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REVIEW

Progress towards achieving new vaccine and vaccination goals

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Abstract
Viral and bacterial vaccines, especially for childhood use, are one of the most successful public health measures of the last two centuries and have a good safety record. However, there are still many diseases that are caused by infectious agents for which vaccines are not available. Our increasing ability to manipulate the immune system offers hope that, in the future, at least some of these infections may be prevented by vaccination. A surprising recent development is the use of vaccine technology to test whether a range of other generally non-communicable diseases can be prevented (or at least controlled) in this way. Investigation of these diseases is still mainly at the experimental level, however the list includes different types of cancers, allergies, drug addiction and neurodegenerative diseases. (Intern Med J 2003; 33: 297–304)

Key words: infections, non-infectious diseases, vaccines, vaccination.

INTRODUCTION

The modern era of vaccination began over 200 years ago, with a procedure to prevent smallpox using prior immunisation with cowpox. It continued during the nineteenth century with the introduction of several more vaccines to control some viral and bacterial infections, but the major contributions occurred in the twentieth century.[1] Over 70 different infectious agents commonly cause disease in humans. Nearly all of these have been (or are now) the target of vaccine development. Currently, there are over 30 registered individual vaccines, approximately half of which are commonly used, mainly to prevent childhood infections and especially in many developed countries. Approximately one dozen candidate vaccines to prevent other diseases have passed stage 2 clinical trials.[2,3]

ACHIEVEMENTS AND CHALLENGES

Approximately 30 years ago, there was some concern about the future of vaccine technology because the number of major manufacturers was declining. However, with the advent of new technologies – especially biotechnology, which saw the start up of many new small companies – the ability to manipulate immune responses and the increasing evidence of the efficacy of many of the new vaccines became apparent and the outlook changed.

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Achievements

Table 1 lists the current vaccines and indicates the type of preparation. This varies from live, attenuated viruses and bacteria to subunits and toxoids. Some countries have kept records of vaccine efficacy based on the changing incidence of some common childhood diseases; in the USA, data from as far back as 1912 are especially impressive. The incidence of disease during an epidemic some years before the vaccine became available has been compared with the incidence in the late 1990s, some years after the vaccine became available. The drop in incidence was: (i) 100% for indigenous poliomyelitis, (ii) >99% for diphtheria and measles, mumps, rubella (MMR) and (iii) >97% for tetanus.[4,5] Two or three doses of many vaccines are needed to achieve maximum protection.[4] Although many vaccines can give side-effects, the great majority are minor and claims to the contrary are usually wrong. For example, claims that the MMR vaccine causes inflammatory bowel disease and autism have not been substantiated in at least 10 epidemiological studies.[4]

The global eradication of smallpox, achieved in 1977 and declared in 1980, is rightly regarded as one of the greatest public health achievements of all time. In the drive to globally eradicate poliomyelitis, three major regions – the Americas, Europe and the Western Pacific – have now been declared free of endemic disease. However, global eradication is proving to be more difficult due, in part, to the occurrence of revertent strains, and the failure in some countries to maintain a high level of vaccination. In addition, prevention of transmission of that highly infectious agent has been achieved in the USA, Canada and Finland following the adoption of a two-dose schedule for measles vaccination.

Another remarkable success in the last decade or so has been the development of conjugate vaccines. Some bacteria have capsular polysaccharides and, although

Table 1 Current viral and bacterial vaccines and type of vaccine

Vaccine	Type
Viral	
Smallpox (vaccinia)	1
Yellow fever	1
Polio (oral polio vaccine)	1
Polio (inactivated polio vaccine)	2
Measles	1
Mumps	1
Rubella	1
Varicella	1
Adeno	1
Influenza	1a, 2, 3
Japanese encephalitis	2
Rabies	2
Hepatitis A	2
Hepatitis B	3
Bacterial	
Bacille Calmette–Guerin (tuberculosis)	1
Salmonella typhi	1, 5, 6†
Bordetella pertussis	2, 3
Vibrio cholerae	1
Bacillus anthracis	2
Coxiella burnetii (Q fever)	2
Borrelia burgdorferi (lyme disease)	3
Clostridium tetani	4
Corynebacterium diphtheriae	4
Streptococcus pneumoniae	5, 6†
Haemophilus influenzae, type b (Hib)	6
Neisseria meningiditis group A.C,W135,Y	5
Neisseria meningiditis group B	3†
Neisseria meningiditis group C	6
Combination	
Diphtheria tetanus whole pertussis	
Diphtheria tetanus acellular pertussis	
Measles, mumps, rubella	
Hib-hep B, DTaP-hep B	

1, live attenuated; 1a, live, reassortant (used successfully in Russia); 2, whole particle, inactivated; 3, subunit; 4, toxoid; 5, polysaccharide; 6, carbohydrate/protein conjugate. †Passed phase 3 clinical trials.

these sugars are poorly immunogenic in infants <2 years of age, they have formed the basis of some vaccines for adults, especially the pneumococcal vaccine for the elderly. The first demonstration that the poor immunogenicity of polysaccharides could be greatly enhanced by conjugating to a protein was in 1931;[6] approximately 30 years before the discovery of T lymphocytes. It also took a long time for the medical community to take advantage of the finding. The first polysaccharide : protein conjugate vaccine is the *Haemophilus influenzae* type b, which prevents subsequent disease in at least 95% of infants.[7] Three more recent successes, which in each case protected more than 90% of infants or children from invasive disease, are: (i) a seven valent (different specificities) *Streptococcus pneumoniae* conjugate vaccine,[8] (ii) a *Neisseria meningitidis*, serogroup C conjugate vaccine[9] and (iii) a *Salmonella typhi* Vi carbohydrate conjugate vaccine.[10] The higher immunogenicity of the conjugates is due to the protein component inducing strong T-helper cell activity in the very young. Other preparations are in the pipeline.

Current and future needs

The World Health Organization has documented the incidence of major diseases, especially those due to infectious agents.[11] Infectious diseases cause approximately 25% of global deaths. When expressed as millions of deaths per annum, the figures are as follows: (i) 2.9 for tuberculosis, (ii) 1.08 for malaria, (iii) 2.5 for diarrhoeal illnesses, especially rotaviruses and (iv) 2.3 (and climbing rapidly) for HIV/AIDS. Levels of infection are much higher: (i) approximately 2 billion latent *Mycobacterium tuberculosis* infections, (ii) approximately 300 million clinically significant malaria infections and (iii) approximately 40 million HIV infections. Respiratory syncytial virus and parainfluenza viruses are the major causes of hospitalization due to respiratory-tract illness in the USA, especially for young children.[12]

In addition, there are increasing levels of resistance to antibiotics by many bacteria, the constant possibility of a major influenza pandemic and the threat of other viruses switching host specificity (i.e. some paramyxoviruses and, more recently, a coronavirus that causes severe acute respiratory syndrome (SARS)). There is, therefore, a strong need to devise improved methods for developing vaccines to the many difficult, and as yet unconquered, infectious agents. Before discussing the new approaches to vaccination, however, it is necessary to understand how vaccines work.

How do vaccines work?

First, a brief outline of the immune system is provided. There are two systems: (i) the innate system, which is common to all multicellular organisms and (ii) the adaptive system, which differs from the former in its great specificity and memory, and is confined to vertebrates. The two systems are intimately connected.

The innate system detects 'danger', and can come into operation within minutes or hours of an infection occurring. It consists of a variety of different cell types. Some cells have receptors that recognize common bacterial products such as endotoxins which, if present, activate the cell to produce and secrete factors harmful to the invader. Other secreted factors include cytokines, such as interferons, and chemokines, which activate and influence the traffic of other cells. Two cell types – dendritic cells (DCs) and macrophages – are a critical link between the innate and adaptive systems because they take up foreign material, process it and express it at the cell surface in a form recognized by T lymphocytes. DCs are called the 'professional' antigen-presenting cells. They have receptors (toll-like receptors), which recognize foreign material such as bacterial DNA (but not vertebrate DNA), and this recognition results in the activation and maturation of the DC.[13]

The adaptive system takes a few days (and sometimes weeks) to be activated and become effective.

Lymphocytes (cells found in the lymph) characterize the adaptive system. There are two types of lymphocytes: (i) B lymphocytes, which make and secrete antibodies and (ii) T lymphocytes, which have several roles. There are two T-cell subclasses, characterized by the cellular differentiation markers (CD4 and CD8). There are two types of CD4+ T-cells: (i) Th-1 and (ii) Th-2. Th-2 cells secrete a number of cytokines (interleukins), whose major task is to help B cells to differentiate and make different classes of antibodies (IgA, IgE and most subclasses of IgG). These cells primarily have a regulatory role (Table 2). Th-1 cells help B cells to make some subclasses of IgG antibodies, however they also secrete a pattern of cytokines which activate a range of other cells, such as macrophages. They are also the major cell type mediating delayed-type hypersensitivity reactions. Thus, they display both regulatory and effector functions. A crucial question that is receiving more attention is what determines the balance between these two responses.

In contrast, CD8+ T cells are called cytotoxic T lymphocytes (CTL) or killer T cells, because they recognize and cause the lysis of cells infected by viruses, bacteria or parasites. Thus, they act as auditors of the body; seeking out and destroying 'dangerous' (i.e. infected) cells. In some situations, Th1-type cells also help in the differentiation and maturation of CTL (Table 2).[14]

A unique property of lymphocytes is that each B cell makes antibodies and each T cell has antigen receptors (TCR) of a single specificity. Only a few thousand B cells make antibody molecules (and T cells) of exactly the same specificity. However, because there are more than a billion different possible specificities, there are a lot of lymphocytes in the body. Following an infection, the appropriate T and B cells are activated, mature and replicate to form many progeny cells, most of which die after the infection is cleared. However, pools of memory cells with those specificities are formed and persist. Over time, B memory cells are activated by contacting retained foreign antigen and become antibody-secreting cells (ASC) or new memory cells. The net result is that, after an infection or immunisation/vaccination, a specific antibody will be continually made for long periods, sometimes many years (decades). Pools of memory T cells are also made following an infection/immunisation, however these persist as such until there is a second exposure to the same (or very similar) infectious agent, when they may rapidly differentiate to a fully effector state.

The sequence of appearance of immune cells following an infection is: (i) regulatory T cells, (ii) effector T cells (usually CTL) and, finally, (iii) ASC. In a rapidly resolving (acute) infection, there may be some overlap, however in another example (human HIV infections), viral titres in the blood rapidly decrease when CTL are first found (2–3 weeks), and neutralizing antibody appears weeks or months later. The evidence from many model systems clearly shows that mainly CTL and sometimes Th-1 cells clear acute infections. Persistent infections occur when the agent evades or subverts the effector T cell response.

Most current vaccines, especially live agent vaccines, induce the long-term production of antibody which can later neutralize a high proportion of the same invading infectious agent, so that small amounts of any escaping agent are dealt with by a normal immune response. However, the antibody approach has not been successful to date with a growing number of agents, especially those displaying considerable antigenic variation like HIV-1. Thus, the amino acids in up to 30% of the envelope antigen (gp120) of HIV may vary, and as many as 10 different amino acids may be found at a few sites in one segment (the V3 loop of gp120 in isolates). After almost 20 years of intensive research, there are now available a few high titre (monoclonal) antibody preparations which neutralize the infectivity of a wide range of antigenically different HIV-1 field isolates. One of these (IgG1b12) prevents the binding of the virus to the main cellular receptor (CD4). The three-dimensional structure of the antibody's CD4 binding site has been determined[15] and this may allow the synthesis of a compound that mimics this critical segment of gp120. Using such a compound as a hapten attached to a highly immunogenic protein carrier could form the basis of an effective HIV-1 vaccine.

In the meantime, because of the urgent need for a vaccine, HIV-1 has become a model to see whether a vaccine that generates a very strong CTL response will clear, or at least effectively control, a subsequent infection. If so, then a vaccinated person who subsequently becomes infected could live a more 'normal' life and

Table 2 Properties of lymphocytes

Cells	Receptors	CD marker	Type	Role	Secreted products
B	IgM, IgD	–	–	Cells are activated via these receptors	–
T	α,β or γ,δ	CD4	Th-2	Helps B cells make antibodies and certain cytokines	IgM, IgG, IgA, IgE Mainly IL –4, –5, –6, –10
	α,β or γ,δ		Th-1	Helps B cells make antibodies and certain cytokines	Some subclasses of IgG IL –2, IFNγ, TNFα, β
	α,β or γ,δ		Th-1	Helps differentiation of CD8+ T cells	
	α,β or γ,δ		Th-1	Mediates DTH responses	
	α,β	CD8		Lyses-infected cells	IL –2, IFNγ, TNFα

DTH, delayed-type hypersensitivity; IFN, interferon; IgA, immunoglobin A; IgD, immunoglobin D; IgE, immunoglobin E; IgG, immunoglobin G; IgM, immunoglobin M; IL, interleukin; TNF, tumour necrosis factor.[10]

be much less infectious for others. If this approach is successful, it will be worthwhile to vaccinate HIV-infected individuals after multi-drug treatment has greatly reduced viral titres in anticipation that drug treatment could then be stopped for at least some time.[16]

MEETING THE CHALLENGES

Novel approaches for vaccine delivery

Although many agents infect via a mucosal route, most vaccines are delivered by injection. Frequent injections and the risk of transmitting diseases through needle reuse remain major concerns for many parents. Two recently described techniques may, in time, change this picture.

Transcutaneous immunisation is a new approach to vaccination. The vaccine is applied with a suitable adjuvant directly to prewashed skin, using a patch.[17] The epidermis is rich in Langerhan's cells (which are highly effective as antigen-presenting cells) delivering processed antigen to the draining lymph nodes. This results in the activation of T cells. The technique has worked well in animals and the first clinical trials using the enterotoxin LT from *Escherichia coli* as the antigen has given durable antibody and cell-mediated immunity (CMI), in both systemic and mucosal responses.

Both antigens from infectious agents and specific antibodies (plantibodies) have been produced in some DNA-transfected plants.[18] When transgenic potatoes expressing the protective protein of gastroenteritis virus were fed to pigs, most were protected from a subsequent challenge of infectious virus. Mice that were orally immunised with hepatitis B surface antigen (HBsAg) in transgenic potatoes gave a stronger immune response than mice immunised with the standard yeast-derived HbsAg. The antigen was bio-encapsulated in the plant and this may have protected it from degradation in the digestive tract.[19] However, the yield of antigen expressed in the plant needs to be increased for this technique to become completely practical.

New technologies for vaccine development

The fact that there are approximately 100 preparations under development for HIV/AIDS vaccines is some indication not only of the tremendous challenge this virus poses to vaccine developers, but of also the variety of current different approaches to vaccine development.[3]

The use of peptides containing amino acid sequences recognized by neutralizing antibodies or binding to selected class I or class II MHC antigens has the advantage that many sequences that might induce autoimmune reactions are eliminated. The chosen sequences can be combined in different ways to improve conformation and ability to react with cells. Candidate vaccines of this nature for rheumatic fever and malaria are in clinical trials.[20,21] Peptide-based preparations require the addition of an adjuvant to enhance immunogenicity. At present, only alum is registered for general medical use, however there is a great range of products being tested, and some are in clinical trials.

Because live attenuated viral vaccines are generally highly effective, the possibility was raised approximately 20 years ago that they could be used as vectors of DNA coding for other antigens. It was shown that up to 10% of the DNA genome of vaccinia virus (a representative of the poxvirus group) could be deleted and replaced by DNA coding for antigens from other infectious agents, for which vaccines were unavailable. Cells infected with the chimeric virus produced the antigen(s) coded for by the inserted DNA. Immunisation of hosts with the chimeric virus induced strong antibody and T cell (including CTL) responses and protected against a challenge by the infectious agent that was the source of the foreign DNA. Table 3 lists the infectious agents that are more frequently used experimentally as vectors. The additional insertion of DNA coding for the interleukins (IL-4 or IL-12) into the vector induced a very strong antibody or effector T cell response, respectively. However, concern was sparked when it was shown that inclusion of DNA coding for IL-4 into ectromelia (mouse pox) virus down-regulated CTL production to such an extent that infection of mice that were normally genetically resistant to ectromelia with this virus caused high mortality. Furthermore, pre-immunisation of these mice was only partly protective.[22] The concern was that this approach, if used by bio-terrorists, could also make the smallpox virus (variola) more lethal for humans.

A recent development is to immunise a host directly with the DNA coding for the antigen of interest, instead of using the antigen itself or the relevant infectious agent.[23] The DNA coding for the antigen(s) is inserted into bacterial plasmids behind a suitable promoter. The chimeric plasmids are then injected intramuscularly, or used in much smaller amounts to coat tiny gold beads which are then injected intradermally using a 'gene gun'. The bacterial DNA component of these plasmids is recognized by receptors on the local dendritic (Langerhan's) cells as being foreign, and this initiates the activation and maturation of the cells. During transit of the cells to the draining lymph nodes, the foreign DNA is transcribed and the protein is processed for presentation to T cells in the node. When tested in mice, there was a surprisingly strong and persistent antibody and CMI response that protected against a challenge infection.

Table 3 Viruses and bacteria commonly used as vectors of DNA (or RNA) from other infectious agents

Viruses
Poxviruses (vaccinia; Ankara strain; New York vaccinia; fowlpox; canarypox)
Other viruses (Adeno,‡ varicella, polio,†,‡ influenza†,‡)
Bacteria
Mycobacterium bovis (Bacille Calmette–Guerin), Salmonella‡ strains

†Would accept small segments of foreign RNA; ‡used for immunisation via a mucosal surface.

Three gene gun injections of DNA coding for malaria parasite antigens to volunteers gave strong T cell responses, and eight of 14 recipients had CTL responses.[24] An improved immune response, for both CTL and antibody levels, has been obtained by adsorbing the chimeric plasmid DNA onto cationic poly (lactide-coglycolide) (PLG) microparticles before injection.[25] The PLG particles are biodegradable and biocompatible. To date, there is no evidence to suggest that immunising with DNA is harmful. For example, the injected DNA does not appear to integrate into the host cell genome which, if it occurred, might result in cancer.

The next surprise was the finding that plasmids containing DNA from an infectious agent were particularly effective at priming the immune system. Immunising (priming) mice first with chimeric plasmids, followed by boosting with a chimeric poxvirus vector containing DNA coding for the same foreign antigen (influenza haemagglutinin), induced unusually high antibody titres. These were as much as 50-fold higher than those seen when two doses of the same construct were administered to mice.[26] This 'prime : boost' approach, as it is now termed, has been applied with success to several of difficult infectious agents in model systems. The main aim has been to generate strong T cell (especially CTL) responses so that a subsequent challenge infection is not prevented but is controlled and possibly cleared. Trials have been carried out in mice and/or monkeys and the list of infectious agents studied in this way includes: (i) HIV-1, (ii) simian immunodeficiency virus, (iii) plasmodia (malaria), (iv) Ebola virus and (v) *M. tuberculosis*.[4] Clinical trials are now in progress.

Currently, several injections are required to administer conjugate vaccines to infants and future prime/boost approaches. The former might be overcome by increasingly combining vaccines. The latter approach is currently being directed to diseases that have such a major impact on human health, especially in developing countries, that, if successful, the benefit is worth the extra effort.

Sequencing the genome of many important bacteria – including Chlamydia and different Mycobacteria, and some parasites such as Plasmodia – should greatly facilitate vaccine development. Structural analysis can indicate which proteins are likely to be membrane bound and partly exposed to the environment. For example, mice immunised with six out of 108 proteins of *S. pneumoniae*, identified from the DNA sequence as having appropriate structural characteristics, were protected from disease when later challenged with this organism.[27] Other proteins will be identified as good sources of different T cell epitopes.

VACCINES TO PREVENT OR CONTROL OTHER DISEASES

An early attempt to use this approach aimed to see whether human fertility could be controlled by immunising females with either a gamete antigen (such as ZP3, one of the antigens expressed on the zona pellucida of the ovum) or a hormone expressed only or mainly during the very early stages after conception. The human chorionic gonadotrophin hormone became the main target.

Both these approaches have worked in animal models however few human trials have been carried out.[28] The areas where there is currently strong interest are: (i) cancer control, (ii) allergic and autoimmune diseases, (iii) drug addiction and (iv) neurodegenerative diseases.

Cancer

From the point of view of vaccine development or immunotherapy, there are two situations: (i) tumours associated with a viral infection and (ii) spontaneous tumours.

Three cancers included in the first category are: (i) primary hepatocellular carcinoma (hepatitis B virus), (ii) genital and squamous cell carcinomas (papilloma viruses) and (iii) Burkitt's lymphoma and nasopharyngeal carcinomas (Epstein–Barr virus). In each case, immunising against viral antigens should prevent tumour development. Early results are promising. Vaccination of infants in Taiwan with the hepatitis B virus vaccine specifically reduced the incidence of the liver cancer in 6–14 year old children by 50% and reduced the incidence of death due to this cancer by 70%.[29] Clinical trials of a candidate vaccine based on the antigen E7 (in the form of virus-like particles) of the papilloma virus type 16 have recently concluded. All 41 cases of new HPV16 infection occurred in the placebo group.[30] Based on this excellent result, a phase III trial is being initiated with a tetravalent vaccine (strains HPV16, 18, 6 and 11). The first two types are responsible for >50% of cervical cancer; the last two types are linked to 90% of genital warts cases.[31] In a clinical trial in China, an aggregate of the L1 antigen of HPV type 6 and HPV type 11 induced strong antibody responses and caused complete regression of genital warts in 22 of 33 subjects.[32] However, there are at least 13 other HPV types that are considered carcinogenic,[33] so much work lies ahead to develop a more broadly protective vaccine. In addition, it now seems likely that vaccine formulations will be forthcoming, which control some of the malignancies caused by Epstein–Barr virus infections such as Burkitt's lymphoma and nasopharyngeal carcinoma.[34]

Control of spontaneous tumours by immunotherapy has proved to be a greater challenge. In 1930, Burnet proposed the concept of 'immunosurveillance' in which he postulated that the immune system would recognize many newly developing malignant cells as foreign, and would destroy them.[35] Tumours that did arise had escaped such recognition by, for example, mutation. Experiments with model systems that were available at the time – such as the 'nude' mouse (having few T cells) – did not support this concept, however it recently became possible to completely inactivate the murine adaptive system. When exposed to carcinogens, such mice develop more tumours than control mice,[36] thus substantiating Burnet's concept. However, this means that the spontaneous tumours that occur naturally are

'immunoselected' to resist an immunotherapeutic attack. Nevertheless, there are some encouraging findings based on developing strong CTL responses to tumour-associated antigens. In small clinical trials in which strong CTL responses to melanoma antigens were induced, partial or complete remissions were achieved in approximately ≤30% of participants.[37,38]

The next task is to discover why the cancers of most trial subjects failed to respond to this intervention, and to devise unique approaches to overcome such resistance. Combining two quite different approaches – such as immunotherapy and antiangiogenesis – could have a marked synergistic effect. Calreticulin (CRT), a transporter of peptides, enhances MHC class 1 expression and has an antiangiogenesis effect. Immunisation of immunocompromised mice with DNA coding for CRT fused to a tumour antigen (E7 protein of human papilloma virus – 16) induced a greater reduction of tumour nodules compared to immunisation with DNA coding for the E7 protein alone.[39]

Autoimmune and allergic diseases

The incidence of autoimmune and allergic diseases in developed countries is increasing. Many immunotherapeutic approaches to control these diseases are currently under trial,[40] and some involve approaches that could be regarded as novel forms of vaccination. For example, in the case of autoimmune diseases, instead of inducing immunity, one approach is to induce specific tolerance to the target antigen by oral administration of the antigen. This approach has worked well in animal models but has been largely unsuccessful in humans. Another unusual approach is to immunise against the amino acid sequences (peptide epitopes) which confer specificity to the T cell receptors that recognize the antigens involved in the main autoimmune diseases. Such approaches are called TCR-based immunotherapy or T cell vaccination.[41] Again, this approach has had some success in model systems.

It was observed that the incidence of autoimmune diseases in ethnic Africans born in Africa was quite low, whereas the level in ethnic Africans born in a developed country was quite high, indicating an environmental effect. One explanation – called the 'hygiene hypothesis' – was that the latter were exposed to fewer natural infections in early life than the former, thus leading to the saying, 'Give us this day our daily germs'.[42] In the absence of such challenges, there could be an imbalance in young children between Th-1 and Th-2 responses, which could be important in later life. Although some autoimmune responses have a Th-1 profile, responses to allergens have a Th-2 profile.

The situation with susceptibility to allergic diseases is becoming clearer. It has been demonstrated that immunisation of BALB/c mice with plasmid DNA containing DNA segments coding for T cell epitopes in mites (*Dermatophagoides pteronyssinus*) induces a Th-1 response. The allergic reaction seen when the immunised mice were later exposed to these allergens was much less than the response of control mice.[43] Non-infectious childhood vaccines, such as diphtheria : acellular pertussis : tetanus (DaPT), preferentially induce a strong antibody response (Th-2), whereas a natural infection induces mainly a Th-1 response. Thus, recovery from natural measles infection reduced childhood incidence of allergic reactions to house dust mites to half that seen in vaccinated children.[44] The neonatal human immune system has a 'Th-2 bias' which slowly changes to a mixed Th-2/Th-1 response. The tetanus-specific cytokine profile was found to be initially Th-2 strong following administration of the DaPT vaccine, however there was increasing IFNγ (a Th-1 cytokine) production over time (≤18 months) in most infants. Those infants who did not switch to the Th-1/Th-2 profile and remained strongly Th-2 biased were found to come from families with a history of allergies.[45] The inoculation of such infants within the first year with one or two vaccines inducing a strong Th-1 response might correct this imbalance and protect from later allergies. A recent retrospective study has shown that children in Europe who live on farms – and are hence exposed to animal stables and farm (non-pasteurized) milk in the first year of life – had a greatly reduced risk of allergic diseases in later life.[46]

Substance addiction

Cocaine abuse is a major medical and social problem world-wide and has reached epidemic proportions in many countries, including the USA. Current methods of treatment that aim to use drugs to block the central neurochemical effects have had limited success in clinical trials and can cause unwanted side-effects. The idea of vaccinating to produce anticocaine antibody to bind free cocaine and so limit or prevent access of the free drug to the brain is attractive, because it should not cause any of the above side-effects. Two recent papers have reported findings that offer some hope that this might be achievable. In one case, a murine model of acute cocaine-induced locomotor activity was used. Mice were immunised with a cocaine derivative/protein conjugate, together with an adjuvant, and challenged several times with systemic cocaine.[47] On each occasion, there was significant reduction of cocaine psychoactive effects. Immunisation of monkeys with a cocaine/protein conjugate also decreased the neurochemical effect of the drug, with a direct relationship between the reduced magnitude of the effect and the serum antibody titre.[48]

Similarly, clinical trials are underway to ascertain whether the antibody formed following vaccination of smokers with nicotine conjugated to cholera toxin induces antibodies that are sufficiently powerful to bind all free nicotine, and so prevent the drug from reaching the brain.[49] It is anticipated that this approach may work best initially for smokers who risk relapsing after quitting smoking.

Neurodegenerative diseases

Alzheimer's disease (AD), Parkinson's disease (PD) and bovine spongiform encephalopathy (BSE) are three neurodegenerative diseases in which changes in a brain protein can be fatal. In AD, a mutant protein, amyloid-β peptide (Aβ42) forms plaques in the brain, resulting in

the loss of mental function. Transgenic mice expressing DNA coding for this protein develop a similar brain pathology and experience loss of memory. Early immunisation of transgenic mice with the protein prevented these changes from occurring;[50] a similar immunisation of the mice after the changes occurred largely 'reversed' the pathological damage.[51] Using a multiphoton microscope, images of the plaques can be seen in the transgenic mouse brains. Direct introduction of a fluorescein-labelled potent Aβ42 antibody into such brains highlighted the deposited protein and showed that it was cleared in approximately 3 days.[52] After testing in a variety of animal models, a phase I clinical trial on patients with mild to moderate AD suggested that vaccination with this protein was safe. A larger trial was suspended recently because of safety concerns.

In PD, a cerebral accumulation of α-synuclein affects motor function. Some patients (the Lewy-body variant of AD) display both cognitive and motor dysfunction, suggesting an interaction between Aβ42 and α-synuclein. To investigate this possibility, mice were made transgenic for: (i) Aβ42, (ii) α-synuclein or (iii) both. Doubly transgenic mice developed motor deficits before the α-synuclein singly transgenic mice, suggesting an interaction between the two proteins.[53]

BSE and scrapie are caused by prions, which are transmissable, pathogenic proteins. The incidence of variant Creutzfeldt–Jacob disease – probably following exposure to BSE – is increasing, particularly in the United Kingdom. It is thought that interaction between the pathogenic prion protein (PrP^{Sc}) and the endogenous cellular prion protein (PrP^{C}) leads to the formation of prions, an infectious form of PrP^{Sc}. Reagents binding to either PrP^{Sc} or PrP^{C} inhibit prion formation.[54] It has now been demonstrated that, when used to treat neuroblastoma cells infected with PrP^{Sc}, antibody fragments specific for PrP^{C} inhibit further PrP^{Sc} formation in an antibody dose-dependent manner.[55] Furthermore, transgenic mice expressing an antiprion protein μ chain developed sustained antiprion antibody titres, which protected against the pathogenesis caused by prions.[56] A practical intervention procedure for humans is a long way off, however these early results are interesting.

CONCLUSIONS

Vaccination, particularly to prevent many common childhood infectious diseases, is one of the most impressive health achievements of the twentieth century. In contrast, making effective vaccines based on preventing infection by antibody against bacteria (such as the tubercule bacillus), parasites (such as plasmodia) and some viruses (HIV-1) has proved to be far more difficult. Our greatly increased ability to manipulate the immune response has now given hope that vaccines can be developed that, by deliberately inducing strong cell-mediated immune responses, will prevent or greatly reduce continuing infection by such agents.

Another remarkable recent development is the increasing application of vaccine technology to the control of 'non-communicable' diseases; ranging from some cancers to different forms of dementia. If only some of these developments are largely successful, this technology will further enhance its reputation over the coming years in the area of public health.

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