Chemotherapy and tumor immunity: an unexpected collaboration

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1. ABSTRACT

Chemotherapy directly targets the transformed tumor cell, and has long been a key component of therapy for most early and advanced cancers. However, its utility is ultimately limited by unavoidable toxicity to normal tissues, and by drug resistance pathways deeply embedded within the biology of the tumor cell itself. These limitations strongly argue for innovative strategies to treat and manage cancer. Engaging the power of the patient’s own immune system is a highly attractive way to complement the activity of standard cancer treatment. Tumor vaccines offer the potential for preventing cancer in those at high risk for disease development, preventing relapse in those diagnosed with early cancer, and treating advanced disease. Notably, the barriers to tumor vaccine efficacy are distinct from the limitations of combination chemotherapy. The ability of vaccines to induce a response robust enough to mediate tumor rejection is limited by the extent of disease burden, the suppressive effect of the local tumor microenvironment, and multiple layers of systemic immune tolerance established to keep the immune response turned off. Chemotherapy can be used with tumor vaccines in unexpected ways, breaking down these barriers and unleashing the full potential of the antitumor immune response.

2. INTRODUCTION

Current cancer therapies are limited either by local modes of action that fail to eradicate distant disease, or by inherent drug resistance integral to the biology of the transformed tumor cell. Many years of refining traditional modes of therapy have maximized their efficacy, but further therapeutic gains of significance are unlikely. More recently, biologically targeted drugs that precisely interrupt signaling pathways indispensable for tumor growth and progression have produced substantial improvement. As examples, the use of Trastuzumab to treat HER-2/neu-overexpressing breast cancer (1) and Imatinib to manage chronic myelogenous leukemia (2) greatly improve patient outcomes. Despite their promise, even precisely targeted therapeutics will ultimately be limited by drug resistance, resulting in disease relapse. This limitation of therapies that target the tumor cell itself is a call for the development of cancer therapies that work in a fundamentally different way.

Tumor vaccines can recruit the power of the patient’s own immune system to actively seek out and destroy transformed tumor cells, and represent a potentially potent complement to standard cancer therapies. Immune-based therapy has a higher level target than the tumor cell...
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itself, re-tooling the host-tumor interaction to induce host cells that favor tumor rejection. Additional unique features of immune-based therapy make it even more attractive. Tumor vaccines can potentially induce a durable antitumor effect by virtue of the immunologic memory response, even in the absence of continued therapy. This memory response also defines immunotherapy as a particularly appropriate strategy for cancer prevention. Despite these unique features, a number of factors pose substantial barriers to cancer vaccine efficacy. First, the extent of the tumor burden frequently overcomes the magnitude of the immune response induced by vaccination. Second, in advanced disease, the tumor mass is a harsh environment for immune effectors to enter and function effectively. Finally, systemic layers of immune tolerance act to keep immune effectors shut down. Integrating immune-based therapy with standard cancer therapies represents one approach for breaking down these barriers.

3. IMMUNE TOLERANCE: A MANIFESTATION OF THE HOST-TUMOR INTERACTION

3.1. Systemic Immune Tolerance

Immune tolerance results from the integration of multiple, overlapping systemic regulatory mechanisms that together prevent the development of immunity to antigens perceived as self (3). The vaccine-mediated induction of immunity to foreign antigens compared to tumor antigens results in T cell responses that are distinct in both quantity and quality. Responses to foreign antigens are routinely of high avidity, with antigen-specific T cell frequencies on the order of 10% or greater. In contrast, responses to self antigens are more typically of very low avidity, with antigen-specific T cell frequencies of 1% or less. These tepid responses to tissue-derived antigens result from distinct mechanisms that control the immune response globally. Additional regulatory mechanisms that map to the tumor microenvironment further conspire to keep antitumor immune responses shut down. At the global level, T cells with the highest avidity for self antigens are deleted either in the thymus, or in the periphery. This process of deletion establishes a peripheral T cell repertoire with lower avidity for antigens that might be perceived as self (3). Moreover, a fail-safe mechanism exists to ensure that any potentially self-reactive T cells that escape the deletion process remain suppressed. A unique T cell population defined in part by expression of CD4, CD25, and the transcription factor FoxP3 (regulatory T cells or Treg) normally function to prevent immune-mediated tissue destruction, and to curtail the normal immune response to foreign antigens (4). This negative feedback is so important in maintaining immunologic homeostasis that Treg activity is complemented by the activity of myeloid suppressor cells and tolerizing dendritic cells to ensure that the system remains under control (3). Notably, progressive tumor growth recruits and expands each of these negative regulatory populations, co-opting the normal host response to facilitate tumor growth and progression (4, 5).

3.2. Local Immune Suppression

When tumors begin to grow, they are so small that they do not access the peripheral lymphoid tissues, thereby “sneaking through” immune surveillance (6). With further growth, the tumor and the immune system begin to influence one another, beginning the process of immunoediting (7). At this point, the potency of immune-mediated tumor rejection is determined by the relative balance between the growth kinetics and physical burden of tumor cells compared to the intensity and diversity of the effector T cells (8, 9). Further limiting the antitumor immune response, the tumor microenvironment is not conducive to the activity of immune effectors that do manage to reach the tumor site. Tumor antigens are presented in a suboptimal fashion, often in the absence of positive co-stimulatory molecules or in the presence of negative accessory molecules for costimulation (10). These alternative contexts for antigen presentation result in weak T cell activation, nonresponsive T cells, or even overt T cell apoptosis (11). The level of tumor antigen itself may be so low that the tumor may simply come to harbor T cells that are functional, but simply fail to see their target and become activated (3).

Tumor cell biology further sets the stage for immune evasion. Tumor cells secrete inhibitory cytokines such as interleukin-10, transforming growth factor-beta, and prostaglandin E2 that serve to further shut down infiltrating T lymphocytes (12). Tumor cells may also express surface fas ligand (fasL or CD95L) or tumor necrosis factor-related apoptosis-inducing ligand (TRAIL), thereby inducing T cell death. This situation becomes even worse as the result of the inherent genetic instability of tumors, which can produce a dynamic plasticity of its antigen expression profile. This promotes the downregulation of tumor antigens either spontaneously, or as the result of a targeted immune-mediated intervention (13, 14). Alternatively, tumors may downregulate various components of the antigen processing machinery (MHC Class I, MHC Class II, proteasome subunits, and the TAP transporter) (12). These mechanisms together provide a means for the outgrowth of antigen loss variant tumors that are resistant to antigen-specific immunotherapy. Even in the absence of immune-based therapy, this phenotype has been correlated with poor clinical outcome (15).

4. CHEMOTHERAPY AUGMENTS TUMOR IMMUNITY

Given the multiplicity of regulatory pathways that shut off tumor-specific immune responses, it is not surprising that most clinical trials testing cancer vaccines as a single intervention in patients with advanced, treatment refractory cancers have failed to demonstrate clinically relevant bioactivity. Since some combination of surgery, radiation, and chemotherapy is routinely used to treat most established cancers, it seems obvious that integrating tumor vaccines with these other modalities could be additive or even synergistic. It is critical to consider the appropriate dose and timing of standard cancer therapy in relation to cancer vaccines, including chemotherapy. First, appropriately sequencing tumor vaccines after these therapies achieve maximal cyto reduction can shift the balance of disease burden and the T cell response in favor of the T cell. Second, chemotherapy can be used to groom
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the tumor microenvironment to better support a productive antitumor immune response. Third, chemotherapy can be used to alter systemic immunoregulatory pathways to optimize immune priming and/or effector T cell expansion.

4.1. Systemic Chemotherapy and Cytoreduction

Studies reveal conflicting results when testing tumor vaccines in close proximity with standard dose cytotoxic chemotherapy, with chemotherapy having no impact, a detrimental effect, or a promoting influence on tumor immunity. Moreover, other studies show that treatment with a tumor vaccine can increase the response to subsequent chemotherapy. One study tested a prime/boost vaccination regimen that primed the antitumor immune response with recombinant vaccinia virus (rVV)-expressing prostate-specific antigen (PSA) admixed with rVV-expressing B7.1, followed by boosting the immune response with recombinant fowlpox virus (rFP)-expressing PSA with and without concurrent Docetaxel therapy in patients with hormone-refractory metastatic prostate cancer (16). Vaccination produced a 3.33-fold increase in PSA-specific T cell precursors by ELISPOT after three months, regardless of concomitant Docetaxel treatment. Median progression-free survival for vaccinated patients on Docetaxel was 6.1 months compared to 3.7 months for a historical cohort treated with Docetaxel alone. Another study tested 3 cycles of standard irinotecan/high dose 5-Fluorouracil/Lucovorin and concurrent vaccination with a carcinoembryonic antigen (CEA)-derived peptide, followed by weekly vaccination alone in patients with newly diagnosed metastatic colorectal cancer (17). Recall antigen-specific CD8+ T cells decreased about 14% in these patients, but almost half demonstrated the induction of CEA-specific immune responses by intracellular cytokine assays. A third study evaluated 17 patients with advanced cancer vaccinated with a CYP1B1 plasmid DNA vaccine encapsulated in biodegradable poly-DL-lactide-coglycolide microparticles (18). Five patients who developed CYP1B1-specific immunity and then went on to receive chemotherapy had a greater, more durable response to salvage chemotherapy than similarly treated patients who did not develop CYP1B1-specific immunity. Together, these studies show that vaccine-induced T cell responses may not be inhibited by chemotherapy, and that previously vaccinated patients may benefit more from subsequent standard dose chemotherapy than those who have never been vaccinated.

At least two studies suggest that standard dose chemotherapy may inhibit vaccine-induced immune responses. In one, the canary pox vaccine ALVAC-CEA B7.1 was given to patients with advanced carcinoembryonic antigen (CEA)-expressing colorectal cancer, and CEA-specific immune responses were measured (19). The number of vaccine-induced CEA-specific T cells was lower in patients who had received a greater number of prior chemotherapy regimens, and in those who had received chemotherapy most recently. A distinct study evaluated vaccination with a granulocyte-macrophage colony-stimulating factor (GM-CSF)-secreting vaccine as part of adjuvant therapy in 14 patients with high risk Stage II and III pancreatic cancer (20, 21). Three of these patients developed vaccine-induced mesothelin-specific CD8+ T cells (as measure by ELISPOP) with one vaccine given after pancreatecoduodenectomy. This immune response became undetectable after the patients completed six months of 5-Fluorouracil (5-FU)-based chemoradiation, and was restored only with three additional monthly vaccinations given after chemoradiation was completed.

The differences between the results obtained across these studies are likely related to significant differences in the extent of tumor burden in treated patients, the sequence in which standard dose chemotherapy and vaccine were given, and the chemotherapy drugs given to the patients. Interestingly, the enhanced clinical response to chemotherapy given to vaccine responders may be due to the triggering of pre-existing immunity by treatment-related apoptosis. Here, chemotherapy-induced cell death may function to release tumor antigens, thereby cross-priming the immune response. This mechanism has been shown in animal models with Gemcitabine (22-24), Doxorubicin (25, 26), Cyclophosphamide (27), and Paclitaxel (28). At least one study provides evidence of this in humans. Neoadjuvant Paclitaxel therapy given to locally advanced breast cancer patients induced tumor infiltrating T lymphocytes (TIL), with the extent of TIL present correlating with clinical response (0% in nonresponsive disease, 25% in disease that partially responded, and 67% in disease characterized by a complete clinical response but residual pathologic disease) (29). Importantly, the magnitude of tumor cell apoptosis with the first neoadjuvant Paclitaxel treatment predicted subsequent TIL and clinical benefit.

4.2. Systemic Chemotherapy and the Tumor Microenvironment

Chemotherapy can modify the tumor microenvironment to promote an effective antitumor response (Table 1). As discussed above, one way that chemotherapy can modulate the tumor microenvironment is to induce frank apoptosis in tumor cells themselves, thus potentially augmenting antigen processing and presentation through cross-priming. Chemotherapy may also augment antitumor immunity in diverse ways by altering the tumor microenvironment to favor a more productive tumor-specific immune response. For example, many chemotherapy drugs have dose- and sequence-specific antiangiogenic activity. Cyclophosphamide, Paclitaxel, Doxorubicin, and Vinblatiletine given at regular intervals preferentially targets the tumor-associated vasculature (30). This may augment tumor immunity both by normalizing the tumor-associated vasculature early in treatment, thereby facilitating the delivery of drugs and T lymphocytes. It may also induce tumor cell apoptosis in those areas of the tumor that do not undergo vascular normalization, thereby enhancing antigen presentation.

Accumulating data suggests that the way that cancer cells die can render them immunogenic (31, 32). Doxorubicin (and other anthracyclines) activate caspases and thereby augment tumor immunity (25, 26). Interestingly, this enhancement is not through increased
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<th>Table 1. Chemotherapy and the immunobiology of the tumor microenvironment</th>
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<td>Tumor Effect</td>
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<tr>
<td>Debunk tumor cells</td>
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<td>Tumor cell apoptosis</td>
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<th>Table 2. Manipulating immune tolerance with chemotherapy</th>
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<td>Immunologic Effect</td>
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<tr>
<td>Abrogate suppressive influence of Treg</td>
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<tr>
<td>Relieve inhibition mediated by myeloid suppressor cells</td>
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<tr>
<td>Enhance dendritic cell migration/maturaton</td>
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<td>Reversal of immunologic skew</td>
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<td>Promote evolution of the memory response</td>
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<td>Promote homeostatic T cell proliferation</td>
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Other cytotoxic drugs can also influence Treg. Standard dose Fludarabine can decrease the number and function of Treg in patients with B cell chronic lymphocytic leukemia (48). When patients with metastatic colorectal carcinoma were treated with the combination of Gemcitabine and FOLFOX 4 (Oxaliplatin, 5-Fluorouracil, and Folinic acid) followed by subcutaneous GM-CSF and Folinic acid, an overall objective response rate of about 69% of patients, with an overall objective response rate of about 70% (49).

There has been little investigation of the impact of chemotherapy on myeloid suppressor cells. However, in addition to promoting cross-priming and T helper type 1 immunity, Gemcitabine can reduce myeloid suppressor cells in mice, thereby enhancing the antitumor activity of CD8+ T cells and natural killer cells (50).

The taxanes, Paclitaxel and Docetaxel, also have an unexpected and multi-faceted immunomodulatory activity. As discussed previously, they effectively induce tumor cell apoptosis, and can thus enhance the T cell response by cross-priming. The combination of Docetaxel and vaccination with a GM-CSF-secreting vaccine has been shown to be effective in murine models of B16 melanoma and Lewis lung carcinoma (51). These drugs also modulate immunity by other mechanisms. Like Cyclophosphamide, Paclitaxel given one day prior to vaccination in tumor-bearing, tolerant neu transgenic mice augments tumor immunity to levels that facilitate tumor rejection (45). This
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effect is in part due to reversal of immunologic skew, with conversion of the tumor-specific immune response from the T helper type 2 to the T helper type 1 phenotype. Paclitaxel has a lipopolysaccharide (LPS)-mimetic effect, binding to toll-like receptor 4 (TLR-4) expressed by murine dendritic cells (DCs) and promoting the secretion of proinflammatory cytokines (52, 53). In humans the LPS-like effect of Paclitaxel is independent of TLR-4 but remains dependent on Myd88, suggesting the involvement of an alternative TLR (54). Consistent with these data, Paclitaxel can augment vaccine-induced immunity when given prior to a HER-2/neu-specific virally-derived vaccine or a genomically-modified fibroblast vaccine in murine models of breast cancer (55, 56).

Doxorubicin also has a dose- and sequence-specific immunomodulatory activity. In tolerant neu transgenic mice, giving Doxorubicin one week after vaccination augments vaccine activity (45). Similarly, Doxorubicin given one week after vaccination in the CT26 model of colon cancer augmented antitumor immunity, resulting in a higher CD8+ T cell response (57). These effects may be due to modulation of tumor cells themselves as discussed previously, with caspase and calreticulin induction rendering them more immunogenic. Alternatively, other mechanisms may also be in play. Supporting this, Doxorubicin could enhance vaccine activity when given prior to vaccination in other systems (56).

The time dependence of the immunomodulatory activity of these chemotherapeutics is striking, and argues for synergy at the time of immune priming for Cyclophosphamide (Treg modulation) and Paclitaxel (DC modulation), and at the time of effector T cell activity for Doxorubicin (apoptosis and cross-priming). Multiple clinical trials testing the ability of low dose chemotherapy to modulate immunity are underway or have just been completed. Two small clinical trials involving patients with metastatic pancreatic cancer (58) or non-small cell lung cancer (59) tested low doses of Cyclophosphamide given one day prior to vaccination with a cell-based GM-CSF-secreting vaccine. These trials showed a trend toward increased vaccine-activated immunity and clinical benefit with Cyclophosphamide-modulated vaccination compared to vaccination alone. There was a transient decrease in Treg numbers with time after Cyclophosphamide treatment in the patients with non-small cell lung cancer. Current clinical trials actively testing these concepts include a Phase I clinical trial of a GM-CSF-secreting breast tumor vaccine given in a specifically timed sequence with Cyclophosphamide and Doxorubicin in patients with stable metastatic breast cancer (60), and a Phase III clinical trial testing a GM-CSF-secreting prostate cancer vaccine with Docetaxel in men with advanced prostate cancer.

High dose chemotherapy can also synergize with tumor vaccines. It is highly effective in debulking established tumor burdens, establishing a state of minimal residual disease. It can eliminate Treg, and sets the stage for lymphopenia-induced homeostatic T cell proliferation (61, 62). Notably, the adoptive transfer of lymphocytes or active vaccination during homeostatic T cell proliferation presents an opportunity to skew the re-established T cell repertoire toward a desired antigenic specificity (63). For example, a highly active population of melanoma-specific T cells developed when lymphopenic, melanoma-bearing RAG-1 deficient mice were vaccinated with a GM-CSF-secreting vaccine, resulting in significant tumor regressions (64). Additionally, vaccine-induced antitumor immunity was increased when tumor-bearing mice were immunized with GM-CSF-secreting vaccines during early engraftment after syngeneic or allogeneic T cell-depleted bone marrow transplantation (65, 66), and can be enhanced by donor leukocyte infusion from vaccinated donor mice (67). In a related strategy, treating tumor-bearing mice with surgical resection followed by nonmyeloablative allogeneic stem cell transplantation and donor leukocyte infusions plus vaccination with a GM-CSF-secreting vaccine developed immune responses capable of rejecting metastatic 4T-1 breast tumors (68). These concepts have been evaluated in clinical trials employing the adoptive transfer of T lymphocytes, and in clinical studies testing immunization combined with the adoptive transfer of primed lymphocytes during immune reconstitution subsequent to myeloablative chemotherapy (69, 70). Significant levels of tumor-specific T cells in the setting of tumor regression were documented in these studies of adoptive cellular therapy. While these approaches have promise, characterizing the kinetics, persistence, and functional quality of tumor-antigen-specific T cells after immune reconstitution will be essential to bring these treatment strategies to routine cancer care.

5. CONCLUSIONS AND PERSPECTIVES

Accumulating data suggests that it will be imperative to combine active immunotherapy with chemotherapy in a rational fashion, capitalizing on additive or even synergistic activity. The mechanisms by which distinct chemotherapeutics can enhance vaccine-induced tumor immunity are highly disparate, and quite dependent on chemotherapy dose, schedule, and perhaps tumor burden. Careful preclinical modeling using the most clinical relevant murine models will be essential for designing the most informative clinical trials, ensuring that tumor vaccines are effectively integrated with standard cancer care to improve clinical outcomes.

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**Abbreviations:** TRAIL: tumor necrosis factor-related apoptosis-inducing ligand; MHC: major histocompatibility complex; TAP: transporter of antigen processing; rVV: recombinant vaccinia virus; PSA: prostate specific antigen; ELISPOT: enzyme-linked immunosorbent assay; CEA: carcinoembryonic antigen; GM-CSF: granulocyte-macrophage colony-stimulating factor; TIL: tumor infiltrating lymphocytes; CTL: cytotoxic T lymphocytes; TLR: toll-like receptor; LPS: lipopolysaccharide

**Key Words:** TRAIL, CEA, rVV, PSA, GM-CSF, CTL, TLR, LPS, Tumor Immunity, Chemotherapy, Cancer Vaccines, Immune Tolerance, Review

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