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Recent Developments in Cancer Vaccines

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The adoptive transfer of cancer Ag-specific effector T cells in patients can result in tumor rejection, thereby illustrating the immune system potential for cancer therapy. Ideally, one would like to directly induce efficient tumor-specific effector and memory T cells through vaccination. Therapeutic vaccines have two objectives: priming Ag-specific T cells and reprogramming memory T cells (i.e., a transformation from one type of immunity to another, for example, regulatory to cytotoxic). Recent successful phase III clinical trials showing benefit to the patients revived cancer vaccines.

Dendritic cells (DCs) are essential in generation of immune responses, and as such represent targets and vectors for vaccination. We have learned that different DC subsets elicit different T cells. Similarly, different activation methods result in DCs able to elicit distinct T cells. We contend that a careful manipulation of activated DCs will allow cancer immunotherapists to produce the next generation of highly efficient cancer vaccines. *The Journal of Immunology, 2011, 186: 1325–1331.*

The question of whether the natural immune response has a role in modulating the progress of spontaneous tumors in patients has been debated for many years. However, it is now accepted that the immune system is able to control cancer both in mice (1, 2) and humans (reviewed in Ref. 3). Perhaps the most compelling evidence of tumor immunosurveillance in humans is provided by the studies in breast cancer and paraneoplastic diseases. Onconeural Ags, which are normally expressed on neurons, can also be expressed in breast cancer cells (4). Some patients develop a strong Ag-specific CD8+ T cell response that controls tumor expansion, but concomitantly results in autoimmune cerebellar degeneration (5), leading to severe neurologic disease.

The molecular identification of human cancer Ags has allowed the development of Ag-specific immunotherapy (6, 7) based on different approaches. In one approach, adoptive T cell therapy (reviewed in Ref. 8), autologous Ag-specific T cells are expanded ex vivo and reinfused to patients. Adoptive T cell therapy appears to be an effective treatment for patients suffering from EBV-associated lymphomas (9) as well as solid tumors (10, 11). The alternative strategy is to expand tumor-associated Ag-specific T cells in vivo through vaccination, a procedure of potentially wider use.

Cancer vaccines: lessons from the past and key recent progress

Vaccination for the prevention of infectious diseases (12) represents a great success of medicine. One example showing great promise with regard to cancer is the prevention of human papillomavirus-positive cervical cancer by vaccinating with a recombinant viral capsid protein (13). The generation of efficacious therapeutic vaccines faces, however, many hurdles, among which the selection of the appropriate target Ags and the quality of pre-existing T cell memory appear critical. Target tumor Ags include unique (mutated) Ags and shared non-mutated self-Ags (3, 14). The choice between these types of Ags for vaccination could be viewed as a choice between inducing immunity (mutated Ags) or breaking tolerance and inducing autoimmunity (self-Ags). The debate about which type of Ag will be more efficient is ongoing. Mutated Ags have potential advantages, such as the following: 1) their T cell repertoire should not be deleted and they should be recognized as nonself by the immune system, as is the case with viral Ags; and 2) their potential resistance to negative selection in case the mutated protein is essential for cell survival (14). These Ags often require priming. For the sake of broadly applicable vaccines, often the targeted cancer Ags are nonmutated self-Ags for which 1) the repertoire of high avidity clones might be depleted through negative selection (15), and 2) the existing memory T cells might be polarized. These often include regulatory T cells (Tregs), either thymus-derived naturally occurring Tregs CD4+CD25<sup>high</sup> (reviewed in Ref. 16) or periphery-induced Tr1 cells, which mainly produce IL-10 (17, 18), and Th3 cells, which mainly produce TGF-β (19). Another important component of existing repertoire are Th2 cells (20, 21). Thus, the existing memory repertoire requires reprogramming from nonprotective immunity toward protective IFN-γ-secreting Th1 cells.
Numerous approaches have been tested for the therapeutic vaccination of cancer patients, including the following: autologous and allogeneic tumor cells modified to express various cytokines, peptides, proteins, and DNA vaccines (reviewed in Ref. 22). The clinical results have overall been of limited impact, although tumor-specific immune response could be measured in some cases. These first generation cancer vaccines have been designed with a limited understanding of the role of dendritic cells (DCs) in the initiation and regulation of immunity.

Cancer vaccines are entering a renaissance era thanks to a series of clinical trials that yielded encouraging clinical outcomes. First, treatment of metastatic prostate cancer with sipuleucel-T (APC8015), a cellular vaccine based on enriched blood APCs briefly cultured with a fusion protein of prostatic acid phosphatase with GM-CSF, resulted in 4-mo–prolonged median survival in phase III trials (23). Sipuleucel-T has been approved by the FDA for treatment of metastatic prostate cancer, thereby paving the clinical development and regulatory path for the next generation of cellular immunotherapy products. Second, a phase III trial in metastatic melanoma testing peptide vaccine in combination with high dose IL-2 versus IL-2 alone showed significant improvement in overall response rate and progression-free survival in patients who received vaccine (24). Third, a phase III trial in patients with follicular lymphoma showed that idiotype vaccine therapy (BiovaxID) significantly prolongs the duration of chemotherapy-induced remission (25). Furthermore, a randomized phase II trial of a poxviral-based vaccine targeting prostate-specific Ag (PROSTVAC) in men with metastatic castration-resistant prostate cancer showed an improved overall survival in patients when compared with patients receiving control product (an observed difference in median survival of 8.5 mo) (26).

Naturally, these first successful trials bring many new questions. For example, which APCs are present in the sipuleucel-T product and which APCs are targeted by the poxviral-based vaccine in vivo? Also, to what extent can the observed improvement in patients’ survival be linked to enhanced prostatic acid phosphatase-specific T cell immunity? Yet, these studies will help define the basic principles of cancer vaccines that set this treatment modality apart from chemotherapy, radiotherapy, targeted therapies, and even adoptive T cell transfer.

These, and earlier phase I/II studies, bring forward two critical questions: 1) how to measure vaccine efficacy, and 2) how to define the correlates of therapeutic immunity (27). In this context, a major conceptual shift has recently taken place in the assessment of clinical efficacy in vaccine and immunomodulation trials (27, 28). Indeed, the use of conventional response evaluation criteria in solid tumor measures to judge efficacy has been challenged by recent clinical trials testing anti-CTLA4 (ipilimumab) in patients with stage IV melanoma. There, in a randomized phase III clinical trial, a 2-fold improved overall survival in patients who received anti-CTLA4 with or without Ag was observed, as compared with single short-peptide vaccine (29). Interestingly, there were no early indications of tumor shrinkage in anti-CTLA4-treated patients, an observation that might be interpreted as the slow buildup of antitumor immunity. During this phase, tumors might progress before they actually regress, including the appearance of new lesions, a point at which according to standard criteria patients need to be taken off the study. Furthermore, tumors might appear clinically enlarged because of inflammation associated with active immune responses and lymphocyte infiltration. Along the same lines, sipuleucel-T and PROSTVAC failed endpoint of improved progression-free survival (30). Yet, the treatment resulted in prolonged median overall survival (30). In all of these studies, the analysis of survival curves shows the separation only after 4 mo, and even up to 12 mo, suggesting a delay in the treatment effect, just as one would expect if efficacy could occur only after the induction or reprogramming of antitumor immunity.

Thus, it may be tempting to conclude that overall survival may be the only true parameter of clinical efficacy. However, surrogate markers are needed because trials based on overall survival can be exceedingly long and costly and would prevent the performance of innovative trials by academic investigators. The need for objective, quantifiable response criteria cannot be overemphasized. In this context, a number of studies in small groups of cancer patients demonstrated that a success or failure of therapeutic vaccination is correlated with the immune response to vaccination defined by the expansion of Ag-specific effector T cells (31). Thus, Ag-specific immune responses should remain among the key parameters of efficacy. A better understanding of how effective vaccines, for example, influenza vaccine or yellow fever vaccine, stimulate protective immune responses (32, 33) might contribute to a better understanding of immune parameters of vaccine efficacy in cancer.

Reprogramming the immune system in cancer via DC subsets

Understanding of how DCs induce, regulate, and maintain T cell immunity (12, 34) is essential for the design of novel cancer vaccines with improved clinical efficacy. Indeed, DCs play a critical role in T cell priming, direct and cross-priming, as evidenced by studies in mice (for review, see Refs. 35 and 36). DCs also regulate the immune response. Thus, in the steady state, nonactivated (immature) DCs present self-Ags to T cells, which leads to tolerance through different mechanisms (37). Once activated (mature), Ag-loaded DCs are geared toward the launching of Ag-specific immunity (38), leading to T cell proliferation and differentiation into helper and effector cells. DCs are also important in launching humoral immunity partly due to their capacity to directly interact with B cells (39) and to present unprocessed Ags (40).

Two critical features, subsets and functional plasticity, allow DCs to mount functionally distinct types of responses. The two major DC subsets are the classical DCs (cDCs), also referred to as myeloid DCs) and the plasmacytoid DCs. Plasmacytoid DCs are considered the front line in antiviral immunity owing to their capacity to rapidly produce high amounts of type I IFN in response to viruses (41). cDCs are composed of subsets displaying different phenotype and functions. In human skin, epidermis hosts only Langerhans cells (LCs), whereas the dermis contains two cDC subsets, CD1α+ DCs and CD14+ DCs, as well as macrophages (42, 43). CD14+ dermal DCs are efficient in the generation of humoral immunity through different mechanisms (43, 44). In contrast, LCs are potent for priming of high avidity Ag-specific CD8+ T cells (45) (Fig. 1). Induction of potent CTL response by LCs is observed in mouse studies by s.c. injections of peptide-loaded epidermal LCs (45). Mouse LCs can actually cross-present Ags to CD8+ T cells in vivo (46).
In contrast, several mouse studies, for example, models using HSV, have questioned the contribution of LCs to the induction of Ag-specific responses in vivo. These studies attribute the HSV-specific immunity to CD8α+ DCs, rather than to LCs (47). Thus, it remains to be determined whether these differences with regard to the function of LCs between mice and humans derive from the differences in their immune systems. Another cDC subset, BDCA-3+ DCs, was recently proposed to be the equivalent of mouse CD8α+ DC subset (reviewed in Ref. 48) that efficiently cross-presents Ags to CD8α+ T cells.

Ex vivo generated DCs have been used as therapeutic vaccines in patients with metastatic cancer for over a decade (for detailed reviews, see Refs. 34 and 49). It was concluded that DCs can expand T cells specific for nonmutated self proteins that are overexpressed in cancer. The analysis of immunological and clinical responses yields three patient groups: 1) one group showing neither clinical nor immunological responses; 2) one group showing some immunological response, but no clinical responses; and 3) one group composed of a few patients showing both immunological and clinical responses. These later patients are essential for performing in-depth mechanistic studies that will eventually permit us to understand the immune responses that lead to control tumor growth and eliminate established tumors.

In the context of ex vivo derived DC-based vaccines, the combination of cytokines used to differentiate monocytes into DCs might play a critical role in determining the quality of the elicited T cell responses. For example, DCs generated with GM-CSF and IFN-α are highly potent in priming T cells (50). DCs generated with GM-CSF and IL-15 display the phenotype and characteristics of LCs. In particular, they are more efficient in priming melanoma-Ag–specific CD8+ T cells in vitro than DCs derived with GM-CSF and IL-4 (51). Thus, vaccination with IL-15 DCs might elicit stronger CD8+ T cell responses that may lead to improved clinical responses. The selection of methods for activating DCs also represents a critical parameter in the design of DC vaccines (Fig. 2). Indeed, injection of immature GM-CSF/IL-4 monocyte-derived DCs results in expansion of Ag-specific IL-10–secreting T cells (52). These observations yield an explanation for earlier clinical studies in which peptide immunization in adjuvant was more immunogenic than peptide-pulsed immature GM-CSF/IL-4 monocyte-derived DCs (53). Injection of mature Ag-loaded DCs allows expansion of Ag-specific T cells (54). However, not all DC maturation signals are equal. For example, IL-4 DCs activated with a mixture of IFN-α, polynosinic-polycytidylic acid, IL-1β, TNF, and IFN-γ induce up to 40 times more melanoma-specific CTLs in vitro than DCs matured with the standard mixture of IL-1β/TNF/IL-6/PGE2 (55). Studies with the new generation of ex vivo DC vaccines will permit us to assess the type of immune responses elicited by human DCs generated in different cytokine environments in vivo.

Another, novel, approach to cancer vaccines via DCs is based on the delivery of Ags directly to DCs in vivo using chimeric proteins made of anti-DC receptor Ab fused to a selected Ag (DC targeting). Studies in mice demonstrate that the specific targeting of Ag to DCs in vivo results in considerable potentiation of Ag-specific CD4+ and CD8α+ T cell immunity. The induction of immunity is observed only when the DC maturation signal is provided (56, 57), as, otherwise, tolerance ensues (57). Studies in animal models demonstrate that targeting of tumor Ags to DCs and LCs (58, 59) can induce the generation of therapeutic antitumor immunity (60, 61).
Targeting tumor Ags to human DCs ex vivo can lead to generation of CD4+ T cell (62) and CD8+ T cell (63, 64) responses. The BDCA-3+ cDCs might be of special interest with respect to their potential for priming CD8+ T cell responses. Importantly, certain lectins, including Dectin-1 and DC-specific intercellular adhesion molecule-3–grabbing non-integrin, as well as other DC surface molecules (e.g., CD40), also provide activation signals (65, 66). They can thus be exploited for both Ag delivery and activation pathway in a single targeted vaccine. The therapeutic success of these vaccines will build on the recent knowledge and progress in our understanding of the biology of human DC subsets, cutaneous cDCs in particular.

A major challenge of this approach will be to elicit T cell responses that are sufficiently robust and long lasting so as to be clinically active. Indeed, the efficacy of DC targeting in vivo needs to be established in clinical trials in patients, and early studies targeting mannose receptor and DEC205 are ongoing. Selection of an appropriate adjuvant is also a critical parameter for the induction of the immunity of the desired type. For instance, TLR2 ligation, which promotes the induction of Tregs rather than Th1 or Th17 cells (67), does not appear to be a preferred option for cancer vaccines. Thus, the challenge is to match the molecular target on DCs with the desired immune outcome, mimicking in many ways the natural role of these DC receptors to fine-tune responses appropriate to the infection. It is also likely that targeting distinct DC surface molecules will lead to specific types of immunity. Clinical correlates of such immune responses will require extensive studies over the next decade. The interpretation of these studies will require a deeper understanding of the biology of human tissue resident DCs, lymphoid DCs, as well as tumor-infiltrating DCs.

**The quality of immune responses**

At least four components of the immune response appear critical for that response to be of therapeutic potential, as follows: 1) the quality of the elicited CTLs; 2) the quality of induced CD4+ Th cells; 3) the elimination and/or non-activation of Tregs; and 4) the breakdown of the immunosuppressive tumor microenvironment. Indeed, the immune responses elicited by the first generation DC vaccines might not be of the quality required to allow the rejection of bulky tumors. For example, the induced CD8+ T cells might not migrate into the tumor lesions (15, 68), necessitating a better understanding of the trafficking into tumors of the elicited effector T cells. Furthermore, low avidity CD8+ T cells might not be able to recognize peptide-MHC class I complexes on tumor cells and/or to kill them (15). Ideally, elicited CD8+ T cells should express high levels of granzyme and perforin, molecules essential for cytotoxic activity against cancer cells (15, 43). Finally, the tumor microenvironment might inhibit effector CD8+ T cell functions, for example, by action of myeloid-derived suppressor cells and Tregs as summarized in recent reviews, respectively (69, 70).

Besides the quality of CD8+ T cells, the quality of CD4+ T cells represents another key parameter of immune efficacy for antitumor immunity (71). CD4+ T cells act through dif-
ferent mechanisms, including the following: 1) provision of help in the expansion of tumor Ag-specific CTLs (72); 2) activation of macrophages at tumor sites (73); and 3) active killing of tumor cells (74). Furthermore, it is now well established that Ag-specific CD4+ T cells are fundamental for the induction of long-term memory CD8+ T cells (75). However, CD4+ T cells can also be detrimental. There, regulatory/ suppressor T cells dampen elicited CD8+ T cell responses (17). Furthermore, type 2 cytokine-secreting CD4+ T cells counteract antitumor immunity by promoting tumor development (21) and/or by polarizing tumor-associated macrophages (76). Clearly, understanding DC biology in tumor environment will provide rationale for their modulation, be it with targeting Abs or with adjuvants. This in turn will allow reprogramming of the immune environment at the tumor site, thereby facilitating tumor rejection.

Combining cancer vaccines with other therapies

In view of the remarkable diversity of regulatory/suppressive pathways present in patients with metastatic cancer, any durable clinical response elicited by vaccination is already an achievement. However, to improve the outcomes, DC vaccines will need to be combined with other therapies that might target various components of tumor development, such as tumor cell apoptosis, angiogenesis, tumor stroma, and inflammation, to offset the suppressive environment created by tumors (22). Such combination regimens will involve several intervention strategies that target different pathways (Fig. 3).

In particular, blocking Abs or soluble receptors can be exploited for the blockade of cytokines in the tumor microenvironment that either suppress the effector T cells directly or act via macrophages and myeloid-derived suppressor cells. Candidates include IL-10 (77), IL-13 (78), and TGF-β (79). Blocking Abs can also be used to offset immune-inhibitory signals in lymphocytes. This can be best illustrated by anti-CTLA-4 (80) in which the intrinsic T cell regulatory pathway can be blocked or by anti-programmed death ligand 1 to block extrinsic T cell inhibitory pathways driven by ligands expressed on tumors or DCs (81, 82). In contrast, agonistic Abs (83) might further promote costimulation of effector T cells, for example, with anti-CD137 (84), a ligand for 4-1BB (85). Just as different tumors are currently treated with different combinations of cytostatic drugs and targeted therapies, we foresee the development of clinical protocols combining DC vaccines with individualized adjunct therapies.

Last but not least, recent studies suggest that the immune system is likely to contribute mechanistically to the clinical efficacy of other therapeutic modalities in cancer, including the following: 1) radiotherapy and some chemotherapeutic agents, for example, anthracyclins (86), as well as 2) Ab therapy such as Herceptin (87). Thus, cancer vaccines might be more efficient, when combined with such treatment modalities.

Conclusions

There has never been a better and more exciting time to work on developing cancer vaccines. The considerable progress made in the understanding of DC biology as well as effector/Treg biology clearly opens avenues for the development of vastly improved clinical protocols (Fig. 4). Importantly, rather than the quantity of IFN-γ–secreting CD8+ T cells, the goal is to generate high quality and high avidity polyclonal and poly-functional effector CD8+ T cells able to reject tumors and long-lived memory CD8+ T cells able to prevent relapse. The capacity of LCs and CD14+ DCs to preferentially prime, respectively, cellular immunity and humoral immunity has significant implications, most particularly in the context of novel cancer vaccines. Therapeutic vaccination in patients with nonresectable metastatic cancer will require combination therapies. These will be tailored to the patient and to the specific suppressive pathways that the patient displays. These pathways will be determined using system biology approaches incorporating genomic studies on blood and tumor samples, as well as polychromatic flow cytometry and assessment of Ag-specific T cell repertoire (88, 89). Lessons learned from the current studies will lay the ground for preventive vaccination, for example, in neoadjuvant setting.

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Disclosures

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