ORIGINAL ARTICLE

Johannes Vieweg · Andrew Jackson

Modulation of antitumor responses by dendritic cells

Abstract The discovery that dendritic cells (DC) play a key role in regulating antitumor immunity has prompted considerable efforts in developing DC-based cancer vaccines for use in clinical oncology. Early translational trials using antigen-loaded DC have established clear evidence of vaccine safety, and demonstrated bioactivity by stimulating immunological and even clinical responses in selected subjects. Despite these encouraging results, the vaccine-induced immune responses achieved to date are not yet sufficient to attain a robust and durable therapeutic effect in the cancer patient. Therefore, further improvements are required to enhance vaccine potency and optimize the potential for clinical success. This article presents a set of emerging concepts that, together, form a framework for a multi-pronged approach that will further enhance the efficacy of DC-based vaccination by either directly improving DC-mediated T cell activation or by inhibiting mechanisms that suppress the induction of an effective antitumor response. The clinical translation of these concepts will result in new opportunities to successfully modulate immune responses in clinical settings.

Keywords Dendritic cells · Antitumor immunity · Immunosuppression · Regulatory T cells · CD4$^+$ T cell immunity

Overview

Over the past decade, the discovery of a network of antigen-presenting cells (APC) that regulate the development of immunity and tolerance [2] has allowed profound insights into the complex relationship between the malignant process and the immune system. Dendritic cells (DC), the most potent APC, play a central role in the presentation of antigens to naïve T cells and in the induction of primary immune responses. DC are typically located at sites of pathogen entry and are uniquely efficient to (1) acquire antigens from

J. Vieweg (✉) · A. Jackson
Genitourinary Cancer Immunotherapy Program, Division of Urology, Department of Surgery,
Duke University Medical Center, MSRB, Suite 455, P.O. Box 2626, Durham, NC, 27710, USA
e-mail: j.vieweg@duke.edu · Tel.: +1-919-6849949 · Fax: +1-919-6817414

pathogens or pathogen-infected cells, and (2) to process these antigens for both class I and class II presentation. Upon antigen encounter, the tissue resident DC, termed immature DC, undergo a differentiation process called maturation, whereby they up-regulate their capacity to present captured antigens to T cells, then migrate to draining lymph nodes, and finally encounter and activate cognate T cells. This maturation step allows DC migration from the injection site to the draining lymph nodes and facilitates the activation of cognate T cells to generate potent Th1 CD4$^+$ T cells and cytotoxic T lymphocyte (CTL) responses. Aside from protection against pathogens, DC also play an important role in the stimulation of tolerance development, and antitumor immunity [3]. The ability of antigen-loaded DC to generate both protective and therapeutic antitumor immunity has been documented in many animal models, establishing a scientific rationale for evaluating tumor antigen-bearing DC as therapeutic vaccines in humans [11].

DC-based cancer immunotherapy holds the promise of a new treatment modality that is tumor specific, bears little toxicity, and, once fully developed, could have a long-lasting effect. The results of numerous DC-based clinical trials have been published, providing initial indications of their potent immunostimulatory capacity in man [8]. DC vaccination has also been associated with tumor regression in selected cancer patients, but at this point, no firm conclusions regarding the clinical and even immunological efficacy of DC-based vaccination can be drawn. Factors hampering the development of DC-based vaccines include: (1) the small patient cohorts enrolled into early phase clinical trials, (2) the non-comparative nature of the clinical trial design employed, and (3) the lack of fully standardized immunological monitoring assays [18], thus precluding systematic assessment of the multiple DC vaccination strategies yet to be explored.

In view of the early developmental stage of DC-based vaccination, it comes as no surprise that all studies to date have demonstrated only limited clinical impact. There are, as yet, no conclusive data regarding the patterns of clinical activity, tumor remissions, duration and survival. Furthermore, DC therapy is labor and resource intensive, making process simplifications and further standardization a high priority for successful clinical development. Cumulatively, these issues have led to increasing skepticism among many investigators regarding the ultimate benefit and utility of employing DC-based vaccines, and have called into question the competitiveness of DC therapy when compared with other new approaches to cancer therapy currently being explored.

However, given the early developmental stage of DC-based immunotherapy, the lack of demonstrable clinical benefit in exploratory phase I trials should not warrant the premature abandonment of DC-based vaccine strategies. Several indications demonstrate that immunological manipulation can be effective in controlling malignant disease, not only at an experimental, but also on a clinical level. For instance, the graft-versus-tumor effect observed after non-myeloablative allogeneic peripheral blood stem cell transplantation is one example of the way in which the immune system can mediate tumor regression [48]. The response patterns seen with this approach indicate that immune-based strategies, at this stage of development, may attenuate, but not completely eliminate tumor growth. Consistent with this observation, the immunomodulatory effects of Bacillus Calmette Guérin (BCG) effectively control recurrence of highly malignant superficial bladder cancer cells that, if left untreated, would quickly progress and eventually disseminate [1]. Finally, the lessons we have learned from monoclonal antibody development, suggest that continued scientific discovery will eventually overcome the current obstacles that hamper DC-based vaccination, and eventually will improve these approaches so that a clinically effective immune response can be achieved. As we have gained a clearer understanding of

the cellular and molecular events that modulate antigen presentation and T cell activation in vivo, new strategies have emerged, allowing the development of more potent "second generation" DC vaccines.

In this review, we outline the requirements for an effective immune response and discuss several novel concepts that have provided new avenues to further enhance the antigen-presenting function of DC-based vaccines. These strategies include: (1) modulating the DC maturation process, (2) enhancing the CD4+ T-cell arm of the immune system, (3) inhibiting regulatory pathways, and (4) targeting antigens with critical roles in oncogenesis. Without question, complex diseases such as cancer will require a combination approach. The concepts described, if justified, can be applied simultaneously, sequentially, or in combination with other strategies to generate superior immunization protocols that will have a therapeutic impact.

Requirements for effective immune responses

The goal of DC-based vaccination is to activate tumor antigen-specific T cell responses that trigger a therapeutic effect in the cancer patient. Most clinical trials using DC vaccines have demonstrated the stimulation of CTL responses in the peripheral blood, lymphatic tissues, or tumors; however, vaccine-induced antitumor immunity has only sporadically correlated with clinical response [18]. One likely explanation for this lack of correlation is that the magnitude of the vaccine-induced immune response has been insufficient to result in significant tumor regression. Alternatively, tumor-mediated factors may weaken the immunostimulatory capacity of DC-based vaccines. From infectious disease settings, we have learned that T cell frequencies of approximately 3–9% of the total CD8+ T cell pool cells are required to clear infections [62]. However, the stimulation of CTL frequencies of a comparable magnitude has rarely been reported in DC-based clinical trials. Conversely, many experimental studies have demonstrated that the induction of high frequencies of cancer-specific CD8+ T cells alone often fails to provide a demonstrable clinical benefit, suggesting that not only the magnitude, but also the quality of the vaccine-induced T-cell response is critical. Further complicating matters, the persistence of the vaccine-induced T cell response will certainly play a crucial role in impacting vaccine potency, although this issue has been investigated only sporadically on either an experimental or a clinical level thus far. Therefore, combining DC therapy with other complementary approaches designed to enhance persistence of immunity, in particular the CD4+ T cell arm of the immune system, may be of considerable benefit.

In summary, the major challenge in DC vaccine development is to design strategies that effectively enhance the magnitude, quality, and persistence of the vaccine-induced T cell response. The concepts described in this review have resulted in clinically applicable solutions that address these important issues in one or more ways.

Improving the antigen-presenting function of DC

Vaccination with antigen-loaded DC involves a complex and labor-intensive manufacturing process that hampers widespread development in academic and industry settings. Therefore, further process simplification, while maintaining efficacy, would represent a

major advancement of this field. The DC maturation step is one key element in the process of generating immunopotent monocyte-derived DC, currently the most widely used DC generation protocol for clinical applications. For cancer immunotherapy, DC are cultured and matured ex vivo to a stage in which they can migrate to the lymph nodes, thereby acquiring the capability to activate the cognate T cells. While the list of reagents that facilitate DC maturation is growing, the exact sequence of events and the combination of factors required for the effective differentiation of the DC so that they will prime effective T cell responses is not yet known.

These uncertainties are reflected by recent reports demonstrating that, under some conditions, in vitro-generated DC can either favor the induction of a Th2 CD4$^+$ T cell response and/or tolerize cognate T cells, as reviewed in [17, 47]. Studies have shown that with excessive exposure to maturation reagents, DC become "exhausted" and favor the generation of Th2 CD4$^+$ T cells [25]. Suboptimal antigen presentation also favors Th2-biased T cell responses, presumably through the failure of inducing CD40L expression on T cells [53]. Additionally, tolerance can be induced by immature DC [9], DC cultured with IL-10 [63], and TNF-α [34]. Finally, in the absence of appropriate costimulatory signals encountered at the lymph node, and delivered via CD40/CD40L interactions, activation of CD8$^+$ CTL will be suboptimal [50].

In brief, it is well established that multiple external stimuli, delivered in certain combinations and using appropriate sequences, influence the development of the DC through various pathways. However, the exact nature of the stimuli and sequence of events leading to DC differentiation in vivo, including DC capable of optimally stimulating Th1 CD4$^+$ and CD8$^+$ T cell responses, is not yet known.

One of the best-characterized protocols for generating mature human DC involves the differentiation of DC from CD14$^+$ monocytes. In this protocol, monocytes are cultured for 5–7 days in the presence of GM-CSF and IL-4 to generate immature DC, followed by 1–2 days of culture in the presence of TNF-α, IL-6, IL-1β and PGE$_2$ to induce their maturation [51]. Upon maturation, these DC express high levels of HLA antigens and the maturation marker CD83, stimulate potent T cell responses, and possess migratory function in vitro. Clinical trials have shown that DC matured in this manner and loaded with melanoma-derived HLA class I or class II-restricted peptides, stimulated both CD8$^+$ and CD4$^+$ T cell responses in patients with advanced disease [55]. Nevertheless, it is questionable whether these conditions represent the optimal modality for ex vivo maturation of DC. This concern stems from observations suggesting that PGE$_2$, required to facilitate DC migration, inhibits responsiveness to CD40L-mediated signaling encountered at the lymph node [28]. Furthermore, defects in CD40L signaling resulted in the activation of IL-10-secreting regulatory T cells [29].

An alternative and potentially more physiological approach to induce DC maturation is to inject antigen-loaded immature DC into "primed" or "preconditioned" cutaneous tissues. "Priming" or "tissue conditioning" can be accomplished through reagents that recapitulate the physiological conditions occurring during pathogen infection by creating a microenvironment in which DC would encounter the necessary signals that facilitate the up-regulation of costimulatory molecules and chemokine receptors. Through these signals, DC acquire the ability to present antigens to T cells and to migrate to the draining lymph nodes.

Murine studies have shown that pre-injection of the proinflammatory cytokines TNF-α and IL-1-α into cutaneous tissues enhanced migration of antigen-bearing DC to the regional lymph nodes [30]. In a second study, injections of bone marrow-derived DC into

skin exposed to adjuvants were as effective or superior to ex vivo-matured DC in acquir-ing migratory capacity, stimulating CTL responses, and inducing potent antitumor immu-nity in tumor-bearing mice [38]. In the same study, immature human monocyte-derived DC injected into the intradermal layer of skin pretreated with the immunomodulator im-iquimod acquired the capacity to migrate to the inguinal lymph nodes as effectively as ex vivo-matured DC [38]. Therefore, injection of immature DC into "primed" skin sites can induce migration, induce potent antitumor immunity, and thus may represent a potent strategy for cancer immunotherapy.

For active immunotherapy, the imidazoquinolines imiquimod or resiquimod may both represent highly effective compounds for facilitating tissue priming, since they provide maturation signals to DC through Toll-like receptors (TLR) 7 and 8. TLR signaling through NF-κB and MAP kinase pathways facilitates secretion of multiple proinflammato-ry cytokines that contribute to a Th1-polarizing microenvironment [67, 69, 73]. Notably, application of imiquimod to the skin of mice also induced the migration of the resident Langerhans cells to the application site, resulting in enhanced allergic contact hypersensi-tivity [67]. Recruitment of endogenous APC into primed skin sites may have important implications for DC-based immunotherapy since the recruited endogenous APC that encounter antigen at the injection site, would likely enhance the vaccine-mediated immunomodulatory effect by presenting tumor antigens to T cells through the indirect presentation pathway. It has been argued that indirect presentation of tumor antigens is a prerequisite for effective priming of naïve tumor antigen-specific T cells [78].

Thus, the in situ priming strategy may offer considerable advantages over the currently established methods of ex vivo maturation. This strategy more closely resembles the phys-iological conditions for DC maturation, and hence may lead to a more desirable outcome, namely a more potent immune response. In situ priming eliminates an in vitro DC culture step, which, in the setting of patient-specific cell therapy, represents a considerable pro-cess simplification. Finally, in situ priming obviates the dependence on expensive biologi-cal reagents used for ex vivo DC maturation such as TNF-α, IL-6, IL-1β and PGE$_2$ [15]. Therefore, injecting DC into pre-primed skin sites may not only prove superior to ex vivo-matured DC, but is also more physiological and circumvents the need for maturing DC ex vivo.

Enhancement of CD4^{+} T cell immunity

One major focus in cancer immunotherapy has been to design and apply immune-based strategies that promote the induction of a potent CD8^{+} CTL response in the cancer patient [33, 52]. However, accumulating evidence strongly suggests that the CD4^{+} T cell arm of the immune response also plays a critical role in mediating antitumor immunity [45]. CD4^{+} T cells are known to provide important functions for the induction, persistence and expansion of CD8^{+} CTL [16]. CD4^{+} T cells, via secretion of effector cytokines such as IFN-γ, sensitize tumor cells to CTL lysis via up-regulation of MHC class I molecules and other components of the endogenous presentation pathway, stimulate the innate arm of the immune system at the tumor site, and, as was recently suggested, inhibit local angiogene-sis [49]. CD4^{+} T cell responses can be generated by simultaneously pulsing DC with pro-tein/antigens such as keyhole limpet hemocyanin (KLH) as well as tumor antigens. The problem with this approach is that the activated/effector or helper CD4^{+} T cells are avail-

able only during CTL priming at the lymph node draining the site of immunization, but will be absent at the tumor site and thus unavailable to expand the activated effector CTL, or to exert their intrinsic effector functions. Thus, it would be advantageous to induce tumor-specific, as opposed to KLH-specific, CD4+ T cell responses. The importance of the CD4+ T cell response in tumor immunity was highlighted in several murine studies showing that CD4+ T cells can eradicate tumor in the absence of CD8+ T cells [13, 36] or constitute a more dominant effector arm when compared to CD8+ T cells in the antitumor response [14]. It is, therefore, conceivable that an optimal antitumor immune response will require the concomitant activation of both the CD4+ and CD8+ T cell arm of the immune response.

As a potential solution to improve MHC class II antigen processing, it was recently shown that routing cytosolic tumor antigens to the endosomal/lysosomal compartment can profoundly improve the in vivo therapeutic potency of recombinant vaccines [27, 77]. Translating these concepts into human settings, chimeric transcripts containing both the model antigen telomerase reverse transcriptase (TERT) and the lysosomal targeting signal of LAMP-1 (LAMP) have been shown to direct antigen processing into the class II pathway. DC transfected with mRNA encoding chimeric LAMP/TERT protein exhibited significant enhancement in their ability to stimulate CD4+ T cell responses in vitro, while allowing for concomitant induction of TERT-specific CD8+ T cell responses [65]. This strategy was recently investigated in a clinical trial in which patients with metastatic prostate cancer were vaccinated with TERT- and LAMP/TERT mRNA-transfected DC (manuscript in preparation). This study revealed several striking observations including that the expansion of TERT-specific CD8+ T cells in the peripheral blood of study subjects was reproducibly measured, with 0.9% to 1.8% of CD8+ T cells exhibiting antigen specificity. Also, patients immunized with the chimeric LAMP/TERT vaccine developed higher frequencies of TERT-specific CD4+ T cells than subjects receiving DC transfected with the unmodified TERT template. Finally, this increased LAMP-driven CD4+ T cell response facilitated the development of central memory T cells in the vaccinated subjects, as evidenced by the acquisition of IL-2 secretion by vaccine-induced CD8+ T cells. These findings may have important implications for determining the optimal time for boosting, since sufficient numbers of central memory T cells should be present before a booster immunization is given.

Recent studies performed in murine models demonstrated that only central memory, but not effector memory, T cell subsets have the ability to: (1) rapidly proliferate after re-exposure to antigen, (2) produce IL-2, and (3) persist long term in vivo by undergoing homeostatic proliferation in response to IL-15 and IL-7 [56, 76].

In summary, enhancing CD4+ immunity, in particular by facilitating access to the endocytic/lysosomal compartment, appears to be highly advantageous by enhancing the magnitude as well as the persistence of a vaccine-mediated T cell response. However, routing antigens through the class II presentation pathway, where class II-restricted peptides can be generated alone, may not be sufficient to elicit high frequencies of antigen-specific CD4+ T cells in vivo.

Another complementary strategy for enhancing the CD4+ T cell arm of the immune system is to facilitate costimulation though OX40/OX40L interactions. OX40, a member of the TNF receptor superfamily, profoundly impacts CD4+ T cell immunity by protecting the newly activated Th1, as well as the Th2 CD4+ T cells from activation-induced T cell death in concert with other extrinsic factors, such as IL-12, which determines the polarization fate of the activated CD4+ T cells [24]. Triggering OX40 costimulation has been

shown to translate into a sustained CD4^{+} T cell response and a larger memory T cell pool [12, 32]. OX40 also stimulates the proliferation of both Th1 and Th2 CD4^{+} T cell lines in vitro [12], and leads to a Th1-polarized CD4^{+} T cell response in vitro and in vivo [75]. Administration of either soluble OX40L-Ig fusion molecules or OX40 agonist antibodies in conjunction with active immunotherapy protocols produced potent adjuvant effects in multiple murine tumor models, including models for sarcoma, breast carcinoma, glioma, melanoma, and colon cancer [19, 75].

One major obstacle to the translation of this concept into the human setting, is the fact that human monocyte-derived DC lack OX40L cell surface expression in the absence of CD40 signaling, regardless of their maturation state [42]. A potential approach to overcome this obstacle and facilitate expression of OX40L by monocyte-derived DC is to transfect the DC with the corresponding mRNA. Generation of clinical-grade mRNA is a relatively simple and cost-effective task, thereby eliminating the chronic dependence on expensive and often unavailable reagents from commercial sources. Our group has recently shown that transfection of monocyte-derived DC with OX40L mRNA effectively improved the immunostimulatory function of monocyte-derived DC at multiple levels; OX40L mRNA co-transfection (1) augmented allogeneic as well as epitope-specific CD4^{+} T cell responses, (2) improved the induction of antigen-specific CTL responses in vitro without interfering with the PGE_2-mediated migratory function of the DC, and (3) led to IL-12p70-independent polarization of naïve CD4^{+} T-helper cells toward a Th1 phenotype, without stimulating a Th2 response. Finally, vaccination of tumor-bearing mice using OX40L mRNA co-transfected DC resulted in significantly enhanced survival due to in vivo priming of a tumor-specific Th1-biased CD4^{+} T cell response (manuscript in preparation).

In summary, recent research suggests that triggering costimulation through the OX40 pathway may represent one effective approach to enhance and prolong antigen-specific T cell responses in vitro, forming a scientific rationale for further clinical investigation.

Reversal of tumor-mediated immunosuppression

Murine studies have identified subsets of T cells, which function in a regulatory capacity by suppressing the function of other T cells. Specifically, CD4^{+} T cells that constitutively express the IL-2 receptor α-chain (CD25) act in a regulatory capacity by suppressing the activation and function of other T cells [58, 59]. It is believed that the physiological role of such regulatory T cells is to dampen chronic immune responses, especially against tumors and self antigens. These regulatory T cells exhibit a partially activated phenotype but are hypo-responsive to activation and proliferation stimuli. Furthermore, they are antigen specific in the sense that their generation and activation to effector function (i.e., suppression of other T cells) require the presence of antigen and TCR signaling. However, their function appears not to be antigen specific, but rather local, and they can inhibit both CD4^{+} and CD8^{+} T cells. The important role of CD4^{+}CD25^{+} regulatory T cells in mice was shown in several studies demonstrating that transient depletion of the regulatory T cells using anti-CD25 antibodies can enhance antitumor immunity [44, 60]. Recently, researchers have identified a similar subset of regulatory T cells in humans [58, 59]. Moreover, it was shown that antibody-mediated elimination of the CD4^{+}CD25^{+} regulatory T cells performed in conjunction with standard immunotherapy could dramatically enhance

antitumor immunity [66]. Therefore, it has been hypothesized that treatment with compounds that lead to the preferential depletion of $CD4^+CD25^+$ regulatory T cells, such as agents which target and kill cells expressing the IL-2 receptor CD25, may enhance the immunostimulatory potency of DC-mediated vaccination protocols.

In this context, the diphtheria fusion protein $DAB_{389}IL-2$ is currently being intensely investigated as a reagent to eliminate CD25-expressing regulatory T cells from the PBMC of cancer patients [43]. Studies conducted in our laboratory have provided a proof of concept that $DAB_{389}IL-2$-mediated regulatory T cell depletion resulted in enhanced stimulation of proliferative and cytotoxic T cell responses in vitro. Furthermore, an ongoing clinical trial suggests that administration of $DAB_{389}IL-2$ followed by vaccination with RNA-transfected DC significantly improved the stimulation of tumor-specific T cell responses in cancer patients, when compared to vaccination alone. Although these early data await further confirmation, it is reasonable to expect that this strategy, if successful, will influence the design of modern vaccine approaches that may incorporate this strategy to ultimately achieve T cell responses with therapeutic impact.

Another important mechanism by which tumors can escape immune attack is through the expression or secretion of factors that suppress the immune response by actively interfering with the differentiation, function and/or survival of DC and immune effector cells. For example, up-regulation of STAT-3 [72], secretion of transforming growth factor-β (TGF-β), IL-10, PGE_2, and vascular endothelial growth factor (VEGF) by the growing tumor have shown to subvert DC differentiation and down-modulate their stimulatory function. DC exposed to these factors can trigger inappropriate responses, such as the stimulation of Th2-cytokine-producing T cells or perhaps even regulatory T cells. Therefore, disruption of these regulatory pathways appears to be a key requirement to enhance vaccine function and to promote clinical responsiveness and regression of malignant disease. It was recently shown that tumor-bearing animals and cancer patients alike exhibit defects in myelopoiesis, which results in the accumulation of immature myeloid cells (ImC) (reviewed in [57]). ImC induce a profound state of immune suppression by interfering with the function of tumor-specific T cells through the production of reactive oxygen species such as nitric oxide and/or arginine depletion [4, 22]. Thus, there is increasing interest in developing strategies that allow targeting and eliminating ImC in immunotherapy protocols. One clinically applicable method to overcome ImC-mediated immunosuppression would be to induce their differentiation to a mature phenotype by using growth factors or differentiation agents such as all-trans retinoic acid (ATRA). Studies in mice suggested that systemic treatment with ATRA is capable of completely eliminating the inhibitory potential of these cells in vivo, providing an exciting opportunity to improve the efficacy of cancer immunotherapy [21]. Methods to characterize and track human ImC are currently being developed, and clinical trials to study the effects of ATRA in cancer patients, either alone or in context with DC-based vaccination, are ongoing at several institutions.

Novel targets for cancer immunotherapy

The efficacy of novel vaccine approaches is not only determined by the potency of the individual vaccination protocol, but also by the antigen used in the vaccine formulation. Systematic searches for human tumor antigens recognized by T cells have generally been limited to studying melanoma. The recent identification of broadly expressed so-called

"universal" tumor antigens may provide new opportunities to design more effective cancer vaccines. Antigenic targets such as human TERT [39, 65, 70, 71], p53 [68], survivin [61, 79], or oncofetal antigen (immature laminin receptor) [7] are commonly over-expressed in a wide variety of solid and hematopoietic cancers, and provide critical functions to tumor cells through promoting their survival or by maintaining the oncogenic phenotype. Thus, it has been hypothesized that the inclusion of epitopes derived from these proteins would make it more difficult for tumors to escape immune recognition by down-regulating antigen expression.

Aside from the above-mentioned targets with critical roles in oncogenesis, recent studies have suggested that the tumor stroma, especially proteins involved in the angiogenic process, may offer a broad range of molecular therapeutic targets, such as VEGF and its receptors, or basic fibroblast growth factor, both of which are proteins involved in endothelial cell differentiation, vessel assembly, and metastatic behavior [10]. Recently, new genes encoding increasingly specific targets for tumor endothelium have been identified, and include the tumor endothelial markers TEM1, TEM5 and TEM8 genes, all of which are abundantly expressed on tumor endothelium, but absent from normal adult vessels [5]. Other stromal targets include fibroblasts, which can be targeted using the matrix metalloproteinases (MMP) or fibroblast activation protein-α (FAP-α), both molecules involved in tumor/extracellular matrix interactions, tumor invasion, metastasis, and angiogenesis [40]. One important aspect of such strategy is that the tumor stroma is genetically more stable than the tumor itself, thereby minimizing the development of tumor escape variants. Moreover, murine studies demonstrated that concomitantly targeting TAA and stromal antigens provides a synergistic effect in controlling tumor growth [37].

Support for the concept of immunologically targeting the tumor stroma was recently provided by a study in which a vaccine based on chicken MMP-2 as a model antigen could induce both protective and therapeutic antitumor immunity in tumor-bearing mice. In addition, angiogenesis was effectively inhibited within the tumor following vaccine treatment [64]. Mediators of angiogenesis have also been examined as potential antitumor targets. Experimental approaches have employed immunization with paraformaldehyde-fixed xenogeneic endothelial cells [74], VEGFR-2 protein-loaded DC [26], VEGFR-2 cDNA-encoding salmonella-based vectors [41], or DC transfected with VEGR-2, Tie-2 or VEGF mRNA [37]. In all studies, tumor growth was significantly inhibited without induction of detectable autoimmune pathology.

In summary, emerging knowledge in the molecular pathways influencing the oncogenic process has led to the discovery of novel targets that will make cancer vaccines more practical, applicable and potentially more effective. It is likely that continued identification of novel targets in concert with more effective vaccination protocols will eventually produce vaccination strategies with clinical impact.

Concluding remarks

Recent insight in the field of immunotherapy has reinvigorated interest in the development of cancer vaccines in academic and industry settings. Among the many developmental vaccination strategies, DC-based immunization appears to be one of the most promising to date. Proof of concept studies using antigen-loaded DC have been performed establishing clear evidence of vaccine safety and bioactivity by stimulating immunological and even

clinical responses in patients with advanced cancers. Continued progress on a basic science and biotechnology level has resulted in many exciting opportunities to more fully develop the therapeutic potential of DC-based vaccines. Traditionally, the translation of basic science concepts into meaningful clinical trials represents the bottleneck for the development of clinically useful DC-based immunotherapies. Considering the rapid pace of basic science discovery, and the multiple pathways that provide new targets for immunological intervention, it will be important to consider which approach holds the greatest promise for the success of DC-based immunotherapy, and which lend themselves to the most expedient and meaningful testing. Since cancer vaccines, particularly autologous products, are inherently safe, randomized phase II trials with extensive biological correlations may likely move the field forward most expeditiously. Nevertheless, with the development of increasingly potent immunotherapy protocols, the development of pathological autoimmunity in clinical trials must be seriously considered, as suggested recently [46].

Successful therapy for cancer will require a combination approach that includes multiple therapeutic steps. For example, antibody-mediated CTL-A4 blockade [46], co-administration of CpG immunostimulatory oligonucleotides [20], adjuvant therapy with soluble CD40 ligand [35], or silencing genes with immunosuppressive roles through small interfering RNA (siRNA) [23] or aptamer technology [54] are technologies that have shown to improve the effectiveness of cancer immunotherapy in experimental animals or human systems. Finally, conventional therapeutic approaches such as radiation- or chemotherapy appear to have synergistic effects in context with DC-based immunotherapy, mainly by rendering tumor cells more susceptible to CTL-mediated lysis [6] or by modulating the tumor microenvironment through release of cytokines or by recruitment of DC [31]. With research continuing at its current pace, it is reasonable to expect that substantial progress with DC-based cancer vaccines can be achieved in the near future.

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