

Vaccines, coming of age after 200 years

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Abstract

An overview on the short, only 200 years, past history and future expectations in the field of vaccines is presented. The focus is on development trends and potential rather than individual vaccines. While the first vaccines were a result of keen observation, the further development has been tightly dependent on the development of microbiology to provide both the knowledge basis and the technology for new vaccines for new purposes. The post-genomic era just starting therefore promises an exponential increase of vaccine research and new vaccines, both improved vaccines with a greater efficacy and less adverse effects to replace old ones and vaccines for prevention of diseases for which no vaccines exist. Furthermore, fully new applications to prevention or treatment of chronic diseases not traditionally associated with infections are expected. © 2000 Federation of European Microbiological Societies. Published by Elsevier Science B.V. All rights reserved.

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1. Introduction

One of the most effective and most versatile means to combat infectious diseases is immunization, also called

vaccination in honour of its first successful application. The history of vaccines is thus short. Edward Jenner published his method of preventing smallpox by inoculation with pus from cowpox (containing the virus *Vaccinia*) only

200 years ago, 1798 [1]. (It is true that this vaccination had been preceded, for centuries, with variolation, i.e. inoculation of a small amount of pus from a patient with smallpox, but a fair part of those inoculated developed smallpox and the method was not widely used.) Although vaccination against smallpox was rapidly accepted throughout Europe, it took nearly 100 years before the appearance of the next vaccines: killed attenuated *Pasteurella multocida* for animals in 1880 [2], anthrax for animals in 1881 [3] and rabies vaccine in 1885 [4], all developed by Pasteur, bacterial cell vaccines for typhoid fever (Salmon and Smith in 1886 for animals [5], Pfeiffer and Kolle, and Almroth Wright for humans in 1886), cholera (Kolle 1896) [6] and plague (Haffkine, 1987) [7]. In the following century, the rate of new vaccine development has still been quite modest, bringing in improvements of existing vaccines and extending the number of vaccine-preventable diseases to some 25. What do we expect of the next century? No modesty in the expectations: new vaccines for new applications, entirely new targets, new modes of administration, new ways of development and production all appear possible with the new biotechnology and new research methods, resulting in new understandings of the immunology of vaccination and the pathogenesis of the diseases.

Vaccination against smallpox has another distinction: it is the first and so far only procedure that has fully eliminated/eradicated the disease, with the important consequences that we do not need to worry about the disease and, furthermore, that this vaccine is no more needed, and all smallpox-related costs are saved globally [8]. Under favorable circumstances, a vaccine can indeed be expected to have these results. There are, however, strict constraints to this. The primary prerequisite for disease eradication is that the agent causing the disease only multiplies in man and the eradication goal is deemed important enough to justify the considerable costs and effort in carrying out a comprehensive vaccination program, including specific surveillance to ensure disappearance of the disease and its agent [9]. Polio is now, only about 50 years after the introduction of the vaccine, a good example of a target suitable for eradication and indeed, an eradication program was launched by the World Health Organization with the final goal set to the year 2000 [10]. Despite the remarkable success achieved in virtually eliminating the virus from the Americas and in considerably reducing it everywhere, the goal needs to be pushed forward by several years, but it still seems fully attainable [11].

What will be the next target is still discussed and debated, measles and perhaps rubella being high on the list. In addition to the spectacular success of eradication of the first world-wide scourge, many success stories can be told of a remarkable reduction and local elimination of life-threatening diseases: tetanus, diphtheria, measles, rubella, *Haemophilus influenzae* type b. But we have also seen that a disease believed to be well under control by a general

vaccination program can resurge if the program is relaxed or circumstances otherwise change to favor the spread of infection or reduce the resistance of the population, for example, the recent diphtheria epidemic affecting the countries of the former Soviet Union at the time of the transition [12].

2. Characteristics of vaccination

2.1. Use of the body's own immune defense system

The system of acquired immunity has specifically evolved to defend the body against the onslaught of microbes or bad consequences of the infection. However, it needs to be taught to recognize the bad guys before it can attack them efficiently. In nature, this happens through an infection but with the risk of a fatal outcome. Only if the host wins, it has learned, the hard way, to know this microbe and to be prepared for its attack. The vaccines can do the same but without the risk of the disease. They only teach the cells of the immune system about the possible attack by microbes or their toxins. Thus, the vaccinated individual will produce antibodies to serve as a front line of defense and continue to have expanded clones of B- and different kinds of T-cells that recognize the target microbe and are ready to combat and further to augment the response when the real attack comes. If a long time elapses after vaccination before the attack, the immunological preparations are slowly dismantled but leave a memory that allows for a quick response when needed.

Many microbes have evolved ways to avoid the immune defenses, e.g. by coating their surface with molecules that avoid initiating an immune response to infection or suppressing the T-cell response by downregulating essential intermediary molecules, so that an infection does not lead to immunity.

Vaccines have a possibility of overcoming these problems. A continuing task for vaccine research is to identify them case by case for each pathogen and then develop a vaccine circumventing the particular problem. A recent example of such vaccine development are vaccines against encapsulated bacteria like Hib or the pneumococcus. They are based on the bacterial capsular polysaccharides that are poorly immunogenic in young children but overcome this restriction by conjugating the polysaccharide to a protein [13]. Thus, they use the basic mechanisms of immunity but fool them in a detail. Correspondingly, it is possible to modulate the immune response to favor one or another of the alternative pathways of maturation. For example, the realization of the essential role of a number of cytokines has opened the possibility of altering their balance and thereby strengthening or reducing the activity of the required type of the effector cells. Thus, such research is currently focused on the application of the recently discovered dichotomy of T-helper cells: either a type I activ-

ity, characterized by production of IL-12 and γ -interferon and stimulation of cytotoxic T-killer cells, or type II activity, characterized by IL-4 and IL-10 and promotion of maturation of B-cells and antibodies [14]. Which activity is wanted depends on the microbe to be prevented (type I basically required for intracellular microbes, type II if antibodies are the primary mechanism of immune protection). Although microbes themselves may use the same tricks, directing the immune response to a pathway from which they are protected, vaccines can be designed to avoid this trap.

2.2. Specificity of vaccines

The second characteristic of vaccination is the specificity of the immunity induced. This is of course also dictated by the basic mechanisms of immunology, but very important to realize when designing a vaccine or when informing the public of the benefits of vaccination. Expectedly, both bacteria and viruses take advantage of this high degree of specificity to evade immunity. Even a small change in the target molecule can make it unrecognized by the immune mechanisms. This is well-known as antigenic variation and its importance is underlined by the numerous different mechanisms utilized by the microbes to effect it: the large number of serogroups and types in populations of pathogenic bacteria and viruses, elaborate mechanisms to allow for new serotypes to emerge in any clone even in the course of ongoing infection and finally, mechanisms to turn off or on genes or gene clusters responsible for an antigen. These variations most commonly affect the binding of antibodies (and thus structures exposed to them during infection, e.g. by their location on the surface of the microbe), but can also be of importance to recognition by T-cells. An extreme example is the evasion of immunity by the HIV virus, which, by virtue of its reverse transcriptase, continuously undergoes random variation of its nucleotide sequence at such a rapid rate that the viral population in each infected individual always consists of tens of variants [15].

2.3. Prevention of diseases

One further aspect has up to now been associated with vaccination, namely its preventive action. In this, it would differ in a very basic way from treatment of a disease with drugs and this difference has had important consequences to the development of vaccines. In their prophylactic use, they are administered to a large number of healthy persons, often young infants, to give them protection from diseases as early as possible. This use requires the vaccines to be very safe; mild fever and slight discomfort at the injection site are tolerated, but really serious, even rare, adverse effects not, and the clinical trials required to assure this also make vaccine development slow and expensive. However, vaccines will in the near future no more be

restricted to prevention of infections. Their application together with drugs (or perhaps even alone) to the cure of diseases is very actively studied and promising results have already been obtained. The applications may concern infectious diseases, e.g. the chronic infections of hepatitis B or C viruses (HBV, HCV), HIV, leishmaniasis and even tuberculosis (in which some bacteria may remain dormant for decades in the macrophages at the primary infection site). What is expected of the new vaccines is improvement over the normal infection, induced immunity to specifically circumvent the means by which the pathogens evade it. However, applications are also planned to harness immune mechanisms to attack cancer cells or, on the other hand, to downregulate the immune mechanisms that have gone astray and are attacking the host's components (autoimmunity) or leading to exaggerated inflammation (asthma).

3. The vaccines by the end of the 20th century

The development of vaccines has been determined on one hand by their need, e.g. by the severity and incidence of the disease to be prevented, and, on the other hand, by the state of knowledge and technology at the time. Thus, the first vaccines, vaccinia, anthrax and rabies, were primarily a result of keen observation and experimentation, whereas the burst of the next ones at the end of the 19th century already benefitted from the development of immunological theory, antibodies by Behring in 1890 [16], the antitoxin concept by Ehrlich at the same time, cell-mediated immunity being understood much later, and of the bacteriological technology, especially the use of solid media to obtain pure cultures of bacteria (Koch, 1881) [17]. When methods were learned to grow viruses in embryonated eggs [18] and later in cell cultures [19], development of killed viral vaccines became possible. These techniques were applied to killed vaccines against influenza and polio, as well as to the development of several attenuated vaccines. The revolution of microbiology through the genetic and molecular approaches starting in the 1950s and in full blow since the 1980s produced the first recombinant vaccine for human use, the hepatitis B vaccine, in 1984 [20], and a burst of research, promising a great deal in the next decades.

3.1. Live, attenuated vaccines

The two basic types of current vaccines, presenting the microbe in an attenuated but live form or as an inactivated, killed vaccine, were included already among the first vaccines. The attenuation was achieved by the use of a related virus (the cowpox) or, more generally, by propagation of the microbe under conditions different from those in the infected host and hopefully unfavorable to its growth. Obviously, this method could not guarantee

attenuation in the hoped for way, not too little for safety, but not too much for efficacy. It is in fact amazing that the first attenuated vaccines worked. Later on, the technologies have of course been improved and elaborate tests carried out to ensure, as far as possible, safety and efficacy, but the safety still remains an issue. Largely because of this problem, the vaccinia vaccination was stopped very quickly after it had become clear that smallpox was indeed eradicated.

A more rational approach to attenuation would be targeted to microbial components and genes known to be important for their virulence. An attenuated typhoid fever vaccine was developed on this principle, aiming at a conditional elimination of the bacterial O antigen (outermost part of the cell surface lipopolysaccharide (LPS) molecule), which resulted in good attenuation of the related *Salmonella typhimurium* in the mouse model of salmonellosis [21]. The *galE* mutant *Salmonella typhi* isolated had, as expected, lost its O antigen (unless provided with external galactose), was found attenuated for humans and developed into a vaccine licensed in many countries [22]. However, subsequent work has shown that a *galE* mutation and conditional loss of O antigen does not sufficiently attenuate *S. typhi* and the vaccine strain Ty21a contained a separate, initially unrecognized mutation which made it avirulent [23]. Despite of this warning example of targeted attenuation, careful analysis of genes needed for the virulence of the microbe will allow for very safe attenuated strains to be developed. The targeted gene should not only be mutated but fully deleted and multiple pathways blocked to safeguard against accidental acquisition of the corresponding functional DNA segment from other bacteria in the normal flora of the site where the vaccine is applied. Cholera vaccines based on this principle are in an advanced stage of clinical development and many others following [24].

3.2. Killed, inactivated microbes as vaccines

The inactivated bacterial vaccines were welcome at the time when they were developed in the 1890s and there were no other means to prevent or treat the serious diseases. This was especially the case with typhoid fever, which was rampant all over the world. The same principle was applied to develop vaccines for other bacterial diarrheas, whooping cough, meningococcal disease, etc., but the successes were less. The problems with the killed vaccines concern both their safety and efficacy. The efficacy of some of them could never be proven (e.g. cholera, meningococcal vaccines) and promising a great deal is even for the best of them not as high as hoped for. The protection is rather short-lived and adverse reactions, largely due to the endotoxin in the bacteria, are common. Only the killed typhoid and pertussis vaccines have survived in a fairly wide use up to this time and for both of them, there are now improved vaccines. The record of inactivated viral

vaccines is better, but not as good for the efficacy as of the attenuated vaccines. An interesting development, of which we may see more in the future, is the mixed use of attenuated (more effective, less safe) and inactivated (less effective, extremely safe) polio vaccine. When the vaccination of a child is started with the inactivated vaccine, the immunity developed is expected to protect from the potential rare ill effects of the attenuated vaccine, which in turn is expected to ensure more long-lasting immunity [25].

3.3. Purified microbial component vaccines

A third type of vaccines introduced as vaccines against diphtheria and tetanus in the 1920s is a much more sophisticated product, a purified bacterial component. In both these cases, it is a protein toxin previously demonstrated to be an essential cause of the disease. For the vaccine, the toxin is chemically modified to yield the non-toxic toxoid. Both these vaccines have performed extremely well in terms of both safety and efficacy, demonstrating that the theory behind them was correct.

Purified single component vaccines have many attractions: the immune stimulus is maximally directed to the molecule relevant for protection and additional components that could cause adverse reactions or other, unwanted but unknown problems are avoided. However, it may often not be possible to identify such an essential component to which protective immunity should be directed and many pathogens would have evolved extensive antigenic variation of such a component or the component may not be immunogenic. The surface polysaccharides of encapsulated bacteria are a case to demonstrate these latter points. For both meningococci and pneumococci, it was shown in patients and in experimental animals that antiserum to the capsular polysaccharide could cure the disease and the effect was specific to the capsular serogroup/type [26,27]. Thus, the several antigenic types (altogether 90 in the pneumococcus, only three major ones in the meningococcus) had to be taken into account when planning a vaccine based on the capsule.

The polysaccharides are easy to purify in quantity and safe when injected in experimental animals as well as humans. The first efficacy trials with group A and C polysaccharides in military recruits were promising [28,29], whereas a trial in Africa showed that the polysaccharide vaccine was unstable at the temperatures prevailing there, a problem that could be easily remedied by refrigeration or use of lyophilized vaccine [30]. However, two major problems appeared: the group B polysaccharide was not immunogenic at all [31] and the group C polysaccharide poorly so in infancy up to the age of 2 years [32]. The inability of group B polysaccharide to immunize is most probably due to immunological self-tolerance. The polysaccharide is identical in structure to polysaccharide chains of the protein N-CAM, a cell surface molecule of

actively growing neural cells in man and several animal species [33,34]. This is in fact a very good example of molecular mimicry to evade the immune defenses of the host.

The inability to induce an immune response in infants is a common characteristic of many, but not all, polysaccharides. It is clearly of advantage to the bacteria: those serogroups/types that have this kind of polysaccharide capsule are 'pediatric types', especially common among infants and young children compared to adults (e.g. *H. influenzae* type b, pneumococci of serogroups 6, 19 and 23). The gap in our immune system that is responsible for the inability to respond to these pediatric polysaccharide types is associated with the general inability of all polysaccharides to use T-cell help (T-cells basically recognize short peptides presented in association with the major histocompatibility complex (MHC) molecules on the surface of other, antigen presenting cells). Instead, the polysaccharides can directly stimulate B-cells for activation, multiplication and antibody production, because of which they are called TI, T-cell independent, antigens. What is special of the pediatric polysaccharide types is that only mature B-cells, which develop only slowly in the first years of life, can be stimulated by polysaccharide [35]. Further development of vaccines for the pediatric type polysaccharides continued. Initially, it was hoped that the problem could be overcome by immunological adjuvants, e.g. the generally fairly good adjuvant pertussis vaccine, but this did not help [36].

The answer came by deliberately turning the polysaccharides to a form that the immune system handles as proteins capable of reacting with T-cells; this was done by chemical conjugation to a protein antigen. Such conjugate vaccines were first prepared and studied with *H. influenzae* type b and proved to be a great success: they could immunize infants starting in the first weeks of life, they behaved like the carrier protein by inducing a T-cell dependent (TD) type of response characterized by increased responses to further doses of vaccine based on immunological memory and by predominance of IgG class antibodies and they prevented not only the serious invasive infections (meningitis, epiglottitis, etc.) caused by *H. influenzae* type b but also its colonization in the upper respiratory tract [13]. This in turn reduced the chances of infection and thus protected even children who had not been vaccinated, thus causing herd immunity [13].

As a result, *H. influenzae* type b disease has been practically eliminated in many countries of the western world and the World Health Organization is recommending expanded use of the vaccine in other parts of the world. However, here, one more problem arises: the vaccine is, because of the multistep procedure of production, more expensive than the basic vaccines now used world-wide and expensive in relation to the health sector budget in poor countries. Therefore, precise knowledge of the incidence of the infection and the cost effectiveness of vacci-

nation would be needed to justify the expenditures, but such epidemiological information is generally lacking in developing countries.

Encouraged by the success of the *H. influenzae* type b vaccine, the same conjugate technology has been applied to the pneumococcal polysaccharides, selecting in the cocktail polysaccharides of the types most commonly causing disease. These include pediatric types that are common all over the world and a few types that are much more common in developing countries than elsewhere [37]. The diseases targeted by infant immunization include bacteremia, pneumonia (the most important cause of infant death in developing countries) and otitis media (very common in the industrialized world). The vaccine is currently tested in efficacy trials for all these targets and the first trial in California has shown an extremely good protection from bacteremia [38].

3.4. Recombinant vaccines

Microbes that cannot be grown or can be grown only with difficulty in vitro pose a special problem to vaccine development. These include several viruses (HBV, HCV the most important), bacteria like *Mycobacterium leprae*, and to a degree *Chlamydia pneumoniae*, and many parasites, of which the *Plasmodia* causing malaria are most important in this context. A real breakthrough for vaccines against them was the genetic technology that allows for their genes to be transferred to and expressed as 'recombinant' products in easily grown organisms, *Escherichia coli*, *Bacillus subtilis* and yeast. This possibility found a rapid application in the production of a recombinant HBV vaccine. The surface protein of HBV was produced in yeast cells and, when purified, formed ball-shaped particles that looked like the viral particles found in the plasma of infected individuals. The candidate vaccine prepared from these particles was shown to elicit antibodies that reacted with those viral particles and to protect from infection [20].

The advantages of the possibility of producing vaccine antigens in a selected heterologous host organism are many, not only ease of growing the host but also the possibility to upregulate the production of the recombinant protein and concomitant ease of purification. Furthermore, the absence of other components of the pathogen makes the recombinant proteins useful as antigen in immunoassays. On the other hand, the recombinant product may not assume in the foreign host its native conformation, which is often necessary for the induction of protective antibodies. Furthermore, if post-translational modifications, e.g. glycosylation, are needed for the native antigenicity, conformation or stability in the vaccinated individuals, the selection of the host is restricted. Thus, most bacterial proteins are not glycosylated and can be produced in other bacteria, whereas viral proteins are as a rule glycosylated according to the scheme of their human

host cells and the recombinant products either remain without the glycosyl tags (when grown in bacteria) or are glycosylated according to the patterns of yeast or the insect cells sometimes used to avoid the lack of glycosylation in bacteria.

3.5. Adjuvants and vaccine formulation

It was learned early on that proteins presented in solution were less immunogenic than proteins presented in the particulate form. On this basis, the soluble toxoids of diphtheria and tetanus were absorbed to calcium phosphate (mainly used in France) [39] or to an alum gel (constituted from aluminum hydroxide or phosphate) to act as an adjuvant, i.e. to improve the immune response to the vaccine antigens. The alum gel has up to now been the only adjuvant generally approved for use in human vaccines despite much research towards other, more potent but safe adjuvants. Many lipid mixtures and bacterial components, e.g. LPS, muramyl dipeptide from bacterial cell walls and killed mycobacterial cells, have been and continue being used as adjuvants in experimental animal research, but many cause local or general adverse reactions and have, because of the very strict safety requirements of vaccines, not been licensed for human use. A 'virosome' adjuvant-containing vaccine against hepatitis A has, however, been licensed recently. The adjuvant consists of liposomes containing the glycoproteins hemagglutinin and neuraminidase from influenza virus [40]. At the same time, the mechanisms by which the alum adjuvant affects the immune response are still not fully understood, although the bias of the response associated with its use to increase maturation of antibodies suggests that it favors the type II helper response (indeed, it may inhibit the generation of cytotoxic responses [41,42]).

While the adjuvant may increase the expected immune response, stability of the antigen from manufacturer to the end user is essential for the activity of the vaccine. This is not a trivial point, considering that most vaccines in the world are used in tropical conditions in developing countries. The World Health Organization has done a lot of work with its EPI (Expanded Programme of Immunization) to persuade all countries to establish a vaccine delivery system, including refrigerated storage and cold transport, that would assure that vaccines given to infants in the most remote villages are still potent. Still, the more heat-labile live virus vaccines (polio and measles in this case) are in a danger zone when the cold chain fails or is not properly observed. The challenge to vaccine development to improve the stability of the vaccines is well-recognized but not easily met.

3.6. Route of administration

The basic means of getting the vaccine antigen in contact with the immune system is by injection and still all the

commonly used vaccines with one exception use this route. The requirement of sterile syringes and needles is a problem in developing countries and a subject of emphasis in the EPI's efforts. The pain of the injections is minor, but nevertheless a consideration especially in connection of the many vaccines given multiple times in infancy. The simple way to reduce the number of injections is to combine several antigens in one vaccine. Thus, the combinations of diphtheria, tetanus and pertussis vaccines and of the live measles, rubella and mumps vaccines are the vaccine forms generally used. However, further combinations, of which several are also on the market, raise questions of possible reduced immune responses when multiple antigens are competing for the machinery and also of expense. Each new combination will have to undergo extensive clinical trials, resulting in more expensive products. This becomes a serious concern as all children in the world, even, and especially, the poorest, should be vaccinated.

The exception pointed at above is the live polio vaccine, given by mouth. Polio virus multiplies in the intestinal epithelium and thus this is the natural way for also the attenuated form to gain access to the body. It is believed to have the additional advantage of engaging the immune system of the intestinal mucosa, the natural first line of immune defense, and thus giving a more effective protection than injected vaccine. However, increasing attention is being devoted to the fact that most infections are naturally acquired through the mucosae, either in the respiratory tract, the intestines or the genital tract, and therefore, local mucosal defenses might be the most effective. The mucosae in fact have a highly developed immune surveillance system in which specialized cells (called M-cells in the intestine) sample the antigens flooding them and mediate them to antigen presenting dendritic cells and the lymphocytes under them. How all the food components and the resident microflora avoid getting into this pathway of immune stimulation is not known. Self-tolerance, called here 'oral tolerance', is one explanation, but what decides which pathway is taken, an immune response or tolerance development, is not understood [43].

Many antigens are known to have a special predilection to the M-cells. These include the enterotoxins of *Vibrio cholerae* and *E. coli* (CT and LT respectively) and several bacteria (*Vibrio cholerae*, *Salmonella*, *Yersinia*, *Shigella*) and some viruses (most notably polio). Efforts at developing vaccines to be given by the mucosal route (avoiding injections) to induce immunity at the mucosa take advantage of this affinity. The vaccine antigen given together with CT or LT, or even with their M-cell-binding component without the toxic part of the molecule, gives strong mucosal immune responses [44]. Attenuated *Salmonella* vaccines do the same, both in respect of the native *Salmonella* antigens and eventual foreign antigens whose genes have been inserted in the *Salmonella* [45,46].

3.7. Vaccination schedules

In order to provide protection as early in life as possible, most vaccines are administered in infancy, starting at 6–8 weeks of age. This is a compromise since at this early age, the immune system is not fully developed and especially the antibody responses are not as good as later in life. However, the T-cell dependent immunological memory mechanisms operate rather well already at birth and giving the infants a second and third dose as the 'primary course of vaccination' builds on to this memory. Some problems remain: the inability of the infants to respond to T-cell independent polysaccharide antigens, now circumvented by the protein-polysaccharide conjugate vaccines as described above, and the sensitivity of the live measles vaccine to antibodies that the newborn has received transplacentally from the mother. Because of the latter, the measles vaccine cannot be given as early as would be needed to prevent the disease especially in developing countries. Again, a compromise has been made by giving the vaccine at 9 months of age in areas with still a lot of measles and at 14–18 months in areas where measles is rare (because of efficient vaccination).

Building up immunological memory is essential for all vaccination schedules. Therefore, children who received their primary course in infancy are usually given booster doses, the timing of which is determined by the risk of infection in the area and the estimated rate of decline of immunity. In areas with a high prevalence of the disease, it may be expected that the child is likely to meet the infecting agent often enough to booster the memory while still immune to the disease. Because long-lasting memory is best established and boosted by repeated encounters with the antigen, most vaccines, even when given to adults, are given as two or three doses with several weeks or months in between. Some live vaccines that are very potent inducers of T-cells and memory do not require the boosters. And again, the polysaccharide vaccines are an exception because they cannot establish T-cell dependent memory.

4. What do we expect in the next decades?

A general answer to this question is: a revolution in vaccine technology, changing most aspects of vaccination and extending its application to fully novel targets. The essential basis for this revolution is the new biotechnology that makes it possible to handle, sequence and transfer genes almost at will and, on the other hand, the deeper knowledge of mechanisms of pathogenesis of the microbes and of the immune defenses of our own bodies. Furthermore, the fact that the entire genomes of most viral and bacterial pathogens are already sequenced (and even that of the malaria plasmodium is close) will enormously facil-

itate the task of identifying the best antigens to be included in the vaccines.

4.1. A new generation of vaccines

Most likely, the vaccines based on the inactivated microbe principle will disappear at least for bacterial vaccines and will be replaced with more efficacious vaccines causing less adverse reactions. The attenuated vaccines may remain, at least for quite some time because of their generally high efficacy. In fact, alternative methods to stimulate long-lasting, efficacious immunity, based on cytotoxic cells, required for many intracellular pathogens, have not yet been identified despite of intensive research efforts. New live vaccines will be designed on the basis of knowledge of the genome sequence and the virulence determinants of the pathogens to maximize their safety.

Most of the new vaccines will contain a few selected antigens of the pathogen. Thus, antigens that are irrelevant from the point of view of protection are left out, allowing for a more focussed response to the target antigens. The vaccines are likely to contain a few antigens rather than one in order to avoid resistance based on variation of the target antigen and rare gaps in the immune response repertoire of the human population. How these antigens are presented cannot be predicted now because of the many possibilities associated with different advantages and drawbacks. The basic possibilities include presentation as purified protein (or protein-polysaccharide conjugate), as a peptide mixture or as nucleic acid expected to initiate the transcription and translation of the gene product in the host. The nucleic acid in turn can be presented as naked nucleic acid or as part of a vector, i.e. a virus or bacterium which itself is capable of limited replication as well as expression of the intended antigen. Proteins as such are good at stimulating antibodies and a type II helper immunity. Their advantages include long experience of the technology of vaccine production as well as of their use as human vaccines. There is much less experience of the use of peptides, but they might be a rational choice when one wishes to specifically stimulate an immune response consisting of cytotoxic CD8+ T-cells by selecting the vaccine peptides that bind to the MHC class I molecules, one can assure that the stimulation is directed to the CD8+ T-cells. In some cases, stimulation of CD4+ T-cells, which takes place via MHC class II molecules, would cause extensive inflammation at the site of infection (when the vaccinated individual meets the pathogen) and exacerbate it. Omitting peptides binding to the class II molecules would be a way to avoid this. The peptides could be fully synthetic, an advantage for the production of the vaccine. On the other hand, a fairly large number of peptides for each wanted antigen would be required to be included in the vaccine because of the variety in alleles of MHC class I molecules (HLA-types)

present in the human population. Furthermore, at present, peptide immunization is considered fairly inefficient, requiring a good adjuvant, but this should not be taken as information totally killing the peptide vaccine approach before more specific research also in humans.

The naked nucleic acid-based approach is the newest and almost no experience in humans is yet available [47,48]. The principle is beautifully simple, but many details are so far unknown. The gene encoding the vaccine antigen is inserted between appropriate eukaryotic promoter and end of reading sequences in a DNA plasmid that can be replicated in a bacterial but not in a human host. The plasmid DNA is purified from the *E. coli* culture and injected as such usually intramuscularly (but other applications are also possible). The DNA is taken up by the muscle cells and enters the nucleus where the antigen gene is transcribed, the mRNA transported to the cytoplasm and translated. The approach has many attractions. The antigen is produced in the cells of the vaccinated individual and thus directly introduced into the MHC class I antigen presentation pathway for stimulation of cytotoxic CD8+ T-cells. In this, the vaccination resembles that with live virus vaccines, which represent so far the best way to stimulate cytotoxicity. The antigen would also be glycosylated by the host cell machinery and thus acquire the glycosylation pattern of the native virus that has multiplied in a human host. The plasmid DNA furthermore provides internal adjuvant action through its 'immunostimulating sequences', i.e. unmethylated CpG [49]. Although the plasmid does not replicate in the animal host cells, it nevertheless persists for weeks or months and provides continuous production of antigen. The DNA vaccines would be easy to produce, with the advantage that essentially the same production technology could be applied to many vaccines. And, of course, the whole process, up to protection from disease, works in mice with many different pathogens.

What are the problems, then? First, the total lack of experience with this approach in humans and the still fragmentary understanding of how and by which cells the plasmid is taken up and whether and how professional antigen presenting cells, especially the dendritic cells, are involved. The latter could be of importance to the cytokine environment if the antigen is presented by the first, e.g. muscle, cells producing it and consequently to the qualitative and quantitative aspects of the immunity produced. Theoretical worries about the foreign DNA interacting with the host's genomic DNA are probably not founded because of a lack of sequence homologies. The amount of DNA needed to immunize a mouse is quite high and preliminary experience suggests that it will be much higher still for man. Nevertheless, so much effort is being put in this approach that the picture will surely clarify and change in the next few years. At the moment, the big question seems to be whether this is a viable approach for human vaccines or not. However, apart from

these questions, DNA vaccines have already proven to be important tools in vaccine research, making it possible to look for protective antigens starting with a large number of genes, eventually representing the whole genome, of the target microbe.

The insertion of the genes encoding the wanted antigens into a live virus or bacterial vaccine as a vector to take those genes more reliably and controllably into the host can be seen as a modification of the naked DNA approach, although research on the vector approach is older. Attenuated viral and bacterial vaccines (or candidate vaccines, not yet in use for their primary purpose) have been used with success in animal experiments [45,46,50,51]. Whether or not the recombinant vector vaccines would serve a dual purpose, immunizing against both the microbe from which the vector was developed and the introduced neo-antigen would be a question of convenience and need. A possible drawback to the use of this approach to a broad range of vaccine antigens arises from immunity to the vector that would at least reduce and in the worst case abolish the immunizing potential of the neo-antigen that depends on the replication of the vector. Vectors that look promising include vaccinia virus further attenuated to get rid of the serious adverse events rarely associated with its use in smallpox vaccination (a potential problem is that the older generation is still immune to vaccinia, the vaccinations having been stopped in the 1980s) [50], *S. typhi* or *typhimurium* [45,46] and BCG [52], but many others are being tested in animal experiments. It is expected that some of these will enter human clinical trials very shortly and will produce essential knowledge required for selecting the most promising one for further development.

4.2. Profiling the immune response

The application of the broad arsenal of molecular biology to pathogenesis of infections has shown a much wider variation in the role of the immune response in both their natural course and protection from them than ever expected. Both the quality and the quantity of the immune response are important determinants of the survival of the infecting agent as well as of the damage caused by the disease process. Likewise, detailed knowledge of these factors is important for our ability to prevent the infecting agent from establishing disease and also to prevent the associated damage. Initiation of the specific immune response requires recognition of the free antigen (by the immunoglobulin on B-cells) or the processed antigen presented as peptides bound to MHC class I or II on antigen presenting cells (by T-cells) and stimulation by the chemokines and cytokines in turn stimulated by the microbes. These cytokines also determine to a large extent how the lymphocytes mature and produce both effector and memory cells. Learning to know these interrelationships is expected to allow us to manipulate the response by provid-

ing the wanted cocktail of co-stimulants. How this will be done in practice is still largely open, but possibilities are many, from the use of microbial components (once we know which cascade of cytokines they stimulate) to the addition of specific cytokine genes to a DNA vaccine. It still remains to know what the characteristics are of the response associated with protection but not causing excessive inflammation or other damage. This will be important both for the design of the new generation of precisely targeted vaccines but also for the possibility of testing vaccine candidates without extensive protection studies and assuring quality of the final products.

4.3. Fewer or no injections?

Administering the vaccines by the mucosal route, orally or as a nasal spray, or through the intact skin as is done with many drugs, would be the most user friendly way of vaccination. At least the number of injections would not frighten anyone away or restrict the number of diseases targeted by vaccines. This possibility seems quite realistic but cannot be effected just now because the basic rules of the game are not known. In fact, many years of work will be needed to know how long the protection elicited lasts. There is also the possibility of inducing tolerance to the vaccine antigens, a very serious handicap until we know how to avoid it.

What the optimal formulations, adjuvants, doses and schedules are needs to be worked out, with specific data on different types of antigens and immune responses wanted. The idea to produce vaccine antigens as protein components of edible plants and use these as oral vaccines sounds attractive: the production in transgenic plants is indeed feasible [53] and immune responses have been obtained in mice fed the plant [54]. However, it is hard to believe in it as a realistic goal just because in vaccination, much more than the antigen needs to be controlled. It is not justified by the ease of mass production either since the amounts of protein antigen in a vaccine dose are relatively small.

Could we reduce the number of doses for any vaccine, injectable or not? This has been set as a target of vaccine development by the World Health Organization, which is concerned of the cost of administering the multiple doses and of the fact that compliance with the schemes requiring many health service contacts is hard to maintain. Acknowledging the need to induce memory and the fact that multiple doses are a way to ensure it, the approach selected has been to construct a single dose vaccine of compartments from which the antigen would be liberated at the wanted intervals [55]. Microencapsulation technology seems able to do this, theoretically, since microspheres consisting of polymers (e.g. polylactide/polyglycolides) are degraded in the body at different rates with release of the entrapped antigen [56]. Practically, there have been problems: the release of antigen did not take

place as distinct peaks but rather continuously (and we do not know how this will affect the stimulation of memory); the protein antigen in the vaccine was itself degraded in the body after injection (rather to be expected, considering that few vaccines would be stable at 37°C) [57].

4.4. Improved vaccines to replace current ones?

This will certainly happen. Improved properties looked for are greater safety, fewer, even minor adverse effects, a better defined product (few identified antigens for each microbe), higher efficacy, improved stability, fewer or no injections. From a public health point of view, a cheaper price would be an important goal but the opposite is likely to happen, because of the high development costs of new vaccines. Examples of this are already there, with both old and new vaccines in use: a pertussis vaccine based on 1–5 isolated components [58–60] rather than whole bacteria, a recombinant HBV [20] vaccine rather than one isolated from human plasma, capsular polysaccharide (Vi) [61] vaccine or attenuated live vaccine [23] against typhoid fever rather than whole killed cell vaccine.

An improved, more efficacious, vaccine against tuberculosis is currently the target of a large research effort. Improved influenza vaccines that would provide longer lasting protection and a better protection than the current ones in the elderly population as well as in infancy would be of importance, especially in face of a possible new influenza pandemic. One approach to such a vaccine is live attenuated vaccine to be administered as a nasal spray. Such vaccines can be easily developed by reassortment of genome segments in cells infected with a parent attenuated virus and a new virulent virus of whichever viral variant wanted [62].

4.5. New infectious disease targets

This is clearly the improvement most expected from vaccine research. There are still a large number of infectious diseases that are serious or common enough to make prevention by vaccination a worthwhile goal. The foremost infections in need of a vaccine include malaria, dengue and HIV. The infectious cycle of malaria is complicated and the role of immunity at the different phases is, despite of intensive research, poorly understood. The plasmodium also seems to interfere with the immune response, a fact not making vaccine development any easier. Some candidate vaccines have been considered promising enough to be tested in clinical trials, but all have been disappointing in the end. A truly new approach seems to be needed or will malaria turn out to be a disease that has overridden the possibilities of human immunity? Dengue is also a widespread and complex disease spread by mosquitoes. No vaccine is available but so far the disease has not been high in the priority list of research to be funded. By

contrast, huge amounts of money and manpower have been spent on the quest for an HIV vaccine. The virus indeed seems to use and fool the immune system to the extent that one truly wonders whether a vaccine will be possible at all.

Much less serious but very common infections, the prevention of which would improve the wellbeing of the most of us and most probably also reduce health expenditures, include a number of respiratory infections, the common cold as the foremost, bronchitis, sinusitis and otitis media (in young children) closely following. Globally, diarrhea continues to be a serious problem especially for young children. The prevention of the sexually transmitted bacterial diseases gonorrhoea and *Chlamydia trachomatis* infection would probably also be high on the list of vaccines. However, these are not easy targets for vaccine development because of the variety of infectious agents and so far rather poor general success in prevention of mucosal infections. The possibility of vaccination would furthermore be welcome against urinary tract infections, against borreliosis, herpes simplex virus and *Staphylococcus aureus*. A vaccine for group A streptococci has been long on the list of vaccines needed, but with little progress, at least partly due to fears of causing autoimmunity to proteins in the heart [63].

4.6. Novel targets

This area will move the vaccines outside their primary definition as tools in the combat of infectious disease. The use of the human immune system remains the common characteristic of the new and the traditional uses. The new uses include vaccines to prevent or cure infections not primarily because of the infection itself (which may be very mild) but because of its serious sequelae. The HBV vaccine may be an early example here, its use motivated as prevention of the liver cancer developing years later in a part of the infected individuals [64]. The HCV is also a cause of liver cancer and in need of a preventive vaccine. The current example is *Helicobacter pylori*, which infects, often without symptoms, the mucosal lining of the stomach of a large fraction of the entire population of the globe, but causes in some of them ulcer and gastric carcinoma [65,66]. A vaccine would be most welcome and research towards it is approaching the clinical trials state. Theoretically, there are many problems, first and foremost how the effectors of the immunity would function in the difficult environment of the stomach. A vaccine against the human papillomavirus serotype 16 causing cancer of the uterine cervix is likewise approaching clinical trials. Such a vaccine could not have been developed without modern biotechnology since the virus cannot be grown in vitro nor isolated in quantity from infected individuals. Now, the genes of the viral capsid proteins can be inserted in naked DNA or vaccinia vectors to be used as vaccine or

used to produce virus-like particles in yeast or insect cells [67–69]. Other diseases in which the connection to the infectious agent is not as firmly established are juvenile diabetes, associated with preceding enterovirus infection inducing antibodies to the cells of the pancreas responsible for insulin synthesis [70], and coronary heart disease (myocardial infarction) associated with *C. pneumoniae* [71]. Vaccination to prevent these chronic, serious and common diseases would open up an entirely new dimension in their treatment.

The use of immunity to treat cancer, not only cancer associated with an infectious agent like those discussed above but basically any cancer, means an even further extension of the vaccination concept. However, surveillance and early action against cancerous cells is one of the basic functions of the immune system and thus enhancing its activity to conquer even established tumors is a very proper function of vaccination. But, how to do this since obviously the tumor was established despite of the immune vigilance? Tumors are likely to have at least some differences from healthy cells, e.g. to the virus that caused the tumor or peptides corresponding to the mutated oncogene [72]. On the other hand, tumor cells may evade the immune response by downregulating their surface MHC molecules or part of their antigen processing machinery [73,74]. Several approaches have been used in the research, aiming at inducing cytotoxic cell-based immunity to the tumor. These include immunization of inactivated tumor cells of either the patient or pooled from several donors, modifying them by introduction of certain cytokine genes (e.g. IL-2 or IL-12) or by treating them with γ -interferon to upregulate their surface MHC molecules, as well as many others [75–78].

A further, exciting new possibility being explored is the use of vaccines to prevent disease due to autoimmune or hyperreactive immunity. Such diseases are common, chronic and without satisfactory treatment. Asthma may be taken as an example. It is characterized by a hyperreactive response to many antigens based on IgE antibodies that represent an extreme maturation of a normal antibody response. Knowing now that such antibody maturation is driven by T-cell help of the type II variety, a logical approach would be to find ways to shift the reactivity towards type I. The allergic tendency is inherited, which may say that some individuals have a tendency to react with a type II response to common antigens, which in others would induce a more balanced response. Could we change this? Both types of responses are self-perpetuating, i.e. the production of γ -interferon through the type I mechanisms will drive new T-cells towards this type and vice versa. Thus, a simplistic approach might be to provide, e.g., IL-12 as part of the usual infant vaccines as a type I adjuvant or to find a general adjuvant that would favor this pathway. However, these are so far only speculations far from reality.

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