ACTIVE IMMUNIZATION IN POPULATION

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Abstract

The active immunization of population is a very important subset of the 21th century. The immunization is acted out with vaccines which are made of seedy bacteria or viruses, the same microorganisms which cause the variety of disorders in human organism. The immune system, after the entrance of these factors,is mobilized and produces antibodies in the same way as when a disease comes out. Antibodies destroy the seedy factors of the vaccine and the human body develops immunity. A very important factor is that the immunity is relatively permanent for a very long period, so when the individual is affected by the same factors causing the same disorder the immune system reacts more quickly for the elimination of the antigen by producing specific antibodies, and in this manner the immune system empowers and provides protection against future infections. Vaccines are also used in confrontation with various lethal diseases such as malignant tumors. In recent years, several researches have been acted out in developing new more efficient treatment options**.** Increased efforts have been made to apply immunomodulatory strategies in antitumor treatment. Such novel approach is the development of DNA vaccines for the destruction of malignant tumor by inducing humoral immune responces. The induction of specific immune responses directed against antigens expressed in tumor cells and displayed e.g., by MHC class I complexes can inhibit tumor growth and lead to tumor rejection. The use of different DNA delivery techniques and coadministration of adjuvants including cytokine genes may influence the pattern of specific immune responses induced. This might be the key to the future treatment of malignant tumors, increasing the life quality of patients and protecting them from relapses. Also very encouraging are the results from the clinical trials in animal models, the future studies may be focused in this way in order to render DNA vaccines safe for clinical use.

Key words: active immunization, DNA vaccination, adjuvants, tumor-antigen

Introduction

Throughout human history, infectious diseases have caused measureless misery and death. This rampage was unchecked until the twentieth century, when immunization was introduced on a wide scale. This led to the global eradication of smallpox, the elimination of polio from the Americas, and has almost eliminated tetanus, diphtheria, mumps, and the horrible congenital rubella syndrome. Immunization has greatly reduced the occurrence of measles, pertussis, and meningitis. Millions of deaths and other tragedies have been prevented by using specific vaccines.

Vaccines and related biologic products constitute an important group of agents that bridge the disciplines of microbiology, infectious diseases, immunology, and

immunopharmacology[1]. Active immunization consists of the administration of antigen to the host to induce formation of antibodies and cell-mediated immunity. Immunization is practiced to induce protection against many infectious agents and may utilize either inactivated (killed) materials or live attenuated agents . Desirable features of the ideal immunogen include complete prevention of disease, prevention of the carrier state, production of prolonged immunity with a minimum of immunizations, absence of toxicity, and suitability for mass immunization (eg, cheap and easy to administer). Active immunization is generally preferable to passive immunization, in most cases because higher antibody levels are sustained for longer periods of time, requiring less frequent immunization, and in some cases because of the development of concurrent cell-mediated immunity. However, active immunization requires time to develop and is therefore generally inactive at the time of a specific exposure [eg, for parenteral exposure to hepatitis B, concurrent hepatitis B IgG (passive antibodies) and active immunization are given to prevent illness][2].

Until recently, the "mechanism of action" of vaccinations was always understood antigen, thus preventing "infection" with that bacterial or viral antigen. In recent years science has learned that the human immune system is much more complicated than we firstly thought[3]. It is composed of two functional branches which may work together in a mutually cooperative way or in a mutually antagonistic way depending on the health of the individual. One branch is the humoral immune system (or Th2 function) which primarily produces antibodies in the blood circulation as a recognizing function of the immune system to the presence of foreign antigens in the body. The other branch is the cell-mediated immune system (or Th1 function) which primarily destroys, digests and expels foreign antigens out of the body through the activity of its cells found in the thymus, tonsils, adenoids, spleen, lymph nodes and lymph system throughout the body[4]. This process of destroying, digesting and discharging foreign antigens from the body is known as "the acute inflammatory response" and is often accompanied by the classic signs of inflammation: fever, pain, malaise and discharge of mucus, pus, skin rash and etc. In the same way, the Th2 branch of the immune system recognizes and even remembers foreign antigens and the Th1 branch of the immune system digests and eliminates the foreign antigens from the body. But the repeated stimulation of the recognizing humoral immune system by an antigen will inhibit and suppress the digesting and eliminating function of the cellular immune system. In other words, overstimulating antibody production can suppress the acute inflammatory response of the cellular immune system[5]. This explains the polar opposite relationship between acute discharging inflammations on the one hand and allergies and auto-immune inflammations on the other hand.

A growing number of scientists believe that the increase in America, Europe, Australia and Japan in allergic and auto-immune diseases (which stimulate the humoral or Th2 branch of the immune system) is caused by the lack of stimulation of the Th1 branch of the immune system from the lack of acute inflammatory responses and discharges in childhood[4]. There is need to identify the factors which cause this shift in the function of the immune system or which cause allergies and auto-immune diseases in childhood to increase.

A vaccination consists of introducing a disease agent or disease antigen into an individual’s body without causing the disease. If the disease agent provoked the whole immune system into action it would cause all the symptoms of the disease. The symptoms of a disease are primarily the symptoms of the acute inflammatory response to the disease. So the trick of a vaccination is to stimulate the immune

system just enough so that it makes antibodies and "remembers" the disease antigen but not so much that it provokes an acute inflammatory response by the cellular immune system and makes individuals sick with the disease which trying to prevent. Thus a vaccination works by stimulating very much the antibody production (Th2) and by stimulating very little or not at all the digesting and discharging function of the cellular immune system (Th1).Vaccine antigens are designed to be "unprovocative" or "indigestible" for the cellular immune system (Th1) and highly stimulating for the antibody-mediated humoral immune system (Th2). Perhaps it is not difficult to see then why the repeated use of vaccinations would tend to shift the functional balance of the immune system toward the antibody producing side (Th2)[6].

The wise use of vaccinations would be to use them selectively, and not on a mass scale. In order for vaccinations to be helpful and not harmful, it must be known beforehand for each individual to be vaccinated whether the Th1 function or the Th2 function of the immune system predominates. In individuals in whom the Th1 function predominates, causing many acute inflammation because of the overreactivity of the cellular immune system, a vaccination could have a balancing effect on the immune system and be helpful for that individual. In individuals in whom the Th2 function predominates, causing few acute inflammations but rather the tendency to chronic allergic or autoimmune inflammations, a vaccination would cause the Th2 function to predominate even more, aggravating the imbalance of the immune system and harming the health of that individual.

Adverse events in vaccination

Advances in development, production, and control of vaccines facilitate the increasing standards of vaccine safety and tolerance. Comprehensive pre-clinical and clinical tests as well as modern manufacturing and testing methods ensure that vaccines marketed nowadays are safe[7]. As a rule, clinical trials performed before granting the marketing authorisation identify the most frequent adverse events and these results are used to evaluate the safety of the product. Such trials can identify relatively rare adverse events, which occur with a frequency of 1:1,000 to 1:10,000 of all vaccinated individuals. These adverse events will then be included in the summary of product characteristics (SPC) for the vaccine. Even after comprehensive clinical trials of vaccines, it is possible that very rare adverse events may be observed for the first time during the general use of a vaccine[8]. In recent years concern over real and alleged risks of vaccines relative to their benefit has grown in many countries including Germany. One reason for this is the fact that most infections that were previously feared have now faded from memory. This situation can be ascribed in part to the success of vaccination. In recent years an increased awareness of substantiated and assumed risks following immunization has been reported in Germany as well as many other countries. In part this may be due to the absence of infectious disease-related mortality and morbidity and to the fact that the severity of vaccine-preventable diseases is no longer observable. Consequently, rare and hypothetical adverse events attain undue public attention[9]. As vaccination willingness diminishes, a resulting lower vaccination rate renders the population susceptible to the natural wild type infection with concomitant increases in mortality and morbidity of vaccine-preventable diseases. Thus, very rare or even unproven adverse events have attracted public attention. Declining vaccination rates resulting from these fears may result in a renewed increase of vaccine-preventable diseases[7-9]. Adverse events following immunization (AEFI) need to be recognized and adequately assessed.

A new generation of vaccines

In recent years, increasing efforts have been made to use vaccination strategies, including genetically modified tumor cells, dendritic cells either pulsed or transduced with tumor-associated antigens, immunization with soluble proteins or synthetic peptides, recombinant viruses or bacteria encoding tumor-associated antigens1. All of these antitumor vaccination approaches aim to induce specific immunological responses to tumor associated antigens, destroying tumor cells and protecting patients from relapses. The antitumor immune memory is based on the induction of expanded populations of T or B lymphocytes, which first recognize and then react against tumor-associated antigens with specificity and high destructive potential [2].One novel and powerful strategy for antitumor vaccination is the direct inoculation of plasmid DNA encoding tumor-associated antigens. This technique, called DNA immunization, is known to induce both antigen-specific cellular as well as humoral immune responses 3-6. The generation of T cell-mediated cytotoxicity against tumor cells can inhibit tumor growth and lead to tumor rejection.

Target	Tumor	Antitumor immune response
Human gp100	Melanoma	Decreasement of lung metastases by 50% and 50% long-term tumor-free survivors
Human gp75	Melanoma	Significant protection from lung metastases and 86% decrease in lung nodules
Human TRP-2	Melanoma	Significant tumor protection
HER-2/neu	Breast cancer	Significant reduction of tumor development
Folate receptor α	Ovarian carcinoma	Significant delay in tumor growth, enhancing of survival time, and reduction of number of lung metastases
hCGβ subunit	Myeloma expressing free hCGβ protein	Marked reduction of tumor size and 30% long term survivors
Tyrosine hydroxylase	Neuroblastoma	Protection from lethal tumor challenge

Table I. Examples for DNA vaccination against tumor agents in animal models

More analytically

The construction of DNA vaccines involves cloning of the gene of interest into a plasmid under the control of a viral promoter, e.g., cytomegalovirus immediate early

promoter. In cell nuclei, the plasmids persist as circular nonreplicating episomes, and they are not integrated into the host's genome[10], resulting in long-term expression of the encoded proteins by the host's cells [10,11]. Gene expression in the skeletal muscle can be detected for up to 19 months after injection [11]. Therefore, DNA vaccines provide a stable and persistent source of the encoded antigen leading to a permanent stimulation of the immune system and the generation of long-lasting immunity[10]. This antigen persistence may contribute to the efficacy of DNA vaccination in antitumor immunotherapy. The major advantage of DNA immunization is that both cellular (including CD4+ and CD8+ T cells) and humoral immune responses can be induced because the encoded antigen is processed through both endogenous and exogenous pathways, and peptide epitopes are presented by major histocompatibility complexes (MHC) class I as well as class II complexes. The uptake of plasmid DNA containing the gene of interest by host cells results in the in vivo synthesis of the encoded protein[10, 11, 12]. The endogenously produced protein is processed into peptides by the proteasome. Membrane-associated transporters of antigeneic peptides (TAP) move these peptides into the endoplasmatic reticulum [13] where they associate with MHC class I molecules. The MHC class I-peptide complex is transported to the cell surface where it can be recognized by CD8+ T cells . Once activated, CD8+ T cells acquire antigen-specific cytotoxic functions. These CD8+ cytotoxic T lymphocytes (CTL) can kill tumor cells through the recognition of antigeneic peptides presented by MHC I molecules on the surface of the tumor. CTLs are known to play an important role in the protection against tumors and in the induction of antitumor immunity. Therefore, an important goal for the development of an effective antitumor vaccine is the generation of a specific CTL response. The induction of CTL responses following DNA vaccination depends on the presentation of the antigen of interest by antigen-presenting cells (APC) [14, 15] displaying costimulatory molecules on their cell surface. APC are the predominant cell type capable of inducing T cells to become effector cells that can recognize and kill tumor cells [16]. The CD28 molecule on the T cell membrane can interact with costimulatory molecules like B7-1 on APC. This interaction appears to be crucial for effective T cell activation and proliferation. One attractive feature of DNA vaccines is provided by the fact that bacterial plasmid vectors contain immunostimulatory nucleotide sequences, unmethylated CpG islands, capable of causing maturation and activaion of APC [17– 21]. Bone marrow-derived APC have been shown to be responsible for stimulating naive CTLs following intramuscular DNA immunization and gene gun bombardement of the skin [14, 22, 23]. After DNA administration, APC either acquire antigen by being directly transfected[14, 24–26] or by the uptake of antigens released from other transfected cells [23, 27]. Lysis of transfected cells expressing an antigen or secretion of the antigen lead to the release of protein, which is taken up by APC. In lysosomes, the antigen is proteolysed into peptides. These peptides bind to MHC class II molecules and travel to the cell surface. The MHC class II-peptide complex is recognized by CD4+ T helper cells secreting cytokines like interleukin-2 (IL-2) that may facilitate tumor cell destruction in the effector phase of immune responses. There is now increasing evidence that CD4+ T cells are an important component of a successful antitumor immune response. Tumor-specific CD4+ cells can not only provide help for the induction of specific CD8+ CTL, but they may also be critical in activating macrophages and eosinophils to produce nitric oxide and superoxides that participate in the destruction of tumor cells[28– 30]. However, neither macrophages nor eosinophils have an intrinsic capacity for tumor specificity. Instead, the tumor specificity of these effectors is based on their activation by neighboring tumor specific CD4+ T helper cells [30]. In addition, CD4+ T

helper cells may provide help to activate B cell antibody production. Humoral immune responses result from the secretion of antigen from transfected cells or by release of antigens as a result of cell lysis.

Coadministration of adjuvants for enhancing immune response using DNA vaccination

It is known that the route of application of plasmid DNA [31] as well as the immunization schedule [32] can determine the quality of the immune response generated. Therefore, attempts to increase immune responses following DNA immunization include varying the vaccination regime. Combining different routes of vaccination was shown to enhance the immunogenicity of encoded antigens. In addition, there exists the potential to influence the immune response generated by a DNA vaccine via codelivery of an adjuvant. A common strategy to further enhance DNA-based immunization is to employ cytokine genes as adjuvants . Several studies indicate that codelivery of vectors encoding cytokines such as IL-2, IL-12, interferon-γ (IFN-γ), or granulocyte macrophage-colonystimulating factor (GM-CSF) is able to direct the nature of the resulting immune response and augments the efficacy of DNA vaccines [33]. The benefit of cytokine gene adjuvants might depend on the intrinsic properties of the antigen used and the immunologic cell types involved [34]. However, several studies confirm that especially GM-CSF has the capacity to potentiate DNA immunization [35]. The inclusion of a GM-CSF encoding plasmid with a tumor antigen encoding DNA vaccine was shown to allow a reduction in the tumor antigen-encoding plasmid dose required for antitumor efficacy in animal model [36]. It is suggested that GM-CSF enhances the initiation of immune responses by recruiting APC to the site where antigen is expressed [37]. GM-CSF stimulates the proliferation and the activity of APC , induces differentiation from immature APC to mature APC , and increases the expression of MHC class II molecules in APC , thus augmenting their antigen-presenting ability. It has been shown that the application of GM-CSF-encoding plasmid by gene gun results in APC accumulation within draining lymph nodes of tumors (48). Another cytokine that is important for the generation of APC and augmenting their function and quantity is Fms-like tyrosine kinase 3 (Flt3)-ligand. Recently published data indicates that fusion of a gene encoding the extracellular domain of Flt3-ligand to an antigen gene can greatly enhance the potency of DNA vaccines [38]. It is remarkable that is not only possible to coadministrate cytokine-encoding vectors to antigen-encoding ones, but also to link the cytokine gene directly to the DNA vaccine or to insert DNA coding for an immunomodulatory peptide of a cytokine [38]. A novel alternative possibility for enhancing the immunogenicity of DNA vaccines is the use of plasmid DNA vectors containing replicons derived from viruses.

Recent experiments pointed out that these plasmids launch a self-replicating RNA vector that in turn can direct the expression of a model tumor antigen. Leitner *et al.* [39] have shown that plasmid DNA replicons induce stronger immune reponses than conventional DNA vaccines and effectively treated tumor-bearing mice. In addition, attempts to enhance the efficacy of DNA vaccines include coexpression of costimulatory molecules.

Cytokine gene	Enhancement of immune response

GM-CSF	Cellular and humoral immune responses
IFN-γ	Th1 cells, CTL activity, and IgG2a antibody production
IL-12	Th1 cells, CTL activity, and IgG2a antibody production
IL-2	Th1 cells, CTL activity, and IcG1and IgG2a antibody production
IL-4	Th2 cells and IgG1 antibody production
Fit3-lingand	CTL activity and antitumor immune response

Table II. Coadministration of Cytokine genes in DNA vaccination

These approaches may counteract immune escape mechanisms of tumors because one feature of tumor cells explaining their failure to stimulate effective CTL responses is their lack of expression of the costimulatory molecules B7-1 and B7-2 [40]. These molecules are ligands for CD28 and CTLA4, providing the second signal that is required for the activation of T cells [42]. It has been shown that vaccination of animals with plasmids encoding an antigen and B7-1, but not B7-2, can induce immune responses against a transfected malignant tumor expressing the antigen . CD40 ligand (CD154) as well can serve as a genetic adjuvant capable of augmenting humoral and cellular immune reponses to antigens encoded by plasmid DNA expression vectors [43]. Another strategy for increasing the potency of DNA vaccines represents the linkage of tumor antigen gene to *Mycobacterium tuberculosis* heat shock protein 70 gene or to the translocation domain of *Pseudomonas aeruginosa* exotoxin A gene. These fusions have been shown to increase the frequency of specific CTL by at least 30-fold and to convert less effective vaccines into ones with significant potency against tumors expressing the antigen [40-43]. You *et al*. [45] described a novel DNA vaccination strategy for enhancing uptake and presentation of antigens by APC. The authors developed a DNA vaccine including an antigen fused to an IgG Fc fragment. After DNA vaccination, the produced antigen-Fc fusion proteins are secreted and efficiently captured and processed by APC via receptormediated endocytosis. Using this strategy, a broad enhancement of DNA vaccine potency, including all arms of the immune system, could be achieved [45].

Concluding remarks

The current use of vaccinations in medicine today is essentially a shotgun approach which ignores differences among individuals. In such an approach some individuals may be helped and others may be harmed. Future researches must be focalized in making vaccines more individualized for each person separately. Vaccinations are usually effective in preventing an individual from manifesting a particular illness. Epidimiologic studies have shown that as families improve their living conditions the risk of acute infectious and inflammatory diseases very much decreases. As regards as for the new generation of vaccines, they constitute novel therapies in confronting of various life-threating diseases such as mallignant tumors. Conventional therapies such as chemotherapy, surgery and radiation are highly invasive and sometimes have only a palliative effect. In recent years immunizations with naked plasmid DNA encoding tumor-associated antigens have revealed a number of advantages. By DNA

vaccination, antigen specific cellular as well as humoral responses can be generated. The improvement of vaccine efficacy has become a critical goal in the development of DNA vaccination as antitumor therapy. Also from previous studies have been shown that polyimmunization with a mixture of tumor-associated antigen genes may have a synergestic effect in tumor treatment and induce complete protection. Preclinical studies in animal models are promising that DNA immunization is a potent strategy for mediating antitumor effects in vivo.

Ending DNA vaccines may offer a novel treatment for tumor patients. Also may be useful in the prevention of tumors with genetic predisposition. However immunization with xenogenetic DNA to induce immune responces against self-molecules is under intensive investigation. By DNA vaccinations preventing infections and the development of viral induced tumors may be avoided. These type of vaccines in the future may be the key to confronting malignant tumors,thus saving a very large percentage of persons who suffer from viral infections associated with tumor formation.

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