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review

A brief overview of the tumor vaccines through the last decade

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How to destroy cancer cells without damaging the normal cells? How to make conventional methods of systemic cancer treatment that predominantly comprise cytotoxic drugs more selective and prevent the development of drug resistance? There is an abundance of such questions that do not have simple answers. If, a few years ago, unselective cytotoxic drugs were the method of choice for the treatment of cancer, in the last 25 years we are witnessing the rapid transition of immunotherapy from the laboratories to the clinics. Among the most attractive and promising immunotherapies for cancer, a special place is reserved for tumor vaccines. Exploiting the latest knowledge in immunology, tumor physiology, as well as in molecular biology, many outstanding approaches for the creation of tumor vaccines have been developed. With no intention to be comprehensive, in the present article some of those approaches are reviewed.

Key words: neoplasms; gene therapy; cancer vaccines

Introduction

In the last few years, we have witnessed a great progress in the fields of immunology, tumor physiology and molecular biology. Namely, the basic facts about the recognition of various structures by the immune system through the cooperation of MHC have been explained. The structures of MHC class I, and MHC class II have been studied and their function analyzed quite thoroughly. The complex mechanisms of antigen presentation and the role of presenting cells have been investigated in details.[1,2] Various cell receptors (especially T cell receptors) have been discovered, and the methods of signal transduction and the activation of T lymphocytes (the major performers of the cellular immunity) have been elucidated.[3,4] The production of monoclonal antibodies towards different well-defined structures has become a routine procedure, which facilitates the transition of such antibodies into clinical praxis.[5] Today, we are also familiar with the structure of and are equipped to produce various immunomodulatory cytokines, which in turn, assists their application in the treatment of some malignant diseases (hairy-cell leukemia, malignant melanoma, renal cell carcinoma).[6-8] On the other hand, also the methods for precise determination of different genes and their transduction into mammalian cells have been

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extensively studied. Thus, the researchers created various vector systems that can be divided into two large groups: (1) viral vectors (retroviral, adenoviral, adeno-associated viral vectors, herpesviral vectors)[9,10] and (2) nonviral vectors (calcium-phosphate precipitation, liposomes, microinjections, electroporation, poly-lysine conjugates, receptor-mediated endocytosis, gene gun).[9,11-16] These discoveries have become the groundwork for the renewed and new biological approaches towards the treatment of malignant diseases.

Tumor vaccines

The first tumor vaccines were created on the principles of classical immunology and comprised irradiated tumor cells and nonspecific immunomodulators. Further approaches towards the creation of tumor vaccines base on the principles of molecular immunology and quite often it is hard to distinguish them from the classical gene therapy approaches. The newer vaccines also include autologous or allogeneic cells, with the difference that various genes coding for proteins involved in the stimulation of immune response (e.g. genes coding for growth factors and cytokines, as well as genes coding for co-stimulatory molecules) are introduced into these cells.[17,18] Instead of whole cells, also certain specific structures that are responsible for the antitumor immune response can be used.[19,20]

Classical tumor vaccines

It was not until recently that we gained some information about the specific antigens that are present on the surface of the tumor cells, and about the co-stimulatory molecules that are necessary for the activation of the immune system, so the first true tumor vaccines were composed of (1) autologous or allogeneic irradiated tumor cells, (2) tumor cell lysates with viral antigens or (3) tumor cells with nonspecific immunomodulators (*Corynebacterium parvum, Bacillus Calmette-Guerin*).[21-27] These first-generation vaccines that base on the principles of classical immunology were termed classical tumor vaccines.

The classical tumor vaccines were applied predominantly for the treatment of malignant melanoma and in the following text this tumor type will be used as an example of the advances in the last years. The most extensively studied vaccine was the CancerVax that was prepared using three viable allogeneic malignant melanoma cell lines (MHC haplotype matches with 95 % of melanoma patients) chosen for their high immunogenicity.[28] As the non-specific immunostimulatory agent the BCG was given with the first two doses. When patients with malignant melanoma stage IV were treated with CancerVax in a clinical study, the five-years survival was as high as 25 %, which can be regarded as quite successful because the surviving fraction in the control group was only 6 %.[29]

In a different clinical trial, the melanoma patients received a classical tumor vaccine composed of autologous tumor cells and BCG. This therapy resulted in a complete response in four out of 40 malignant melanoma patients, and in a partial response in one patient.[30,31]

Worth mentioning are also the studies of Mitchell *et al*. In the first study the authors applied the tumor vaccine composed of the allogeneic cell lysate and Detox (i.e. the detoxified endotoxin from *Salmonella minesota*, cell wall skeletons of *Mycobacterium phlei*, squalane oil and emulsifier), while in the second they applied the Melacin (i.e. the lyophilized version of melanoma lysate vaccine and Detox). The first trial resulted in a partial or complete remission in 20 out of 106 melanoma patients, with the median duration of response of 21 months. The survival of the melanoma patients treated with Melacin in the second study equaled the survival of patients

treated with chemotherapy. As expected, the response rate was lower in the vaccinated group but the toxic side effects were much more pronounced in the chemotherapy group.[32,33]

Additionally, the results of our pre-clinical study with a syngeneic melanoma vaccine proved the high efficacy of the prepared vaccine. In the case of the aggressive intraperitoneal malignant melanoma tumor model we successfully protected more than 40 % animals from tumor development with just a single application of sublethally irradiated tumor cells admixed with MVE-2 (nonspecific immunomodulator). When such a vaccine was applied twice, the number of the protected animals rose to as much as 90 %. Concomitantly, we confirmed that our vaccine induced the long lasting protective immunity, since over 60 % of the former survivors that were rechallenged with the tumor cells survived again without any further treatment.[34,35]

Genetically modified and recombinant tumor vaccines

The group of second-generation vaccines that base on the principles of molecular immunology comprises *genetically modified* and *recombinant tumor vaccines*.

Just like the first-generation vaccines also the second-generation vaccines can be divided into the ones that utilize whole autologous or allogeneic cells, and ones that utilize only certain specific structures. Yet, unlike the first-generation vaccines that are prepared strictly with autologous or allogeneic tumor cells, the second-generation vaccines employ either tumor cells or non-tumor cells (mostly autologous) as fibroblasts and dendritic cells. Regarding the approach towards a better recognition of the tumor cells by the immune system, the second-generation vaccines can be subdivided into the (1) vaccines prepared with genetically modified tumor cells, (2) vaccines prepared with genetically modified non-tumor cells (most frequently dendritic cells and fibroblasts), and (3) recombinant vaccines.

The approach towards the creation of tumor vaccines on the base of genetically modified tumor cells includes different modes of preparation. The more promising modes of preparation are the transfection of tumor cells with genes coding for antigens that are being presented through MHC class I and II, with genes coding for co-stimulatory molecules, and with genes coding for various cytokines.

With the transfer of genes coding for the structures that are being presented through MHC class I into the tumor cells we expect to accomplish an enhancement of the presentation of specific antigens for the activation of CTL, while the transfer of genes coding for the structures that are being presented through MHC class II should augment the activation of helper T cells. Such studies were encouraged by the discovery of different specific tumor antigens that are quite often underexpressed in tumor cells. The transfer of genes coding for the above mentioned structures in different studies resulted in an augmented activation of autologous CTL.[36,37] Similarly to the transfer of genes into the antigen presenting cells (described below), the genes coding for tumor specific antigens from the group of MAGE, MART, MUC-1 and CEA were transferred in these studies.[38]

The transfection of tumor cells with genes coding for B7 ligand should enable a direct activation of CTL, thus bypassing the role of antigen presenting cells. Namely, sole tumor antigens on the surface of tumor cells are insufficient for the activation of the effector cells and can even trigger the development of a complete immune tolerance. Therefore, an additional signal is needed for the activation of the cytotoxic T cells and is mediated through the co-stimulatory molecules (B7.1 – CD80 and B7.2 – CD86) that bind to CD28 and CTLA-4. In humans, the B7 is expressed

only on antigen presenting cells – it is expressed constitutively on dendritic cells but can be induced also on activated B, T and NK cells, as well as on macrophages. The studies that applied the transfer of gene for B7 into tumor cells demonstrated the transition of non-immunogenic tumor cells into highly immunogenic tumor cells which resulted in tumor rejection *in vivo*.[39,40] Quite similar was the effect of the transfection of tumor cells with genes coding for other co-stimulatory molecules as ICAM-1 and LFA-3. For that reason, it can be concluded that the presence of co-stimulatory molecules is obligatory for the activation of T lymphocytes while these molecules are not needed for the function of already activated CTL, since the achieved immunity against the transfected cells was preserved also in the case of non-transfected cells.

The cytokines are expected to induce such a vigorous antitumor response that bystander native tumor cells would also succumb to it. Therefore, genes for various cytokines are being transferred into tumor cells in order to achieve a higher level of production of the cytokines that are involved in the complex immune reactions including stimulation of CTL activation, acceleration of the multiplication of activated cells (the cytokines act as growth factors), triggering of the expression of various cell receptors, cytokine cascades and antibody production and in some cases attaining of a direct cytostatic/cytotoxic effect on tumor cells. In contrast to the activities of exogenous cytokines, the cytokines produced in genetically changed tumor cells mimic the activities of natural endogenous cytokines (underlie to some extent control mechanisms of the organism), which on one hand improves their effectiveness and on the other hand minimizes their toxic side effects. When preparing tumor vaccines, different researchers introduced genes for numerous cytokines or growth factors (IL-1, IL-2, IL-3, IL-4, IL-6, IL-7, IL-10, IFN-γ, TNF-α, GM-CSF and G-CSF), respectively, into tumor cells.[41-45] Preclinical results in various tumor models *in vivo* primarily confirmed the expectations concerning the action of this kind of vaccines on the immune system. It was shown clearly that vaccines containing an imunomodulatory cytokine are indeed capable of CD4+ and CD8+ T lymphocyte activation[46-49], activation of macrophages and neutrophils[50,51], as well as stimulation of differentiation of precursor blood cells and dendritic cells (important antigen-presenting cells for T lymphocytes).[52,53] Considering all mentioned facts, a slightly different effect was achieved on tumors than was expected. Even though the cytokine vaccines showed the potential to protect the animals from challenge with wild type tumor cells, none of these vaccines was efficient enough to cure the established tumors in a convincing proportion of experimental animals. The best protection, as well as the most pronounced antitumor activity against formed tumors, has been ascribed to vaccines created of tumor cells bearing gene for GM-CSF, while the most effective combination of genes for preparation of tumor vaccines comprised genes for IL-2 and GM-CSF.[52,53] The results of clinical trials are variable: there are reports about complete or partial tumor remissions after the treatment with cytokine vaccines, but also about an inadequate or missing clinical response.[20,27] Regarding the mechanisms of action of these vaccines in humans it is likely that they are pretty similar to those in animal tumor model: the vaccines should attack the tumor cells by activating the CD8 T cells, NK cell response, dendritic cells and macrophages. The most impressive antitumor (antimelanoma) immune reaction was displayed by the patient who received the GM-CSF vaccine.[54]

The purpose of the vaccination with genetically modified non-tumor cells is also the transfer and the mediation of immune active structures/substances to the effector cells in the organism. This mode of preparation beca-

me especially attractive after the discovery of specific tumor antigens and after the elucidation of the role of antigen presenting cells for the activation of naive T cells. The dendritic cells – DC (i.e. the most potent antigen presenting cells in the organism) that express the co-stimulatory and adhesion molecules (e.g. CD58, CD54, CD50, CD80 – B7.1, CD86 – B7.2) and MHC class I and II are employed in these reactions. The surface structures of DC bind to adequate structures on T cells (e.g. CD28) and through them mediate the signals for triggering of the primary immune response. This approach is thus opposite to the ones where the tumor cells were transfected with the genes encoding costimulatory molecules: if, with the transfection of tumor cells with B7 ligand the intention was to bypass the function of the antigen presenting cells, with this very approach it was intended to achieve the activation of specific CTL in the absence of tumor cells.

The first approaches using DC were based on *in vitro* activation of these cells with specific proteins as for example the OVA peptide, gp100, and MelaA/MART.[55,56] The DC prepared in this way were capable of triggering the CTL response leading to lysis of target cells that contained the corresponding antigen structures. Later on, the researchers in order to achieve the presentation of these structures by the DC, rather transfected the DC with the genes for tumor (more or less) specific antigens (instead of growing the DC *in vitro* together with certain antigen structures). Using various transfection techniques they succeeded to transfect the DC with MART-1, MUC-1, 2-galactosidaze gene[57-60], and demonstrated that the transfection of DC with genes coding for certain structures is a method that is superior in achieving the expression of these structures on the surface of antigen presenting cells to the *in vitro* activation of these cells with the very structures.[60] The latest approach in the field of DC application is the preparation of hybridomas, that is the fusion of DC with tumor cells. The resulting cells include all of the tumor antigens as well as all of the co-stimulatory molecules. In one study, where the researchers fused MC38 tumor cell line with DC from the bone marrow of the experimental animals, the vaccination with such vaccine prevented the development of lung metastases in 90 % of the treated animals.[61] In another research, 17 patients with renal carcinoma were treated with hybridomas created of autologous tumor cells and allogeneic DC. Thirteen months after the vaccination four patients were in complete remission, one in partial remission, and in two patients there was a less than 50 % reduction of the tumor burden.[62]

The group of recombinant vaccines comprises predominantly the peptides, fusion proteins and immunoglobulins. In this context, a question arises, if it is more rational to transfer the genes coding for tumor associated antigens into the antigen presenting cells and then apply these cells as tumor vaccines, or to apply such tumor antigens directly in the form of proteins and peptides as tumor vaccines. The immune response can be triggered by two main groups of peptides. Peptides with 8 to 11 amino acid residues that are bound in the MHC class I of the antigen presenting cells trigger through binding to the cell receptors of T lymphocytes (CD8+) the activation of CTL (cells responsible for the antitumor immunity).[63,64] The second group of peptides with 11 to 15 amino acid residues is expressed through MHC class II of the antigen presenting cells. These peptides are responsible for the activation of CD4+ lymphocytes that, in turn, produce cytokines involved in the activation of CD8+ cells.[65] The effectiveness of such vaccines depends upon if the treated organism carries the same MHC allele that will code for the recognizable paratopes on lymphocyte receptors. Also oligopeptides that include both epitopes (the MHC class I epitope and the MHC class II epitope) can be used as tumor vaccines. Most

of the specific oligopeptides represent malignant melanoma specific antigens (MAGE-1, MAGE-3, MART-1, gp100, tyrosinase, gp75), colorectal carcinoma specific antigens (CEA), breast cancer specific antigens (MUC-1). Since there are many different epitopes on these oligopeptides, some studies demonstrated that these antigens trigger the immune response (activate CTL) regardless of the type of the MHC present (MHC-unrestricted manner).[66,67] So far, only some of the above mentioned antigens were tested in clinical studies. In one of these studies, Rosenberg *et al*. achieved the development of immunity in 90 % of the patients vaccinated with the vaccine that included one of the peptides derived from gp100 (i.e. one of the epitopes). In 13 out of 31 patients with metastatic melanoma, that received IL-2 beside the vaccine, they observed an objective clinical response to treatment.[68] The preparation of fusion proteins and immunoglobulins is based on the use of monoclonal antibodies. Through the binding of the prepared monoclonal antibodies onto specific cell receptors the researchers aim to selectively influence the CTL and the antigen presenting cells. An example of such application is the preparation of monoclonal antibodies to CTLA-4 in order to reduce the weakened activation of CTL. Another example, already employed in the clinical practice, is the production of antibodies (Herceptin) to HER-2 (receptor for the growth factor in various types of carcinoma). These antibodies block the binding sites for the growth factor needed by the tumor cells and thus prevent the growth of the tumor.[69] The second mode of antibody application is the production of fusion proteins with cytokines. The most promising model is the employment of monoclonal antibodies bound to the IL-2 molecule. In both preclinical and clinical studies this fusion protein successfully activated the tumor infiltrating lymphocytes.[69]

Prospectus and conclusion

I intend to start the conclusion like I began the abstract with one of the general questions related to the cancer: Do we know enough about the tumor cells and their relations to the host? The answer is not simply YES or NO. Yes, we do know a lot about the tumor cells: their physiology, morphology, signal transduction, about gene susceptibility....We also do know a lot about the relations to the other – normal cells in the organism. Yet, I fell like we have all parts for the simple clock and some additional for an extremely sophisticated one. All the time we are discovering more and more additional parts belonging to the sophisticated clock, but unfortunately, there are still some missing parts that unable us to complete the clock. So instead of putting together the parts of the simple clock and constructing the usable device, we are trying to construct a complicated apparatus with many functions that are less important for daily determination of time.

Something similar is happening to us when we are trying to develop a systemic drug or therapy against cancer: we are targeting a single ultra-specific process offering the tumor cells plenty of time to rebuild the damaged functions. The researchers are discovering more and more details concerning the tumor cell but no one is open-minded enough to orchestrate all these pieces of information into a global prospect of surviving and proliferation of tumor cells in the host organism. Carcinogenesis as a process occurs in the living organisms much more often that could be concluded on the basis of real tumor incidence. Fortunately, only few of these malignantly transformed cells succeed to develop into tumor cell colonies and tumors. Majority of them are being recognized by the immune system and are destroyed before the tumor mass becomes clinically detectable. Considering the diverse origin of the transformed tumor cells (tumors of epithelial, me-

senchymal and other origins) that are recognized by the one immune system in the organism, it is clear that the immune system has the potency to distinguish between different types of tumor cells and control their proliferation. Once when we recognize the tiny borderline in the relation between the host immune system and the tumors the balance would be tilted in favor of the host. So speaking about the novel systemic cancer treatments, I am convinced that, rather than approaches where the therapeutics are acting directly against tumor cells, the approaches that propose the mobilization of protective mechanisms in the host will turn out to be more effective. That implies the development of the tumor vaccines that would be capable of triggering an antitumor immune response and preparing the host for a long lasting control of tumor growth and metastasis. At the moment, it is difficult to predict what kind of vaccine is going to be the most successful. It seams that the most effective one is going to be the vaccine that includes the tumor presenting cells armed with some genes encoding tumor antigens and immunostimulatory cytokines. On the other hand, the advantage will be given to the vaccine that comprises the elements of adjuvant and standard therapy allowing the application as a single adjuvant therapy after the surgical removal of the tumor, or in combination with the therapies that aggressively act directly against the tumor cells.

In conclusion, I should emphasize that classical tumor vaccines (first generation vaccines) are many times more potent than modern tumor vaccines (genetically modified second generation vaccines). The reason probably lies in the concepts used for the preparation of genetically modified vaccines (they are too specific in their activity). For that very reason the future of modern tumor vaccines is the preparation of vaccines that would be constituted of different major structures necessary for the triggering of the immune system or in combining of the currently available vaccines.

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