Tumor antigen-specific T cells and cancer immunotherapy: current issues and future prospects

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Abstract

The critical role of antigen-specific T cells in the eradication of cancer has been demonstrated in numerous animal models. Data compiled from both in vitro systems and human clinical trials indicate that T cells can be identified that recognize antigenic fragments derived from gene products expressed by tumors. Nonetheless, results from clinical trials have been for the most part disappointing, since vaccine protocols designed to elicit anti-tumor T cell activity have, in the majority of cases, failed to result in tumor eradication and enhanced patient survival. The focus of this review article is to summarize the current status of antigen-specific tumor immunotherapy and provide insight into potential future strategies for the successful activation of T cells for the immunotherapy of cancer.

Keywords: Cancer; T cells; Immunotherapy

1. Introduction

The essential requirement for antigen-specific T cells in the eradication of cancer has been demonstrated in multiple animal models. A landmark in the field of T cell-based cancer immunotherapy of cancer was the identification by Boon and co-workers of MAGE-1, a tumor-specific gene product recognized by T cells derived from a melanoma patient [1]. The discovery of MAGE-1 established the potential for vaccine-based treatment of cancer by demonstrating that T cells existed in the peripheral circulation that were capable of recognizing specific proteins expressed and processed by tumor cells. The subsequent identification and characterization of a number of gene products that were expressed aberrantly by tumor cells, as well as continued progress in unraveling the intricacies of the immune system, have precipitated the development of a variety of antigen-specific vaccination strategies for the activation of tumor antigen-specific T cells.

Results from in vitro systems have demonstrated that T cells can be identified in peripheral blood that recognize peptide fragments derived from a number of gene products expressed by tumors. Furthermore, analyses of cancer patients treated with tumor-specific vaccines have provided evidence for the in vivo stimulation of tumor-specific T cells, both de novo and as a result of vaccination strategies. However, results from clinical trials against cancer have been for the most part disappointing, since the majority of patients undergoing cancer treatment do not mount successful anti-tumor responses.

The focus of this article is to summarize what is known of the role for antigen-specific T cells in cancer immunotherapy, highlight current approaches for both the identification and delivery of tumor vaccines, and finally provide insight into potential future strategies for the successful activation of T cells for the immunotherapy of cancer.

2. Role of antigen-specific T cells in the eradication of cancer

2.1. Animal model data

Numerous mouse tumor models have been developed to examine the role of T cells in the eradication of tumors. The overwhelming conclusion from these models is that both CD8+ and CD4+ T cells play a role in the effective eradication of tumors. The critical role for CD8+ and CD4+ T cells in the eradication and or inhibition of tumor growth has been demonstrated using adoptive transfer, antibody-based T cell depletion and transgenic mouse experiments [2–7]. Historically, CD8+ T cells have been considered to be the primary mediators of T cell anti-tumor activity, at least in part because CD8+ T cells are cytolytic, which has been considered the classical effector function, and the great
metastatic melanomas were observed in conjunction with been associated with prolonged survival, and regressions of proliferative capacity of CD8+ in lymph node metastases has been associated with reduced control of tumor growth. The presence of tumor infiltrat-
cancers have revealed evidence of a role for T cells in the pre- and prospective analyses of patients with early stage in the absence of measurable T cell activity. Nonetheless, conversely, when remissions are observed they often occur of cases following vaccination, there is a poor correlation sion of antigen-specific T cells has be observed in a number that contain defined CD8+ of melanoma, primarily because tumor associated antigens have been disappointing [18,19] . Although in vivo expansion of antigen-specific T cells in cancer patients. In the case of tumors that present naturally to CD8+ T cells [21,22].

2.3. Tumor antigens under consideration for T cell vaccines

A large body of literature exists on the identification of gene products with potential for use as cancer vaccines. The overwhelming majority of gene products expressed by tumors are also expressed at significant levels by normal cells and thus are not reasonable candidates for the develop-
ment of T cell-based immunotherapy protocols. However, tumor cells also express a number of gene products that are not expressed by normal tissues and thus could serve as attractive targets for the T cell arm of the immune system. The neoplastic phenotype is associated with expression of viral antigens expressed by viruses, differentiation antigens, as well as gene products whose expression is normally re-
stricted to fetal and/or privileged tissues (cancer/testis anti-
gen). Specific mutational events in normal cellular genes that result in point mutations, such as CDK-4 and bcr-abl, deletions, and translocations of normal cellular genes have also been associated with specific tumors types. Tumor cells have been shown in a number of instances to over-express specific gene products (Her-2/neu, p53, MUC-1), as well as transcribe unique RNA species derived from intronic sequences or from alternative reading frames of known genes (NYESO-1, R2, TRP-2). In addition, tumor cells can aberrantly modify and glycosylate proteins, and such proteins have the potential to be differentially processed and presented to the immune system.

In a number of cases, the presence of CD8+ and/or CD4+ T cells that recognize tumors that present naturally processed epitopes derived from the antigen candidates has been demonstrated, supporting the use of those antigens as vaccines. Immunologically validated tumor-specific vaccine candidate genes have been identified for a wide variety of tumor types, including melanoma, breast, prostate, colon, ovarian, cervical, pancreas, lung, uterine, leukemia, andrenal and hepatocellular carcinomas. Although a comprehensive listing of human tumor antigens recognized by T cells has been recently published [23], new potential cancer vaccine antigens continue to be identified and immunologically validated.

2.4. Current clinical strategies to activate antigen-specific T cells

Over the past 10 years, a number of clinical trials have been initiated to examine the potential for the activation of tumor antigen-specific T cells in cancer patients. In the case of tumor types for which well characterized tumor antigens have been identified, approaches have generally involved ad-
ministration of the antigen either as whole recombinant pro-
tein or in the form of peptides that have been demonstrated to be immunologically relevant. Defined antigens have been
delivered alone, in conjunction with potent adjuvants or cytokines, or loaded onto DC [24–30]. Approaches that do not depend on the identification of defined tumor antigens have involved vaccination strategies with allogeneic or autologous whole tumor, irradiated and in some cases modified to express cytokines or co-stimulatory molecules or tumor cell lysates, either in conjunction with adjuvant or loaded ex vivo onto DC [31–33]. As discussed above, although encouraging results have been reported from individual patients, results from clinical trials have to date been on the whole disappointing.

The lack of success in to date clinical trials can be attributed at least in part to the fact that early stage trials are conducted on advanced stage patients with, in most cases, severely compromised immune systems as a result of disease and/or treatment regimens. However, it is apparent that a number of variables still need to be optimized for the effective application of T cell-based vaccines against cancer. In particular, methodology and route of administration, use and type of adjuvants, readouts for effective immunization, and clinical endpoints differ widely among clinical trials, and thus the possibility to comparatively assess methodologies has not been possible [17,34]. The development of objective universally accepted parameters to vaccinate and assess the success of vaccinations is an important consideration to address in future trials.

2.5. Future prospects for T cell therapy

The primary limitations of current vaccine strategies against cancer can be identified as: (i) lack of optimal antigens to which a vigorous T cell response can be generated, (ii) lack of appropriate delivery systems for the effective activation of an anti-tumor immune response, and (iii) the inability to effectively circumvent tumor induced immuno-suppression and T cell energy/tolerance for most tumor antigens.

2.5.1. Lack of optimal antigens to which a vigorous T cell response can be generated

Since the initial discovery of MAGE-1, a large number of antigens have been described that could serve as T cell targets. The majority of these antigens have been discovered based on the presence of pre-existing antigen-specific T cell or antibody responses. Although pre-existing T cell responses might be considered to be advantageous for tumor eradication, it is possible that antigens identified on the basis of pre-existing anti-tumor T cell responses may not be optimal for use as vaccine candidates for tumor immunotherapy, since these T cell responses generally were insufficient to eradicate the corresponding tumors.

A variety of approaches have been recently developed to identify tumor-specific or enriched genes on the basis of mRNA and/or protein expression. Such techniques include proteomics-based approaches such as peptide elution from tumors, as well as molecular approaches such as serial analysis of gene expression (SAGE), subtraction hybridization, microarray analysis, and PCR-based differential display. A number of novel and known genes have been identified to be over-expressed by tumor cells using these approaches, and a subset are currently being evaluated for their potential as vaccine antigens. Candidates that show tumor-specific expression or significant over-expression by tumors, and for which potent CD8+ and CD4+ T cells as well as antibody responses are shown to exist in vivo are most likely to ultimately show efficacy as cancer vaccines.

2.5.2. Lack of appropriate delivery systems for the effective activation of an immune response

Most clinical trials to date have utilized DC generated from patient PBMC-derived monocytes by differentiation ex vivo in the presence of GMCSF and IL-4, loaded with recombinant protein or peptide, and subsequently introduced into patients [35]. However, such DC, referred to as “immature”, may not be optimal for presenting epitopes to T cells or for migrating to tumor sites and proximal lymph nodes; furthermore, such DC rapidly de-differentiate in the absence of cytokines. The co-ordination and use of maturation regimens that allow for efficient uptake, processing and presentation of antigenic epitopes to effectively stimulate T cells will be a critical area to optimize for the development of successful immunotherapy protocols.

The potential to genetically modify APC to express vaccine antigens, cytokines, or co-stimulatory factors is likely to allow for more efficient antigen presentation to the immune system. A number of attenuated virus delivery systems, including vaccinia, retroviruses, adenoviruses, lentiviruses, and alphaviruses have been considered or are in early clinical trials to mediate expression of vaccine candidates in DC [36–38]. Although virus delivery systems have great promise, a critical issue to resolve with the use of such vectors is the potential of anti-virus T cell and antibody responses in patients.

New methodologies for vaccine antigen delivery that include the use of other APC such as epidermal Langerhans cells, heat shock proteins, microsphere encapsulation, liposomes, or mechanical delivery approaches, as well as the identification of optimal prime-boost strategies, will be critical for efficient delivery and activation of anti-tumor immunity.

Identification of the optimal routes of administration for vaccine delivery is another critical area for research. Although most clinical trials have involved intravenous administration of vaccines, evidence from both mouse models and clinical trials suggests that intranasal or intradermal administration of vaccines is superior in terms of eliciting T cell responses.

Finally, the identification of novel adjuvants that can elicit a potent and appropriate immune response is likely to be critical for effective anti-tumor vaccination. In this regard, oligonucleotides containing CpG motifs, cationic poly-amino acid complexes, bacterial cell wall components...
or lipopolysaccharide derivatives such as Klebsiella pneumoniae P40, monophosphoryl-lipid A (MPL®) adjuvant, and TLR-4 agonists/antagonists are being tested in both animal models trials for the ability to mediate effective immune system activation.

2.5.5. The inability to effectively circumvent tumor induced immuno-suppression and T cell tolerance

Growth of tumors in vivo is accompanied by strong selective pressures for the development of mechanisms to evade the host immune response. Tumors have evolved multiple mechanisms for evading the immune response, including antigen loss, down regulation of MHC, and production of immuno-suppressive factors. Additionally, tumors lack expression of co-stimulatory molecules critical for the activation of naive T cells. Finally, because the majority of tumor antigens are also expressed in some normal tissues, high affinity antigen-specific T cells are likely to be deleted, and tolerance mechanisms are operative in vivo to prevent T cell activation in response to those antigens. These issues present a formidable challenge for the development of vaccination strategies to elicit effective tumor-specific T cell activity.

Strategies have been developed to enhance the ability of tumors cells to act as APC, primarily by expression of co-stimulatory, adhesion, and cytokine genes. A major limitation with these approaches is the requirement for tumor cells that can be cultured in vitro and efficiently modified to express transgenes in a stable fashion. The development of culture protocols as well as efficient gene transfer methodologies will have significant impact on the efficacy of such strategies in the future.

Numerous transgenic mouse models have been develop-ed to study tumor antigen-specific tolerance. Three important conclusions can be drawn from these models: (i) T cells specific for self antigens expressed by normal tissues can be found to exist, (ii) such T cells are generally non-responsive to antigen-expressing cells (i.e. are ignorant and/or tolerized), and (iii) if tumor antigen-specific T cells can be activated, then tumor eradication may occur in the absence of autoimmunity. In the context of these observations, the development of in vivo approaches to robustly activate tumor-specific CD8+ and CD4+ T cells in the context of a strong tolerogenic environment will be critical for the ultimate effectiveness of vaccine strategies in cancer immunotherapy.

One promising non-vaccine-based approach for T cell-based immunotherapy of cancer involves adoptive transfer of antigen-specific T cells into cancer patients [39]. The best example of the efficacy of adoptive transfer protocols in the eradication of cancer is illustrated by allogeneic stem cell transplantation. The strong correlation between the development and severity of graft versus host disease (GVHD) and tumor regression has led to the conclusion that adoptively transferred T cells, specific for minor histocompatibility antigens expressed on patient tumor cells are responsible for tumor eradication. To adoptive transfer of antigen-specific T cells has been applied with limited success in the tumor immunotherapy field [40]. Adoptive transfer overcomes at least some of the issues associated with T cell tolerance, since antigen-specific T cells are activated ex vivo, prior to re-infusion into patients. The recent observation that ex vivo culture of tolerized T cells results in activation of effector functions and subsequent tumor eradication upon re-infusion supports the development of adoptive transfer protocols for the eradication of tumors to which endogenous T cell responses are tolerized [41].

Two major limitations are associated with the widespread use of adoptive transfer in the cancer setting: (i) most tumor antigen-specific T cells identified to date are not particularly effective as effector cells, in part due to their moderate affinity for tumor cells and in part due to their tolerized state, and (ii) due to precursor frequency and limitations with in vitro expansion protocols, it is difficult and time consuming to reproducibly obtain tumor antigen-specific T cells from each patient.

A relatively novel approach to overcome the difficulty with generating antigen-specific T cells from every patient involves transfer of T cell specificity via TCR transfer [42,43]. Methodologies have been developed for the efficient cloning and transfer of TCR into primary T cells [44–46]. Thus, once a tumor antigen-specific T cell is identified, TCR α and β chains could be cloned and introduced at will into T cells from patients that share the relevant MHC molecule. A number of methodologies are currently under development to allow the generation of tumor antigen-specific T cells that express TCR with high affinity for self antigens expressed by tumors. One approach with particular promise involves the isolation of TCR from high affinity tumor antigen-specific T cells generated in the context of alloreactivity [47–49].

3. Conclusions

Over the past decade, significant effort has been applied toward the discovery, development, and clinical application of T cell vaccine strategies against cancer. A number of potential antigens have been identified as vaccine candidates to stimulate tumor-specific T cells, and clinical trials have been initiated in a number of cases, with overall disappointing results. Continued progress in understanding the intricacies of the immune system has revealed at least some of the critical obstacles, such as T cell tolerance and tumor-driven immuno-suppression, that need to be overcome for the successful application of vaccine approaches to tumor immunotherapy. The development of new methodologies for the discovery and effective delivery of cancer vaccine antigens, and the development of adoptive T cell transfer-based approaches to activate and modify tumor-specific T cells provide new avenues to pursue for the ultimate success of T cell-based immunotherapy of cancer.
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