

review

## Tumor vaccines

Mojca Frank, Alojz Ihan

*Institute of Microbiology and Immunology, Faculty of Medicine,  
University of Ljubljana, Ljubljana, Slovenia*

---

Tumor vaccines have several potential advantages over standard anticancer regimens. They represent highly specific anticancer therapy. Inducing tumor-specific memory T-lymphocytes, they have potential for long-lived antitumor effects. However, clinical trials, in which cancer patients were vaccinated with tumor vaccines, have been so far mainly disappointing. There are many reasons for the inefficiency of tumor vaccines. Most cancer antigens are normal self-molecules to which immune tolerance exists. That is why the population of tumor-specific lymphocytes is represented by a small number of low-affinity T-lymphocytes that induce weak antitumor immune response. Simultaneously, tumors evolve many mechanisms to actively evade immune system, what makes them poorly immunogenic or even tolerogenic. Novel immunotherapeutic strategies are directed toward breaking immune tolerance to tumor antigens, enhancing immunogenicity of tumor vaccines and overcoming mechanisms of tumor escape. There are several approaches, unfortunately, all of them still far away from an ideal tumor vaccine that would reject a tumor. Difficulties in the activation of antitumor immune response by tumor vaccines have led to the development of alternative immunotherapeutic strategies that directly focus on effector mechanisms of immune system (adoptive tumor-specific T-lymphocyte transfer and tumor specific monoclonal antibodies).

*Key words: cancer vaccines; antigens, neoplasms; immunotherapy*

---

### Introduction

Development of tumor vaccines is based on the researches that have shown that many tumors express tumor antigens and are able

to elicit tumor-specific B- and T-lymphocyte responses. Tumor vaccines have several potential advantages over standard anticancer regimens. They are directed against tumor antigens and represent highly specific anticancer therapy. Inducing tumor-specific memory T-lymphocytes, they have potential for long-lived antitumor effects. Side effects of tumor vaccines are rare, in most cases limited to local reactions with minimal systemic toxicity (transient elevated body temperature, flu-like symptoms). Autoimmune reactions are also rare (vitiligo with melanoma vaccines).

Received 29 September 2006

Accepted 19 October 2006

Correspondence to: Mojca Frank, Institute of Microbiology and Immunology, Zaloška 4, Faculty of Medicine, University of Ljubljana, Ljubljana, Slovenia. Phone: +386 1 5437493; Fax: +386 1 5437401; E-mail address: mojca\_frank@yahoo.com

## Tumor antigens

Genetic and epigenetic changes characteristic of carcinogenesis make cancer cells antigenically distinct from normal human cells.<sup>1</sup> Cancer cells express tumor-specific antigens and tumor-associated antigens. *Tumor-specific antigens* are ideal targets for antitumor therapy. They are protein products of mutated normal cell genes and are expressed only by cancer cells. They are foreign to immune system and therefore elicit high-affinity antitumor T-lymphocyte responses with low probability of intercurrent autoimmune reactions.<sup>1-4</sup> Their main disadvantages are that they are highly heterogeneous and expressed only by certain types of tumors, therefore they cannot be used as a universal antigen in a cancer vaccine. A special subgroup of tumor-specific antigens are idiotypic sequences of B-cell membrane immunoglobulins or T-cell receptor.<sup>5,6</sup>

*Tumor-associated antigens.* Most antigens expressed by tumor cells are normal, non-mutated self-molecules to which immune tolerance exists.<sup>3,7</sup> There are several classes of tumor-associated antigens: *tissue-specific antigens* (PSA, melanocyte antigens), *oncofetal proteins* (normally expressed only during fetal development, foreign to immune system, reactivated in undifferentiated tumors), *cancer testes antigens* (normal testicular proteins, overexpressed on cancer cells, foreign to immune system - spermatocytes do not express MHC molecules), *overexpressed normal cell proteins* (HER2 Neu in breast carcinoma) and self-proteins with abnormal *posttranslational modifications* (overglycosylated mucins, as MUC1 in breast carcinoma, changes in glycosylation can expose cryptotopes, foreign to immune system).<sup>1,9,10</sup>

*Viral oncoproteins* (human papilloma virus proteins E6 and E7) are a special group of tumor antigens, playing a critical role in malignant transformation of infected cells.

Being foreign to a body, they can induce high-affinity T-lymphocyte responses.<sup>11,12</sup>

## Antitumor immune response

Tumor-specific cytotoxic T-lymphocytes are central effector cells of antitumor immune response. They are the only cells capable of efficiently killing cancer cells, inducing their apoptosis or lysing them by action of perforines and granzymes. They are produced in cross-priming of naive CD8+ T-lymphocytes, mediated by mature dendritic cells (DC). The process is essential for induction of antitumor immune response and involves cross-presentation of antigenic peptides originating from extracellular proteins through MHC I molecules on the surface of DC to naive CD8+ T-lymphocyte.<sup>9,13</sup> The pathway involves endosome to cytosol shuffling of antigenic peptides from extracellular proteins mediated by TAP transporters, and is a major pathway of cross-presentation under physiological conditions.

Efficient cross-activation of naive CD8+ T-lymphocytes requires 3 types of cells, mature DC, naive CD8+ T-lymphocyte and helper T-cell, and 2 signals. *Signal 1 (specific antigenic signal)* arises from interaction between antigenic peptide, presenting on dendritic cell MHC I molecule, and antigen specific T-cell receptor (TCR) of naive CD8+ T-lymphocyte; *signal 2 (costimulatory signal)* is mediated by costimulatory molecule B7 on the surface of DC and its CD28 receptor on naive CD8+ T-lymphocyte (two signal hypothesis).<sup>9,13</sup> For activation of naive CD8+ T-lymphocyte to be effective, licensing of DC is necessary. It is mediated by an interaction between CD40 ligand (CD40L) of DC and its receptor CD40 of helper T-cell, specific for antigenic peptide presented on MHC II molecule of DC. Interaction CD40 - CD40L results in an upregulation of cos-

stimulatory molecules B7 on the surface of DC - DC licensing. B7 interact with CD28 receptor, providing a dominant costimulatory signal for the activation of naive CD8+ T-lymphocyte.

### **Immune tolerance for tumor antigens**

Existence of tumor-specific lymphocytes and antitumor antibodies before and after vaccination has been found in many cancer patients; however, there has been no correlation with clinical improvement so far.<sup>1</sup> There are many reasons for inefficiency of tumor vaccines. Tumor vaccines contain mainly weakly immunogenic tumor-associated (self) antigens and elicit weak antitumor immune responses. Frequency of tumor-specific lymphocyte precursors, that arise during tumor vaccination, is small ( $\leq 1\%$ ) compared to the frequency of lymphocyte precursors ( $\geq 10\%$ ) against the infectious agents arising during classic vaccination.<sup>8</sup> Even more importantly, the population of potentially tumor-reactive lymphocytes is represented by low-affinity T-lymphocytes, as high-affinity self-reactive T-lymphocytes have been deleted in a process of self-tolerance.<sup>8</sup> Self-tolerance protects the body from autoimmune reactions and plays an essential role in inefficiency of tumor vaccines.<sup>1</sup>

### **Mechanisms of tumor escape**

Tumors evolve many mechanisms to evade actively or silence antitumor immune response, what makes them poorly immunogenic or even tolerogenic.<sup>14</sup>

#### *Tumor cells inefficiently present antigens to effector T-lymphocytes*

Genetic instability of tumors results in changing the tumor antigenic profile.<sup>15</sup>

Mutations of immunodominant tumor epitopes can prevent the recognition of a tumor cell by immune system.<sup>1</sup> Level of tumor peptide presentation through MHC I molecules can be so low that the tumors remain undetected by specific T-lymphocytes.<sup>8</sup> Mechanisms of antigen presentation and processing are defective in many tumors. Low levels of surface MHC I molecules are characteristic of many tumors and correlate with worse prognosis. However, total absence of surface MHC I molecules makes a tumor cell more susceptible for lysis by natural killer cells.<sup>14</sup>

#### *Induction of tolerance - anergy or deletion- of tumor specific lymphocytes*

Tumor cells actively participate in the induction of immune tolerance of tumor-specific lymphocytes. They interfere with maturation of DC, express surface Fas ligand inducing apoptosis of Fas positive tumor-specific lymphocytes, produce immunosuppressive cytokines (IL-10, TGF- $\beta$ ) and redirect immune response in the development of regulatory CD4+CD25+ T-lymphocytes that inhibit the action of effector T-lymphocytes.<sup>1,13-17</sup>

#### *Tumor interference with function of dendritic cell*

It has been found that DC are numerically and functionally defective in cancer patients.<sup>9</sup> Adoptively transferred tumor-specific T-lymphocytes in mouse tumor model become anergic soon after their transfer to a mouse that has already developed the tumor.<sup>16</sup> Anergy is caused by interaction of unmaturing DC, lacking a costimulatory signal, with tumor-specific T-lymphocytes. Level of DC maturation is essential in directing immune response either in antitumor immunity or in unresponsiveness to tumor antigens.<sup>9</sup> Mechanisms of tumor

cell interference with the function of DC involve an early and a late inhibition of DC maturation.<sup>13</sup> The early inhibition of DC maturation is a result of cytokine mediated redirection of granulo/monocyte precursors from DC line to monocyte/macrophage line, decreasing the number of circulating DCs and increasing the number of circulating monocytes/macrophages which are inefficient antigen-presenting cells in antitumor immunity.<sup>13</sup> The late inhibition of DC maturation is a result of tumor mediated suppression of antigen cross-presentation and DC costimulatory molecules expression. Many tumors downregulate the expression of heat shock proteins that participate in endosome to cytosol shuffling pathway and provide maturation signals for DC. IL-10 limits availability of lysosomal proteases that are essential for the production of antigenic peptides.<sup>13</sup> Phagocytosis of early apoptotic melanoma cells, rich in IL-10, inhibits the induction of DC costimulatory molecules.<sup>13</sup>

#### *Peripheral deletion of tumor-infiltrating lymphocytes*

Peripheral deletion of tumor-infiltrating lymphocytes is mediated by Fas ligand on the tumor cells inducing apoptosis of Fas receptor positive tumor-specific T-lymphocytes.<sup>8</sup> Expression of Fas ligand on esophageal carcinoma cells is an early sign of disease progression.<sup>14</sup>

#### *Immunoregulatory CD4+CD25+ T-lymphocytes*

Immunoregulatory CD4+CD25+ T-lymphocytes are important negative regulators of immune response and represent 5 to 10% of peripheral T-lymphocytes. They induce anergy of high-affinity self-reactive T-lymphocytes that have escaped central deletion process, and protect the body from au-

toimmune diseases and overdriven normal immune responses against microbes.<sup>8,18</sup> An increasing number of evidence show that they significantly suppress the anti-tumor immune response and participate in the induction of immune tolerance to tumor antigens.<sup>5,19</sup> Simultaneous use of anti-CD25 monoclonal antibodies and tumor vaccine in a mouse tumor model increases the efficiency of the vaccine and prolongs the survival of the experimental animal. The ratio of peripheral immunoregulatory CD4+CD25+ T-lymphocytes correlates negatively with the prognosis of gastrointestinal malignancies.<sup>23</sup> Cancer patients (23 ± 4%) have increased numbers of peripheral immunoregulatory CD4+CD25+ T-lymphocytes compared to healthy (6 ± 3%) volunteers.<sup>5,19</sup>

#### *Immunoregulatory control points*

The high-affinity inhibitory CTLA-4 receptor expressed on activated T-lymphocytes competes with the lower-affinity stimulatory receptor CD28 for binding the B7 costimulatory molecules on DC. It is implicated in the induction of self-tolerance and regulates the amplitude of normal T-lymphocyte responses. Similar actions are mediated by the B7-H1 and B7-H4 molecules that are frequently over-expressed in pancreatic carcinoma. Monoclonal antibodies directed against the B7-H1 and B7-H4 molecules are already in development.<sup>17</sup>

#### *Production of immunosuppressive cytokines and cytokine immunostimulation of tumors*

Many cytokines (especially IL-10 and TGFβ), produced by tumor cells or tumor-infiltrating lymphocytes, have several different immunosuppressive actions.<sup>14</sup>

Cytokines can also accelerate tumor growth. The immunostimulatory actions of cytokines are seen mainly in hematologic

malignancies. However, most solid tumors express the low-affinity IL-2 receptor  $\beta\mu$  (IL2R  $\beta\mu$ ) which correlates with the increased therapeutic resistance of a tumor. Cytokines should therefore be used cautiously in cancer patients as they could have detrimental effects on the survival of patients.

#### *Tumor microenvironment*

Infiltration of a tumor by tumor-specific lymphocytes is highly dependent on local tumor microenvironment.<sup>20</sup> Tumor microvasculature represents significant barrier for lymphocytes. Although peritumor regions are rich in lymphocytes and tumors are usually well vascularized, lymphocytic infiltration of tumor remains poor.<sup>20</sup> High-endothelial venules with activated endothelium that are important for entrance of lymphocytes in an inflamed tissues are rarely present in intratumor regions.<sup>20</sup> Tumor cells actively suppress expression of endothelial adhesion molecules by local secretion of angiogenic factors and cytokines. Poor lymphocyte infiltration is characteristic of many tumors and bears poor prognosis.

### **Tumor vaccines**

*Ideal tumor vaccine* is a specific-tumor antigen expressed only by tumor cells (cannot induce autoimmune reactions) that participates in carcinogenesis and is crucial to tumor cell survival (preventing selection of immunoresistant clones during immunotherapy). It must be expressed in high levels at all stages of the disease and must be common (universal) to different tumors. It is foreign to immune system and elicits high-affinity cellular and humoral immune responses with long-lived antitumor immunological memory.<sup>5,9</sup>

The real situation is far from being ideal. Most tumor antigens are self-molecules tol-

erated by immune system.<sup>7,21</sup> Tumor-specific lymphocytes isolated from cancer patients are rare and mainly anergic. Antitumor immune response imposes selective pressure over a genetically unstable tumor and accelerates the emergence of immunoresistant clones. Simultaneously, the tumor develops many strategies to evade successfully antitumor immune response.<sup>16,21</sup> Novel immunotherapeutic strategies are therefore directed toward breaking the immune tolerance to tumor antigens, enhancing the immunogenicity of tumor vaccines and overcoming the mechanisms of tumor escape.<sup>16,22</sup> There are several different immunotherapeutic approaches, all of them unfortunately still far away from an ideal tumor vaccine that would reject a tumor.

The present role of tumor vaccines in cancer therapy is minor. They are used mainly as adjuvant treatment in the patients with advanced cancer. However, best results with tumor vaccines could be expected in a state of minimal residual disease after the majority of tumor burden has been removed by surgery or chemotherapy as the probability of immunoselection of resistant clones is the smallest and the immunosuppressive effects of tumor are least pronounced.

#### *Cellular vaccines*

Cellular vaccines are either autologous or allogenic and contain tumor cells or their lysates.

*Classic autologous cellular vaccines* contain attenuated patients' own tumor cells (requiring surgical resection of a sample of the patient tumor). Their primary advantages are that they contain all antigens of the patient's tumor, can be specifically tailored for each patient and in every moment match the tumor's changing antigenic profile.<sup>23,24</sup> Previous identification of tumor antigens is not required and there are no



limitations concerning the patient's HLA haplotype.<sup>15,23</sup> They induce polyclonal anti-tumor immune response that more readily overcomes several tumor evading strategies and imposes smaller immunoselective pressure over the tumor.<sup>15</sup> However, as the identity of tumor antigens is unknown, there are difficulties with the standardization of vaccine production protocol and measurement of postvaccination immune responses. Besides, variable immunogenicity of tumor antigens among different patients influences the efficiency of immune response elicited by vaccine.<sup>15,24,25</sup>

*Allogenic cellular vaccines* are based on the idea that tumors of the same type from different patients share many common antigens. They are prepared from cultured tumor cell lines, standardized, readily available and can be applied to many patients.<sup>15,25</sup> Canvaxin and Melacin are allogenic cellular vaccines, approved in adjuvant therapy of metastatic melanoma, and induce regression of melanoma lesions in 5 to 10% of treated patients.<sup>22,25</sup> The main disadvantage of cellular vaccines is their weak immunogenicity that can be improved by transfection of tumor cells with the genes that code for different immunostimulatory molecules (cytokines, chemokines, adhesion, MHC and costimulatory molecules) or by hybridizing tumor cells and DC.<sup>15,16,25</sup> Genetic modifications have been shown to increase importantly immunogenicity of tumor vaccines, however clinical improvement has remained poor.<sup>16</sup>

#### *Peptide vaccines*

Peptide vaccines are intended to stimulate T-lymphocyte responses to tumor-specific antigenic peptides presented on the surface of tumor cells through MHC I molecules. Namely, most tumor antigens originate in tumor cell cytosol or cellular organelles and present themselves in the complex with the

surface MHC I molecules. Peptide vaccines have several advantages; they are easily produced, inexpensive, safe, synthesized in big quantities and represent a standardized, well defined antigen, allowing post-vaccination immune response monitoring.<sup>2,23</sup> Their main disadvantage is MHC I allotype restriction that makes them useful only in the patients matching MHC I allotype.<sup>2,23,27</sup> Vaccination of cancer patients with one or two antigenic peptides has so far induced specific immune response in as many as 80% of patients; however, clinical improvement has been found only in 10 to 20% of patients.<sup>2</sup> It has been proposed that a combination of many peptides would be necessary to achieve clinical results.

#### *Dendritic cell vaccines*

DCs are professional antigen-presenting cells essential in cross-presentation and differentiation of naive tumor-specific CD8+ T-lymphocytes in efficient cytotoxic cells. The basis for the development of DC vaccines has been established with the protocols for *ex vivo* preparation of DC.<sup>28</sup> DC can be prepared from CD34+ precursor cells isolated from bone marrow or peripheral blood after their incubation with different cytokine combinations, as are TNF $\alpha$ , GM-CSF, Flt3 ligand, CD40 ligand and TGF $\beta$ .<sup>28</sup> Mature low-phagocytic DC are produced, expressing high levels of membrane costimulatory molecules. Alternatively, DC are prepared from peripheral blood monocytes in culture with GM-CSF and IL-4. These DC are immature, highly phagocytic and efficiently take up tumor antigens (tumor cells, peptides, proteins, tumor exosomes, heat shock proteins) they are incubated with.<sup>15,16,28</sup> Another possibility of DC antigen loading is transfection of DC with cDNA or mRNA, coding for tumor antigens, mediated by viral vectors, electroporation or lipofection.<sup>16,28</sup> Still better method

is the transfection of DC with total tumor mRNA. After antigen loading of immature DC is finished, it is necessary to induce DC maturation, mainly by TNF $\alpha$ , Toll-like receptor agonists (CpG oligonucleotides), IL-1 $\beta$  or IL-6.<sup>16,21</sup>

#### *Tumor heat shock protein vaccines*

An increasing number of evidence show that heat shock proteins (HSP), as are GP96 in HSP70 isolated from tumor cells, can induce a specific antitumor immune response. HSP are able to bind the antigenic peptides arising in a tumor cell, to be actively taken up by DC in a process of receptor mediated endocytosis and to induce DC maturation through the interaction with DC Toll-like receptors.<sup>23,29</sup> After HSP internalization, the antigenic peptides are released from HSP and enter antigen processing and cross-presentation process, finally emerging as a complex with MHC I on the surface of APC. There are many advantages of HSP vaccines. They contain many, if not all tumor antigenic peptides, induce polyclonal antitumor immune response, bring antigens to DC and induce their maturation.<sup>23,29,30</sup> The disadvantages of HSP vaccines are time consuming isolation of peptide-HSP complexes and unknown antigenic profile.<sup>23,29</sup> Many animal models have confirmed *in vivo* immunogenicity of HSP70 or GP96 vaccines. They have proven efficient in the induction of prophylactic and therapeutic antitumor immune responses in many preclinical trials and are now being tested in first- and second-phase clinical trials.<sup>33</sup> In a study by Castelli *et al.*, the vaccination of colorectal carcinoma and melanoma patients by GP96 induced statistically significant antitumor T-cell immune response in 59% of melanoma patients and 47.8% colorectal carcinoma patients. A complete regression of melanoma lesions was achieved in 18%

of patients and the survival of colorectal carcinoma patients was prolonged.<sup>29</sup>

#### *Nanovesicular vaccines - tumor exosomes*

Exosomes (nanovesicles) are small membranous vesicles, originating from late endosome. They bud from the membrane of subcellular multivesicular bodies, fuse with plasmalemma and are released extracellularly, where they fuse with the neighbor cells' membranes. Exosomes are composed of different cytosolic and membrane proteins and have dual function. They represent a vehicle for removing redundant cellular proteins and are a pathway for trafficking proteins between cells, thereby participating in a complex intercellular communication.<sup>31</sup> The DC exosomes are enriched in adhesion proteins, costimulatory molecules, MHC I and MHC II molecules together with antigenic peptides - they have immunomodulatory capacity. The tumor cell exosomes are enriched in native tumor proteins and are constitutively secreted by tumor cells. They bring tumor antigens to DC and, through action of surface HSP70, accelerate self-internalization in DC. The incubation of DC with the tumor exosomes *in vitro* and *in vivo* in mouse tumor models results in the activation of specific cytotoxic T-lymphocytes. Because of important role in antitumor immune response and proven preclinical antitumor efficiency, the DC and tumor cell exosomes are being tested in the first-phase clinical trials.<sup>31</sup>

#### *Idiotypic vaccines*

Idiotypic vaccines use the variable region of B-lymphocyte membrane immunoglobulin as an antigen. The variable region contains epitopes unique to malignant B-lymphocyte clone and is therefore highly specific for the tumor.<sup>6</sup> Idiotypic epitopes elicit polyclonal immune responses. Multiple myeloma patients have antiidiotypic antibodies and

idiotype-specific T-lymphocytes in their blood. *In vitro* experiments and animal tumor models have shown that antiidiotypic immune response can destroy malignant myeloma cells.<sup>5</sup> Polyclonal nature of antiidiotypic immune response strongly reduces immunoselective pressure and the resultant emergency of immunoresistant myeloma cells. Idiotypic vaccines could therefore represent a promising immunotherapeutic antitumor strategy.<sup>22</sup> Their main disadvantage is their weak immunogenicity as idiotype is a self-protein. It has been shown that the conjugation of an idiotype with a strongly immunogenic adjuvant is necessary for eliciting an efficient antiidiotypic immune response.<sup>23</sup> Specific idiotypic protein can be produced from hybridoma cells or can be synthesized by methods of recombinant gene technology. Total idiotypic protein can be produced, or better, only its single-chain variable fragment avoiding harmful reactions against the immunoglobulin constant region.<sup>23</sup> Lately, idiotype fusion DNA vaccines have emerged. They are composed of cDNA coding for heavy or light chain variable region linked to bacterial DNA or cDNA coding for the tetanus toxoid C fragment.<sup>5</sup>

### *Viral vaccines*

Cervical carcinoma is caused by persistent infection of cervical epithelia with cancer-associated types of human papilloma virus (HPV). The genome of cancer-associated HPV is found in 99% of cervical malignant lesions.<sup>12</sup> HPV infects the basal cells of cervical squamous epithelia. HPV proliferation and assembly are intimately linked to epithelial cell differentiation program; infective virions are produced only in fully differentiated epithelial cells.<sup>12</sup> As tissue damage with HPV infection is minimal and double helical RNA, an effective APC activator, is not produced during HPV

cycle, the spontaneous anti-HPV immune response is weak.<sup>11</sup> Despite that, most HPV infections spontaneously disappear in few years.<sup>11,12</sup> As cervical carcinoma is caused by HPV16 or HPV18 in two thirds of patients, prophylactic and therapeutic vaccines are directed mainly against their antigens. Prophylactic vaccines contain recombinantly produced HPV16/HPV18 capsid antigens L1, forming virus-like particles, and are entering in clinical use.<sup>12</sup> Two important randomized placebo controlled studies that included young sexually active women have shown the vaccination with HPV16/HPV18-like particles to be safe and effective and protects against persistent HPV infection and development of precancerous cervical lesions.<sup>11</sup> Therapeutic HPV vaccines are directed against HPV proteins E6 and E7 and are mostly experimental with limited clinical efficiency.<sup>11,12</sup>

Difficulties in activation of antitumor immune response by tumor vaccines have led lately to the development of alternative immunotherapeutic strategies directly focusing on the effector mechanisms of immune system. Such approaches include adoptive tumor-specific T-lymphocyte transfer and tumor-specific monoclonal antibodies.

### **Adoptive T cell transfer**

Autologous tumor-specific T-lymphocytes isolated from the patient's peripheral blood, tumor or tumor-infiltrated lymph nodes are activated and multiplied *ex vivo* in the presence of specific T-cell epitopes and then returned to the patient.<sup>16,32</sup> There are several advantages of the adoptive T-cell transfer. It provides large numbers of tumor specific T-lymphocytes which are activated in the absence of inhibitory and tolerogenic tumor actions.<sup>7</sup> Compared to tumor vaccines, it is a better immunotherapeutic option for the patients with widespread disease and



high tumor burden.<sup>32</sup> However, the identity of tumor antigens used in the *ex vivo* activation of antitumor T-lymphocytes has to be known and the process of antigen identification is difficult and time consuming. Main disadvantages are decreased ability of transferred lymphocytes for tumor infiltration and their shorter survival that can be partially improved by adding IL-2.<sup>16,32</sup> Lymphodepletion with the resultant removal of regulatory T-lymphocytes, preceding adoptive T-cell transfer, is an important factor in achieving an efficient antitumor immune response mediated by the transferred tumor-specific lymphocytes.<sup>7,21</sup> Adoptive transfer of Melan-A/MART1 epitope-specific T-lymphocytes or GP100-specific CD8+ T-lymphocytes induced regression of melanoma lesions in metastatic melanoma patients; however, target melanoma epitopes were eventually lost with the resultant overgrowth of immunoresistant melanoma cells.<sup>21</sup>

### Monoclonal antibodies

Antitumor monoclonal antibodies (mAb) are an alternative form of effector immunotherapy. Rituximab (anti-CD20 mAb) and Herceptin (anti HER-2 Neu mAb) are successfully used in the treatment of B-cell non-Hodkin's lymphoma (NHL) and breast carcinoma patients, respectively.<sup>3</sup> There are increasing numbers of novel antitumor mAb that are tested in preclinical and first-phase clinical trials.<sup>33</sup> Rituximab (MabThera®) is a chimeric IgG1 kappa mAb, produced by recombinant gene technology methods. It is used in the treatment of III-/IV-stage chemoresistant follicular NHL and its relapses.<sup>34</sup> CD20 antigen is expressed by healthy B-lymphocytes and more than 90% of B-cell NHLs, but not by plasma cells. Rituximab quickly depletes CD20+ B-lymphocytes with the restoration

of their numbers only 9 to 12 months after treatment.<sup>34</sup> Mechanisms involved in the depletion of B lymphocytes are antibody mediated cell cytotoxicity, complement dependent cytolysis and induction of apoptosis.<sup>34</sup> As the plasma cells are not affected, the production of immunoglobulins is practically normal.<sup>34</sup> Monoclonal antibody efficiency can be improved by their conjugation with toxins, radionuclides or cytotoxic drugs. Mylotrag, anti-CD33 immunotoxin, is used in the treatment of CD33 positive acute myeloic leukemia in older patients and shows comparable antileukemic efficiency to chemotherapy with fewer side effects.<sup>3</sup>

### Conclusion

There are three main requirements for cancer immunotherapy to be effective. *First*, there must be enough high-affinity tumor-specific lymphocytes; *second*, tumor-specific lymphocytes must successfully infiltrate the tumor and *third*, the tumor infiltrating lymphocytes must effectively kill tumor cells. Real situation is totally different. *First*, potentially tumor-reactive T-lymphocyte population is represented by a small number of low-affinity T-lymphocytes. Tumor vaccines can elicit only weak immune response against tumor antigens. *Second*, local tumor microenvironment is an important barrier for T-lymphocyte infiltration of the tumor. *Third*, tumor cells develop several strategies to evade successfully antitumor immune response. Although tumor vaccines arose as promising anticancer strategy with several potential advantages over standard anticancer regimens, the results of clinical trials on tumor vaccines have so far been disappointing. Considering all the barriers that the immune system must overcome to reject the tumor, disappointing results are all but surprising.

## References

1. Mosolits S, Ullenhag G, Mellstedt H. Therapeutic vaccination in patients with gastrointestinal malignancies. A review of immunological and clinical results. *Ann Oncol* 2005; **16**: 847-62.
2. Singh-Jasuja H, Emmerich NP, Rammensee HG. The Tübingen approach: identification, selection, and validation of tumor-associated HLA peptides for cancer therapy. *Cancer Immunol Immunother* 2004; **53**: 187-95.
3. Huber CH, Wolfel T. Immunotherapy of cancer: from vision to standard clinical practice. *J Cancer Res Clin Oncol* 2004; **130**: 367-74.
4. Wong KK Jr, Chatterjee S. Vaccine development for chronic myelogenous leukaemia. *Lancet* 2005; **365**: 631-2.
5. Harrison SJ, Cook G. Immunotherapy in multiple myeloma-possibility or probability? *Br J Haematol* 2005 Aug; **130**: 344-62.
6. Kunkel LA. Idiotype vaccines in the treatment of B-cell non-Hodgkin's lymphoma. *Cancer Invest* 2004; **22**: 97-105.
7. Rosenberg SA. Shedding light on immunotherapy for cancer. *N Engl J Med* 2004; **350**: 1461-3.
8. Emens LA, Jaffee EM. Leveraging the activity of tumor vaccines with cytotoxic chemotherapy. *Cancer Res* 2005; **65**: 8059-64.
9. Turtle CJ, Hart DN. Dendritic cells in tumor immunology and immunotherapy. *Curr Drug Targets* 2004; **5**: 17-39.
10. Adam JK, Odhav B, Bhoola KD. Immune responses in cancer. *Pharmacol Ther* 2003; **99**: 113-32.
11. Frazer IH. Prevention of cervical cancer through papillomavirus vaccination. *Nat Rev Immunol* 2004; **4**: 46-54.
12. Mahdavi A, Monk BJ. Vaccines against human papillomavirus and cervical cancer: promises and challenges. *Oncologist* 2005; **10**: 528-38.
13. Cho HJ, Bhardwaj N. Against the self: dendritic cells versus cancer. *APMIS* 2003; **111**: 805-17.
14. Pawelec G. Tumour escape: antitumour effectors too much of a good thing? *Cancer Immunol Immunother* 2004; **53**: 262-74.
15. Rousseau RF, Brenner MK. Vaccine therapies for pediatric malignancies. *Cancer J* 2005; **11**: 331-9.
16. Mapara MY, Sykes M. Tolerance and cancer: mechanisms of tumor evasion and strategies for breaking tolerance. *J Clin Oncol* 2004; **22**: 1136-51.
17. Laheru D, Jaffee EM. Immunotherapy for pancreatic cancer - science driving clinical progress. *Nat Rev Cancer* 2005; **5**: 459-67.
18. Velders MP, Markiewicz MA, Eiben GL, Kast WM. CD4+ T cell matters in tumor immunity. *Int Rev Immunol* 2003; **22**: 113-40.
19. Terabe M, Berzofsky JA. Immunoregulatory T cells in tumor immunity. *Curr Opin Immunol* 2004; **16**: 157-62.
20. Chen Q, Wang WC, Evans SS. Tumor microvasculature as a barrier to antitumor immunity. *Cancer Immunol Immunother* 2003; **52**: 670-9.
21. Mahnke YD, Speiser D, Luescher IF, Cerottini JC, Romero P. Recent advances in tumour antigen-specific therapy: in vivo veritas. *Int J Cancer* 2005; **113**: 173-8.
22. Antonia S, Mule JJ, Weber JS. Current developments of immunotherapy in the clinic. *Curr Opin Immunol* 2004; **16**: 130-6.
23. Campoli M, Ferrone S. T-cell-based immunotherapy of melanoma: what have we learned and how can we improve? *Expert Rev Vaccines* 2004; **3**: 171-87.
24. Frankenberger B, Regn S, Geiger C, Noessner E, Falk CS, Pohla H, Javorovic M, Silberzahn T, Wilde S, Buchner A, Siebels M, Oberneder R, Willimsky G, Pezzutto A, Blankenstein T, Schendel DJ. Cell-based vaccines for renal cell carcinoma: genetically-engineered tumor cells and monocyte-derived dendritic cells. *World J Urol* 2005; **23**: 166-74.
25. Demierre MF, Allten S, Brown R. New treatments for melanoma. *Dermatol Nurs* 2005; **17**: 287-95.
26. Cheuk AT, Chan L, Czepulkowski B, Berger SA, Yagita H, Okumura K, Farzaneh F, Mufti GJ, Guinn BA. Development of a whole cell vaccine for acute myeloid leukaemia. *Cancer Immunol Immunother* 2006; **55**: 68-75.
27. Baxevasis CN, Sotiriadou NN, Gritzapis AD, Sotiropoulou PA, Perez SA, Cacoullos NT, Papamichail M. Immunogenic HER-2/neu peptides as tumor vaccines. *Cancer Immunol Immunother* 2006; **55**: 85-95.
28. Grunebach F, Muller MR, Brossart P. New developments in dendritic cell-based vaccinations: RNA translated into clinics. *Cancer Immunol Immunother* 2005; **54**: 17-25.

29. Castelli C, Rivoltini L, Rini F, Belli F, Testori A, Maio M, Mazzaferro V, Coppa J, Srivastava PK, Parmiani G. Heat shock proteins: biological functions and clinical application as personalized vaccines for human cancer. *Cancer Immunol Immunother* 2004; **53**: 227-33.
30. Stebbing J, Bower M, Srivastava P. Kaposi's sarcoma as a model for cancer immunotherapy. *Trends Mol Med* 2004; **10**: 187-93.
31. Li X, Zhang Z, Beiter T, Schluesener HJ. Nanovesicular vaccines: exosomes. *Arch Immunol Ther Exp (Warsz)* 2005; **53**: 329-35.
32. Knutson KL, Wagner W, Disis ML. Adoptive T cell therapy of solid cancers. *Cancer Immunol Immunother* 2006; **55**: 96-103.
33. Finley RS. Overview of targeted therapies for cancer. *Am J Health Syst Pharm* 2003; **60 (24 Suppl 9)**: S4-10.
34. White CA. Rituxan immunotherapy and zevalin radioimmunotherapy in the treatment of non-Hodgkin's lymphoma. *Curr Pharm Biotechnol* 2003; **4**: 221-38.
35. Villinger F. Cytokines as clinical adjuvants: how far are we? *Expert Rev Vaccines* 2003; **2**: 317-26.

## **Tumorska cepiva**

**Frank M, Ihan A**

Tumorska cepiva imajo številne prednosti v primerjavi z drugimi oblikami zdravljenja raka. Predstavljajo visokospecifično protitumorsko terapijo in jih lahko usmerimo proti antigenom, ključnim za proces maligne preobrazbe. Imajo edinstven potencial za trajni protitumorski učinek zaradi nastanka dolgoživega, za tumor specifičnega imunskega spomina. Kljub velikim pričakovanjem so dosedanja klinični poskusi cepljenja bolnikov z rakom s tumorskimi cepivi v glavnem prinesli razočaranje. Vzroki za neuspešnost tumorskih cepiv so številni. Potencialno protitumorsko populacijo limfocitov T predstavljajo nizkoafinitetni in maloštevilni periferni limfociti T. Večina tumorskih antigenov namreč predstavlja lastne antigene, za katere je imunski sistem toleranten. Vzporedno tumorji razvijajo različne mehanizme, s katerimi se izogibajo imunskemu sistemu in so kot taki slabo imunogeni ali celo tolerogeni. Novejše imunoterapevtske strategije so usmerjene v premagovanje imunske tolerance na tumorske antigene, povečevanje imunogenosti tumorskih cepiv in nasprotovanje mehanizmom tumorskega izogibanja imunskemu sistemu. Pristopi so številni, a še vedno daleč stran od idealnega tumorskega cepiva, ki bi uspešno zavrnilo tumor. Težave pri aktivaciji protitumorskega imunskega odziva s tumorskimi cepivi so privedle do razvoja alternativnih imunoterapevtskih strategij, ki neposredno vključujejo efektorske mehanizme imunskega odziva (adoptivni prenos limfocitov T in monoklonska protitelesa).