Science, medicine, and the future

Vaccines and vaccination

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Vaccines and vaccination are at a turning point. Recent advances in microbial genetics and in immunology have greatly increased our understanding of microbial pathogenesis and of host defence mechanisms. As a result, within the next 10-15 years, a whole set of new preventive vaccines should become available. These will not only be used to prevent infectious diseases but also for preventing neoplasms such as stomach and endocervical cancer. Also under development are therapeutic vaccines to treat autoimmune diseases and allergic disorders. This review outlines some of the advances in biology, development of new vaccines, and vaccination strategies and discusses the factors that will determine the extent of their use in the future.

Possible futures

- Prevent additional infectious, neoplastic, and even autoimmune diseases by vaccination
- For young children, preventive strategies against severe lower respiratory infections and meningitis (immunisation against *Streptococcus pneumoniae*, *Neisseria meningitidis*, and respiratory syncytial virus added to that against *Haemophilus influenzae* type B and pertussis)
- For young children, particularly in developing countries, preventive strategies against diarrhoeal diseases (rotavirus, shigellosis, enterotoxigenic and enterohaemorrhagic *Escherichia coli*, and, in some areas, cholera)
- For elderly people, combined prevention of respiratory infections (respiratory syncytial virus, influenza, pneumococcal pneumonia)
- Therapeutic immunomodulation of chronic diseases (tuberculosis, chronic hepatitis, certain cancers, allergies, diabetes)

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Disease prevention and therapy through vaccination

Although vaccination has been shown to be the most effective way to prevent infectious diseases, its major impact on public health has been restricted to the control of certain human diseases such as smallpox, poliomyelitis, neonatal tetanus, diphtheria, pertussis, and measles. The eradication of smallpox was the first result of the appropriate use of vaccination at a global level, but efforts made in the past 20 years by the WHO Expanded Programme on Immunisation to increase coverage with routine childhood vaccines (from 5% to about 80%) have probably saved over three million lives annually.

Vaccines currently in clinical use have been developed through relatively simple, largely empirical approaches, but new vaccine strategies are emerging based on an understanding of microbial pathogenesis and host defence mechanisms. More than nine million deaths could be prevented annually through the use of vaccines against a few important infectious diseases such as pneumonia, meningitis, diarrhoeal diseases, tuberculosis, malaria, and schistosomiasis.[1] We may even dream of an AIDS vaccine. In addition, since the introduction of hepatitis B immunisation and its proved preventive effects against liver cancer,[2] the benefit of vaccination should be extended to preventing cancers associated with *Helicobacter pylori* (stomach cancers) and papilloma viruses (endocervical cancers). Some other cancer vaccines based on the use of tumour antigens (such as melanoma) have now entered clinical trials.

Vaccines against diseases due to immunological dysregulation are also emerging. Some therapeutic peptide vaccines that aim at restoring a "normal" immune response to major allergens (such as cat antigens and house dust mite) are in development, while preventive and therapeutic peptide vaccines for autoimmune diseases (such as insulin dependent diabetes and multiple sclerosis) are on the drawing board.

Microbial genetics and vaccine development

Selective deletion of virulence genes for avirulent live vectors

Progress in microbial genetics and advances in genetic engineering are essential in the revolution in vaccine development. Defining the molecular basis of microbial virulence and identifying antigens essential for the induction of successful host defence mechanisms allows the construction of "intelligent" vaccines. These include genetically engineered attenuated microorganisms, formed by selective deletion of virulence genes, or live vectors carrying foreign genes relevant for

Some important diseases that may be controlled with new vaccines

Infectious diseases
- Pneumonia and otitis media (*Streptococcus pneumoniae*, conjugate vaccines, respiratory syncytial virus)
- Infant meningitis (*S pneumoniae*, *Neisseria meningitidis*, conjugate vaccines)
- Diarrhoeal diseases (rotavirus, cholera, shigella, enterotoxigenic *Escherichia coli*)
- Malaria, schistosomiasis
- Tuberculosis (adults)
- AIDS

Neoplasms of infectious aetiology
- Gastric cancer (*Helicobacter pylori*)
- Cervical cancer (papilloma virus)
- Hepatocarcinoma (hepatitis C, complementing existing hepatitis B vaccine

Diseases due to immunological dysregulation
- Allergies (to house dust mite, cat antigens)
- Autoimmune diseases (insulin dependent diabetes, multiple sclerosis)

protection. For example, selective deletion of virulence genes in *Shigella* sp, enterotoxigenic *E coli*, *Salmonella typhi*, and *Vibrio cholerae* led to the production of attenuated strains, which can be used as live candidate vaccines or as vectors carrying foreign antigen genes into their bacterial genome.[3] In consequence, the development of a "vaccine package" against diarrhoeal and other enteric diseases, which would contain a mixture of enteric vaccines and would be administered orally, is becoming conceivable.

Genome sequencing to identify vaccine candidates

Deciphering the entire genome sequence of the most important human pathogens should have a marked impact on vaccine development. Genes of specific interest for vaccine design are identified by computerised analysis of genomic sequences and search for sequence homologies between different micro-organisms.

This approach is of particular interest for developing a new vaccine against tuberculosis (still causing about 2.8 million deaths a year). Complete sequencing of the genome for *Mycobacterium tuberculosis* is expected by the end of 1997, and it may have an unprecedented impact on vaccine development (fig 1). It will help to identify genes involved in mycobacterial virulence, as well as genes present in *M tuberculosis* but absent in BCG vaccine.[4] This will make it possible to construct attenuated strains of *M tuberculosis* by specifically altering sequences involved in bacterial virulence (site-directed mutagenesis), or to design a new BCG vaccine through inserting some of the specific mycobacterial genes to optimise its immunogenicity. These modified mycobacteria will also contribute to a better understanding of the complex host-parasite relationship that prevails between humans and mycobacteria, and they may lead to the production of new attenuated candidate vaccines.

In a different approach the systematic screening of the entire genome for antigens capable of eliciting protective immune responses is now being done by construction and injection of cDNA libraries, using the DNA vaccine technology (see below). This should help to identify antigens capable of inducing a desired pattern of immune response. A similar approach is now being initiated towards malaria vaccines.

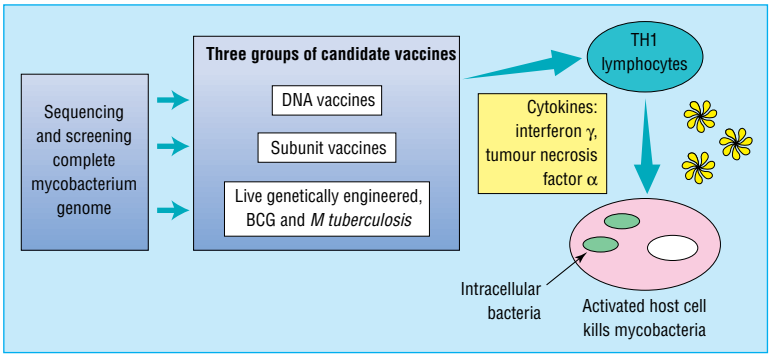

Fig 1 Impact of genome sequencing on development of new tuberculosis vaccines. Identification of genes involved in mycobacterial virulence is expected to lead to various candidate vaccines: DNA vaccines, secreted proteins, or genetically modified mycobacteria. These will be evaluated for their capacity to induce in vivo the production of interferon γ and tumour necrosis factor α by T cells which seem to be required for killing of mycobacteria by activated macrophages.

Immunological advances and vaccine design

Defining mechanisms of immunological protection

In order to be effective, vaccines should be designed to elicit appropriate protective immunological effects. Understanding protective mechanisms—that is, the molecular processes involved in the immunological recognition of microbial antigens and in the differentiation of cells that mediate effector mechanisms—is useful for the design of new vaccines against diseases for which an empirical approach to vaccine development has failed.

Whereas antibody responses are sufficient to protect from infections by pneumococci or meningococci, additional cellular responses are usually needed to prevent diseases caused by microorganisms able to hide and survive within infected cells—such as viruses, chlamydiae, certain bacteria (mycobacteria, salmonella, shigella, listeria), and parasites (toxoplasma, plasmodium, leishmania). Given that a subset of T lymphocytes (CD4 TH1 helper T cells) which produce appropriate cytokines such as tumour necrosis factor α and interferon γ or cytotoxic T lymphocytes are required for the clearance of such infected cells, vaccines directed against intracellular microorganisms should induce these patterns of immune responses. The development of tuberculosis vaccines has been directly influenced by these conceptual advances.[5]

Designing vaccines with optimal protective capacity

It is now becoming feasible to transform immunological concepts into real vaccines through designing vaccine formulations that can direct immune responses towards appropriate effector pathways. Three recent developments are contributing to this advance.

New immunological adjuvants are emerging after decades of stagnation, when aluminium salts were the sole adjuvant for human use, and will influence our ability to induce protective responses. For example, mixing the same candidate vaccine for malaria (part of the circumsporozoite protein of *Plasmodium falciparum* inserted into a recombinant hepatitis B surface antigen) with three different adjuvant mixtures produced markedly different levels of protection in challenged human volunteers: the vaccine containing

an adjuvant mixture composed of monophosphoryl lipid A (MPL), a water-in-oil emulsion, and QS21 (a derivative of saponin) was effective in six of the seven volunteers subsequently exposed to a malarial challenge, whereas vaccine formulations containing either aluminium hydroxide and monophosphoryl lipid A or the water-in-oil emulsion alone did not confer any significant protection despite good antibody responses.[6] This suggests that appropriate T cell responses are needed to prevent malaria and that future malaria vaccines should be designed accordingly. Such new adjuvants will also probably be used in the development of therapeutic vaccines aimed at redirecting immune responses either towards particular cell mediated protective mechanisms (chronic infections and cancer) or towards non-pathogenic responses (allergic and autoimmune diseases).

Live viral or bacterial vectors which express vaccine antigens can be used to generate cytotoxic lymphocytes for the specific recognition and destruction of infected cells. For example, vectors derived from herpes and pox viruses have been engineered to express HIV-1 antigens.[7] In preclinical studies such modified organisms have induced strong HIV-1 specific cytotoxic lymphocytes in immunised mammalian hosts. Despite their potential efficacy, however, the future of live vector vaccines will depend on their potential adverse effects in patients with variable levels of immunocompetence, and only non-replicating or self destructing vectors could meet global acceptance.

DNA vaccines encoding a number of different vaccine antigens (fig 2) have been shown in animal models to have a remarkable capacity to induce strong protective immunological responses, TH1 and cytotoxic lymphocytes, against intracellular microorganisms.[8] This revolutionary concept of vaccination could be of particular interest in preventing human or veterinary diseases—including scourges such as malaria, HIV, and tuberculosis—that require the clearance of organisms inside infected cells. We do not yet know whether this approach will prove efficient in humans, but initial clinical trials with DNA vaccines encoding HIV-1 glycoprotein have just been started. In terms of safety, considerable efforts will have to be made to ensure that DNA vaccines do not become integrated into the host genome, with the associated risk of delayed oncogenic effects.

The new biology and vaccine production

Genetic engineering has profoundly influenced the vaccine industry. The production of hepatitis B vaccine is currently accomplished with microorganisms such as yeast which have been modified by genetic recombination, thus avoiding the use of plasma from hepatitis B carriers as a source of vaccine antigen. Several new vaccines could not be produced without this technology, which is still rapidly evolving. A challenging development is the use of transgenic plants designed to express vaccine antigens as a source of vaccines. Transgenic bananas expressing hepatitis B surface antigen and potatoes expressing antigenic components of rotavirus have already been developed, but the potential of this approach for high yield antigen production or, in an ideal world, as a direct source of "edible vaccines" remains to be seen.

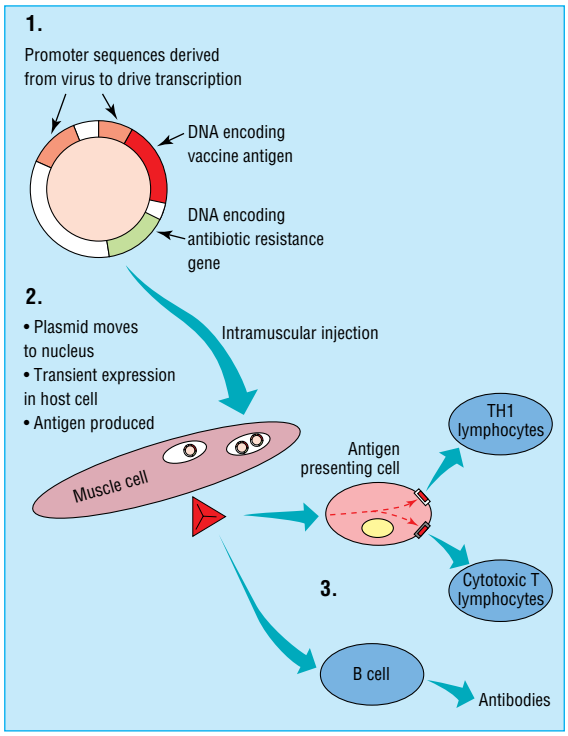

Fig 2 Principles of DNA vaccines. (1) Genes coding for one or several microbial antigens are inserted into a bacterial plasmid (a circular piece of DNA) together with promoter and regulatory elements, and this bacterial vector expressing foreign genes of interest is amplified into *E coli* and purified from bacterial contaminants. (2) Host is immunised by direct intramuscular or intradermic injection of recombinant DNA plasmid: the DNA enters some cells, in which RNA transcription and protein production occur. (3) After antigen expression by transfected cells, specialised cells of the immune system (dendritic cells) take up vaccine antigens, transport them into draining lymph nodes, and initiate immune responses against them.

Will new vaccines be used in clinical practice?

Predicting the impact of new vaccines on clinical management is a delicate task. The increasing availability of vaccines has raised new questions and issues that already limit their introduction into routine clinical use. Even vaccines that have already been shown to be both efficient and safe—such as pneumococcal and influenza vaccines—are not yet being used optimally by doctors to protect those who need it most, such as elderly patients. Whether this implies that routine universal immunisation will be the only way to protect high risk groups who are otherwise difficult to reach, as shown for hepatitis B vaccines, will have to be determined.

However, parents are already becoming concerned about the high number of vaccines given to their young children. Although considerable efforts are being made to facilitate vaccine administration (combined vaccines, oral or nasal administration), infant immunisation schedules are already quite full and may not readily allow the addition of many new vaccines. Thus, the capacity to implement routine immunisation practice beyond infancy and early childhood is likely to become a key determinant of future use of new vaccines.

The public acceptance of new vaccines and their practical impact will greatly depend on the perception of their usefulness by doctors, nurses, and other public

health officers. As adverse events related to vaccines become more obvious than some diseases prevented by vaccines, efforts to maintain a high level of vaccination coverage for the benefit of the community are at risk of being jeopardised by small but active groups of opponents to immunisation. For example, failure to reach or maintain the vaccine coverage required to interrupt measles virus transmission in a few Western European countries could, in the not too distant future, hamper successful measles control on other continents as a result of persisting reservoirs for the measles virus.

Last but not least, a major obstacle towards the global use of new vaccines is their cost, particularly in developing countries. In view of the investments made in research and development, new vaccines will have to be more expensive than older ones. However, their cost effectiveness will generally be high, and the exceptional value of vaccination as a preventive intervention should be properly compared with the costs of increasingly expensive therapeutic interventions.

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Lesson of the week

Lumbar puncture still has an important role in diagnosing subarachnoid haemorrhage

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Lumbar puncture is necessary in suspected subarachnoid haemorrhage when a computed tomogram is normal or unavailable

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An early diagnosis of subarachnoid haemorrhage is essential if patients are to undergo appropriate investigation and treatment. It is increasingly common for patients with suspected subarachnoid haemorrhage to initially undergo computed tomography. Although the procedure is sensitive and non-invasive, it does not detect every instance of subarachnoid haemorrhage. Lumbar puncture should be considered in patients who have an acute onset of headache and who are fully conscious but have a normal scan or when the hospital does not have access to a computed tomographic scanner. We report on two patients in whom an early diagnosis of subarachnoid haemorrhage was missed because they did not undergo lumbar puncture.

Case reports

Case 1—A 58 year old man had lost consciousness for one minute. On recovery he had a severe headache and bloodstained vomitus. He was admitted to hospital and given intramuscular opiates for pain relief. Haematemesis was provisionally diagnosed. His initial symptoms were attributed to possible alcohol withdrawal and treatment with diazepam was started. Two days later his headache had not improved. Subarachnoid haemorrhage was suspected, and he was booked in for a routine computed tomography, the earliest appointment being two days later. On the next day, however, he became unconscious and had a fixed, dilated left pupil. An emergency scan showed extensive subarachnoid haemorrhage, and he was transferred to a neurosurgical unit. Before further investigations and treatment could be performed he died, presumably of a rebleed.

Case 2—A 17 year old woman attended her doctor because of a history of headache associated with nausea and vomiting for one week. She was referred to hospital and underwent computed tomography to rule out subarachnoid haemorrhage. The scan was normal, and she was reassured and discharged home. Two days later she awoke with a sudden severe headache, vomited, and then collapsed. On arrival at hospital she was in a coma but reacting to pain. She was intubated and ventilated and transferred to a neurosurgical unit. Computed tomography showed a subarachnoid haemorrhage and a cerebral angiogram showed a terminal carotid artery aneurysm. During angiography her condition deteriorated, both pupils became fixed and dilated, and she subsequently died. The original tomogram was reviewed and did not show any evidence of subarachnoid haemorrhage.

Discussion

Subarachnoid haemorrhage should be suspected in all cases of severe headache of sudden onset. In the first case diagnosis was delayed while the patient awaited computed tomography. In the second case a normal scan was considered sufficient to exclude subarachnoid haemorrhage. In both cases an early diagnosis would have been possible had a lumbar puncture been performed. Although an earlier diagnosis would probably not have altered the outcome in these two patients, it could be important in other cases.

In a large prospective study scans were normal in 5% of patients subsequently shown to have had a subarachnoid haemorrhage.[1] In a recent study in which computed tomography was performed with third generation scanners within 12 hours of the onset of headache, two out of 119 patients with subarachnoid haemorrhage (2%, 95% confidence interval 0.25% to 6%) had a normal scan.[2] As extravasated blood can dis-

perse quickly it may be undetectable by computed tomography after 12 hours. Thus if a scan was delayed there would be more chance of a subarachnoid haemorrhage being missed. In a series of 181 patients investigated for suspected subarachnoid haemorrhage the sensitivity of computed tomography performed within 12 hours of the onset of headache was 93.1% and after 12 hours was 83.8%, giving an overall sensitivity of 91.2%.[3] The decreasing sensitivity of computed tomography with increasing time from the onset of headache was shown in a series of 2940 patients presenting with subarachnoid haemorrhage. The sensitivity of scanning decreased from 92.1% on the day of the bleed to 57.6% on day 5.[4] Thus the sensitivity of computed tomography cannot reliably exclude lumbar puncture in patients who have symptoms suggestive of subarachnoid haemorrhage.

Lumbar puncture is necessary in cases of suspected subarachnoid haemorrhage when a computed tomogram is normal or unavailable. Subarachnoid haemorrhage can be detected by the presence of xanthochromia between six and 12 hours after haemorrhage.[5] To ensure that xanthochromia is detected after subarachnoid haemorrhage, lumbar puncture should be deferred until 12 hours after the onset of headache, necessitating admission in most cases. In cases of late presentation after subarachnoid haemorrhage all patients have xanthochromia up to two weeks after haemorrhage, 70% after three weeks, and 40% after four weeks.[5]

The risk of neurological deterioration after lumbar puncture is disputable. In 1982 seven cases of neurological deterioration after lumbar puncture were reported in 55 patients investigated for suspected subarachnoid haemorrhage.[6] All seven patients had moderate to severe headache and neck stiffness and two had a focal deficit and confusion. As lumbar puncture is contraindicated in the presence of papillo-oedema, focal deficit, or reduced consciousness it may have been inappropriate in some if not all of these patients. In a retrospective review of 123 cases of subarachnoid haemorrhage, 91 patients underwent lumbar puncture with no evidence of deterioration from the procedure. In the same study 22 out of 24 patients with an intracerebral haematoma on computed tomography had focal neurological signs and severe impairment of consciousness. The authors concluded that lumbar puncture in patients without these signs was safe.[7]

The Society of British Neurological Surgeons has recently circulated guidelines to its members on the initial management of subarachnoid haemorrhage. The guidelines emphasise that computed tomography detects subarachnoid haemorrhage in some but not all patients. When a computed tomography scanner is available computed tomography should be performed and lumbar puncture carried out if the results are normal. A lumbar puncture should be performed if a diagnosis is in doubt and computed tomography is unavailable but the patient is oriented and obeying commands. Lumbar puncture should not be performed in patients with papillo-oedema or focal neurological signs.

One study proposed that reports of neurological deterioration after lumbar puncture might lead physicians to abandon the procedure in cases of suspected subarachnoid haemorrhage.[8] Even with the increasing availability of computed tomography, lumbar puncture should still be performed in appropriate cases.

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Correction

Science, medicine, and the future: Obesity treatment

An author's error occurred in this article by John Wilding (18 October, pp 997-1000). In the first paragraph on page 998 it was wrongly stated that no drug is licensed for treating obesity in Britain. In fact, phentermine is still licensed for use, but it is not recommended for the routine management of obesity.

A lesson learnt

Asking the wrong questions

Shortly after the second world war my teaching hospital created a metabolic ward to further the rapid advances in endocrinology. The hospital was old, draughty, and without central heating or air conditioning, but here the air was controlled and, for all I know, may even have been weighed and measured. Everything that passed in or out was chemically analysed. Dozens of specimens went hourly to the laboratory. Normal visiting was not allowed. We were continually told how expensive the unit was, and how valuable to science, to patients, and to our education.

I remember two patients who shared a room. Each had a rare and puzzling disorder. One of them continually excreted more calcium than she ingested. The other failed to gain weight in spite of a high calorie diet. Despite innumerable investigations, no one could discover why.

The pair were eventually discharged with their problems unsolved. It fell to me to clerk the patient with the calcium problem when she came to the follow up clinic. I asked her how she had enjoyed her stay in the metabolic ward.

"It was lovely," she said, then added, "The lady in the other bed used to give me all her milk."

I did not have the nerve to tell the learned and rather formidable consultant endocrinologist what the patient had said, but I sometimes wonder what would have happened if I had.

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