Leveraging the Activity of Tumor Vaccines with Cytotoxic Chemotherapy

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Abstract

Engaging the power of the patient’s own immune system to actively seek out and destroy transformed cells holds great promise for cancer therapy. Tumor vaccines offer the potential for preventing cancer in high-risk individuals, preventing disease relapse after diagnosis and initial therapy, and shifting the balance of the host-tumor interaction to mitigate the progression of advanced cancers. The therapeutic activity of tumor vaccines is limited by the sheer physical burden of the cancer itself, pathways of local immune tolerance and escape active within the tumor microenvironment, and superimposed potent systemic mechanisms of immune tolerance. In this review, we describe how cytotoxic chemotherapy can be integrated with tumor vaccines using unique doses and schedules to break down these barriers, releasing the full potential of the antitumor immune response to eradicate disease. (Cancer Res 2005; 65(18): 8059-64)

Introduction

Surgery, radiation, and chemotherapy are the mainstay of cancer management. Surgery and radiation therapy are relatively precise and used to achieve local control. In contrast, cytotoxic chemotherapy exerts a systemic effect and is used to cytoreduce established tumors and eradicate micrometastatic disease. When properly sequenced, these treatment modalities can cure a substantial number of hematologic malignancies and a smaller subset of various early-stage solid tumors. However, despite the use of combination chemotherapy regimens that incorporate several drugs with complementary mechanisms of action and nonoverlapping toxicities, a significant percentage of cancers remains incurable. Furthermore, the imprecise nature of combination chemotherapy results in collateral damage to normal tissues, resulting in significant side effects that adversely affect the patient’s quality of life. The associated toxicities and limited success of traditional treatments in maximizing cure rates is a clear mandate for developing innovative therapeutic strategies that capitalize on emerging insights about tumor biology and the host-tumor interaction. Immune-based approaches that recruit the host antitumor immune response to the therapeutic effort are a particularly attractive strategy for improving clinical outcomes in malignant disease.

Recent progress in biotechnology and molecular medicine has accelerated the development of immune-based drugs, and a number of therapeutic monoclonal antibodies are in standard use today. Progress in manipulating the cellular arm of the immune response has been slower, but viable cancer therapies based on the adoptive transfer of lymphocytes or the de novo induction of an effective antitumor immune response by active vaccination are under intense investigation. Immunization with tumor vaccines in particular offers advantages that other cancer therapies do not. First, it is highly specific and can target antigens integral to the process of transformation. Second, it is well tolerated, with minimal side effects limited primarily to injection site reactions and minor systemic toxicities (transient fever and flu-like symptoms). Third, vaccination offers the unique potential for a durable antitumor effect due to the phenomenon of immunologic memory, potentially obviating the need for prolonged, repetitive cycles of therapy.

Immune Tolerance as a Major Barrier to Effective Immunization against Cancer

Despite these advantages, the clinical efficacy of immune-based therapeutics can be severely curtailed by the burden of established cancer compared with the magnitude of the immune effectors in play. This is reflected by the dynamic interplay between progressive tumor growth and the immune response. When tumors are small, they grow without accessing peripheral lymphoid tissues and are thought to “sneak through” immune surveillance at its earliest stages (1). With progressive growth, the tumor and the immune system engage one another, setting the stage for immunoeediting (2). At this point, immune-mediated tumor rejection is determined by the relative balance between the growth kinetics and physical burden of tumor cells compared with the intensity and diversity of the effector T-cell response induced (3, 4). Ultimately, the tumor simply overwhelms the developing immune response, resulting in relentless disease progression. Superimposed on this dynamic, both active immune tolerance and the genetic instability of tumor cells themselves further discourage an effective antitumor immune response. These observations, together with recent revelations into the molecular basis of immune tolerance and tumor biology, suggest strategies for circumventing some of these limitations, thereby facilitating the recruitment of immune effectors to mediate successful tumor rejection.

Immune tolerance results from the integration of multiple, overlapping regulatory mechanisms that have evolved to prevent the development of immunity to antigens perceived as self-antigens (5). Vaccination targeting foreign antigens to prevent infectious disease can induce antigen-specific T-cell precursor frequencies of ≥10%. In contrast, tumor vaccines typically induce a tepid immune response to endogenous self-antigens, with antigen-specific T-cell
T cells (Treg) can shut down those high avidity T cells that do escape therapy. Importantly, this phenotype has been correlated with mechanisms together providing a means for the outgrowth of antigen-II, proteasome subunits, and the TAP transporter; ref. 11). These components of the antigen-processing machinery (MHC class I, MHC class II, and two at 5 × 10^8 cells) developed evidence of delayed-type hypersensitivity (DTH) to autologous tumor cells after one vaccination. These three patients also developed mesothelin-specific CD8^+ T cells after one vaccination as measured by ELISPOT; none of the other 11 patients with negative DTH to autologous tumor developed evidence of mesothelin-specific immunity (17). Notably, after completing 6 months of adjuvant therapy, the mesothelin-specific CD8^+ T-cell response was undetectable in these patients and was only restored after three additional vaccinations.

**Cytotoxic Chemotherapy Can Augment Tumor Vaccine Activity**

Because cytotoxic chemotherapy is widely used to treat most malignancies, integrating tumor vaccines with standard chemotherapeutic drugs is highly attractive. Carefully choosing the dose and timing of chemotherapy in relation to immunization with tumor vaccines maximizes the potential for capitalizing on potential synergy between these treatment modalities. The importance of dose and timing is often overlooked but is a pivotal consideration in the design of treatment regimens that effectively combine cytotoxic drugs and tumor vaccines. Rational treatment strategies that combine tumor vaccines with cytotoxic drugs can be integrated in at least three ways. First, chemotherapeutics can be combined with surgery and radiation to achieve a state of minimal residual disease, thereby altering the balance of the disease burden and the vaccine-induced T-cell response in favor of the T cell. Here, standard drug doses are typically used, and immunization must be timed to occur either during or after drug reconstitution. Second, chemotherapy can be used to groom the local tumor microenvironment to optimally support a productive immune response. Here, chemotherapy must be given at doses that modulate immunologically relevant features of the tumor cells (e.g., antigen expression) at the time that vaccine-induced immune effectors come into play. Finally, chemotherapy can be used to set the stage for a robust vaccine-induced immune response by globally altering immunoregulation within the host, subsequently permitting a robust vaccine-induced immune response. Here, chemotherapy is used in a dose and schedule designed to abrogate specific mechanisms of immune tolerance, or to facilitate skewing of the T-cell repertoire during immune reconstitution. These concepts are illustrated in Figs. 1 and 2, and specific examples are provided below.

**Cytoreduction and Tumor Vaccines.** Traditional drug development has typically begun in heavily pretreated patients with extensive disease. This approach has initially been carried over into cancer vaccine development, with emerging evidence of adverse consequences on vaccine-induced immunity. For example, both a greater number of prior chemotherapy regimens and close juxtaposition to a prior chemotherapy treatment was reported to limit the induction of carcinoembryonic antigen (CEA)-specific T-cell precursors in patients with advanced colorectal carcinoma immunized with the canary pox vaccine ALVAC-CEA (15). Jaffe et al. also reported the negative effect of standard cancer therapy on vaccine-induced immune responses. They conducted a phase I vaccine cell dose escalation trial of a granulocyte macrophage colony-stimulating factor (GM-CSF)-secreting allogeneic pancreatic tumor vaccine integrated with primary surgery followed by adjuvant chemoradiation in 14 patients with high-risk stage II and III pancreatic cancer (16). After pancreaticoduodenectomy, patients received one immunization, and then went on to 6 months of aggressive chemoradiation. Those who had no evidence of disease recurrence after adjuvant therapy then received three additional immunizations at monthly intervals. Three individuals who received the highest doses of vaccine cells (one at 1 × 10^9 cells and two at 5 × 10^8 cells) developed evidence of delayed-type hypersensitivity (DTH) to autologous tumor cells after one vaccination. These three patients also developed mesothelin-specific CD8^+ T cells after one vaccination as measured by ELISPOT; none of the other 11 patients with negative DTH to autologous tumor developed evidence of mesothelin-specific immunity (17). Notably, after completing 6 months of adjuvant therapy, the mesothelin-specific CD8^+ T-cell response was undetectable in these patients and was only restored after three additional vaccinations. These data provide clear evidence for the inhibitory effect of standard therapy in this patient population and suggest that it can be overcome with appropriate boosting schedules for subsequent vaccinations. These observations provide a strong argument for integrating therapeutic vaccination with standard cancer therapies in schedules that optimize the activity of each modality. Further, the likelihood of imbalance between the magnitude of tumor burden and the intensity of the vaccine-induced antitumor immune response in advanced disease strongly suggests that...
patients with minimal residual disease are the more appropriate target patient population for combining therapeutic cancer vaccines with traditional treatment modalities.

Modulation of the tumor microenvironment. Cytotoxic drugs can be used to modify the tumor microenvironment, thereby making it more receptive to an effective immune response. The direct cytolytic effect of some cytotoxic drugs, such as doxorubicin, 5-fluorouracil (5-FU), gemcitabine, and paclitaxel, can enhance antigen presentation by inducing tumor cell apoptosis (18, 19). This mechanism of therapeutic synergy has been shown with CY, doxorubicin, or paclitaxel when given with dendritic cell–based vaccines (20, 21). Notably, one clinical study showed that, even in the absence of concurrent vaccination, the first dose of neo-adjuvant paclitaxel induced an apoptotic response within the tumor that correlated with the induction of TIL in 67% of locally advanced breast cancer patients who developed a complete clinical response (22). Paclitaxel has other potential mechanisms of immunologic synergy. For example, it can activate dendritic cells through the toll-like receptor signaling pathways, thus engaging the innate immune response. Paclitaxel also induces cytokine production patterns typical of the T helper type I phenotype, thereby promoting effective cytotoxic T-cell responses (23–26).

Another way in which cytotoxic drugs can make the tumor microenvironment more conducive to an effective immune response is by restoring the expression of tumor antigens or MHC molecules that have been lost during tumor progression. 5-Aza-2’-deoxycytidine can reinduce the expression of these molecules on tumor cells in vitro thereby restoring melanoma- and renal cell carcinoma–specific CTL activity (27). Similarly, 5-FU restores CTL activity against treated colon and breast carcinoma cells (28). Some chemotherapeutics (melphalan and Mitomycin C) up-regulate the expression of costimulatory molecules (B7-1 and B7-2) thereby rendering the tumor cells themselves more efficient antigen presenting cells. Others (5-FU and cisplatin) sensitize tumor cells to CTL-mediated apoptosis through Fas- or perforin/granzyme-mediated pathways (29). Finally, metronomic scheduling of a variety of chemotherapeutics can modulate the structure and biochemical nature of the tumor-associated vasculature thereby altering T-cell trafficking and activation within the tumor microenvironment (30). These mechanisms offer numerous opportunities for potential therapeutic synergy with tumor vaccines.

Host milieu and tumor vaccines. Cytotoxic drugs can also potentiate the vaccine-induced immune response by mitigating systemic mechanisms of active immune tolerance, or by otherwise
altering the global immunologic milieu in which the antitumor immune response develops. Many chemotherapeutics can either amplify or diminish the antigen-specific immune response depending on the drug dose and timing in relation to the antigen exposure (31). CY given in immune-modulating doses at the time of T-cell priming (usually 1-3 days before antigen exposure) enhances humoral and cellular immunity and abolges immune tolerance. Conversely, CY given with or subsequent to antigen inoculation induces immune tolerance. Importantly, accumulating data suggests that properly scheduled CY augments immunity by abrogating the activity of CD4+CD25+ Treg (7, 32, 33). Like paclitaxel, CY (and melphalan) promotes the induction of the T helper type 1 response characteristic of effective antitumor immune responses (25, 34). CY also up-regulates type 1 IFNs, facilitating the evolution of a CD44hi memory T-cell response (35). Although the mechanisms remain unclear, other chemotherapeutics also affect immunity. Doxorubicin given 3 to 5 days before antigen exposure augments adaptive immunity in some models (31). In others, it must be given a week after antigen exposure (at the time of T-cell expansion) to augment the CD8+ T-cell response (25, 36). In one study, gemcitabine inhibited humoral immunity while potentiating the cell-based immune response (37). In contrast, we have shown that gemcitabine inhibits the activity of GM-CSF-secreting cell-based vaccines in HER-2/neu-transgenic mice.

Multiple groups have shown that the immunomodulatory activity of cytotoxic drugs can be harnessed by using them as vaccine adjuvants in immunization regimens for cancer. In one exploratory study, 11 cytotoxic agents were systematically compared to assess their ability to enhance immune induction by GM-CSF-secreting vaccines in the CT26 model of colorectal cancer (36). CY with or without vaccination cured between 30% and 35% of tumor-bearing mice. Vaccination followed by doxorubicin cured 40% of mice with established tumors, whereas doxorubicin alone cured 10% of tumor-bearing mice. The mechanism by which doxorubicin enhanced vaccine-induced immune responses was not explored in this study. The other cytotoxics tested in this model clearly reduced vaccine efficacy.

Machiels et al. extended these studies to the neu transgenic mouse model of spontaneous breast cancer (25). Neu mice represent a stringent and clinically relevant laboratory model for developing potent immunization strategies that can overcome immune tolerance. Due to mouse mammary tumor virus–driven expression of the rat neu proto-oncogene, they spontaneously develop breast cancers histologically similar to human mammary tumors (38) in the context of profound immune tolerance specific for HER-2/neu (39). Ercolini et al. recently characterized the HER-2/neu-specific immune response at the molecular and cellular level in both nontolerized parental FVB/N mice and in tolerized neu transgenic

![Figure 2](image_url)
mice (7, 25, 40). Parental FVB/N mice vigorously reject large, established HER-2/neu-expressing tumors after immunization with HER-2/neu-targeted, GM-CSF-secreting vaccine cells. This immune response is characterized by both substantial HER-2/neu-specific antibody titers and a robust population of high avidity CD8+ T cells that are almost exclusively specific for the immunodominant epitope of rat HER-2/neu, RNEU420-429 (40). In contrast, neu mice mount a very weak response to vaccination, with no discernible difference in tumor outgrowth rates between mice immunized with mock vaccine compared with the HER-2/neu-targeted, GM-CSF-secreting vaccine cells (25). The HER-2/neu-specific immune response is characterized by minimal HER-2/neu-specific antibody titers and a diverse CD8+ T-cell population that is small, of low avidity, and contains rare CD8+ T cells specific for the immunodominant epitope RNEU420-429 (7, 25, 40).

Neu transgenic mice develop a more robust antitumor response to immunization when preceded by CY (100 mg/kg) or paclitaxel (20 mg/kg) given 1 day before vaccination (at the time of T-cell priming; ref. 25). In addition, sequencing immunization with doxorubicin (5 mg/kg) 1 week later (at the time of T-cell expansion) augmented the antitumor response (25). Others have also validated the immunomodulatory activity of doxorubicin and paclitaxel in neu mice vaccinated with either plasmid-based or viral vaccines specific for HER-2/neu (41). Reversing the sequence of the drugs and immunization in our model inhibited vaccine activity. Vaccine activity also diminished as increasing drug doses resulted in decreasing peripheral T-cell counts (25). Importantly, the combination regimen of CY (day – 1), vaccination (day 0), and doxorubicin (day 7) given in a specifically timed sequence was most effective, curing up to 40% of neu mice of preestablished tumors.

These studies revealed two mechanisms by which CY augments vaccine activity in neu-transgenic mice. The first mechanism is that CY reverses immunologic skew, favoring the development of a productive HER-2/neu-specific T helper type I response as measured by ELISPOT; paclitaxel exerts a similar effect (25). The second mechanism is that CY abrogates the suppressive influence of cycling CD4+CD25+ Treg, enabling the recruitment of otherwise latent, high avidity CD8+ T cells specific for the immunodominant epitope RNEU420-429 to the antitumor immune response (7). Importantly, high avidity CD8+ T cells specific for RNEU420-429 are detectable only in those neu mice that received CY, vaccine, and doxorubicin and that were cured of their tumors. These data have important implications for the clinical development of tumor vaccines. They suggest that functional, high avidity, antigen-specific T cells capable of effecting the most potent tumor rejection may be present within the host and recruited to the antitumor immune response if the appropriate vaccination regimen is used. Together, these observations highlight the importance of drug schedule and dose in relation to immunization. These concepts are currently undergoing "proof-of-principle" testing in early clinical trials (42).

Cytotoxic drugs can also alter the host milieu in which an antitumor immune response develops in other ways. Lymphopenia-induced homeostatic T-cell proliferation is a recently described mechanism for restoring the memory T-cell compartment (43). Manipulating the T-cell repertoire by immunization during immune reconstitution after lymphoablative treatments might skew the T-cell repertoire towards a particular antigen specificity (44). Supporting this idea, the induction and expansion of active, melanoma-specific T cells was achieved in RAG-1-deficient lymphopenic tumor-bearing mice vaccinated with a GM-CSF-secreting melanoma vaccine, resulting in significant tumor regressions (45). In more clinically relevant models, vaccine-induced antitumor immunity can be enhanced by immunizing tumor-bearing mice with GM-CSF-secreting cancer vaccines during early engraftment after syngeneic or allogeneic T cell-depleted bone marrow transplantation (46, 47). Similar studies revealed that tumor-bearing mice treated with surgical resection followed by nonmyeloablative allogeneic stem cell transplantation and then donor lymphocyte infusions plus vaccination with a GM-CSF-secreting tumor vaccine developed immune responses capable of lysing metastatic 4T1 mammary tumors (48). Importantly, the phenomenon of homeostatic proliferation in humans has been suggested by the adoptive transfer studies of Rosenberg et al. (49–52). Because many standard cancer therapies result in lymphopenia, characterizing the kinetics, persistence, and functional quality of tumor antigen-specific immune reconstitution will be required for the effective application of cancer vaccines to the lymphopenic setting. A number of clinical trials testing cytotoxic agents in sequence with cancer vaccines are ongoing.

Conclusions and Future Directions

It is now clear that standard cancer therapies, including chemotherapy and radiation therapy, can have a profound pharmacodynamic influence on the vaccine-induced antitumor response. These interactions can influence the magnitude, quality, and efficacy of the tumor-specific T-cell response, as well as other variables of the immune response. Advances in molecular immunology have provided the tools for identifying the immunoregulatory pathways that form the basis of therapeutic synergy or antagonism. Certain chemotherapeutic agents have been shown to modulate some of these checkpoints of immunoregulation. Agents that target other checkpoints are also under development. These advances are clearing the path for rationally designed combinatorial cancer vaccine trials that capitalize on the strengths of diverse therapeutic modalities.

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