
Vaccines – current status and future needs

This paper has been prepared on behalf of the Academy of Medical Sciences by a group comprising:

Professor Peter Lachmann (Chairman)
Professor Jangu Banatvala
Professor Jennie Blackwell
Professor George Griffin
Professor Adrian Hill
Professor Thomas Lehner
Professor Andrew McMichael
Professor Richard Moxon
Professor Geoffrey Smith
Professor Peter Smith
Professor Freda Stevenson
Professor Herman Waldmann
Professor Arie Zuckerman

Introduction

Prophylactic immunisation against infectious disease has been the most successful medical intervention yet in reducing mortality. From the introduction of vaccination against smallpox at the end of the eighteenth century, there has been continuous progress in producing safe and effective vaccines against a number of common diseases. These vaccines were bacterial toxoids (diphtheria and tetanus); killed whole organisms (e.g. typhoid, cholera, pertussis and the Salk polio vaccine); or live attenuated organisms (e.g. BCG, yellow fever, the Sabin polio vaccine, measles, mumps and rubella).

Since the advent of biotechnology in the 1970s, it has become possible to make new vaccines using the novel techniques of molecular biology. The first and strikingly successful recombinant protein vaccine was hepatitis B surface antigen expressed in yeast. This vaccine has been highly effective in preventing hepatitis B and thereby has become the first vaccine that is also capable of preventing a human cancer; the hepatocellular carcinoma associated with early-acquired, persistent hepatitis B infection.

Effective vaccines have yet to be made against many microbial diseases. In addition, the use of vaccines is now being extended to purposes other than the prophylaxis of infectious disease. Vaccines are being developed to control existing, persistent infectious disease. This approach is being explored as a way to control HIV and other persistent viral infections. Desensitisation against antigens that have produced IgE-mediated allergic reactions has been undertaken for many years but has not worked particularly well for allergens that enter the body by the mucosal route. Therapeutic vaccination against tumour-associated antigens has long been investigated as a form of tumour therapy and the approach now shows some promise. Contraceptive vaccines directed against

human chorionic gonadotrophins have been developed and shown to be effective. Attempts to counter drug addiction by vaccination with drug immuno-conjugates have been reported. The use of immunisation to counter autoimmune diseases is also being studied.

Vaccine research is now very active.

Improved Vaccines

Advances in immunology have led to a clearer understanding of what is required of an effective vaccine.

Effective **prophylactic** vaccination requires, in almost all cases, a strong and long lasting antibody response. Because T cells see antigen in the context of the major histocompatibility antigens (MHC) they do not recognise pathogens that have not become associated with host cells and, for this reason, cannot prevent infection, though they may eliminate an infection before it becomes chronic. High affinity IgG antibody responses are the best for preventing parenteral infections but good levels of IgA are required to protect mucosae. The latter are best stimulated by oral immunisation and techniques for achieving oral immunisation by using living vectors that are effective orally or by conjugating antigens to the beta (mucosal-localising) subunit of cholera or *E. coli* enterotoxin are being explored. Improved adjuvants to increase immunogenicity are also being developed. For raising antibody titres, experimental studies have shown that coupling antigens to oligomers of C3d is a powerful adjuvant (2).

To control existing infections – **therapeutic** vaccination – the situation is quite different. Here T-cells are the main effectors; and for viruses, it is the CD8-positive cytotoxic lymphocyte that is particularly important. Vaccine strategies directed at generating these cells are being developed. Incorporating antigens by recombinant DNA techniques into vaccinia virus is one such way and using such vectors to boost responses after an initial immunisation with a plasmid containing the same antigen (the "plasmid prime - vaccinia boost" regime) has been shown to be particularly effective (9).

Traditional, replicating vaccinia virus based vaccines have given rise to safety concerns since vaccinia virus itself gave significant side effects in around 1 in 10,000 people when used as a vaccine against smallpox. However, considerable progress has been made in removing from vaccinia the genes responsible for its virulence and non-replicating strains of such viruses (e.g. modified virus Ankara) are now being tested against AIDS and malaria. A vaccinia based anti-rabies vaccine has been highly effective in reducing the spread of rabies in the wild. Bait infected with the vector is dropped from aeroplanes and is eaten by the foxes that, in Europe, are the main carriers of this dangerous virus.

Other living microbial vectors are being investigated. These include salmonellae and lactococci for oral immunisation and a number of other poxviruses (such as avipoxviruses) as an alternative to vaccinia.

DNA vaccines (which it is preferable to call plasmid vaccines since this is what they are and the term is less emotive) are a major innovation with great potential (3). These vaccines are made up entirely of DNA which does not replicate in mammalian cells. They contain the signals that allow them to be grown in bacteria and those that allow the inserted antigens to be expressed in mammalian cells. They have several great advantages. Plasmids themselves carry no viral genes, so that pre-existing immunity to, for example, vaccinia, will

not affect vaccine performance. They are potentially much cheaper to produce than recombinant protein vaccines and any plasmid vaccine can be manufactured by, essentially, the same process. They do not require cold storage and are therefore much easier to transport and use, especially in developing countries; and it is relatively simple to introduce multiple variants of an antigen into a single batch of plasmid vaccine.

Candidate microbial antigens can now be selected from genomic sequences and plasmid vaccines permit much easier exploitation of this new data than the alternative of developing a good expression system for each candidate antigen and then preparing the recombinant protein. This is a major advantage for therapeutic vaccination against cancer antigens which may be identified only as DNA sequences generated from mapping human and cancer genomes.

The main disadvantage of plasmid vaccines is a, generally, low level of immunogenicity. Good adjuvants will be needed to overcome this disadvantage. One solution is to incorporate into the plasmid, genes for those cytokines that promote immune responses (for example GM-CSF or IL-4); or for oligomers of C3d as an adjuvant for B-lymphocytes. Other possible solutions include a subsequent booster immunisation with the corresponding antigen incorporated in a vaccinia vector or as a protein.

There remain some lingering doubts about the safety of plasmid vaccines, which are almost certainly ill founded, but which will be fully evaluated as these vaccines enter clinical trials. No evidence for either germ line incorporation or for oncogenicity has been found in any studies done so far.

Another approach to the production of antigens is the use of synthetic peptides. This is an attractive concept and carries no worries about safety – but has not lived up to its early promise. One problem is that peptides tend to be of low immunogenicity and need to be coupled to a carrier that is a good T-cell antigen. The most powerful of such carriers is PPD (purified protein derivative of tuberculin) (6) but this is a difficult material to handle and has been used only experimentally for this purpose. Tetanus toxoid is more frequently used for this purpose in man.

Increasingly, it is realised that microorganisms have evolved mechanisms for protecting themselves from the immune response. As these become delineated, they may also be circumvented by suitable techniques. The most important and difficult evasion mechanism is antigenic variation where organisms carry major important antigens often in a large number of variants so that an immune response to one variant simply allows another to grow. This problem is at the root of the difficulty of making effective vaccines against, for example, HIV, influenza and the blood stages of malaria. It is likely that a variety of strategies will be required to overcome this problem of which making vaccines containing multiple variants is the most obvious.

The opportunities for creating new vaccines are therefore very good. The need is also very great.

Vaccines for Infectious Diseases

Viruses

HIV

There is a very urgent need for an effective prophylactic vaccine against HIV. The problems lie both in extreme antigenic variability and in the fact that lentiviruses are able to establish latent infections by incorporating their genome in the host cell genome. These latently infected cells cannot be eradicated by immune mechanisms unless they express some viral product. It may be, therefore, that a universal prophylactic immunisation programme will be needed to eradicate this disease. Since the virus enters frequently across mucous membranes, mucosal as well as systemic immunity will be needed. There is much international activity in trying to produce a satisfactory, prophylactic HIV vaccine generally using multiple components of HIV (7).

Attempts to boost T-cell immunity to the virus in those already infected are also in progress. Trials are beginning with the "plasmid prime - vaccinia boost" regime (4). The aim here is to stimulate specific CD8 positive cytotoxic lymphocytes and thereby to limit viral replication subsequent to infection, thus preventing or delaying the appearance of AIDS and other HIV-related diseases. Clinical trials to evaluate this strategy will need to extend over many years.

Herpes viruses

Herpes viruses achieve latency as episomes and are difficult or impossible to eradicate after infection has taken place. The major members of this group infecting humans are herpes simplex types I and II, cytomegalovirus, varicella zoster virus and Epstein Barr virus. Cytomegalovirus is an important cause of psychomotor retardation and of deafness in infancy and an effective vaccine would be of great value. Preventing infection with the Epstein Barr virus could prevent Burkitt's lymphoma and the naso-pharyngeal cancer associated with infection with this virus in China. It might also have an impact on some auto-immune diseases although this is controversial. An effective vaccine against varicella zoster may help to prevent shingles in the elderly. Two more herpes viruses have been described more recently; human herpes virus 6, a candidate virus for multiple sclerosis; and human herpes virus 8 which causes Kaposi's sarcoma.

No vaccine is generally, clinically available for any of these viruses in the UK. Replication-defective living virus is being explored for therapeutic vaccination for example in recurrent genital herpes. Recombinant proteins are also being studied as antigens for prophylactic vaccines.

Hepatitis viruses

Good vaccines exist to prevent hepatitis A and hepatitis B. A therapeutic hepatitis B vaccine that would eliminate infection and reduce the risk of liver cancer in the 350 million carriers of this virus is a global health priority. Currently clinical trials are assessing plasmid vaccines and plasmid prime- vaccinia boost regimens as possible therapeutic vaccines.

Hepatitis C is a major worldwide problem as a cause of chronic liver disease and liver cancer. There is much antigenic variation and attempts are being made to develop a plasmid vaccine, which would cover multiple antigenic types, as well as a protein-adjuvant vaccine.

A vaccine against hepatitis E would also be valuable where this water-borne virus, which is dangerous in pregnant women, is common (e.g. India) and for travellers.

Papilloma viruses

These viruses cause warts and cancer of the cervix. They are an important target for vaccines to prevent - and even to treat - this common cancer. Effective vaccines have been developed against papillomavirus infections in animals and there is the prospect of developing human vaccines against those types of human papilloma virus (HPV) that are associated with cervical cancer. The first large scale trial of a human HPV vaccine is starting in Puerto Rico.

There is also an unfilled need for vaccines against many other viruses. These include rotaviruses, parvovirus B19, haemorrhagic fever viruses such as Ebola, dengue and respiratory syncytial virus (RSV). In the latter two examples the disease is to some extent due to immunopathology and great care has to be taken that immunisation does not make the disease worse as happened with the early, killed RSV vaccines.

Some existing vaccines also need substantial improvement. The current influenza vaccines are effective against the currently occurring variants but a vaccine effective against all influenza A strains would be very valuable, particularly in protecting against a new pandemic strain. There would also be considerable benefit to having an efficient killed vaccine against measles, which could safely be given very early in life.

Bacteria

There are major needs for effective vaccines against enteric infections, perhaps particularly against shigellosis and enterotoxigenic *E. coli*, as well as more effective vaccines against cholera and salmonellae. Better vaccines are needed against pneumococci. Trials of a conjugate vaccine in the US have shown high efficacy and large-scale trials in developing countries, where pneumococci are an important cause of child mortality, are in progress. Better vaccines are similarly needed against meningococcus group A (where the current polysaccharide vaccine is inadequate and the infection is responsible for periodic devastating epidemics in parts of Africa); and against group B (where the major carbohydrate is sialic acid and is effectively autoantigenic in man). A prophylactic vaccine against *helicobacter pylori* could have a major effect on duodenal ulcers and on gastric cancer incidence. Vaccines against chlamydia and gonococci would make a large contribution to reducing the burden of sexually transmitted disease.

Perhaps the most pressing global need is for a more effective vaccine against *mycobacterium tuberculosis* to replace BCG. The efficacy of BCG in the UK has been shown to be around 70-80% and in most geographical areas it seems to protect against tuberculous meningitis; but in some studies in the US and in developing countries efficacy has been absent or disappointingly low. Interestingly, it appears to protect children of Asian origin in the UK against tuberculosis whereas a large trial in South India showed no protection in that population. The reasons for this variation are unknown but the most plausible explanation is that it is related to the extent to which individuals are exposed to other mycobacteria in the environment. A variety of new approaches are at the pre-clinical stage of development but none in clinical testing. A prophylactic vaccine that confers solid, life-long immunity would have a great impact on global infectious disease mortality, not least in those infected with HIV, who are at high risk of tuberculosis (of the order of 10% a year in some studies).

Protozoa

Malaria is the major target. Anti-plasmodial vaccines based on antigens from the blood stages of the disease are being developed and preliminary field trials have shown modest success. Antigenic variation and the rapid waning of even the immunity acquired through infection are major hurdles.

Recently, very encouraging progress has been made with new pre-erythrocytic stage vaccines that target the very early sporozoite and liver-stages of the life cycle. This type of vaccine could prevent malaria in travellers as well as reducing the current deaths from malaria of 1-2 million children a year in sub-Saharan Africa. A protein-adjuvant vaccine has been shown to prevent malaria in about 50% of a small number of experimentally challenged volunteers and has shown about 65% efficacy in preventing malaria infection in adults in a trial in Africa. However, the short duration of the protective effect, only 2 months, would be of little value in most malaria endemic populations and improved formulations are needed. Plasmid vaccines combined with a booster immunisation with a vaccinia vaccine, an approach pioneered in the UK, show good promise and are now in clinical trials both in the UK and in West Africa.

There are cogent reasons for believing that the best way to approach the final eradication of malaria (though this is a very ambitious goal, given the adaptability and antigenic variability of the parasite) is the use of vaccines that block transmission, such as sexual-stage vaccines, probably combined with vaccines against other stages of the parasite and other control measures (such as insecticide-impregnated bed-nets) (10). Antibodies against sexual-stage antigens are taken up by the mosquito with the blood meal and prevent the development of the parasite in the mosquito and hence the mosquito's ability to infect a new host. Such vaccines are directed against antigens expressed only in the mosquito phase of the parasite's life cycle and have the advantage that the antigens concerned are not seen by the vertebrate host during infection and therefore have not given rise to antigenic variation. However, a major problem with such transmission blocking vaccination is that it becomes effective only when most of the population at risk has been immunised and there has, as yet, been little development of this approach to the stage of field trials.

The tools for the eradication of malaria, by vaccination combined with other, existing, control measures such as bed nets, are likely to become available in the near future but this endeavour will require an act of political will comparable to, or even greater than, that required for the eradication of smallpox.

Vaccination against dental caries

Streptococcus mutans is the main causative agent of dental caries. Monoclonal secretory IgA antibodies to the streptococcal adhesin have been shown to prevent colonisation with *Streptococcus mutans* and, when made in plants can be used as a passive vaccine against caries (8).

Vaccines as a precaution against bioterrorism

There is a danger that certain micro-organisms might be used as agents of biological warfare or by terrorists. Smallpox virus and anthrax bacteria are two examples. It would seem prudent to maintain adequate supplies of vaccines against such agents as a precaution.

Therapeutic vaccination for cancer treatment (11)

Much effort is being put into identifying antigens that are effectively tumour-specific. Operationally this means that they are not expressed on normal cells to an extent where an immune response against them would produce cell damage. On the other hand an immune response to a tumour-specific antigen can destroy the tumour cell. Candidate tumour specific antigens can now be identified by comparing expression libraries from cancers with those from the corresponding normal cells. Melanomas and lymphomas have been among the initial target tumours. There is active investigation in this country into using plasmid vaccines against the idiotype of B cell tumours - with promising results.

An attraction of vaccination is that, for several cancers, patients can be successfully brought into remission with first line treatment, but they then relapse due to the emergence of residual tumour cells. The aim is to activate the immune system to suppress these cells on a continuing basis.

Several experimental vaccines are being developed for solid tumours using mucin antigens, proto-oncogenes such as her2/neu and carcinoembryonic antigen for epithelial tumours.

There are also encouraging reports of the treatment of both colon cancer and breast cancer by passive immunisation with monoclonal antibodies given at the time of minimal tumour burden. More experimentally, the transfer of T-cells reactive to tumour associated antigens is also being explored.

Vaccines For Other Purposes

Immunisation against Alzheimer's disease

The reports that antibodies against β amyloid peptides can accelerate the removal of the Alzheimer's amyloid from the brain in mice (5) has encouraged investigation into the question of whether the immune response against β amyloid vaccination could arrest or even reverse Alzheimer's disease. Theoretically, it might also prevent it but trials on this topic would seem rather further away. It has to be said that this whole field remains quite controversial. If, however, it can be confirmed that antibodies against amyloid deposits can facilitate their removal, this could have important implications for other forms of amyloid, not least for variant CJD.

Immunomodulatory Vaccines

Immunisation with increasing doses of antigen (desensitisation) to treat anaphylactic sensitivity against pollens, dust allergens and insect stings goes back to the work of Loveless in the 1930s and continues to be practised. This concept of immunomodulatory vaccines has been extended in recent years on the basis of an increased understanding of the immune response. There is now increasing evidence that T-cells can regulate immune responses in a beneficial way. In the past few years, evidence for the existence of a subpopulation of CD4 T-cells in mice, rats and humans suggests that it may be possible to "vaccinate" so as to potentiate the ability of these cells to prevent autoimmune disease and transplant rejection. What is especially interesting is that "vaccination" against

one set of antigens can allow effects on responses directed to other antigens in the same tissue (linked suppression). This will soon become an important area for the evolution of specific immunosuppressants (13).

Contraceptive vaccines

The idea of producing long-lasting but reversible contraception by vaccination with fragments of human chorionic gonadotrophin has long been investigated, particularly in India and by WHO (12). It is an attractive idea, particularly for use in developing countries.

Vaccination against drug addiction

There is experimental evidence that antibodies raised against cocaine reduce the psychotropic effects of the drug in animals (1). This raises the intriguing possibility of using immunisation with drug-conjugates as a way of treating drug addiction.

Vaccine Safety

The public is extremely averse to any risk arising from vaccination even where the overall risk benefit analysis is hugely favourable. This can be attributed, in part at least, to the fact that a child actually or allegedly damaged by a vaccine is an identifiable person, who was healthy at the time of vaccination, whereas the children who do not get the disease in consequence of vaccination are generally unidentifiable and their number is derived through statistical calculations.

For example, an effective vaccine was developed against rotavirus but was withdrawn after a small number of vaccinated infants developed a life-threatening bowel complication. While it was argued that the global burden of mortality due to rotavirus would justify the use of a vaccine even if it had a low level of serious adverse effects, this argument did not carry the day. It is clear that any vaccine that is given to healthy infants will be acceptable only if the rate of associated serious adverse events is extremely small.

Vaccine scares such as the reported association of hepatitis B vaccine with multiple sclerosis in France and the recent furore in this country about the reported association of MMR vaccine with autism and inflammatory bowel disease discourage vaccine take up, even though the evidence for the purported adverse effect may be very poor. The evidence put forward for any association in both these examples is without any convincing scientific substance. No matter how good the next generation of prophylactic vaccines are, they will not have their full effect if their uptake is incomplete.

With the public so concerned about vaccine safety, it should be emphasised that all vaccines have to undergo extensive testing, which is required to include testing in animals, before they are released. It also has to be recognised that most vaccines are associated with some adverse effects, usually minor, but very occasionally severe. A consequence of the efficient deployment of highly effective vaccines is that the diseases they prevent may become very rare and thus the only impact of vaccination that is apparent to the population is the occasional adverse effect.

With the record linkage possibilities in the UK, we are in a strong position to set up routine and systematic monitoring systems to detect and to assess both short- and long-term adverse effects of vaccines. This would seem to be an important

priority, not least to enable a rapid and informed response to allegations of vaccine-associated adverse effects that are bound to recur and that have detrimental effects on vaccine uptake even when they have little substance.

References

1. Carrera MRA, Asjleey JA, Wirsching P et al (2001) A second-generation vaccine protects against the psychoactive effects of cocaine. *Proc Natl Acad Sci* 98: 1988-1992.
2. Dempsey PW, Allison ME, Akkaraju S et al (1996) C3d of complement as a molecular adjuvant: bridging innate and acquired immunity. *Science*. 27: 348-50.
3. Gurunathan S, Klinman DM and Seder RA (2000) DNA vaccines: immunology, application and optimization. *Ann Rev Immunol* 18: 927 – 97.
4. Hanke T and McMichael AJ (2000) Design and construction of an experimental HIV-1 vaccine for a year-2000 clinical trial in Kenya. *Nat Med* 6: 951-955.
5. Janus C, Pearson J, McLaurin J et al (2000) A beta peptide immunization reduces behavioural impairment and plaques in a model of Alzheimer's disease. *Nature* 408: 979-82.
6. Lachmann P J (1994) The use of purified protein derivative of tuberculin (PPD) as a carrier molecule for immunisation particularly with peptides. IN: *Recombinant and synthetic vaccines*. Eds G P Talwar, K V S Rao and V S Chauhan. Narosa Publishing House, New Delhi, India. 415 - 424.
7. Lehner T, Bermeier L, Wang Y et al (1999) A rational basis for mucosal vaccination against HIV infection. *Immunological Reviews* 170: 183 – 196.
8. Ma J K-C, Hikmat BY, Wycoff K et al (1998) Characterization of a recombinant plant monoclonal secretory antibody and preventative immunotherapy in humans. *Nature Medicine* 4: 601-606.
9. Schneider J, Gilbert SC and Blanchard TJ (1998) Enhanced immunogenicity for CD8+ve T cell induction and complete efficacy of malaria DNA vaccination by boosting with modified vaccinia virus. *Ankara Nat Med* 4: 397 - 402.
10. Sinden RE (1993/1994) Antimalarial transmission-blocking vaccines. *Sci Prog*. 77: 1-14.
11. Stevenson FK (1999) DNA vaccines against cancer antigens. *Annals of Oncology* 10: 1-6
12. Talwar GP (1999) Vaccines and passive immunological approaches for the control of fertility and hormone-dependent cancers. *Immunol Rev*: 171:173 - 92.
13. Waldmann H and Cobbold S (2000) Current Opinions in Organ Transplantation 5: 83 -89.

