Clinical review

Science, medicine, and the future

New vaccine development

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Vaccines are hailed as one of the most important public health achievements of the 20th century.[1] In the next five to 15 years, new vaccines and new vaccine delivery technology will fundamentally change how clinicians prevent and treat disease, with a substantial impact on public health. This review describes recent developments in the basic science underpinning the development of new vaccines and summarises the potential of these vaccines to treat and prevent a wide range of infectious and non-infectious diseases.[2–5] In addition, research is being carried out on much needed vaccines for the developing world for diseases such as malaria, hookworm, dengue, enterotoxigenic *Escherichia coli*, shigella, and tuberculosis, but these are beyond the scope of this brief review.

Summary points

New prophylactic and therapeutic vaccines will prevent and potentially cure disease by providing people with the necessary immunological tools

Advances in current vaccines such as conjugated pneumococcal vaccines for adults, nasal spray vaccines for influenza, and adult acellular pertussis vaccines will provide an efficient way to produce longlasting protective immunity

Development of vaccines against non-infectious diseases (such as cancer, diabetes, and Alzheimer's disease) and nicotine and cocaine dependence will provide alternative treatments

Vaccines against biological weapons will be possible by advances in DNA vaccines

New vaccine delivery technology will provide easier delivery routes (such as transcutaneous, depot, nasal, and oral delivery) without compromising efficacy

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BMJ 2002;324:1315–9

Methods

We searched PubMed and Medline databases (1995-2001), as well as our own libraries, for articles of relevance to this brief review.

New vaccines against infectious diseases

Development of DNA vaccines

One approach generating great interest is that of inducing protective immune responses by injecting engineered DNA sequences from infectious organisms against which protection is desired. If an antigen can be identified it is possible to insert the DNA sequence coding for the protein antigen into a carrier genome (such as several of the poxviruses or alphaviruses). Once delivered into the host, the organism (and hence the inserted DNA) undergoes limited replication, the protein of interest is produced, and the host develops an immune response against the protein.

In a related strategy, so called naked DNA is injected directly into the host to produce an immune response (fig 1). Naked DNA is simply sequences of DNA inserted into bacterial plasmids (simple, extrachromosomal rings of DNA found in bacterial cells) and injected into the host. These have been effective in animal models, but intramuscularly injected DNA in humans has failed to generate vigorous immune responses, although transdermal or intradermal delivery of DNA has been more encouraging. A clinical trial of transdermally delivered microscopic gold beads coated with DNA coding for hepatitis B surface antigen generated protective levels of antibodies to the antigen.[6] This vaccine has also generated CD8 cytotoxic lymphocytes.[6] Although efforts have been successful in animal models of vaccines against several pathogens, progress in humans has been much slower. To date, only DNA vaccines against hepatitis B[6] and malaria[7] have induced immune responses thought to be protective in humans.

Development of therapeutic vaccines

Traditional vaccination is the prevention of a specific infectious disease by delivering an immunogenic antigen derived from the surface of the infectious agent, resulting in immunity against the foreign organism replicating and establishing an infection. A therapeutic vaccine, however, can limit or eradicate an already present and established infectious agent or condition. The development of therapeutic vaccines has depended in part on the ability of DNA vaccination to induce both humoral and cell mediated immune responses by inoculation of plasmid DNA containing sequences for transcription and translation, resulting

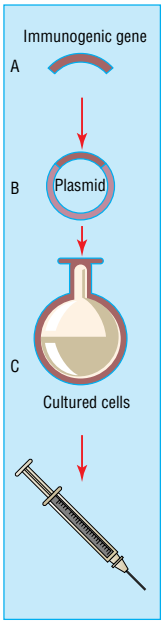

Fig 1 Principle of DNA vaccination. An immunogenic gene is inserted into an expression plasmid (A), which is inserted into cultured cells (B). The cells are screened for expression of the gene protein and then cultured. The plasmid DNA is then extracted from the cells and purified before being used to immunise a host (C)

in the in vivo synthesis of an immunogenic peptide or protein.

Attempts are being made to develop a therapeutic vaccine against HIV that will induce virus-specific cytotoxic T lymphocytes against HIV, with the goal of having activated T cells destroy latently infected cells. Other efforts include developing therapeutic vaccines against *Helicobacter pylori*, mucosal candidiasis, herpes viruses, and human papillomavirus. DNA vaccination for hepatitis B virus has shown great promise. The delivery of viral DNA sequences can induce longlasting humoral and cell mediated immunity in mice infected with hepatitis B virus.[8 9] In transgenic mice, at least, there is a decrease in or clearance of the hepatitis B surface antigen, with evidence of induction of antibodies and proliferation of CD4 T cells.[10] Clearly, the capabilities of the immune system to eliminate an infectious agent even after an infection or disease is established could substantially improve human health.

Other important examples of therapeutic vaccine development include the development of vaccines against certain cancers,[11] which is discussed later.

Advances in current vaccines

The bacterium *Streptococcus pneumoniae* and influenza viruses account for considerable morbidity and mortality worldwide. Now approved in several Western countries, *S pneumoniae* conjugate vaccines should help reduce the number of cases of invasive *S pneumoniae* disease (bacteraemia, meningitis, and sepsis) in infants and young children. A live, attenuated influenza virus vaccine is nearing approval in the United States. This vaccine, administered as an intranasal spray, should stimulate both systemic and mucosal immunity, while decreasing reliance on the use of parenteral injections (see box for a list of potential vaccines).

Streptococcus pneumoniae

Multivalent polysaccharide vaccines for *S pneumoniae* have been available in the United States since 1977, but they produce a poor or inconsistent immune response in children, especially those less than 2 years old. Polysaccharide vaccines induce antibodies primarily by mechanisms independent of the T cells and are not long lasting and do not induce an immune memory response. For these reasons, a protein carrier conjugated to a polysaccharide antigen of *S pneumoniae* has now been developed, which causes the immune response to be T cell dependent, allowing infants and children to respond better to the vaccine. The US licensed heptavalent *S pneumoniae* conjugated polysaccharide vaccine contains the seven serotypes (4, 6B, 9V, 14, 18C, 19F, and 23F) most commonly associated with invasive disease among infants and young children. The new vaccine is also expected to have the benefit of reducing nasopharyngeal carriage of these seven *S pneumoniae* serotypes.

Influenza virus

The only influenza vaccines currently licensed in the United States are parenteral inactivated influenza virus vaccines prepared in chick embryos. Because of changes in the influenza viruses circulating each year (antigenic drift), protection of high risk individuals requires annual vaccination.

A live attenuated influenza virus vaccine being proposed for US approval contains recombinant cold-adapted strains of influenza A and B and is given by intranasal spray. Several studies have examined the use of live attenuated influenza vaccines in children and adults.[12–14] In seronegative children more than 15 months old antibody responses to the influenza A and B components after a single dose of vaccine indicated an overall efficacy of 93%.[12] Use of a live attenuated trivalent vaccine in adults significantly reduced the occurrence of illness, visits to healthcare providers, and days of work lost.[14]

New vaccines against non-infectious diseases

When correctly targeted, an immune response can be used to eliminate cells with aberrant behaviour (dysplasia) or aberrant genomic function (malignancy) or to reduce the amount of inflammation affecting a specific organ (such as in diabetes).[15 16] This raises the possibility of developing vaccines against diseases not known to be related to infectious agents. Two of the most exciting and promising areas in this regard are vaccines against cancer and autoimmune diseases.

Cancer

The identification of specific tumour antigens (tumour associated antigens) that are present only in cancer cells—such as those found in leukaemia, breast cancer, melanoma, prostate cancer, and colon cancer—provide immune targets for which immunogenic vaccines may

Potential vaccination in the 21st century (adapted with permission from Plotkin (2001)[5])

New maternal vaccines—Group B streptococcus, respiratory syncytial virus
New vaccines for neonates—Respiratory syncytial virus, hepatitis B
New vaccines for infants aged 2-6 months—Paediatric combinations (acellular pertussis (DtacP), *Haemophilus influenzae* type b (Hib), hepatitis B, pneumococcal, meningococcal, hepatitis A, etc), otitis (non-typable *Haemophilus influenzae*, *Branhamella catarrhalis*), rotavirus (new), meningococcal conjugate
New vaccines for the developing world—Enterotoxigenic *Escherichia coli*, shigella, malaria, dengue, tuberculosis
Vaccines for children aged 1-2 years—Measles-mumps-rubella-varicella (MMRV), influenza (intranasal)
Vaccines for children aged 4-6 years—MMRV booster, paediatric combination booster, *Streptococcus mutans* (oral) (anti-caries), Lyme disease and tick-borne encephalitis (endemic areas)
Vaccines for children aged 11-13 years—HIV, human papillomavirus, herpes simplex virus 2, *Neisseria gonorrhoeae*, cytomegalovirus, parvovirus, Epstein-Barr virus
Vaccines for young adults—Tetanus and diphtheria toxoids, acellular pertussis, *Helicobacter pylori* (anti-ulcer), *Chlamydia pneumoniae* (anti-atherosclerosis)
Travel vaccines—Therapeutic vaccines against diabetes, multiple sclerosis, meningococcal conjugate
Vaccines for people aged ⩾ 50 years—Influenza (subcutaneous and intranasal), pneumonococcus (protein and polysaccharide), herpes zoster, cancer (prophylactic and therapeutic vaccines)

conceivably be designed. For example, the expression of protein GPI-B7-1 transferred onto membranes from a murine thymoma tumour cell protects mice against this kind of tumour.[17] In humans it is possible to stimulate T cell responses using isolated membranes surgically removed from human tumour tissues that express major histocompatibility complex (MHC) class II molecules, suggesting the possibility of establishing an immune response that could specifically target and eliminate tumour cells.[18]

Other efforts include therapeutic vaccines against melanoma, colorectal cancer, leukaemia, and other cancers.[19 20] The ability of DNA vaccines to deliver precise and specific nucleotide sequences representing target genes—such as the ALVAC gp100 gene for melanoma and the ALVAC CEA-B7.1 gene for colorectal cancer—and specific protein fragments such as the HER2/Neu peptide found in breast cancer cells[21 22] have been studied as a potential means with which to induce an immune response.[19 23]

Autoimmune diseases

Diseases related to pathological immune activation, such as autoimmune diseases and allergies, might be treatable or preventable with vaccines. Efforts are being made to develop vaccines against rheumatoid arthritis, multiple sclerosis, myasthenia gravis, food allergies, and especially type 1 diabetes because of its associated substantial morbidity and mortality.

In the case of type 1 diabetes, lymphocytes infiltrate the pancreatic islets and selectively destroy the insulin secreting β cells. One strategy for vaccine development is to reduce the pathological lymphocytic infiltration by tolerisation.[15 16 24] Tolerisation involves the administration of small amounts of the same antigens that are the target of the aberrant immune response, which, in the absence of cytokine costimulation, fuels the activation of T cells, which reduce inflammation.

In disorders such as Alzheimer's disease, it may be possible to target the β amyloid protein that is responsible for the neurodegenerative plaques observed in this disorder. In murine models vaccines have been shown to reduce and prevent plaque formation, with some improvement in cognitive function.[25] Other examples of potential vaccine development include vaccines to prevent cocaine and nicotine addiction. With the use of immunopharmacotherapy, antibodies can be designed to neutralise a drug rather than target the receptors in the brain. Efforts are also being made to develop vaccines against atherosclerosis and to prevent conception.[26–28]

Vaccines against biological weapons of mass destruction

Interest has increased in biological weapons of mass destruction as terrorists look for methods with which to inflict harm on the greatest number of people, with the lowest possible cost and technology needs, while creating mass panic. While vaccines have been licensed against smallpox, plague, anthrax, and others, only limited amounts of anthrax vaccine are being produced in the United States for specific risk groups. Limited and ageing stockpiles of smallpox and plague vaccine are available but are insufficient for large numbers of people.

Because of the ability of biological weapons to infect and kill large numbers of people, and the risk of person-to-person transmission, vaccines are likely to be the only practical means of protection.[29 30] Second generation vaccines against anthrax, smallpox, and plague are being developed, and vaccines against other agents of bioterrorism such as the haemorrhagic fever viruses and others are also in development. However, major obstacles in producing such vaccines for public use include the need for a financially viable market, the impossibility of conducting human efficacy trials, the intangible risk:benefit ratio at the public health level, and governments' reluctance to face the reality of bioterrorism.

New vaccine delivery technology

Virtually all recommended immunisations require parenteral administration, and many require a series of injections. To be effective, vaccines for some diseases will need to enhance mucosal immunity as well as systemic immunity. For these reasons, new vaccine delivery methods, specifically alternatives to injections, are being sought. Topically applied (transcutaneous) vaccines, transgenic edible plants that contain genes for human vaccine antigens, and controlled delivery depot systems with vaccine antigens encapsulated in biodegradable polymers are possibilities currently under study. Such new delivery methods could decrease reliance on repeated injections, the need for trained healthcare workers, and perhaps the need for a stringent cold chain for vaccine storage.

Transcutaneous immunisation

Animal studies have shown the production of both systemic and mucosal antibodies after topical vaccine application. Agents such as cholera toxin and the heat labile enterotoxin of *Escherichia coli*, in combination with a vaccine antigen such as tetanus toxoid, act as an adjuvant and produce protective antibodies after being applied to the skin of animals.[31] Non-toxic mutants or subunits of cholera toxin and *E coli* enterotoxin would be needed, however, for any application on to human mucosal surfaces. Various other adjuvants besides cholera toxin and *E coli* enterotoxin (including bacterial ADP-ribosylating exotoxins, interleukin ¯1β fragment, interleukin 2, and tumour necrosis factor -α) have also been shown to produce an immune response after topical application.[32]

Transgenic edible plants to deliver vaccines

The development of plants capable of expressing vaccine antigens is a novel and promising strategy (fig 2). Such genetically engineered plants would produce vaccine antigens in their edible parts and would, like subunit vaccine preparations, contain no genes capable of replicating a whole infectious organism.[33] Because food plants can be regenerated rapidly, it may be possible that crops containing vaccine antigens could be produced indefinitely and on a local basis. Potato and tomato plants have synthesised antigens from Norwalk virus, enterotoxigenic *E coli*, *Vibrio cholerae*, and hepatitis B virus. A recently completed human study has shown that a recombinant bacterial antigen, subunit B of heat labile enterotoxin, produced in a potato and eaten resulted in production of both serum antibodies (IgG and IgA) and mucosal antibodies (sIgA) to the antigen.[34]

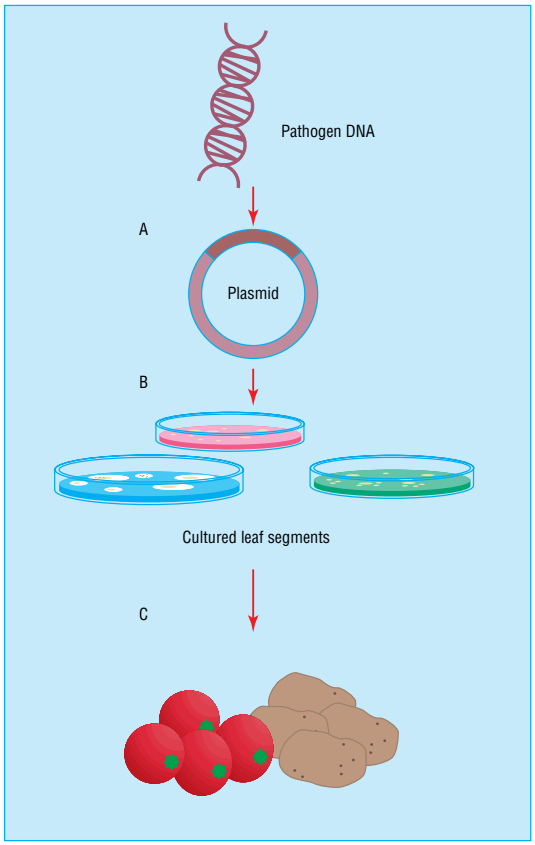

Fig 2 Principle of delivering vaccines in edible plants. A gene from a human pathogen is inserted into a bacterium that infects plants (A). The bacterium then infects cultured leaf segments of the selected food plant (B), which sprout into whole plants containing the human pathogen gene (C). Once the plant is eaten, it triggers an immune response to the pathogen

Other plants, such as bananas, and other vaccine antigens, including tetanus and diphtheria toxoids, may be included in future studies.

Controlled delivery depot systems

The use of controlled delivery of vaccine antigen, or depot vaccine technology, reduces the number of parenteral injections while potentially mimicking natural infection. Various vaccine antigens have been encapsulated in microspheres composed of biodegradable polymers such as poly (lactic/glycolic) acid (PLGA), which can be targeted to various cells in the immune system or can form a depot at the injection site, allowing slow release of the antigen over time.[35] The release profile of vaccine antigen depends on the particle size of the delivery vehicle, and a combination of large and small microspheres can create a pattern that mimics the antigen concentration profile in conventional immunisation, combining both primary and booster injections. A recent study in animals found that encapsulated tetanus toxoid or *Haemophilus influenzae* type b polysaccharide elicited high antibody levels that persisted for months.[36]

Additional educational resources

- Centers for Disease Control and Prevention (www.cdc.gov/)
- World Health Organization (www.who.int/home-page/)
- Merck Vaccines (www.merckvaccines.com/)
- National Vaccine Information Center (www.909shot.com/)
- DNAvaccine.com (www.dnavaccine.com/) a global platform for vaccine research
- Food and Drug Administration (www.fda.gov/default.htm)
- National Foundation for Infectious Diseases (www.nfid.org/)
- American Society for Microbiology (www.asm.org/)
- Infectious Diseases Society of America (www.idsociety.org/)

Conclusions

The future of vaccinology provides tremendous promise for controlling diseases. Vaccines will be delivered orally, by nasal spray, or transcutaneously by a minimally trained layperson and in a manner that does not require expensive equipment. However, despite rapid advances in the development of new vaccines, concerns about vaccine safety and a rise in anti-vaccine sentiment adversely affect immunisation coverage, the willingness of manufacturers to develop new vaccines, and the willingness of individuals and healthcare workers to use them.[37 38] As advanced vaccines and vaccine technologies become available, massive public education efforts will be required to alleviate these concerns. This is particularly true for DNA vaccines, combination vaccines, vectored vaccines, and vaccines administered in a parenteral depot fashion. The more distant potential for person-specific vaccines based on individual genotyping (vaccines against a specific malignancy in a specific individual) will also raise serious concerns. None the less, the prospect of both preventing and treating many serious diseases by the use of vaccines portends an exciting era in public health and vaccinology.

We thank Kim Zabel for her excellent editorial assistance in the development of this review. DM's current address is Pediatric Infectious Diseases, Medical College of Georgia, Augusta, GA, USA.

Funding: GAP and this work was supported in part by a grant from the Centers for Disease Control and Prevention (AVA 001) and grants from the National Institutes of Health (AI 33144 and AI 48793).

Competing interests: None declared.

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Lillian and New Year's Eve

It was some 20 years ago that I moved to New Orleans to complete my medical training. A fellow at the hospital told me about an old blacksmith's shop that had been converted into a piano bar down in the French Quarter. I was far from home and it was New Year's Eve, so I decided to join my friends and go to listen to this remarkable lady "that plays the piano throughout the night."

At about 10 pm, the remarkable lady, called Mrs Lillian, appeared. She was petite with delicate features and hands that seemed to be ageless. She politely said good evening and took her seat at the old piano. It was like a scene from an old Bogart film with clouds of smoke surrounding the dimly lit area where she began to play. I had to laugh when her first selection turned out to be a tune from the movie *Casablanca*, which got things off to a wonderful start.

As she finished her set, she turned to me and asked me where I was from. "Argentina," I said. Suddenly, a wonderfully familiar sound filled my ears. Mrs Lillian was playing a famous tango and singing it in perfect Castillian. Little did she realise how close to home she had taken me. The name of the song was *Caminito*. She nudged me to join in, and I reluctantly began to sing in a low voice as my mind drifted back home to my father—*Caminito* happened to be his favourite song. When the song ended, she sweetly talked to me, wanting to know all about me. I'll never forget how wonderful she made me feel.

When midnight arrived, she wished everyone a Happy New Year, many by their first name. Being so far from home, I somehow had this marvelous sense of being made part of a very special family, all because of Mrs Lillian. In the years that followed, we forged a friendship.

One day she called me at the hospital and told me that she was not feeling well. I knew that she had a history of hypertension and was taking treatment. When she arrived at my office she complained of weakness, nausea, and lack of appetite. Studies revealed that she had renal failure and would probably need dialysis soon. It struck me deep down she was my dear friend. I took care of her from then on. Whenever she came to the office the same effusiveness she so generously gave to me was also shown to my staff.

Despite her illness, she continued to work in the old blacksmith's shop. And every New Year's Eve, I was the first person she would call with best wishes. No matter where I was, she was the first moment of every New Year for me.

One day, just three days before New Year's Day, I received a telephone call from her husband; Mrs Lillian had died while sleeping. For the first time, Mrs Lillian's greetings did not come, the first moment of the new year was not shared with her. The old blacksmith's shop would somehow seem silent.

This New Year's Eve feels so hollow. The clock strikes midnight, and I find myself humming *Caminito*. "Happy New Year, Mrs Lillian."

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We welcome articles up to 600 words on topics such as *A memorable patient, A paper that changed my practice, My most unfortunate mistake,* or any other piece conveying instruction, pathos, or humour. If possible the article should be supplied on a disk. Permission is needed from the patient or a relative if an identifiable patient is referred to. We also welcome contributions for "Endpieces," consisting of quotations of up to 80 words (but most are considerably shorter) from any source, ancient or modern, which have appealed to the reader.