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# Vaccines to Treat Cancer-An Old Approach Whose Time Has Arrived

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**Abstract** There are extensive DNA changes in tumor cells and the genes of tumor cells continuously mutate at a high rate. While this can provide therapeutic targets, it makes it unlikely that an agent that is selective for a single target will work against all cells in a tumor. However, it may be possible to use tumor epitopes as sentinels to engage adaptive and innate immunological mechanisms and create a tumor destructive environment effective also against variant cells that have lost a given antigen or their ability to present it. We hypothesize that therapeutic tumor vaccines, in combination with the targeting, to tumors, of costimulatory molecules such as anti-CD137scFv, or lymphokines such as GMCSF, will expand anti-tumor responses for therapeutic benefit when used as an adjunct to surgery and chemotherapy. J. Cell. Biochem. 102: 291–300, 2007. © 2007 Wiley-Liss, Inc.

**Key words:** cancer vaccines; immunotherapy; targeting

The use of vaccines for treatment of cancer is based on the finding that tumors in both humans and experimental animals, such as mice, express targets that can be recognized by T cells and/or antibodies. In difference to vaccination against infectious agents where vaccines are used to induce neutralizing antibodies that act prophylactically, the objective of therapeutic cancer vaccination is to induce and expand immune responses that can cause the destruction of established tumors. Vaccines against HPV recently became available to prevent cervical cancer, and there will most likely be future vaccines to prevent other human cancers where infectious agents play a role.

## WHY CANCER VACCINES?

There are extensive DNA changes in tumor cells including gene mutations, translocations, and amplifications, and the genes of tumor cells continuously mutate at a high rate [Bielas et al., 2006]. While this provides many therapeutic targets, some of which have high tumor specificity, it makes it unlikely that an agent that is selective for a single target will work against all cells in a tumor, since variants that have lost a given target are likely to occur. However, also these cells may still be accessible to immunotherapy, for example, via "bystander effects" mediated by activated NK cells and macrophages and by lymphokines such as TNF$\alpha$ and IFN$\gamma$. By using tumor epitopes as sentinels to create a tumor destructive environment, it should be possible for the immune system to also kill neighboring variant cells that have lost a given antigen or their ability to present it. The fact that the immune system has memory provides another advantage. The impact of the immune system on cancer can no longer be ignored, and more interactions between cancer immunologists and cancer biologists are likely to bear fruit [Prendergast and Jaffee, 2007].

In this article, we will express our personal views and focus on work with which we have been involved. We make no attempts to cover the field, for which we refer to five reviews including one of our own [Boon et al., 1994; Rosenberg et al., 2004; Palena

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et al., 2006; Emens, 2006; Hellstrom and Hellstrom, 2003].

## TUMORS EXPRESS TARGETS FOR CANCER VACCINES

Pioneering studies several decades ago by Gross, Prehn, Klein, Sjogren, and others demonstrated that syngeneic mice can be immunized to tumors that are induced by chemical carcinogens or certain viruses, so they reject a small number of transplanted cells expressing the immunizing antigens. The "rejection antigens" in chemically induced tumors were found to be unique for each tumor, while tumors induced by the same oncogenic virus expressed shared antigens. In contrast, spontaneously occurring mouse tumors were not rejected and were hence referred to as non-immunogenic. However, when more effective approaches were introduced, for example, by immunizing with tumor cells that had been modified by exposure to a mutagen or infected with a virus, also naturally occurring mouse tumors were found to possess antigens that can both induce and be targets of an immune response, that is, to be immunogenic.

The same tumor commonly expresses both shared and individually unique tumor antigens, which differ in the abilities to immunize a naïve or tumor-bearing host. A simple explanation for the unique antigens is that they are caused by some of the many random genetic changes in cancer cells. Such changes may also explain why chemically induced mouse tumors induced by a large dose of a carcinogen are more "immunogenic" than tumors induced by a small dose. Because of their relative specificity, individually unique tumor antigens may be excellent targets for immunological intervention, as long as it does not have to be tailor-made for each patient. The existence of "epitope spreading", offers hope that a strong immune response induced to one antigen may lead to increased recognition also of other antigens expressed by the tumor, and there may be several approaches to further expand the immune response also to antigens different from those vaccinated against.

It has also been known for a long time that human cancer patients form both cellular and humoral immune responses to their tumors [Hellstrom and Hellstrom, 1969], and a very large variety of human tumor antigens that can be recognized by T lymphocytes and antibodies has been characterized over the past 15 years. These include cancer/testis (CT) antigens, the first human tumor antigens identified as T cell targets [Boon et al., 1997], which are shared by tumors and germ cells in the testis, as well as oncofetal antigens, mucins, differentiation antigens shared by tumor cells and their normal counterparts, so-called universal cancer antigens like hTERT and survivin, antigens encoded by amplified or mutated cellular oncogenes, growth regulatory genes or viral genes, and antibodies to many hundreds of tumor-associated antigens have been identified using the SEREX technology. The question whether human tumors express antigens as a possible vaccine target has thus been answered with a resounding "yes". Among all of them, antigens encoded by genes that are involved in the neoplastic transformation, for example, the HPV E6 and E7 proteins in cervical cancer and Her2/neu in breast and ovarian cancer, are of particular interest because it is less likely for antigen-negative tumor cell variants to survive.

Early studies showed that lymphocytes and sera from cancer patients more selectively recognized tumor cells that were of the same type, suggesting that there were tumor antigens selective for melanomas, breast carcinomas, etc. [Hellstrom and Hellstrom, 1969]. However, few if any such tumor-type specific antigens have been identified using MAbs or tumor-reactive T cell clones as probes. This suggests that the tumor-type specific immunological reactivity was directed against a combination of antigens that is characteristic for a given tumor, for example, for melanoma. Vaccines targeting such antigen combinations may have increased efficacy by focusing the immune response on the tumor and decreasing the response to normal cells which share fewer targeted epitopes with the tumor.

CD8+ T lymphocytes with CTL activity, Th1 type CD4+ helper cells, NK cells, and macrophages all play important roles as effector cells involved in the immunological destruction of tumors, and antibodies have anti-tumor activity by mediating antibody-dependent cellular cytotoxicity and by interfering with signaling via growth factor receptors. The relative role of CTL as compared to CD4+ Th1 type lymphocytes is different against different tumors, and Th2 type lymphocytes can have the opposite effect by producing immunosuppressive lymphokines

such as IL10. Since epitopes that are targets for CTL, or a tumor cell's ability to present them, are sometimes lost (see below), it is crucial that vaccination also generates and expands populations of CD4+ lymphocytes that produce Th1 type lymphokines such as IFN$\gamma$ and TNF$\alpha$ which activate NK cells and macrophages and which also can have a direct inhibitory activity on the tumor cells and their blood supply.

For a tumor-destructive immune response to be induced, antigen presentation to the T cell receptor (TCR) must occur together with costimulatory signals, of which those between CD80 and/or CD86 on the APC and CD28 on the T lymphocytes appear to be most important [Chen et al., 1992]. Antigen presentation by CD80/CD86 negative APC does not cause an effective immune response and can induce anergy via Treg cells [Dhodapkar and Steinman, 2002] and perhaps via other mechanisms as well. Mature dendritic cells (DC) express CD80 and CD86 at high levels, while immature DC do not. Most tumors do not express these ligands [Chen et al., 1992], and a tumor-destructive immune response is therefore not induced until tumor antigen is taken up and presented by mature DC in tumor-draining lymph nodes. This may explain why neoplastic cells are often not recognized by the immune system until a tumor has reached a certain size and is likely to contribute to immunosuppression at the tumor site.

Additional (co)stimulatory signals have been identified and found to facilitate the induction of a tumor-destructive immune response, working alone or in combination. They include, inter alia, CD2 [Li et al., 1996]; CD40 [Todryk et al., 2001], CD137 [Melero et al., 1998; Ye et al., 2002], and CD83 [Yang et al., 2004].

## TUMORS EASILY ESCAPE FROM IMMUNOLOGICAL CONTROL

The ability of tumor cells to escape from immunological destruction constitutes the primary reason why therapeutic cancer vaccines have not been more successful.

Many escape mechanisms have been identified [Kiessling et al., 1999]. We shall first discuss escape mechanisms mediated by the host whose physiological role is to protect against autoimmunity via central and peripheral tolerance. Those mechanisms are likely to be evolutionarily more important than any mechanism protecting from cancer, since most cancers occur first at the end of the reproduction period after a series of events has converted a cell from normal to neoplastic. Continuous presence of antigen is needed to maintain tolerance to normal tissues, and antigen release from tumor cells is likewise needed to prevent an effective anti-tumor immune response [Vaage, 1972].

Tumor-bearing animals often display concomitant tumor immunity, detected by their ability to reject cells from the same tumor transplanted at a different, tumor-free site and by the demonstration of an anti-tumor response in vitro. It is reflected by the fact that T cell signaling mechanisms among tumor-infiltrating lymphocytes (TIL) are often defective but can recover when the lymphocytes are cultured in vitro, implying that the environment at the tumor site is immunosuppressive, probably because of the high concentration of tumor antigen there and perhaps of other immunosuppressive molecules such as TGF$\beta$ as well. Increased understanding of the immune status at the tumor site, including draining lymph nodes, is likely to contribute to the development of more effective immunotherapies.

Some early insight about tumor-related inhibitory mechanisms came from studies demonstrating that sera from tumor-bearing mice and humans can inhibit ("block") anti-tumor responses as studied in vitro, an effect that was attributed to circulating antigen and to antigen-antibody complexes [Sjogren et al., 1971]. Circulating immune complexes have been associated with a poor prognosis for patients with cancer, and transplanted mouse tumor cells were reported to grow poorly in B cell deficient mice whose relative tumor resistance was abolished if the mice were transplanted with B lymphocytes. This may indicate that antibodies, most likely in the form of immune complexes, facilitate a tumor's escape from immunological control. Notably, engagement of CD137, which has strong therapeutic efficacy against some tumors in mice [Melero et al., 1997], causes the depletion of peripheral B lymphocytes and abrogates T cell-dependent antibody responses [Mittler et al., 1999]. However, administration of the anti-B cell monoclonal antibody, Rituximab, did not demonstrate any beneficial effect among patients with renal carcinoma when applied as an adjunct to therapy with IL-2 [Aklilu et al., 2004].

Gershon et al. [1974] demonstrated in the early 1970s that a combination of antigen and antibodies can induce T lymphocytes with suppressive activity. These cells, nowadays referred to as regulatory T cells (Treg), play key roles in establishing and maintaining peripheral tolerance to normal tissues. Intriguingly, there is accumulating evidence that tumors actively engage Tregs in order to create the tumor-microenvironment as an immunoprivileged site, protected from immunological control by the host [Schevach, 2004]. It remains unclear, however, whether antibodies play a role in the generation of Treg cells, although one may hypothesize, since antibodies can facilitate antigen uptake via the Fc receptors on APC, that immune complexes in antigen excess more effectively than antigen alone can exhaust the supply of mature DC in tumors and their draining lymph nodes to cause antigen presentation by immature DC, an event that can lead to the generation of tumor antigen-specific Treg cells [Dhodapkar and Steinman, 2002]. Antigen presentation by plasmacytoid DC can also lead to the generation of Treg cells [Wei et al., 2005], but it is not known whether plasmacytoid DC take up shed tumor antigen or immune complexes more easily than other DC. Both immature DC [Larmonier et al., 2007] and plasmacytoid DC are present at high amounts in tumors. The fact that many tumor cells can present antigen via MHC but lack CD80 and CD86 may also facilitate their escape from immune destruction by promoting the local generation of Treg cells.

The most selective Treg cell marker is the intracellular transcription factor FoxP3 and appears to be intimately involved with the immunosuppressive activity [Kim et al., 2007]. FoxP3 should thus be an excellent target for drug discovery. FoxP3-positive cells are more prevalent in the blood and tumor microenvironment of cancer patients, and intra-tumor accumulation of FoxP3-positive T cell is associated with a poor prognosis for many tumors [Wolf et al., 2005].

In addition to an antigen-specific suppressive effect that depends on cellular contact, antigen-induced Treg cells often make TGFβ and IL10 which suppress immune responses to many antigens. It is likely, therefore, that some of the antigens expressed on tumor cells will engage Treg lymphocytes because of their similarity/identity to normal "self" antigens and cause suppression also of immune responses to antigens of high-tumor selectivity. Other molecules that downregulate immune responses to tumor antigens include prostaglandins and NO [Kiessling et al., 1999]. Both of these molecules have been shown to be produced by macrophages from tumor-bearing mice.

Another downregulatory mechanism is based on the fact that CD80 and CD86 bind not only to CD28, but with even higher avidity to CTLA-4 on activated T cells. The latter binding induces a negative signal that can terminate the immune response [Egen et al., 2002]. Notably, CTLA-4 is also expressed on the surface of Treg cells.

Tumors can escape from immunological control in many other ways as well. Lost expression of tumor epitopes, as well as loss of MHC class I or a tumor cell's ability to process and present epitopes via class I, are major obstacles for cancer vaccines which exclusively induce CTL [Hellstrom and Hellstrom, 2003]. Another obstacle is the apoptosis of T lymphocytes upon contact with B7-H1, a ligand expressed on many tumor cells [Dong et al., 2002]. Production of TGFβ by many tumors, gliomas in particular provides another escape mechanism.

## TUMOR ESCAPE MECHANISMS CAN BE OVERCOME

As we have previously discussed [Hellstrom and Hellstrom, 2003], it may be worthwhile to recollect some findings made during the early days of kidney transplantation, when a few patients were transplanted with cadaver kidneys from cancer patients that had been contaminated with cancer cells and developed large metastases. As immunosuppression was withdrawn, the metastases were commonly rejected, most likely because of an immune response against their foreign histocompatibility antigens. Gestational choriocarcinomas express foreign histocompatibility antigens and may represent a similar case, since the majority of patients are cured, even in the presence of widespread metastases. Taken together these findings imply that also large tumors can be destroyed by immunological mechanisms and are not inherently protected.

Other evidence that the immune response can be engaged to cure human cancer patients comes from the demonstration that leukemia patients who received allogeneic bone marrow that differed at minor histocompatibility loci

performed significantly much better clinically than patients receiving autologous marrow or marrow from an identical twin [McSweeney et al., 2001]. The presence of foreign histocompatibility antigens on the tumor cells is likely to have engaged a larger part of the T cell repertoire than that only recognizing tumor-associated antigens.

Notably, most patients with advanced seminomas can be cured by chemotherapy, which is in sharp contrast to most patients with cancers of other organs, for example, ovary. The expression of many CT antigens is particularly high in seminomas, and following the destruction of the majority of tumor cells by cytotoxic drugs, we speculate that the many CT antigens expressed on tumor cells recruit a very large number of tumor-reactive T lymphocytes, a situation analogous to that when a tumor expresses foreign histocompatibility antigens.

Some of the beneficial effects of cytotoxic drugs, as well as of $\gamma$-irradiation, may be explained by a favorable effect on a patient's immune response to tumor antigens. Destruction of large tumor masses (including tumor removal by surgery) will decrease the number of tumor cells that needs to be destroyed by the immune system, it removes a source of tumor antigen that can thwart the generation of an effective immune response, and it may also, like radiation and the anticancer drug cyclophosphamide, inhibit suppressive T lymphocytes [Hellstrom and Hellstrom, 2003]. Furthermore, anthracyclins such as doxorubicin, can make tumor cells more immunogenic [Obeid et al., 2007] by translocating calreticulin to the tumor cell surface. For these reasons we speculate that anti-tumor immune responses are an essential component of most conventional anti-tumor therapies and that integration of tumor vaccines into established treatment schedules is likely to enhance their efficacy.

## IMMUNE DESTRUCTION OF TUMORS—THE IMPORTANCE OF BYSTANDER EFFECTS

The high mutability of tumor cell populations can provide novel epitopes as vaccine targets but also makes it likely that tumor variants will evolve that do not express a given epitope. In addition, epigenetic events can influence antigen expression. Consequently, all cells within a tumor are unlikely to express and present those antigens that are commonly the targets for immunotherapy. For tumor vaccination to be clinically successful, also those tumor cells that lack or fail to present the antigen vaccinated against need to be destroyed.

Our group has recently demonstrated that syngeneic melanoma cells transfected to express single chain (scFv) fragments of an immunostimulatory anti-CD137scFv antibody at their surface are rejected when transplanted onto an immune-competent host by a mechanism that involves accumulation at the tumor sites of Th1 type lymphocytes and NK cells. Intriguingly, admixture of the anti-CD137scFv-expressing melanoma cells to wild-type (non-transfected) cells from the same melanoma or from an antigenically unrelated sarcoma caused rejection of the wild-type tumor cells via "bystander effects" [Yang et al., 2007]. Based on this observation, mice with small-established tumors from the low-immunogenic B16 mouse melanoma were given cyclophosphamide systemically to decrease the impact of Treg cells and 4 days later their tumors were injected with an adenovirus vector encoding anti-CD137 scFv. Anti-tumor responses were seen, including complete and partial remissions, while a combination of cyclophosphamide with subcutaneously transplanted MMC-treated B16 cells expressing anti-CD137scFv had no therapeutic effect when given as a systemic vaccine, that is, the immune stimulation by anti-CD137 scFv had to be targeted to the tumor site. An agonistic MAb may be considered the most straightforward approach to engage CD137 [Melero et al., 1997]. However, working in an immune-tolerant transgenic model with carcinomas expressing epitopes encoded by the Her2/neu oncogene, Zhang et al. [2006] found that a vaccine in the form of MMC-treated tumor cells that expressed anti-CD137 scFv was more efficacious and less toxic than systemic administration of anti-CD137 MAb.

It is noteworthy that occasionally dramatic therapeutic effects have been seen in some patients given the anti-colon carcinoma Mab 17.1.A, although it is not highly selective for cancer cells and is not known to interfere with growth factor receptors. We speculate therefore, as have others, that those effects are secondary to an antibody-mediated induction of an active immune response at tumor sites, and clinical responses to some other anti-tumor Mabs may, likewise, be secondary to a locally induced anti-tumor immunity. Combination therapy in an orthotopic renal cell

carcinoma model by giving IL-2 systemically and intratumoral injection of a fowlpox vector encoding three costimulatory molecules (CD80, ICAM-1, and LFA-3) plus GM-CSF reduces tumor burden in mice [Kudo-Saito et al., 2007], another finding that is reminiscent of the "bystander effects" referred to. The successful therapy of non-metastatic bladder carcinomas by local application of Bacillus Calmette Guerin (BCG) as an immunostimulant, and the local treatment of skin tumors by systemic injection of a sensitizing agent combined with its local application [Klein et al., 1976] probably has the same explanation, that is, the accumulation at tumor sites of Th1 type CD4 cells accompanied by activated NK cells and macrophages which themselves have antitumor activity and which, in addition, may favor the generation of an immune response to tumor selective antigens, and the localization of systematically derived immune T cells to tumor.

Because of the frequent loss of tumor epitopes and the tumor cells' ability to present such, it is important that the vaccination makes it possible for epitope-positive tumor cells to act as sentinels promoting the creation of an environment at the tumor site that can destroy also those tumor cells that cannot be killed by CTL. To increase the probability of tumor destruction, we therefore propose that either the tumor vaccine or some immunomodulatory agent, facilitating the influx and expansion of tumor-selective T lymphocytes is delivered to the tumor site for uptake there and in draining lymph nodes to provide a "danger signal". A tumor-seeking, recombinant virus [Gaggar et al., 2003] or some antibody-construct may provide potential vehicles for such targeting of the primary tumor and its metastases. For non-resectable but locally accessible tumor masses, direct intratumoral application of immunomodulatory agents is an alternative option.

Besides providing immunostimulatory signals, the therapeutic efficacy of tumor-site located vaccination will be increased by incorporating antibodies or drugs, which specifically target the immunoinhibitory mechanisms inside the tumor, for example, antibodies which inactivate Tregs or trigger the maturation of dendritic cells. Subsequently, tumor-site located vaccination and counteraction of intra-tumoral immunosuppressive networks can be combined with a vaccine given systemically.

Targeting of immunomodulatory agents may facilitate the generation of immune responses to a variety of epitopes expressed by the given tumor by combining a large number of different epitopes expressed by the tumor cells, including unique ones, with a strong immunological stimulus. Importantly, the side effects observed with a targeting approach are likely to be substantially smaller than those resulting from the systemic administration of a drug, antibody, or tumor vaccine.

## TUMOR VACCINATION FOR SYSTEMIC DELIVERY

We have already emphasized the importance that tumor vaccination not only engages CTL but also CD4+ Th1 type helper cells and that NK cells and macrophages are attracted and activated at the tumor site. Furthermore, vaccination needs to be combined with some agent(s) to counteract the most powerful tumor escape mechanisms, including the one mediated by Treg and perhaps other mechanisms as well, such as molecules made by tumor cells and thwarting tumor-directed T lymphocytes. Mabs to CD25 and to CTLA4 have been used for the latter purpose, as has cyclophosphamide. Some anti-cancer drugs may have effects similar to cyclophosphamide as has a low dose of X-irradiation, and we expect that drugs targeting FoxP3 will be discovered with increased selectivity for Treg cells. A combination between tumor vaccination and adoptive transfer of tumor-reactive T cells should also be considered. Furthermore, the localization to tumor sites of immunostimulatory agents, as discussed in the preceding section, may facilitate the expansion and function of adoptively transferred CD4 and/or CD8 T lymphocytes.

To increase the probability of success, therapeutic tumor vaccines are best administered when the tumor load is minimal following conventional cancer therapy, for example, to a patient with stage III or IV ovarian carcinoma who temporarily is clinically tumor-free but has a high probability for relapse. Beyond the fact that the number of tumor cells that needs to be destroyed is less, the remaining tumor cells are less protected by physical barriers, and escape mechanisms driven by tumor antigen and other molecules made by the neoplastic cells are less pronounced.

Among the approaches taken to vaccinate against established tumors, the probably simplest one is to immunize with killed (e.g., by X-irradiated) autologous (in man; syngeneic, in mice) tumor cells in combination with an adjuvant, to be administered after conventional therapy when the tumor load is small to boost an anti-tumor response with the objective to destroy any remaining tumor cells. Cell-based autologous vaccines comprise the large variety of antigens expressed by the given tumor but have the disadvantage that they have to be "tailor-made" for each patient. A relatively large pool of cells from allogeneic tumor lines should be representative for shared tumor antigens but would not include any unique ones, resulting from mutations within an individual patient's tumor. Some clinical benefits have been reported from studies on patients with melanoma given such pools of allogeneic tumor cells [Morton and Barth, 1996]. However, it is noteworthy that, if sufficient costimulatory signals are not provided, injection of killed tumor cells to a tumor-bearing host can also induce immune-tolerance toward tumor-antigens and thus facilitate tumor growth [Vaage, 1973].

To make cell-based vaccines more effective, tumor cells can be transfected to make certain lymphokines and/or to express certain costimulatory molecules. Encouraging clinical findings have been obtained when tumor cells were transfected to make GMCSF at the vaccination site [Pardoll, 1998]. Our group has focused on vaccines incorporating costimulatory molecules rather than lymphokines, believing that they can generate a cascade of immunostimulatory molecules. Therapeutic effects against established mouse tumors have been obtained following immunization with tumor cells transfected to express various (co)stimulatory molecules, including CD80 [Chen et al., 1992], CD137 ligand [Melero et al., 1998], and CD83 [Yang et al., 2004]. Working with CD137 and CD40 we have been more successful replacing the ligand with the scFv from a Mab recognizing the respective costimulatory receptor [Ye et al., 2002] (and unpublished findings). Vaccines combining more than one costimulatory signal are often advantageous [Palena et al., 2006; Yang et al., 2004].

An alternative to using cell-based tumor vaccines is to vaccinate against one or several molecularly defined antigens that are expressed by the given tumor, either in the form of peptides [Disis et al., 2002] or carbohydrates or of gene(s) encoding known tumor proteins. The advantage is that the antigen is well-defined helping standardization of the vaccine and measurement of the immune response to the vaccine. Although an immune response to a peptide may be narrow, epitope spreading often causes an immune response also against antigens that are not vaccinated. Promising preclinical results have been obtained in transgenic female mice that carried the rat Her-2/neu oncogene and spontaneously develop breast carcinomas by vaccination with a CTL epitope in the form of a peptide from the rat HER-2/neu gene product in combination with a Toll-like receptor agonist adjuvant. Mice that were vaccinated with this approach and also given anti-CD25 Mab to counteract Treg cells did not develop mammary carcinomas and had long lasting CTL responses, also against other HER-2/neu peptides as a result of epitope spreading [Nava-Parada et al., 2007].

Several gene-based cancer vaccines have been tested, including cDNAs [Disis et al., 2003; Qin et al., 2007] and recombinant viruses [Adamina et al., 2005]. A vaccine, PANVAC $^{TM}$-VF, vaccine for pancreatic cancer is in clinical trials. It is composed of a priming dose of recombinant vaccinia virus and booster doses of recombinant fowlpoxvirus expressing carcinoembryonic antigen, mucin-1, and a triad of costimulatory molecules, CD80, IAM-1, and LFA-3 [Petrulio and Kaufman, 2006]. The vaccine is given subcutaneously, followed by 4 days of local injection of GMCSF at the vaccination site.

An attractive alternative is to transfect bacteria, Listeria in particular, to express a given tumor antigen [Sewell et al., 2004] for uptake, processing, and presentation. Most likely, the costimulatory molecules engaged and cascade of lymphokines produced, are the same as normally involved in guarding against infection.

Heat shock proteins HSP70 and GP96 can act as potent adjuvants for eliciting anti-tumor immunity, and HSP-based tumor vaccines have been successful in animal models and are now being tested in man. HSPs function as chaperones for tumor antigen to elicit tumor-specific adaptive immune responses, and HSPs also appear to induce innate immune responses in

an antigen-independent fashion [Facciponte et al., 2006].

Since DC may be deficient in number or function at the tumor site, Song and Levy evaluated the ability of immature naïve dendritic cells to induce antitumor immunity when injected directly into a murine B cell lymphoma. While injection of the DC alone had no therapeutic effect, and systemic chemotherapy alone resulted in only transient tumor regression, a combination of intratumoral injection of DC after chemotherapy led to complete, long-term tumor regression in most of the treated mice with regressions also of tumors that had not been injected with DC [Song and Levy, 2005]. Although DC from tumor-bearers are often deficient in antigen processing/presentation, they can recover from this deficiency when removed from the body [Gabrilovich et al., 1996], after which they are pulsed with antigen or transfected for use as immunogen to either present an array of antigens expressed on a patient's tumor or a selected number of tumor epitopes. This approach has yielded encouraging findings in several mouse models and has for several years been evaluated in man with some promising results [Steinman and Pope, 2002].

Anti-idiotypic antibodies as cancer vaccines attracted some attention several years ago, but there has not been much recent development in this field.

## CONCLUSIONS

There is an abundance of preclinical data showing that experimental animals can be cured from small-established tumors by various strategies involving tumor vaccination, and that spontaneous appearance of tumors can be delayed or even prevented. Clinical responses in some cancer patients, including complete remissions, make it likely that improved approaches to cancer vaccination will become part of the clinical mainstay. However, better strategies are needed to destroy cancer cells that have lost tumor antigens or their ability to present them as CTL targets and to circumvent mechanisms that downregulate the immune response to tumors, most notably those mediated by Treg cells, without causing autoimmune damage to normal tissues.

Drug combinations have made cancer chemotherapy highly successful against some tumor types. We believe that, likewise, combination therapies, involving an immunological approach, will have major impact on cancer therapy. They will probably include combinations of immunotherapy with other forms of cancer therapy, as well as combinations of a systemically applied cancer vaccine with the targeting of immunostimulatory agents to tumors and/or the adoptive transfer of in vitro expanded tumor reactive T lymphocytes.

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