold despite its economic importance and an obvious public demand. This is attributed to the large number of viruses which can cause the disease, including approximately 100 antigenically distinguishable strains of rhinovirus, as well as adenoviruses and coronaviruses. Similarly, while there is good evidence that influenza vaccines afford protection against disease if they match the circulating epidemic strains, the continual drift in the wild-type virus means that maintaining the match requires a large amount of effort. The difficulty of developing vaccines against a polymorphous collection of viruses is also regarded as an important consideration in the development of vaccines against HIV.

In some instances the understanding of the immune response or the pathogenesis of the disease is so limited that vaccine development could conceivably be dangerous. In the 1960s formalin-inactivated, alum-adsorbed vaccines were developed against measles and respiratory syncytial virus, both of which are in the paramyxovirus family. When vaccinees were subsequently exposed to wild-type virus, they experienced serious atypical disease (Fulginiti et al. 1967; Kakak et al. 1993); in the case of respiratory syncytial virus several children died. The effects were long lasting and suggested that some inappropriate cellular immune response had been generated. It is still not clear how this had been effected, but this has severely restricted efforts to develop new measles vaccines which could be given in the presence of maternal antibody, or any kind of vaccine against respiratory syncytial virus, although live attenuated strains are currently receiving close attention.

A further example in which understanding of the protective response is lacking is afforded by herpes virus, where recurrences of herpetic lesions can be a regular and painful consequence of primary infection. Clearly between recurrences the infection is somehow controlled, presumably by an immune response. This has led to the trial of various types of vaccine to stimulate immunity. In view of the amount of immunogen which must be generated during a recurrence it is not clear how effective this approach is likely to be,

and some trials have been clear failures. However, improving the immune response may be what is required.

Thus in general a better understanding of immunity and virulence and pathogenesis is likely to be required to generate better viral vaccines. Specific future developments could include improved adjuvants to generate the appropriate protective immune response and, once this has been identified, various presentation systems including DNA vaccines and the development of therapeutic vaccines, especially vaccines against papilloma viruses, the major cause of cancer of the cervix.

C. Parasite Vaccines

I. Past

Parasite diseases have traditionally been controlled, if at all, by vector control and by drug prophylaxis and treatment. Vaccination has not been an option for virtually any parasitic disease. The single exception is cutaneous leishmaniasis, where the practice of deliberate infection of a sacrificial site – such as the buttocks – with material derived from an active sore has been widespread in the Middle East for centuries (HANDMAN 1986). During this century the use of live cultured promastigote preparations has been introduced, and large numbers of vaccinations have been carried out in the former Soviet Union and Israel (HANDMAN 1986) and recently Iran (ENGERS et al. 1996). However, the difficulties involved in standardizing and controlling what is in effect a naturally virulent strain have been considerable, and attempts to produce attenuated varieties by a range of techniques have tended to result in strains which sensitize but do not protect.

II. Present

Although no vaccines for prevention of parasitic disease in man have yet reached the stage of routine use, significant successes have been achieved in the veterinary field which give grounds for optimism. Thus effective vaccines have been developed against the protozoan parasites *Babesia bovis*, *B. bigemina* and *Theileria parva*, the cestode *Taenia ovis* and the nematodes *Dictyocaulus filaria* and *D. viviparus* (TELLAM et al. 1997).

Prospects for the development of parasite vaccines have been transformed over the last 15 years by the development of recombinant DNA techniques and, even more recently, by the great strides made in the understanding of the immune system. With the exception of leishmaniasis, immunization with attenuated or killed whole parasites is not likely to be a practical option, and attention has concentrated almost entirely on the identification of suitable candidate parasite components and antigenic determinants, informed by a rapidly increasing understanding of the immune response during infection.

1. Killed and Attenuated Vaccines

The only important parasitic disease for which large-scale culture outside a live host is practical is for *Leishmania* spp. A number of trials are proceeding using killed organisms either as prophylactic vaccines or as immunotherapeutic treatment (as an alternative or adjunct to chemotherapy) for a number of different species of *leishmania*, both old-world and new (ENGERS et al. 1996). Most of these trials combine the killed organisms with a low dose of BCG as adjuvant. Phase III trials for single-dose regimes against cutaneous leishmaniasis in Iran and Pakistan and visceral leishmaniasis in Sudan were due for completion in 1997. In addition, there are attempts to produce stable attenuated strains by removing essential virulence genes (ENGERS et al. 1996).

2. Peptide Vaccines

Because of the difficulty of obtaining parasite components for immunization, there have been several attempts to construct synthetic vaccines combining epitopes identified as being possibly protective. Peptide constructs based on the immunodominant repeating sequence of the *P. falciparum* circumsporozooite coat protein conferred slight protection to challenge in volunteers but showed no efficacy in field condition. However, a vaccine based on empirically identified blood-stage epitopes, Spf66, has been extensively clinically tested (AMADOR et al. 1996). Early trials were compromised by poor design, but four recent double-blind trials have given conflicting results, two (in Colombia and Tanzania) showing about 30% protection against clinical malaria and two others (in Thailand and the Gambia) showing none (FACER and TANNER 1997). A further trial amongst those aged under 5 years is in progress in Tanzania.

3. Vaccines Based on Expressed Parasite Proteins

Almost all current efforts to develop vaccines depend on the expression of cloned parasite genes in a suitable system. Of the many under development, one of the most promising is the *P. falciparum* pre-erythrocytic stage vaccine RTS,S, in which the circumsporozooite protein is expressed fused to the HbSAg protein of hepatitis B virus. This construct showed exceptional protection to challenge in immunized volunteers (six of seven protected), but only when formulated with a complex adjuvant containing monophosphoryl lipid A and the saponin, QS-21, in an oil-in-water emulsion (STOUTE et al. 1997). This combination is currently entering clinical trial in the Gambia. There are large numbers of possible candidate blood-stage malaria antigens under development, including the invasion-associated protein MSP-1 (HOLDER 1994).

One interesting approach is the development of transmission-blocking vaccines. A trial is underway of a vaccine based on *P. falciparum* Pfs25, a protein expressed only in the mosquito-specific developmental stages and thus not subject to immune selection in the mammalian host. Antibodies present in

the blood meal may interfere with the development of the parasite in the mosquito gut, thus preventing onward transmission (KASLOW 1997). While such vaccines may not help the recipients directly, they may greatly reduce transmission to other family members. Almost all the possible routes of expression and delivery of recombinant proteins appear to be under investigation for candidate malaria antigens. For other parasite diseases the proteins under investigation include juvenile components, which may be of importance in blocking infection, and also conserved parasite antigens such as glutathione-*S*-transferase, triosephosphate isomerase and actin, immunity to which may significantly limit parasite burden.

4. Live Carriers

Several different approaches to the construction of vaccinia recombinants containing parasite are under investigation. The most spectacular of these is the malaria candidate vaccine NYVAC-7, in which no less than seven genes coding for pre-erythrocytic, blood-stage and mosquito-specific antigens have been incorporated (TINE 1996). NYVAC-7 is at present in phase I and II trials. There is also interest in *Salmonella* as a carrier for protective antigens from *Leishmania* and *Schistosoma* and in the use of transformed BCG as a vehicle rather than simply as an adjuvant for protective antigens (ENGERS et al. 1996; WAINE and MCMANUS 1997).

5. Other Approaches

Since the chief factor underlying development of cerebral malaria seems to be the abnormal production of TNF, stimulated by endotoxin-like *Plasmodium* products, one proposal is to protect against this, the major cause of morbidity in falciparum malaria, by immunization against toxins (PLAYFAIR 1996). A second interesting approach, for which there is already precedent in veterinary vaccines, is to block transmission by vaccinating against the arthropod vector, rather than the parasite, and thus interfere either with ingestion or digestion.

III. Future

Clearly any prediction for the future is made against a much more tentative background than for groups of organisms for which there is an extensive background of successful vaccination. There are uncertainties about such basic issues of what degree of protection is reasonable to expect – high or limited – what kind of protection should be looked for, what effect this would have on the epidemiology (GREENWOOD 1996; RILEY 1997; CHAN et al. 1997), and whether the product is likely to be affordable in the context of the resources available to health care. Mundane issues such as safety, stability and reproducibility have hardly been touched on. Any likely vaccine must probably be expected to take its place as part of a package of disease-management mea-

sures rather than as the main solution. Two areas distinct from those under current development stand out:

1. DNA Vaccines

The experimental use of DNA to induce immune responses shows great promise, but raises technical as well as regulatory issues. Recent results suggest that the combination of DNA priming followed by boosting with the same immunogen delivered by an alternative means can result in immunity which cannot be obtained with either alone. Clearly there is much to be learnt about the way in which DNA vaccines are best used.

2. Adjuvants

This is a complex area, and a recent compendium of adjuvants runs to over 80 pages (VOGEL and POWELL 1995). Nevertheless it is clear, from the example of RTS,S above, that adjuants can be critical in directing the immune response in a productive direction. The use of adjuvants is still highly empirical, but clearly this is an area in which progress is very rapid.

Finally, one of the lessons of the past 10 years is that, just as synthesizing a peptide epitope does not deliver a vaccine automatically, nor does cloning a protein, a better understanding of the interaction of parasites with the immune system is not only an important but an essential part of developing a parasite vaccine. Fortunately, the pace of increase in knowledge of immunity is breathtakingly fast at present.

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