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Old and new vaccine approaches

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Abstract

The conventional, currently available vaccines, though quite successful, suffer from a few shortcomings which hamper future vaccine development. We present herewith some of the new approaches that are presently being pursued, including (1) the development of recombinant, or genetically engineered, vaccines which are based either on the expression of the relevant protective antigen and its formulation into vaccine, or the production of live vaccines, where an appropriate live vector (virus or bacterium) presents the foreign antigen. (2) The development of naked DNA vaccines that include the gene(s) coding for the relevant protective antigen(s). (3) Peptide vaccines that include defined B cell and T cell epitopes, either in a chemically synthesized molecule or in a synthetic recombinant construct. The efficacy of such vaccines is usually dependent on adequate presentation and delivery, namely, carrier/adjuvant technology. (4) Therapeutic vaccines, based on all of the above approaches, may be applied for chronic or long-term infections, or for noninfectious diseases including autoimmune diseases, various neurological disorders, allergy and cancer.

Keywords: Recombinant vaccines; DNA vaccines; Peptide vaccines; Therapeutic vaccines

1. Introduction

The development of vaccines has been one of the most important contributions of immunology to medicine and public health. It was initiated about 200 years ago by Jenner, with the inoculation of the fortuitously cross-protective cowpox discharge, for prevention of smallpox infection. The major breakthrough, occurring a hundred years later, was the preparation by Pasteur, of rabies vaccine, which is based on the intentional attenuation of the pathogen. This paved the way for the development of a whole series of viral vaccines. These ''old'' or conventional vaccines are based on the entire disease-causing microbial agent and consist of the killed or live attenuated organism that does not lead to infection but is capable of inducing protective immunity. They include also the detoxified toxins of some toxin-secreting bacteria, which are effective in preventing the pathology of the bacterial infection. The existing conventional vaccines have been instrumental in either the eradication or drastically diminished incidence and morbidity of a large number of infectious diseases including major killers such as smallpox, polio and diphtheria.

Notwithstanding these tremendous achievements, there are several crucial drawbacks incurred by the

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current procedures for vaccine preparation. For example: (1) The difficulty in preparation of sufficient material for vaccine production in case of some viruses which cannot be cultivated in vitro, such as HBV, or in the case of parasites. (2) Safety considerations—the difficulty of ascertaining adequate killing or attenuation of the vaccine preparation, and the hazard which may be caused by exposure of both the vaccinees and those involved in vaccine production. This consideration is of particular consequences in case of fatal incurable diseases such as AIDS. (3) The genetic variations in viruses, or the frequent recurring variations in the antigenic components of parasites, which result in the evolution of new strains with different serological specificity, for which continuous development of new vaccines is obligatory.

For these reasons and others, new approaches are being considered for vaccine development, which are *not* based on the entire organism. These include (1) the use of recombinant DNA technology for the production of relevant microbial protective protein antigens in bacterial, yeast, plant or animal cells, for vaccine preparation. (2) The use of recombinant DNA techniques for production of live vaccines by introducing the relevant gene(s) into the genome of an adequate vector such as vaccinia virus or *Salmonella* mutants. (3) The use of naked DNA vaccines, consisting of plasmid DNA into which the relevant gene(s) of the microbial agent has been inserted. (4) The utilization of synthetic peptides which constitute the relevant protective epitopes of viruses, bacterial toxins or parasites, for eliciting neutralizing immune response towards the disease-causing agent. (5) A novel means, called synthetic recombinant vaccines, based on synthetic oligonucleotides, which code for the relevant epitope(s), that are inserted into an appropriate vector, for the expression of this external epitope(s). This approach may allow the inclusion of more than one epitope in the desired vaccine.

These various new approaches for vaccine development will be discussed in the following. We will also mention a more recent direction, namely, the development of therapeutic vaccines, that could be applicable for infections that are of long duration or chronic nature, as well as in the case of noninfectious diseases including cancer, autoimmune diseases or allergy.

2. Recombinant vaccines

2.1. Protein-based recombinant vaccines

The finding that both humoral and cellular arms of the immune system recognize and react with specific regions of the pathogen has led to the design of vaccines based on subunits of the pathogen, namely, protein component(s) that lead to protective effect. Such protective proteins have, in some cases, been isolated from the organism, either secreted, as in the case of toxins, or extracted and isolated from the organism after its disruption, e.g. influenza surface antigens, and served as vaccines. Alternatively, such proteins can be synthesized by recombinant DNA technology. One of the major problems facing the development of vaccines against highly hazardous pathogens is the problem of culturing the organism and immunizing with a live attenuated vaccine, as well as the possible reversion of the mutation. Despite the advances in the field of vaccinology, some persisting infections, such as those caused by HIV and mycobacteria, still pose a great challenge to vaccine developers. In such cases, it is preferable to immunize with a recombinant protein that presents an immunogenic part of the pathogen to the immune system, while avoiding the whole hazardous pathogen. Thus, several recombinant HIV-I proteins [24], as well as *Mycobacterium tuberculosis* antigens [12], are under investigation for their protective effect. In other cases, e.g. HBV or various parasites (malaria, schistosoma), the difficulty in growing the organism in culture prompted the development of a vaccine based on a recombinant protein. Intensive research in this direction resulted in the identification and production of recombinant protective antigens of malaria [26] and the SM28 GST of schistosomiasis [8], which led to clinical trials as well. The only recombinant vaccine that proved highly effective and is currently approved for human use is the vaccine against HBV infection. It includes the recombinant hepatitis B virus surface antigen (HbsAg), made by DNA-transfected yeast or mammalian cells. This recombinant vaccine is efficient also when administered immediately after birth, since seroconversion after vaccination has been demonstrated in almost all infants and children [16,19].

Some recombinant proteins have low immunogenicity, and the only immunologic adjuvant currently

licensed for human use, the aluminum salt, is sometimes ineffective. Thus, to increase the effectiveness of such vaccines it is proposed to use the molecular biology methods for endowing built-in adjuvanticity. Much initial work on the rational design of adjuvants has centered on the use of cytokines, and particularly interferon γ (IFN-γ), which is one of the most studied cytokine adjuvants and is effective even when simply mixed with the antigen before immunization. A convenient means of attaching a vaccine antigen to IFN-γ is by production as a recombinant, chimeric protein. Such a fusion protein composed of IFN-γ and the human immunodeficiency virus (HIV) type 1 surface glycoprotein gp120 was used for immunization of mice, which indeed gave rise to enhanced primary antibody responses to gp120, particularly of the IgG2a subclass. In addition, both T cell proliferation and IFN-γ production in response to the antigen were strongly enhanced by primary immunization with the fusion protein [25]. This approach could lead to a new generation of recombinant vaccines.

2.2. Live vector-based recombinant vaccines

Vaccines based on live viruses have traditionally been highly effective and relatively easy to produce. For example, the elimination of smallpox was accomplished through mass vaccination with the live vaccinia virus, a mildly pathogenic animal virus related to smallpox. Live attenuated poliovirus developed by Sabin was also responsible for the eradication of the disease in the Western hemisphere and for its drastic reduction all over the world. The live attenuated vaccines are well tolerated and immunogenic and led to effective vaccine against additional infectious diseases, e.g. yellow fever [11], mumps [14], shigella [21] and others. These vaccines are usually produced by attenuation of the pathogen by physical means or by selection of naturally occurring mutants that lead to infection with abortive replication of the pathogen, while retaining its immunogenicity.

Using molecular biology and DNA manipulation methods, it has also been possible to express protective proteins in adequate live vectors and thus design live vaccines against various pathogens. Thus, the development of reverse genetics systems for the recovery of viruses from cDNA has made it possible to rapidly generate recombinant attenuated derivatives of these viruses by either point mutations or by attenuating hazardous sequences that are included in the vaccine.

The attenuating mutations approach can be exemplified in a very well characterized cold-passaged (cp), temperature sensitive (ts) parainfluenza virus vaccine candidate denoted HPIV3cp45. The efficacy of this vaccine candidate, which includes 15 point mutations, was detected in nonhuman primates. The level of virus replication was restricted but sufficient to provide protection against challenge with the wild-type virus [32].

A live attenuated vaccine that is already evaluated in clinical trials is the vaccine candidate against respiratory syncytial virus (RSV). This virus infects infants in the first several weeks when their immune system is immature. There is no animal model for the disease and therefore evaluation of vaccine candidate in humans is done with great care. Protective antibodies are directed to the fusion (F) and attachment (G) protein and hence most studies concentrate on these antigens. The first generation of live attenuated vaccines consisted of naturally occurring mutant viruses; however, they were genetically unstable. Later it was found that enhanced stability was associated with multiple attenuation mutations (temperature sensitivity, host range and cold-adapted phenotype). Based on this principle and by using chemical mutagenesis, several vaccine candidates were designed and are currently being examined in clinical trials in seronegative infants [13].

Live vaccines can be derived also using genetic engineering techniques since cloning procedures enable the generation of live viruses from plasmid DNA copies containing the whole virus genome. Vaccine candidates can thus be designed by site-directed mutagenesis, gene insertions or deletions and by generation of chimeric viruses. In addition to mutations aimed at neutralizing the pathogen, engineered viruses may bear a phenotype that facilitates the immune response towards it, for example, expression of cytokines by recombinant RSV [9]. Another example for a recombinant live vaccine is the use of the vesicular stomatitis virus (VSV) as a vector that carries HIV antigens. In humans, VSV infection causes only mild flu like symptoms, and hence it is ideal as a vector for the hazardous HIV. It was shown to induce high cellular and humoral responses both to its own proteins and to

the additional proteins encoded by the recombinant VSV. A VSV/HIV recombinant expressing the *env* and *gag* glycoproteins of HIV generated both cellular and humoral responses to both proteins in mice; it could also significantly protect rhesus monkeys from challenge with the virus for up to 14 months after infection [29]. The future will tell if this approach will indeed lead to efficient recombinant live vaccines for human use.

3. DNA vaccines

The most recent advance in vaccine development involves the direct administration of plasmid DNA coding for immunogenic antigens to tissues that are capable of taking the DNA and expressing the foreign antigen in such a way that will lead to an effective immune response. A popular method of DNA delivery includes intramuscular injection of the DNA, or ''gene gun'' delivery of DNA-coated gold beads to the epidermis. Using both methods, the immune response against the antigen persists for about 1 month. In mice, DNA immunization is comparable with, or superior to, whole virus-based vaccines, concerning both the intensity and persistence of humoral and cytotoxic T-lymphocyte (CTL) response [30]. The capacity of DNA vaccines to confer protection was demonstrated for several viral infections in animal models [17]. This technology is exemplified by the development of vaccine against malaria that contains antigens from the different stages of the parasite life cycle and hence is expected to eliminate or reduce parasitemia in both moderately exposed travellers and in people who live under intense exposure in endemic areas. The naked DNA technology in this case offers the advantage of flexibility since the plasmid content can be easily adjusted according to the diversity in HLA in various field settings, so that it will include the most immunogenic antigens and modulators for a specific population. However, despite the encouraging results in animal models that were immunized with plasmid constructs expressing different malarial proteins, in the first two clinical trials, immunization with a DNA plasmid containing the PfCSP gene (a pre-erythrocytic stage protein) did not induce antigen-specific antibody responses in humans. In order to improve the efficacy of the vaccine, a multivalent DNA-based vaccine was designed. Immunization of Rhesus monkeys with a trivalent construct encoding antigens from both the pre-erythrocytic and erythrocytic stages together with three immunostimulatory cytokines resulted in significant protection against *Plasmodium knowlesi* sporozoite challenge [22]. The results of the clinical trials are pending.

There are several possibilities for optimizing the immunogenicity of DNA vaccines: (a) to enhance the expression of the genes that are included in the DNA plasmid. This can be done by employing the human codon usage in order to achieve the optimal expression in the vaccinated individual. (b) To enhance the immunogenicity of the DNA plasmids by addition of cytokine genes such as interleukins IL-12 and IL-2, granulocyte-macrophage colony-stimulating factor (GM-CSF), B7-1, CD40L, and other host genes. It was further shown that when primary immunization with DNA plasmid is followed by boosting immunization with recombinant pox or other viral constructs and/or recombinant proteins, the immunogenicity and protection level were enhanced [36]. Based on these results, the testing of the first multigene DNA vaccine began in August 2000 in a Phase I and IIa study assessing safety, tolerability, immunogenicity and protective efficacy in healthy adult volunteers.

4. Peptide epitope-based vaccines

The main benefit of immunization with peptide or polypeptide-based vaccines is the ability to immunize with a minimal structure, consisting of a well-defined antigen which can be thoroughly characterized with respect to its antigenicity and immunogenicity, in order to stimulate an effective specific immune response, while avoiding potential undesirable effects. For example, antigenic regions that activate suppresser mechanisms or a response against self-antigens can be excluded from the vaccine preparation, thus providing a safer vaccine. However, using a too well-defined peptide antigen may encounter the problem of low immunogenicity as compared with the large number of epitopes included in a protein antigen or in an entire pathogen that is used for immunization in the conventional vaccines. Using the strategy of peptide-based vaccines, it is important to dissect the specificity of antigen processing, the presence of both B cell and T

cell epitopes and the MHC restriction of the T cell response.

Since the cellular immune response in humans is restricted to specific HLAs, any single epitope-based vaccine will probably not be effective in a broad range population. This can be overcome by the use of vaccines comprising several peptides, which would be effective in various sections of the population, as well as in inducing all arms of the immune response. Furthermore, this approach allows the selection of those epitopes restricted to the HLAs which are most frequent in the population of interest and thus the design of vaccines for optimal efficacy according to geographical distribution. It is also possible to design a multi-epitope vaccine that will protect against more than one infecting pathogen and/or several viral strains in one vaccine preparation.

Before considering the use of synthetic peptides as potential immunogens, it should be realized that a prerequisite for the induction of immunity is the presentation of the peptide on the MHC to the T cells and the formation of the ternary complex with the T-cell receptor (TCR). The peptide epitope must possess both MHC recognition motif and TCR recognition elements. In addition to the processing, MHC-binding and direct presentation to the TCR, the activation of the T cell effector mechanisms of immunity requires cross-linking of the TCR by the antigen-MHC complexes aided by costimulatory factors secreted by the T cells, namely, cytokines (e.g. IFN-γ, IL-4). The immunogenicity of exogenously presented antigens could be augmented by externally added cytokines which act as immunomodulators. Furthermore, as mentioned for recombinant protein vaccines, synthetic epitope-based vaccines can also be designed to express cytokines as an integral part of their structure.

Ideally, peptide-based vaccines should contain both B-cell epitopes that are important for protective antibody response and T-cell epitopes that will serve to induce a T helper and a CTL response. The epitopes should be specific for the pathogen and not induce cross-reactions with self-antigens, and should provide a long-lasting immunity not requiring frequent booster doses. Since the immunogenicity of peptides is low, it should be adequately presented and usually formulated within an appropriate carrier or adjuvant, as discussed below. The first generation of peptide vaccines included various B- and T-cell epitopes, sometimes in clusters, either in tandem repeats or as multi-antigen peptides (MAPs) [34]. However, it has been found that these vaccines could be further improved. One of the ways to improve the protective effect of peptide-based vaccine is to refine its affinity to MHC class I molecules. In a study on influenza, aimed at increasing the CTL response towards a nucleoprotein (NP)-derived peptide-based vaccine, a chimeric peptide was designed. It was composed of amino acid residues from a high affinity peptide in positions that interact with the MHC, and those residues from a low affinity peptide that are exposed to the TCR. Vaccination with such a chimeric peptide induced a very efficient protective immunity [35].

Additional considerations were applied in order to develop enhanced vaccines for chronic viral infections such as human immunodeficiency virus (HIV) and hepatitis C virus (HCV). For the design of a vaccine against HIV, peptides from *nef*, *gp120*, *rev* and other viral proteins are under investigation as vaccine candidates. The following criteria were adopted: (1) Selected epitopes were used to avoid potentially harmful immune responses. (2) Linkage between helper and CTL epitopes was found to be important. (3) ‘‘Epitope enhancement’’ was achieved by modifying the sequences of epitopes to make more potent vaccines; it was shown that by modifying a T helper peptide from HIV, its binding to MHC class II molecules was improved, resulting in enhanced proliferation response. (4) CTL avidity was found to be important for clearing viral infections in vivo; such CTL against HIV peptide could kill infected cells early in infection before much viral progeny was produced. Vaccines that selectively induce high avidity CTLs may be more effective than peptides that induce lower avidity CTL. (5) Cytokines were employed as adjuvants to steer immune responses toward desired phenotypes; synergy was shown between IL-2 and GM-CSF or TNF-α in increasing the CTL response and protection against HIV challenge. (6) The route of administration is extremely important. Local mucosal CTL response against HIV was found to be crucial for resistance to mucosal viral transmission and this resistance was enhanced with mucosally delivered interleukin-12 [6].

Immunogenicity can also be augmented by the use of macromolecular carriers to which the desired epitope is either complexed or covalently attached. One recent example is the use of KLH coupled to a

synthetic peptide from group A streptococci streptomycin. This streptolysin is toxic but not immunogenic; the coupling of the 20 amino acids peptide derived from it to KLH enabled the induction of antibodies that completely neutralized the hemolytic activity of the toxin in vitro. This study shows for the first time that it is possible to raise neutralizing antibodies against one of the most potent bacterial cytolytic toxins known [15] and emphasizes the potential of epitope-based vaccines incorporating appropriate carriers.

Alternatively, the carriers could comprise a recombinant fusion product, expressing the desired epitope as a foreign one. A synthetic oligonucleotide coding for the particular epitope is inserted into a gene of a viral or bacterial protein leading to a recombinant organism, which can either serve as an intact "live" vaccine or be used for isolation of the recombinant protein which would serve as a synthetic recombinant vaccine, as described in the following.

5. Recombinant epitope-based vaccines (synthetic recombinant vaccines)

Synthetic recombinant vaccines are expression vectors incorporating defined epitope(s) of microbial agents. They are prepared by inserting synthetic oligonucleotide(s) coding for previously identified relevant epitopes into the genome of the desired vector, using recombinant DNA technology. The results obtained hitherto with several experimental systems indicate that immunization with such vaccines carrying an epitope derived from the pathogen may lead to protective immunity against the respective agent. For example, hepatitis B core protein antigen (HbcAg) has been used as a synthetic recombinant product for the expression of the foot-and-mouth disease virus (FMDV) epitope, VP1 141–160, and led to a response approaching that induced by FMDV particles [10].

Vaccinia virus is one of the vectors most frequently used as a recombinant vaccine expressing foreign antigens, including the expression of single epitopes. Thus, a recombinant vaccinia expressing the immunodominant CTL epitope of lymphocytic choriomeningitis virus (LCMV) completely protected mice from lethal infection [20]. It was also used to present a protective epitope of cytomegalovirus (CMV). Other viruses, including adenovirus and even influenza virus, can also serve as vectors, as summarized in a review article [2]. Thus, for example, epitopes of HIV-1 *env* protein were inserted in frame into influenza HA gene and led to epitope-specific antibodies. LCMV epitopes were similarly expressed in the influenza neuramindase (NA) stalk. *Salmonella* vaccine strains have also served as vectors for expressing foreign antigens, including single epitopes [27]. This vector is particularly suitable for expression of specific epitopes in recombinant vaccines, since it could in turn be used as a live vaccine employing the whole bacteria, for use in the oral administration route. Alternatively, when the epitopes are expressed by the flagellin gene, the flagella can be cleaved from the recombinant bacteria and used as nonlive vaccine, using other routes of immunization including the intranasal route. This vector has been employed in our own laboratory for the induction of protection against the parasite *Schistosoma mansoni* [4], as well as for the development of influenza vaccine, as will be described in the following.

The cumulative results using the various vectors demonstrate the potential of synthetic recombinant vaccines for the induction of adequate humoral and cellular immune responses against various epitopes of viruses as well as parasites, and conferring protecting immunity.

5.1. A case in point: influenza vaccine

The influenza virus is a major health concern; it causes an acute respiratory illness, which exhibits high attack rates especially in newborns, in people with pre-existing cardiopulmonary diseases and among the elderly. Since influenza infection is very widespread, it also results in an economical burden, and hence the need for an efficient vaccine.

The currently available vaccines against influenza are of several types, namely, whole virus vaccines, subunit vaccines and live-attenuated influenza virus vaccines. Only killed (inactivated) and subunit vaccines are currently licensed for human use. The subunit vaccines include the outer membrane proteins hemagglutinin (HA) and neuraminidase (NA) from strains that are expected to be spread in the following year. They do not provide a satisfactory solution of

long-term immunity or protection against new emerging strains; they also fail to confer protection to populations with a declined immune system such as the old and infants.

In our laboratory, we have investigated several influenza epitopes (HA91–108, NP55–69 and NP147–158), each stimulating a different arm of the immune system, for the design of a synthetic vaccine. In earlier studies, we showed that the 18-residue peptide corresponding to the sequence of the HA region 91–108 (HA91–108), which is conserved in all H3 strains, is a very effective epitope. The mice immunized with this peptide were partially protected against challenge infection with several H3 influenza virus. The Th and CTL epitopes from Nucleoprotein (NP55–67 and NP147–158, respectively), which are also highly conserved, induced MHC-restricted immune responses. We have also described the evaluation of the above epitopes when expressed in a chimeric flagellin protein and have demonstrated that this peptide-based recombinant vaccine, administered intranasally without the aid of adjuvant, induced efficient cross-strain protection and long-term immunity against influenza in mice [23]. Furthermore, the combined use of B and T cell epitopes administered as a mixture of recombinant flagella, each expressing individually one epitope, significantly improved the protective efficacy against viral infection, indicating the synergistic effect of priming both arms of the immune system. The same efficacy of the recombinant flagella vaccine was observed also when it was administered to aged (24 months old) mice [3].

This approach was further investigated, aimed at constructing a human influenza vaccine using the same methodology: the above B-cell epitope from the HA surface antigen and three T-cell epitopes that are restricted to the most prevalent HLA molecules in the population were expressed in the flagellin and used for vaccination. The mixture of the recombinant flagella, expressing individually the four influenza epitopes, was intranasally administered to humanized mice, e.g. mice that had been irradiated and then transplanted with human PBMC. The successful results demonstrated that this route enabled the induction of local immune response in the nasal cavity and lungs, which are the primary site of influenza infection. The induction of local immune response in the lungs was achieved, as demonstrated by the presence of specific anti-influenza antibodies in the lung homogenates. The ability of the recombinant flagella to effectively present influenza epitopes to the human PBMC was demonstrated both by the induction of antibodies and by the elevation of $CD8^+$ lymphocytes proportion following administration of the flagella preparations to the chimeric mice. Furthermore, after viral challenge, the immunized mice could successfully and rapidly clear the virus from their lungs, which led to effective protection against both sublethal and lethal viral challenge. Furthermore, the vaccine induced cross strain protection as illustrated in Fig. 1 [5].

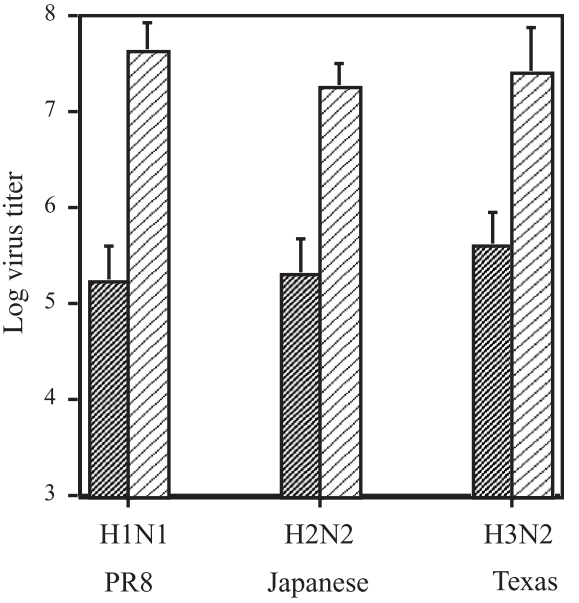

Fig. 1. Cross strain protection effect. Virus titer in lungs of human/mouse radiation chimera transplanted with PBMC and immunized intranasally with a mixture of four recombinant flagella vaccine. Each group of human/mouse chimera was infected 7 days after the immunization with one of three different influenza strains: A/PR/8/34 (H1N1), A/Japanese/57 (H2N2) or A/Texas/1/77 (H3N2). Both transplanted (▨) and nontransplanted (▨) mice were vaccinated with the tetra construct. However, only the transplanted mice were able to resist the infection and the virus titer in their lungs is significantly reduced. Taken from Ref. [5].

6. Therapeutic vaccines

Vaccines are, by definition, prophylactic, but recent years saw an interest in developing therapeutic vac-

cines, in infectious diseases–for diseases such as AIDS, tuberculosis, and peptic ulcer, in cancer–a variety of approaches to combat different kinds of cancer, and in autoimmune diseases–a definite success in developing a drug/vaccine against multiple sclerosis and hopes for myasthenia gravis, lupus and diabetes [31]. Additionally, therapeutic vaccines are being developed against Alzheimer's disease, mad cow disease (immune response to prions) and possibly Huntington's disease. All these efforts are based on the specificity of vaccines–the therapeutic vaccine is closely related chemically to the agent provoking the disease.

In infectious diseases, caused by viruses or bacteria, the duration of the disease is usually short and hence only prophylactic vaccines can be of benefit. However, infections that are of chronic nature, namely, when the duration from exposure to full manifestation and morbidity is long, are amenable for therapeutic vaccination, as illustrated by some recent examples: an obvious case is HIV, and indeed several attempts were made: using the whole killed virus in combination with drug (HAART) treatment; defined HIV proteins, such as p24 and p17 expressed in baculovirus; DNA vaccination with HIV-1 *env* and *rev* with or without HAART; several HIV-1 proteins–*gag*, *pol*, *env* and *ref*–expressed in recombinant pox viruses, combined with HAART; as well as the use of heat shock proteins and combination with immune-based therapies. Success, though limited has been reported, manifested in the reduction of viremia [28]. In the case of hepatitis B, the best means of control is by prevention, using HbsAg vaccine, but once infection has occurred the pathogenic factor is HbcAg, and this antigen is the basis for the development of a DNA-based therapeutic vaccine [7].

In the case of tuberculosis, therapeutic vaccines are needed mainly to combat the multi-drug resistant tubercule bacilli (MDRTB), and this was attempted both by the use of DNA vaccines expressing various TB antigens, or more recently the use of dendritic cells and immunotherapy. Finally, in the case of parasitic diseases such as schistosomiasis and malaria, in view of the lack of any effective vaccine, attempts are directed towards therapeutic vaccination, again based mainly on DNA or recombinant constructs expressing whole protein antigens, or on specific peptide-based vaccines. Several trials, using for example, the Sm28GST DNA vaccine for schistosomiasis, or the SPf66 peptide-based vaccine for malaria, are underway. Gastric ulcer caused by *Helicobacter pylori* is another example for which therapeutic vaccine would be useful. Indeed, vaccination, mostly by oral delivery, with a recombinant *Salmonella* expressing a protective antigen, led to both humoral and cellular immune response in mice, as well as long-lasting protection [33]. Prions-induced diseases are also potential candidates for therapeutic vaccination, but to date the only indication available is that anti-prion antibodies led to beneficial effect by passive immunization.

Therapeutic vaccination has also been an important approach in the search for remedy to several neurological diseases, including Alzheimer's disease (AD) and autoimmune diseases. In the case of AD, since one of the main expressions of the disease is the accumulation of amyloid plaques in the brain, the major target in vaccine development is the amyloid-β peptide (Aβ), a fibular 40–42 residue peptide, generated from the amyloid precursor protein (APP). Two independent studies reported recently that AP vaccination seems to preserve memory and learning ability in plaque-producing mice. Furthermore, the tetrapeptide EFRH, which corresponds to residues 3–6 of the human Aβ peptide, presented via phage display without adjuvant, led to high affinity antibodies.

In regard to autoimmune diseases, there are several examples by now where a substance (peptide or polypeptide) immunologically related to the autoantigen leads to suppression of the autoimmune response, as well as the pathology caused by it. Thus, in the case of multiple sclerosis (MS), the synthetic polymer Cop1, which is immunologically cross-reactive with the encephalitogen myelin basic protein (MBP), was efficient in suppressing both the animal model EAE and MS in patients [1], and has been approved worldwide as a drug/vaccine for the treatment of MS. Several altered peptide ligands related to sequences of MBP are also being evaluated. DNA vaccines encoding encephalitogenic sequences were shown to protect from subsequent EAE in animal models, but no trials were performed on patients as yet. Myasthenia gravis (MG) is another autoimmune disease in which the use of peptides as treatment/vaccine has been investigated, and indeed several peptides related to the acetylcholine receptor (AchR),

representing T-cell epitope analogues, were reported effective in suppressing the animal model EAMG and hence as potential modulators of MG [37]. In two other autoimmune diseases, lupus and diabetes type I [18] relevant peptides have been identified, which led to the arrest of the disease.

Atopic disorders and allergy can also be subjected to therapeutic vaccination. Indeed, the approach of desensitization, namely, a series of injections of the suspected allergens, has been in use for many years. Recent years have seen an advancement in this field, in the purification, and characterization of several allergens, the use of vaccines comprising genetically engineered allergens, or synthetic peptides representing defined epitopes of the allergen, with proven hypo-allergy efficacy, as well as the employment of DNA-based vaccines, to protect against both food allergy and asthma.

Finally, vaccination against cancer is being considered as a means for induction of anti-tumor immune response, to prevent disease in healthy, high-risk individuals or effectively eradicate tumor cells during an on-going disease. This topic is discussed in detail elsewhere in this workshop.

This cumulative information indicates that this recent approach of therapeutic vaccines may become highly relevant in the future for the prevention/treatment of a wide range of diseases.

7. Conclusions

Although the conventional "old" vaccines, which consist of disease-causing organisms in a killed or attenuated form, have been instrumental in the eradication or drastic decrease in the incidence of many infectious diseases and plagues, they have not eliminated the danger from both existing and newly emerging diseases. The new approaches to vaccine development described above, resulting from the renaissance in this field of research, carry the promise of overcoming some of the shortcomings of existing vaccines. They may lead, on the one hand, to the production of safer and more efficacious vaccines that may replace the currently available ones, and, on the other hand, to the development of vaccines against diseases for which no vaccine is available as yet. These include not only infectious diseases (caused by bacteria, viruses or parasites) but also autoimmune disorders, various neurological syndromes (including Alzheimer's disease), allergy and even cancer. The future will tell whether the hopes raised by the accumulated results attained so far will be fulfilled.

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