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On combining antineoplastic drugs with tumor vaccines

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Introduction

Over the past 2 decades, the classical paradigm of trimodal cancer therapy has been expanded to include immunotherapy, encompassing both passive, adoptive T-cell transfer techniques as well as active vaccination strategies. As the mainstay of anticancer therapy, antineoplastic drugs have long been used for their direct tumoricidal properties, while the immunosuppressive adverse effects have been merely tolerated and supported. With the advent of the increasing use of immunotherapy in the clinical setting, investigators have sought to determine ways in which to combine accepted chemotherapeutic regimens with innovative immunotherapeutic techniques, and have discovered that the lymphodepletion that results from antineoplastic drug administration may be, in some cases, advantageous in eliciting clinically relevant responses to cancer immunotherapy. As well, several of these drugs have been found, paradoxically, to actually augment antitumor immunity. There is a paucity of preclinical and clinical data to date on combining chemotherapy and antitumor vaccines, as this is a strategy in its infancy. However, it may ultimately be found that chemotherapy combined with vaccine therapy offers therapeutic advantages over single-modality treatment. Here we will explore the available data regarding the mechanisms behind enhancement of antitumor efficacy through the combination of antineoplastic drugs with tumor vaccines.

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Rationale for combining chemotherapy with tumor vaccines: the immunomodulatory effects of antineoplastic drugs

It has been known for quite some time now that some antineoplastic drugs have inherent immunomodulatory properties. Of all of the chemotherapeutic agents, the immune effects of cyclophosphamide (Cytoxan), an alkylating agent widely used to treat solid organ malignancies as well as lymphoproliferative and autoimmune disorders, have been studied most extensively [11, 12]. Cyclophosphamide has been shown to exhibit both immunosuppressive or immunopotentiating effects depending on the dosage used and the temporal relationship between drug administration and antigen challenge [11, 12]. For example, cyclophosphamide has been shown to suppress antibody and DTH responses when administered concurrently with, or subsequent to, antigenic sensitization [20, 21, 22, 33], whereas these responses are augmented when drug administration precedes antigen challenge [11, 16, 33]. There is also evidence that cyclophosphamide administration can break self-tolerance, which is particularly important if one is to generate T-cell clones that are reactive to tumor-associated self-antigens. Examples of this phenomenon include experiments in which male mice that were pretreated with cyclophosphamide developed delayed-type reactions in response to injection of testicular cells [34], and the inhibition of acquired tolerance to the hapten DNCB through presensitization treatment with cyclophosphamide [27].

Early investigations into the effects of cyclophosphamide on the immune system in tumor-bearing hosts led to the conclusion that cyclophosphamide administration caused a selective reduction in the suppressor T-cell population, thereby permitting antitumor immune activity to occur. To this end, Awwad and North [3]

Table 1 Immunomodulation by chemotherapeutic agents

Drug	Immune effects	Prevaccine administration	Postvaccine administration
Cyclophosphamide	Decrease in antibody and DTH responses when administered with antigen [20, 21, 22, 33]; increase in antibody and DTH responses when administered prior to antigen challenge [11, 16, 33]; pretreatment can overcome innate or acquired tolerance [27, 34]; increase in type I interferon with skewing of T lymphocytes to a memory phenotype [29, 31]	Increase functional/antigen-specific T cells [19]; enhancement of vaccine antitumor efficacy [19]; can break tolerance to tumor-associated antigens [19]	Decrease in effector T-cell function [25]; no demonstrable in vivo antitumor effect with combination therapy greater than chemotherapy alone [19]
Adriamycin (doxorubicin hydrochloride)	Increased primary CTL responses [1, 26]; increased IL-2 secretion by splenocytes [10], and peripheral blood mononuclear cells [1]; monocyte/macrophage activation, increase in tumoricidal activity [18]; increased numbers of monocytes/macrophages in the spleen [26]; IL-1 production by peritoneal exudates cells [18]	No demonstrable in vivo antitumor effect with combination therapy greater than chemotherapy alone [19]	Increase in effector T-cell activity [25]; protective antitumor effects [19]
Taxanes	IL-12 secretion by macrophages [24]; "second signal" to macrophages leading to NO and TNF-α secretion [23, 24]	Increase in functional/antigen-specific T cells [19]; enhancement of vaccine antitumor efficacy [19]; can break tolerance to tumor-associated antigens [19]	No demonstrable in vivo antitumor effect with combination therapy greater than chemotherapy alone [19]

conducted studies utilizing L5178Y lymphoma, a tumor line resistant to direct cytotoxicity by cyclophosphamide. They were able to show that L5178Y tumors grew in B6D2F1 mice, that tumor growth was augmented by cyclophosphamide administration alone, and that adoptive transfer of splenocytes from L5178Y-immunized mice had no effect on tumor growth. However, when cyclophosphamide was administered 1 h prior to transfer of immune spleen cells, complete tumor regression was achieved. These investigators further demonstrated that the antitumor effects of cyclophosphamide and immune lymphocyte transfer could be abrogated through the administration of splenocytes from mice bearing established tumors. It was surmised from these studies that the splenocytes from tumor-bearing animals contained large amounts of suppressor T cells that accounted for the reversal of the antitumor effect.

In subsequent studies by Hoover et al. [14], it was further shown that although cyclophosphamide could eliminate suppressor T-cell populations, the effect was not selective for suppressor cells, as elimination of CTL activity from the spleens from tumor-bearing hosts treated with cyclophosphamide was also observed. This finding prompted further research to determine the mechanism behind the immunomodulatory effects of cyclophosphamide. Recently, studies have suggested that cyclophosphamide exerts its immunologic effects in tumor-bearing animals through the induction of type I interferon, resulting in augmented lymphoproliferation and skewing of T lymphocytes to a memory phenotype—effects which were prevented through the administration of antibodies against interferon [29, 31].

The immunomodulating effects of Adriamycin (doxorubicin hydrochloride) have also been studied, although not to the same extent as those of cyclophosphamide (see Table 1). In 1977, Orsini et al. [26] demonstrated that splenocytes taken from Adriamycin-treated mice exhibited enhanced primary immune responses when cultured with allogeneic leukemia cells. Importantly, this effect was abrogated when the splenocytes were depleted of monocytes and macrophages prior to coculture with tumor cells, illustrating the requirement for these ''accessory cells'' to generate the response. Additionally, it was found that the presence of monocytes was only required for a short time period, as depletion of monocytes 24 h after initiation of the coculture did not diminish the allo-CTL effect. Furthermore, histologic examination of spleens 3–5 days after Adriamycin administration showed a marked increase in the numbers of monocytes. Later, studies were conducted suggesting additional modes of action of Adriamycin including increased IL-1 elaboration and priming of macrophages in peritoneal exudates [18], and increased IL-2 secretion by spleen cells thereby causing activation of CTL [10]. In vivo antitumor immune effects ascribable to Adriamycin were successfully demonstrated by Maccubbin et al. [17] using an EL4 tumor model in C57/BL6 mice. Briefly, prolongation of survival was seen as a result of Adriamycin administration in animals bearing EL4 tumors as well as in animals inoculated with an Adriamycin-resistant tumor, providing additional evidence that the antitumor effects of Adriamycin were not solely due to direct tumor cytotoxicity.

The taxanes are known to elicit their antineoplastic effects through inhibition of cell division via microtubule stabilization. Like cyclophosphamide and doxorubicin, they have also been shown to exhibit dichotomous effects on the immune system. On the one hand, they can act as a ''second signal'' to activate IFN-γ–primed macrophages to secrete nitric oxide as well as TNF-α, and thereby elicit a tumoricidal effect [23, 24]. Conversely, the taxanes have been found to inhibit activation and proliferation of T and NK cells, thereby suppressing lymphocyte-mediated cytotoxicity [6, 7], although IL-12 secretion by paclitaxel-treated, nitric oxide–secreting macrophages may reverse this effect [24].

Combining chemotherapy with vaccine therapy in vivo

To test the effects of chemotherapeutic agents on tumor vaccine efficacy in vivo, Nigam and colleagues [25] vaccinated BALB-C mice with a GM-CSF–secreting cancer cell vaccine followed 1 week later by intraperitoneal administration of one of a variety of anticancer drugs. In vitro analysis of cytotoxic T-cell function was then undertaken, revealing that administration of doxorubicin following tumor vaccination greatly increased tumor-specific CTL activity over that which was seen with vaccination alone. Of the 11 agents tested, doxorubicin was the only drug that increased effector cell activity. While nine of the remaining 10 drugs resulted in either no change or a decrease in effector T-cell function, cyclophosphamide appeared to be the only agent that reduced effector function to the level of the unvaccinated controls. Based on previous studies of the positive immunomodulatory effects of cyclophosphamide, this was somewhat surprising. However, these results could potentially be explained by the timing post-vaccine of the administration of cyclophosphamide, as other studies have demonstrated that cyclophosphamide administration pre-vaccine or pre-adoptive T cell transfer augments antitumor immunity [19, 29, 31].

Through an elegant set of experiments, Machiels et al. [19] set out to closely approximate the conditions of human malignancies, and test the efficacy of cyclophosphamide, Adriamycin, and paclitaxel on GM-CSF–secreting whole cell vaccines. These investigators used *neu*-transgenic mice, which express the *neu* (c-erbB2, ERBB2) proto-oncogene, a gene that has been shown to be present in human breast cancer and whose expression is directly proportional to severity of disease. *Neu*-transgenic mice have been shown to develop spontaneously metastasizing mammary adenocarcinomas [13], and exhibit immune tolerance to *neu*-expressing tumor

cell lines [30]. This animal model is particularly translatable to human breast cancer because, while patients with HER-2/*neu*-positive breast cancers are able to generate HER-2/*neu*-specific T cells, these T cells are often tolerant to HER-2/*neu* as demonstrated by the fact that the tumors continue to grow and spread in their presence [30]. In the studies performed by Machiels et al. [19], it was shown that a whole cell, GM-CSF–secreting vaccine expressing *neu* was able to generate an antitumor immune response capable of both protecting wild-type animals from challenge by *neu*-expressing tumor cells, as well as treating animals with established *neu*-positive tumors. In contrast, vaccine treatment had no effect in treating established *neu*-expressing tumors in *neu*-transgenic mice, and only had a small effect in protecting *neu*-transgenic animals after tumor challenge, thus reestablishing the fact that these mice were *neu*-tolerant. When *neu*-transgenic mice were given chemotherapy (with cyclophosphamide, doxorubicin, or paclitaxel) prior to vaccine administration, it was found that the animals pretreated with cyclophosphamide and paclitaxel both exhibited an increase in *neu*-specific T cells as determined by ELISpot analysis. This effect was not observed in the animals receiving doxorubicin either before or after vaccination, nor was it seen in animals treated with cyclophosphamide or paclitaxel after vaccine administration. These results are in agreement with those from Nigam et al. [25] in that cyclophosphamide and paclitaxel are ineffective when administered post-vaccine. In tumor protection experiments within the same set of studies by Machiels and associates [19], cyclophosphamide and paclitaxel were found to augment tumor protection from *neu*- and GM-CSF–expressing whole tumor vaccines in *neu*-transgenic animals when given prior to vaccination; doxorubicin had a protective effect when given post-vaccination, again correlating with the results from Nigam's group and pointing to a distinct immune effect of doxorubicin compared with cyclophosphamide and paclitaxel. Most importantly, these experiments in *neu*-transgenic mice nicely illustrate the efficacy of cyclophosphamide or paclitaxel treatment prior to vaccine therapy in breaking tolerance to tumor-associated self-antigens.

Rationale for combining chemotherapy with tumor vaccines: the effect of lymphopenia on antitumor immunity

The idea that a lymphopenic state, as would be encountered in a patient following chemotherapy or bone marrow transplant, might enhance tumor vaccine efficacy at first appears counterintuitive. There is a growing body of evidence, however, that this may indeed be the case. First consider the concept of homeostatic proliferation: simply stated, this tells us that the few remaining lymphocytes within a lymphopenic host will proliferate, in response to the recognition of self-peptides presented in the context of MHC class I and II molecules, until the point at which the lymphocyte pool has been replenished to normal levels. Several different investigative groups have sought to take advantage of this phenomenon to cause expansion of cytotoxic T-cell clones that are reactive to tumor-associated antigens. Dummer and associates [9] made use of this concept in a series of experiments in which they induced lymphopenia in C57/B6 mice through sublethal irradiation, and subsequently challenged them with tumor cells. Tumor growth inhibition was demonstrated in the irradiated animals, and was augmented in a dose-dependent manner by adoptive transfer of syngeneic lymph node cells. Subsequent experiments illustrated the fact that these results were not simply due to either a nonspecific effect of the radiation (sublethal irradiation of recombination-activating gene–deficient mice resulted in no antitumor effect) or adoptive lymphocyte transfer alone (non-irradiated mice that received adoptive lymphocyte transfer exhibited no tumor growth inhibition), and that the treatments were effective in both protection and treatment models. Furthermore, when T lymphocytes specific for an irrelevant peptide were adoptively transferred into the irradiated, tumor-bearing animals, there was no further growth inhibition over that seen with radiation alone, suggesting that the antitumor effect was due to the presence and expansion of tumor antigen–specific T cells present within the adoptively transferred cell pool. It was additionally shown by this group [9] that there are restrictions as to where homeostatic proliferation occurs in order to initiate antitumor immunity. As one of the inciting factors of homeostatic proliferation, T lymphocytes in the lymphopenic host must encounter antigen-presenting cells (APCs) carrying antigens within the context of MHC class I or II, an event which occurs in the secondary lymphoid tissues. Using transgenic mice lacking lymph nodes and an organized architecture to the splenic white pulp (lymphotoxin-α knock-out B6 mice), Dummer et al. were able to demonstrate abrogation of the tumor growth inhibition seen after sublethal irradiation and lymphocyte transfer. Similar results were achieved in a related experiment in which the adoptively transferred cells were deficient in β7-integrin and L-selectin, causing them to be unable to migrate into secondary lymphoid tissues. These experiments demonstrate the fact that in order for homeostatically proliferating cells to be primed to tumor-associated antigens, they must encounter APCs within lymphoid tissue.

Combining lymphodepletion with vaccine therapy in vivo

If a lymphopenic environment is conducive to the proliferation of tumor-specific T cells, then it would follow that active immunization strategies employed in a lymphopenic setting may be more effective than those administered to an immunocompetent host. Dendritic

cells are potent antigen-presenting cells that, when loaded with tumor antigens, can prime tumor-specific T cells and effect antitumor responses in vivo [28, 32]. To determine the efficacy of tumor lysate-pulsed dendritic cell (TP-DC) vaccines in a lymphopenic setting, Asavaroengchai et al. [2] used a bone marrow transplant (BMT) model in which mice were lethally irradiated and subsequently infused with bone marrow cells from syngeneic mice. These mice then received 3-weekly TP-DC vaccines starting at 7 days following BMT, and were subsequently challenged subcutaneously and intravenously with viable tumor cells. The antitumor effects of TP-DC administered during the early lymphoid recovery phase of bone marrow transplant were found to be consistently improved over those that were seen when TP-DCs were given to fully immunocompetent animals. Additionally, this treatment strategy resulted in antitumor effects that persisted for greater than 100 days following BMT, and was also found to be effective in treatment models of established lung metastases. In a variation of this schema, Hu et al. [15] used recombination-activating gene-deficient (RAG1 knock-out) mice, which are without T and B cells, and reconstituted them with splenocytes from normal mice. While still lymphopenic, the animals were vaccinated with a GM-CSF–producing whole cell melanoma vaccine. The vaccine-draining lymph node (VDLN) cells were then harvested, activated, and expanded in vitro, and subsequently used in adoptive immunotherapy experiments treating established pulmonary metastases. In comparison to control, immunocompetent animals, the VDLN cells obtained from RAG1 knock-out mice elicited much greater tumor-specific tumor lysis in vitro, and tumor regression in vivo. Importantly, in experiments in which vaccination was performed 1 week after naïve splenocyte transfer, at which time the lymphopenic state was largely reversed via homeostatic proliferation, it was found that the antitumor efficacy of the resulting VDLN was much less robust. These two studies demonstrate that active immunization strategies are significantly more successful when they are employed in a lymphopenic host.

From bench-top to clinic—human clinical trials combining chemotherapy and immunotherapy

Translating experimental work into the clinical setting, Berd et al. [4] in 1986 published the first trial of combined cyclophosphamide and vaccine therapy to treat human patients with melanoma. This study treated 19 patients with metastatic melanoma with an autologous melanoma cell vaccine either alone, or preceded by cyclophosphamide, and demonstrated in vivo immunopotentiation by cyclophosphamide as shown through increased DTH responses from vaccine administration in the cyclophosphamide pre-treatment group. This outward sign of immune responsiveness translated into clinical antitumor responses in some, but not all patients; the lack of uniform clinical effectiveness was attributed to the presence of variability in tumor burden among the patients. Further studies have been published by this group utilizing cyclophosphamide pretreatment to successfully augment antitumor immune responses initiated by vaccine therapy [5]; however, this approach has yet to be widely incorporated into cancer vaccine trials.

In a recently published clinical trial of adoptive immunotherapy in patients with metastatic melanoma, cyclophosphamide and fludarabine were utilized not for the inherent immunopotentiating effects, but rather to effect lymphodepletion before infusion of autologous, ex–vivo expanded, tumor-infiltrating lymphocytes (TIL) [8]. Of the 13 patients treated and discussed in this report, six exhibited clinically demonstrable partial antitumor responses, and four others had mixed responses in which reduction in the size of at least one metastatic focus was noted. Importantly, five of the patients with clinical responses developed vitiligo or uveitis—signs of autoimmune-mediated anti-melanocyte activity that suggest that tolerance to antigens shared by normal melanocytes and melanoma cells was successfully overcome. Furthermore, laboratory evaluations of peripheral blood specimens from the treated patients showed that a majority developed lymphocytosis. In the two partially responding patients tested, it was determined that a high percentage of their circulating lymphocytes were specific for the melanoma tumor-associated antigen MART1, indicating that the adoptively transferred TIL expanded in vivo, successfully migrated to tumor sites, and effected clinically measurable antitumor responses. This is the first study in which lymphodepletion has been used successfully to augment adoptive immunotherapy in humans, and future studies are needed to test whether the inclusion of vaccines in this setting provide additional therapeutic benefit.

Conclusion

Decades worth of research into the direct immune-modulating effects of antineoplastic drugs, and of radiation or chemotherapy-induced lymphopenia, have brought us to a point at which these principles can now be exploited to redirect the immune system to recognize and obliterate malignant tumors by reacting to self-antigens. It is now becoming possible to circumvent and overcome the immune tolerance to self-antigens initially put in place to protect us from autoimmune disease, and to use these tools to combat cancer. Here we have illustrated examples of how the effectiveness of immunotherapeutic techniques, including both adoptive T-cell transfer immunotherapy and vaccine strategies, can be enhanced when used in concert with lymphodepleting chemotherapy. Likewise, we have seen that the direct effects of various chemotherapeutic agents on T-cell and macrophage activity can be effectively utilized to augment in vivo antitumor immunity. This large body

of work leads us now to an era in which these concepts can be tested and refined in the clinic, and ultimately be used to effectively treat patients with minimal residual disease.

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