



Review

Considerations on Clinical Use of T Cell Immunotherapy for Cancer

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Abstract. The recognition by effector T lymphocytes of novel antigenic targets on tumor cells is the premise of specific, targeted immunotherapy of cancer. With the molecular characterization of peptide epitopes from melanoma antigens and, more recently, broadly expressed tumor antigens, there has been considerable enthusiasm for clinical evaluation of peptide tumor vaccines. Immunologic monitoring of vaccinated patients has demonstrated an expansion of CD8⁺ T cells that react with the relevant peptide and, more importantly, with native tumor. In most instances, however, vaccine-induced CD8⁺ T cell responses alone have not been sufficiently robust or sustained to translate into a high percentage of durable clinical responses. Vaccine strategies have also utilized dendritic cells (DCs) that have been modified to present tumor antigens. The superior antigen-processing capacity and co-stimulatory function of DCs convey a powerful stimulatory signal to both CD4⁺ and CD8⁺ T cells. Several strategies are attempting to broaden the immune response beyond single antigens by introducing the entire complement of tumor antigens into DCs. Adoptive immunotherapy is a promising strategy to recover tumor-reactive precursor T cells from patients, stimulate them to induce numerical expansion, and then re-infuse them. *Ex vivo* manipulation of the tumor-reactive T cells also permits cytotoxic therapy to be administered to the patient without damaging the effector cells. Recently, host lymphodepletion prior to adoptive transfer of effector T cells has resulted in an extremely high and sustained frequency of effectors that has achieved therapeutic efficacy against bulky metastatic disease in a substantial fraction of treated patients.

Key words: tumor antigens; T lymphocyte; immunotherapy; dendritic cell.

Introduction

Cancer is predominantly an acquired disease that results from the accumulation of mutations in successive clones, providing for ever-greater survival and proliferative advantage^{58, 69}. The genetic dysregulation that accompanies the neoplastic process results in the expression of mutated proteins, novel chimeric proteins, re-expression of oncofetal proteins, and altered magnitude of expression of normal proteins. This pro-

vides the immune system with the potential to discriminate neoplastic cells from normal tissue. However, because tumors evolve from normal self-precursors, they lack the pathogen-associated molecular patterns that so effectively trigger intrinsic host defenses and initiate a vigorous adaptive immune response^{62, 63}. Despite the existence of tumor antigens, the vast majority of cancers arise in immunologically competent hosts and there are only anecdotal instances of spontaneous induction of a therapeutic immune response to cancer

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once it is established. The role of immune surveillance to control the progression of incipient neoplasms, while of great theoretical interest, is tangential to the clinically important problem of generating a curative immune response against advanced metastatic cancer²³. Therefore, amplification of the immune response to a therapeutic level will require artificial manipulation of the immune system rather than merely unmasking the endogenous response. There are numerous approaches to cancer immunotherapy that feature various components of either the humoral or cellular arms of the immune system, but this review will focus on T cell immunotherapy and strategies to enhance T cell sensitization. The main advantage of the T cell response is that it is extremely adaptive. The extant T cell repertoire can respond to nearly any appropriately presented antigen. More importantly, there is the capacity for tremendous numerical amplification of effector cells. When T cells are generated under appropriate culture conditions, they preserve the ability to traffic to all sites in the body and mediate tumor cell death either through direct cytotoxicity or through orchestration of other effector mechanisms. This review will discuss critical elements that contribute to a therapeutically effective T cell response against metastatic cancer.

Active Immunotherapy through Non-Specific T Cell Stimulation

There are two predominant strategies to elicit a therapeutic T cell response, active immunotherapy and adoptive immunotherapy⁸⁰. Active immunotherapy involves *in vivo* manipulation of the host immune system. On the other hand, adoptive immunotherapy uses passive transfer of preformed effector T cells. The interventions of active immunotherapy can occur at any stage of the response, beginning with the sensitization of naïve T cells to an antigen all the way to bolstering the function of T effector cells within tumors. The earliest strategies of active immunotherapy were focused on augmentation of effector cell function with non-specific immune modulators such as bacillus Calmette-Guérin (BCG) but were largely unsuccessful³⁹. The major difficulty with non-specific immune stimulants is that this strategy does not leverage the specificity of the immune response that is its fundamental advantage. The advent of cloning of many cytokine genes coupled with mass production technology ushered in an era of clinical testing of recombinant cytokines at maximally tolerated doses. This clinical experience has been predominantly unsuccessful with the exception of a subset of patients

with renal cell carcinoma (RCC) or melanoma who respond to high-dose interleukin (IL)-2 or interferon α ^{1, 29, 37, 84}. Other cytokines, such as tumor necrosis factor α , IL-4, and IL-12, were logical choices to pursue in clinical testing because of their great promise in pre-clinical models. However, these cytokines had insufficient efficacy and were associated with toxicity^{5, 38, 102, 103}. Unfortunately, the systemic route of administration of recombinant cytokines does not recapitulate their natural autocrine or paracrine function. Therefore, it is extremely difficult to achieve selective activation of effectors within tumors using cytokines.

Specific Activation of T Cells with Tumor Antigen Vaccines

The advantages of T cell immunotherapy are its exquisite specificity, systemic effects, and duration of action. Specificity can provide a very high therapeutic index by targeting antigens expressed exclusively by tumor cells thereby sparing normal tissues. This specificity results from each T cell clone expressing a unique receptor generated through genetic recombination of the T cell receptor (TCR) loci. Most immunotherapy strategies seek to stimulate classical T cells that express TCR $\alpha\beta$ and recognize short peptide fragments that are bound non-covalently to major histocompatibility complex (MHC) proteins. There are interesting approaches to utilize natural killer (NK) cells, $\gamma\delta$ T cells or non-classical NKT cells, but these are outside the scope of this review and have been discussed elsewhere^{6, 25, 60}. CD8⁺ T cells recognize peptide antigens in the context of MHC class I molecules that are expressed on the majority of somatic tissues, whereas CD4⁺ T cells recognize MHC class II bound peptides displayed by a more limited range of cells with antigen-presenting function. The vast majority of epithelial tumors express MHC class I molecules but fail to express class II, which has focused attention on defining epitopes for CD8⁺ T cells for use as cancer vaccines. However, as will be discussed below, stimulation of a broad response to multiple antigens with full participation of CD4⁺ and CD8⁺ T cells is more likely to induce sustained therapeutic effects.

Single Antigen Peptide Vaccines

The first CD8⁺ T cell-restricted human tumor antigen to be molecularly defined at the gene and peptide level was MAGE-1^{95, 97}. Characterization of other

melanoma antigens soon followed^{17, 47, 48}. The thrust to develop melanoma antigens resulted from the availability of numerous melanoma-reactive T cell clones to use as probes, and the recognition that cross-reactive antigens were expressed by most melanomas⁴⁹. Moreover, clinical responses in melanoma patients to tumor infiltrating lymphocyte (TIL) therapy made melanoma a logical malignancy to target⁸². The initial group of melanoma antigens were assorted into two major categories, the cancer/testis antigens and melanocyte-associated proteins. The cancer/testis antigens are expressed by germ line but not by normal somatic cells and are aberrantly expressed in a broad range of malignancies. Since the initial discoveries, numerous human tumor antigens have been defined with expression in a wide range of malignancies, catalogued in⁷⁸.

The major goal of the search for tumor antigens has been to define reagents that could be developed for widespread clinical use. The ease with which peptides can be manufactured and by which modified variants can be screened for enhanced binding to particular MHC molecules contributes to their appeal and has led to numerous clinical trials. The frequent reactivity of melanoma-reactive T cell lines with the MART-1/Melan A and gp100 proteins and prevalence of HLA alleles HLA A1001 or A2001 led to their choice as reagents for initial testing. Promising results were reported for the injection of a modified gp100 peptide in incomplete Freund's adjuvant. Patients who were treated with combined modality therapy using peptide vaccination and IL-2 experienced a higher rate of clinical responses than with peptide alone. However, the enhancement of clinical efficacy was confounded by other effects of IL-2, which led to difficulty in documenting the enhanced numbers of T effector cells in immune monitoring studies⁸³. The strategy of using peptides derived from widely expressed tumor antigens such as carcinoembryonic antigen (CEA), telomerase (TERT), or Her2/*neu* has considerable theoretical appeal^{19, 26, 99}. Direct injection of peptides is the simplest approach, but effective immune stimulation usually requires an adjuvant, such as incomplete Freund's adjuvant, or cytokines, such as granulocyte-macrophage colony-stimulating factor (GM-CSF) or IL-12^{18, 57}. In aggregate, the studies of single CD8 epitope peptide vaccines have documented safety and feasibility. Amplification of specific T cell responses from undetectable levels pretreatment to precursor frequencies exceeding 1% of CD8 T cells are occasionally seen, but the typical responses are more modest, on the order of 0.01 to 0.1% of CD8 T cells. An important observation from the use of CD8 stimulatory peptides alone is that

the induced response is transient, indicating that methods to stimulate CD4 helper T cells may be required⁵⁴.

Combined CD4 and CD8 Stimulation Enhances the Anti-Tumor Response

CD4 cells participate in the initiation of most CD8 immune responses through provision of signals *in cis* to the identical antigen-presenting cell (APC)⁹. Because the characterization of CD4 tumor antigens lagged behind that of CD8 epitopes, many studies used co-administration of either the pan HLA-DR helper epitope PADRE or KLH along with the class I-binding peptides in an attempt to stimulate helper CD4 T cells during sensitization^{2, 3, 79}. Although non-tumor helper epitopes do lead to strong CD4 responses during priming, the reactive CD4 cells are obviously not available for cognate help upon restimulation of CD8 cells in the tumor or within other lymphoid tissue. This may abrogate the long-term persistence of a robust CD8 response.

The provision of cognate CD4 help has significant theoretical appeal. Moreover, the convincing demonstration of the synergistic effect of cognate CD4 help points out the critical limitation of non-specific CD4 stimulation⁷⁰. This fact has fostered a search for class II epitopes in tumor-restricted antigens that can be co-administered with class I peptides^{94, 100, 101}. Fortuitously, an extended sequence from Her2/*neu* that binds somewhat promiscuously to HLA DR alleles also contains an embedded class I epitope, thereby providing cognate CD4 help within a single peptide^{19, 53}. Interestingly, this peptide induced epitope spreading to additional segments of the Her2/*neu* protein that were not included in the original vaccine. Epitope spreading is indicative of enhanced processing of native Her2/*neu* protein, presumably from tumor cells. Although not investigated or documented yet, the induction of epitope spreading to other tumor antigens, including unique tumor antigens, would be hypothesized to induce a more robust immune response.

MHC class II molecules accommodate greater heterogeneity in peptide length and sequence than MHC class I, raising the possibility of developing peptides that will also be useful for a broader subset of HLA alleles. However, the efficacy of peptide binding to class I and class II alleles that are independently expressed remains a complicated issue in the application of combined peptide approaches³⁰. The processing of an extended-length peptide from the cancer/testis antigen NY-ESO led to the introduction of a family of overlapping trimmed peptides into class I molecules.

However, this resulted in a presentation of epitopes that were suboptimal for generating CD8⁺ T cells that recognize naturally processed NY-ESO epitopes²⁴. Thus, although peptides have the advantage that they can be easily manufactured and administered to patients, there is a general realization that improved methods are needed to enhance and direct their *in vivo* presentation⁷¹.

Immune Response to Active Immunotherapy Using Single Tumor Antigens

A considerable appeal of single peptide epitopes is that they allow quantitative measurement of the induced immune response using highly sensitive and specific assays^{50, 106}. MHC tetramer complexed with a single peptide species has permitted the frequency of specific T cells to be determined before and after vaccination without any *ex vivo* manipulation. Likewise, functional assays, such as ELISPOT or cytokine FACS analysis can also provide a quantitative measurement of the frequency of antigen-specific T cells directly from the patient. This is in contrast to more traditional immune monitoring assays such as proliferation, cytotoxicity, and limiting dilution analysis that require culture activation to reach measurable levels. Although many studies have observed increases in frequency for specific T cells, the response is variable between assays and the overall frequency of CD8 T cells is typically between 0.1–1% and is transient^{54, 104}. Moreover, clinical responses against macroscopic tumors have been sporadic and have not always correlated with response in immune monitoring assays¹⁰.

Delivery of Tumor Antigens with Dendritic Cells

Dendritic cells (DCs) are specialized antigen-presenting cells that mediate antigen uptake from peripheral sites and migrate to secondary lymphoid organs to sensitize naive T cells. They are a logical choice as a vehicle to deliver tumor antigens, and simple methods to generate large numbers of DCs from peripheral blood mononuclear cell (PBMC) collected by apheresis have stimulated many clinical trials for cancer immunotherapy. The simplest approach is to load peptides onto DCs prior to injection^{7, 26, 65, 92}. This strategy, however, is potentially limited by the instability of peptide bind-

ing and by the turnover of MHC molecules on DC. One strategy to overcome this limitation is to transfect the DC with genes encoding single tumor antigens, such as CEA, prostate specific antigen (PSA), or TERT delivered through RNA or recombinant viral vectors^{35, 66, 91}. A considerable theoretical advantage of this approach is that there is a continuous supply of antigen to the antigen-processing machinery. Moreover, multiple epitopes, derived from the full-length protein, can be presented by the endogenous MHC molecules. Early results from phase I clinical trials support the safety of this approach and the generation of immune response, but an analysis of clinical efficacy has not yet been published^{34, 66}.

Active Immunotherapy with Whole Tumor Cell Vaccines

Creating an immune response to the entire array of antigens derived from whole tumor cells has a number of theoretical advantages over single tumor antigens. Although immune responses to single tumor antigens provide ease of monitoring, it is likely that a large number of tumor antigens have the potential to stimulate a host immune response. It is also evident that the antigenic complexity of a tumor cell is orders of magnitude higher than most viral pathogens, where the paradigm of single immunodominant epitopes is accompanied by strong experimental support. At an intermediate level of genetic complexity, protozoan pathogens rapidly shift antigenic structures to subvert an effective immune response. Thus, it should come as no surprise that antigen loss variants exist within a genetically unstable population of tumor cells⁵¹. The emergence of clones resistant to attack by a narrowly focused immune response mirrors the all too common phenomenon of chemoresistance. Fortunately, a byproduct of the type of genetic instability that generates single antigen loss variants is the production of a very large number of abnormalities in gene expression^{4, 59, 90, 98}. Because many of the abnormalities of the autologous malignant cell are likely to be idiosyncratic, it will be difficult to predict which antigens for a given patient will provide strong immune responses. This complicates immune monitoring studies because it is unknown whether T cell responses to well-defined epitopes will be appropriate surrogate markers. Consequently, it may be advantageous to measure T cell responses to autologous whole tumor cell antigen preparations in order to report accurately the aggregate immune response.

Autologous versus Allogeneic Tumor Cell Vaccines

An unresolved question regarding the use of whole tumor cell preparations is the relative immunogenicity of shared versus unique tumor antigens. Animal models using carcinogen-induced tumors have demonstrated that the dominant immune response is directed against unique tumor antigens that do not cross-protect⁷⁶. Considerable data has been derived in our laboratory assessing immune reactivity during the natural sensitization of tumor-draining lymph node T cells by progressive subcutaneous tumors. These experiments clearly demonstrate that unique tumor antigens account for the therapeutic response because adoptive transfer of *ex vivo* activated tumor-draining lymph node (LN) cells is highly specific for the tumor that provided the *in vivo* sensitization¹⁰⁸. Analysis of cross-protection experiments involving the P1A tumor antigen is also instructive in this regard. The murine tumor antigen P1A is the dominant antigen in the methycholanthrene-induced mastocytoma P815 and is naturally expressed by several tumors of various histologic origins⁹⁶. B7-transduced P815 tumors regress spontaneously and induce high levels of cytotoxic T lymphocyte that are cross-reactive with other P1A-expressing tumors. However, P815-B7-cured mice were not cross-protected against challenge with other P1A-expressing tumors⁷⁷. One interesting finding from adoptive immunotherapy models is that an immune response to shared subdominant antigens emerges during the vigorous response associated with regression of established tumors⁴⁴. Furthermore, clinical adoptive therapy melanoma trials have demonstrated that T cell responses against such shared tumor antigens can dominate successful therapy²¹.

Clinically, there is extensive experience with whole tumor cell vaccines derived from three allogeneic melanoma cell lines^{11, 41, 42}. These studies demonstrate therapeutic benefit for patients with melanoma in transit associated with the induction of an immune response. The allogeneic vaccines express nearly all the defined melanocyte antigens and presumably this formulation is sufficiently immunogenic to generate a therapeutic anti-tumor response. There is considerable experimental evidence that tumor antigens are engulfed by host APC and presented in secondary lymphoid tissue⁴³. Whereas BCG was used as the adjuvant in the above clinical studies, another approach is to use allogeneic tumors transfected with GM-CSF to augment their immunogenicity^{45, 88}. The advantages for clinical use of allogeneic cell lines is that they can be selected for the presence of defined tumor antigens, ease of propagation

in tissue culture, and can be modified to enhance their immunogenicity. Moreover, each subject in clinical trials receives a standardized product, facilitating data analysis. The disadvantage is the absence of antigenic epitopes unique to the patient's own tumor.

Other Formulations of Whole Tumor Cell Antigens

One potential limitation of whole tumor cell vaccines is that they may not be the optimal formulation to stimulate tumor-reactive T cells. The depot of irradiated tumor cells may require repackaging within the host, such as infiltration by DCs that subsequently convey antigen to secondary lymphoid tissue. One promising strategy to generate responses against the entire complement of tumor antigens is to amplify mRNA from tumor samples and introduce it directly into autologous DCs by transfection³⁶. Theoretically, a small biopsy sample from autologous tumor can provide a sufficient quantity of mRNA following the use of amplification strategies. However, the instability of RNA requires prompt processing of tumor samples into appropriate preservative solutions.

A related strategy is to use lysate derived from tumor cells to pulse DCs¹³. The advantages of tumor lysate are that it contains a broad array of cytoplasmic proteins from the tumor and incubation of lysate with the DCs recapitulates the process of exogenous antigen uptake. Typically, freeze/thaw preparations are used, thus the insoluble portion of the cytoskeleton, nuclear proteins, and membrane proteins might not be appropriately represented. Moreover, the quantity of lysate is dependent on obtaining a sufficient amount of resected tumor sample, potentially limiting its application to patients with bulky disease. This limitation could be neutralized by culture of autologous tumor cells, or the use of allogeneic tumor lines⁶⁴. An additional consideration is that antigen uptake and processing by DCs is highly dependent on their stage of maturation with immature DC demonstrating much higher efficiency. Clinical studies of lysate-pulsed DCs are ongoing and the feasibility and efficacy relative to other approaches will be available in the future following their completion.

Active Immunotherapy with DC-Tumor Fusion Heterokaryons

An alternative approach to introducing the entire complement of tumor antigens into DCs is to create

heterokaryons of DCs and tumor cells through fusion of their plasma membranes. Polyethylene glycol induces membrane fusion and has been used for many years to produce hybridomas. The efficiency of this method to create stable viable heterokaryons capable of prolonged replication is relatively low; consequently it is accompanied by drug selection of the rare hybrid cells. Several investigators have applied this technique to produce fusions of human tumor cells with DCs^{28, 31, 40, 52}. Recently electrofusion has been used to generate DC-tumor fusion cells with very high efficiency³³. This study rigorously documented the existence of multinucleate heterokaryons and their potent effects *in vivo* for active immunotherapy of established tumors. The major advantage of DC-tumor cell fusion cells is that they retain high expression of MHC class I and class II molecules as well as co-stimulatory and cell adhesion molecules. The fusion cells stimulate both CD4 and CD8 T cells *in vitro* and *in vivo*. Moreover, the entire tumor proteome, including membrane, nucleus and cytoplasm is incorporated into the fusion cells making all antigens available for presentation through class I and class II molecules. Our recent studies have clearly documented that the MHC molecules of the DC but not the tumor determine the antigen presentation to T cells. This finding differs fundamentally from the strategy of using autologous tumor fused with allogeneic DCs that was reported for RCC⁵⁶. Because of technical variability when individual patient tumor materials are employed, the currently preferred strategy employs fusion of autologous DC from patients with a panel of allogeneic tumor lines that express numerous shared tumor antigens. A substantial advantage of the host DC restriction of antigen presentation is that it would overcome the need to establish an autologous tumor cell line for each subject and would utilize the adequate number of DC precursors available even in cancer patients. Moreover, *ex vivo* culture of DC precursors prior to electrofusion could potentially reverse the functional defects documented for cancer patients²⁷. A phase I clinical trial to evaluate a vaccine consisting of DC-melanoma fusion cells will be performed shortly.

Adoptive Immunotherapy

Adoptive immunotherapy uses *ex vivo* production of tumor-specific T effector cells that are passively transferred to the patient. A large body of preclinical experimental work indicates that adoptive transfer of tumor-reactive T lymphocytes, as a single agent, is able to mediate regression of advanced tumors in every anat-

omic location, including immunoprivileged sites such as the central nervous system¹⁶. The principal advantage of adoptive immunotherapy is that the conditions of activation can be controlled in order to optimize proliferation of T cells. For example, powerful mitogenic stimuli such as bacterial superantigens or anti-CD3 monoclonal antibodies mAbs in combination with IL-2 would be toxic if administered directly to patients but can effectively drive rapid and extensive proliferation *ex vivo*. It is possible to prepare doses of T cells that exceed 10^{11} cells, at which point they constitute a substantial percentage of the patient's total lymphocytes. However, an important consideration is that tumor regression depends on the number of tumor-reactive T cells transferred. It is not sufficient to transfer even a large number of non-specific activated T cells. Clinical experience with lymphokine-activated killer (LAK) cells, which are PBMC activated *ex vivo*, with high concentrations of IL-2 did not demonstrate significant therapeutic effects above IL-2 alone in randomized clinical studies⁸¹. Another strategy, designated as autolymphocyte therapy (ALT), uses PBMC that are activated *ex vivo*, with anti-CD3/IL-2 and in some cases with addition of anti-CD28 co-stimulation. ALT does not incorporate a selective enrichment for tumor-reactive T cells as opposed to non-specific lymphocytes by either the method of collection or through antigen-specific activation. ALT therapy has not so far been associated with substantial clinical efficacy^{32, 61}.

Ex Vivo Generation of Tumor Antigen-Specific CD8 Clones

Activation of tumor-specific T cells can be accomplished in several ways. The most logical, and unfortunately the most labor intensive, approach is to perform the entire process of T cell generation *ex vivo*. Generation of tumor-reactive clones begins with sensitization to tumor antigens using APC followed by selection of tumor-reactive cells and then proliferation of candidate clones to therapeutic numbers. This approach has proved technically daunting until recently, however, when the definition of peptide tumor antigens and methods to prepare autologous DCs and purified naïve CD8 T cells have considerably enhanced feasibility. This strategy has been pursued using the MART-1 melanoma antigen for initial stimulation and isolation of CD8 T cell clones reactive with MART-1. The T cell clones are subsequently amplified to high numbers using non-specific stimulation with anti-CD3 and propagation using low concentrations of IL-2. Clinically,

infusion of MART-1 clones was well tolerated and provided some minor therapeutic effects¹⁰⁷.

A different but related approach utilized CD8 cells that were initially sensitized to GP100 peptides *in vivo* before retrieval. The CD8 cells were subsequently cloned and expanded to high numbers *in vitro* with anti-CD3 and high concentrations of IL-2. This clinical study demonstrated the feasibility and tolerability of adoptive transfer, with most toxicity associated with the concomitant high-dose IL-2 infusions. However, the T cells exerted minimal clinical efficacy²⁰. The major difficulty identified in these studies was the short survival *in vivo* of the transferred CD8 cells. This presents a similar problem identified previously in active immunotherapy studies using peptides that only stimulate CD8 cells. Consequently, attempts have been made to extend the survival of CD8 clones by either conditioning the host with systemic immunosuppression or by providing T cell help. Provision of low-dose IL-2 improved the median survival of transferred MART-1 reactive CD8 T cell clones from 6 to 16 days, but this maneuver alone did not provide for long-term survival or result in dramatic improvements in clinical efficacy¹⁰⁷.

An advantage of adoptive immunotherapy as opposed to active immunotherapy, is that it is possible to condition the host with immunosuppression prior to cell transfer. The beneficial effect of host immunosuppression has extensive experimental support in preclinical models^{67, 68}. While the proper use of host immunosuppression has not yet been defined in every clinical situation²², it has dramatically enhanced therapeutic outcome in several studies^{14, 21}.

As with active immunotherapy strategies that target a single antigen, a potential problem is the emergence of antigen loss variants. It is evident that methods to expand the repertoire of antigens recognized and to provide survival signals either through cytokine support or, more likely, through generation of tumor-reactive CD4 T cells will be needed to optimize the efficacy of cloned CD8 cells.

Ex Vivo Activation of *in Vivo* Sensitized T Cells

The process of sensitizing naïve T cells *in vitro* is very labor intensive and has not been optimized for production of a broad polyclonal immune response. Our laboratory has performed several clinical trials using a strategy of *in vivo* sensitization of T cells with whole tumor cell vaccines followed by removal of vaccine-draining LNs. This strategy is supported by exten-

sive preclinical data indicating that tumor-draining LNs are highly enriched for tumor-reactive T cells relative to other sites⁸⁷. An important feature of draining LN cells is that the tumor-reactive T cells are a minor subset of the total T cells and express a phenotype of recent antigen activation⁴⁶. There are significant differences in the properties of tumor-reactive T cells originating in the tumor-bearing host compared with non-tumor-bearing immunized hosts. The most relevant feature is that T cells from tumor-bearing hosts acquire markedly enhanced anti-tumor efficacy only following *ex vivo* stimulation, whereas hyperimmune lymphocytes are fully competent upon immediate adoptive transfer¹⁰⁸. This strategy was investigated in a clinical trial for melanoma or RCC patients. Subjects received *in vivo* sensitization with irradiated autologous whole tumor cell vaccines and BCG, followed by removal of vaccine-draining LNs and *ex vivo* activation with anti-CD3 and IL-2¹². This trial demonstrated objective clinical responses in RCC patients that were associated with the development of positive DTH responses to tumor cells.

T Cell Adoptive Immunotherapy Using Vaccine-Primed LN Cells

We performed a series of clinical trials using adoptive transfer of T cells in patients with malignant glioma, RCC, or squamous cell carcinoma of the head and neck^{72, 73, 74, 93}. For these studies, irradiated autologous short-term cultured tumor cells were used as the source of tumor antigens and GM-CSF was administered locally at the vaccine site as an adjuvant. This vaccine formulation was chosen to provide the unique tumor antigens as well as any shared antigens that are, as of yet, uncharacterized for these diseases. GM-CSF was used with a goal of stimulating APC migration through the vaccine site and was much better tolerated than BCG used in previous trials. Moreover, there was some concern about using of an infectious agent, albeit attenuated, as an adjuvant in the glioma patients because of recent corticosteroid use. Draining LNs in the inguinal region were removed 6–8 days after vaccination as an enriched source of tumor antigen-primed T cells. In many cases, the draining LNs were markedly enlarged and easily palpable. The bacterial superantigen *Staphylococcus aureus* enterotoxin A (SEA) was used to activate LN T cells. Superantigens bind to MHC class II molecules and certain TCR V β families and provide a mitogenic signal to the appropriate T cells. Although superantigens and anti-CD3 mAb provide antigen-inde-

pendent activation to T cells, the vaccine-draining LN is enriched for tumor-reactive T cells compared with other lymphoid tissues or peripheral blood. A particular advantage for the use of SEA or anti-CD3 is that they provide for rapid proliferation of both CD4 and CD8 T cell subsets and preserve the broad polyclonal response that was naturally sensitized within the LN. Subsequent expansion in serum-free medium with a low concentration of IL-2 (10 U/ml) resulted in a median of 42-fold proliferation over 6–8 days and provided a dose of greater than 10^{10} T cells for nearly all subjects. The T cells were administered without concomitant IL-2 treatment and were tolerated as outpatient therapy with minimal toxicity. Thus, the feasibility and safety of T cell adoptive immunotherapy was established in this series of phase I clinical trials and several objective responses indicated the therapeutic potential of this approach.

Adoptive Immunotherapy with TIL

Acquisition of T lymphocytes from tumors theoretically provides highly enriched tumor-reactive effector T cells selected through preferential migration. Clinical use of TIL was supported by murine tumor models that showed 100-fold enhanced tumor-specific activity compared with LAK cells^{8, 89}. The most extensive clinical testing of TIL therapy has been for melanoma or RCC. These studies documented unequivocal durable responses in patients with progressive bulky metastatic disease that was previously unresponsive to IL-2 alone.

TIL therapy requires considerable expertise and effort to successfully obtain therapeutic doses of cells, and generation of greater than 10^{11} TIL from melanoma, renal, breast, or colon carcinoma, was successful in only 45% of the samples¹⁰⁵. For unknown reasons, melanoma and RCC frequently yield adequate TIL, whereas the success for other solid tumor is sporadic. Clinically, the overall response rate for patients with metastatic melanoma treated with TIL and conjunctive high-dose bolus IL-2 was 34%⁸⁵. This included a 6% complete response rate identical to that observed with high-dose bolus IL-2 alone. In keeping with *in vitro* data on tumor reactivity, the clinical efficacy of TIL derived from subcutaneous melanoma was higher (49%) than for TIL from tumor-involved LNs (17%). Other salient features of TIL cultures that were associated with clinical responses included: shorter time in culture, more rapid doubling time, specific production of GM-CSF, and cytotoxicity to autologous tumor⁸⁶.

Recently, adoptive immunotherapy of metastatic

melanoma with TIL has been performed after conditioning the recipients with non-myeloablative chemotherapy consisting of cyclophosphamide and fludarabine²¹. This strategy, coupled with *in vitro* anti-CD3 stimulated hyperexpansion of the TIL prior to adoptive transfer, led to dramatic *in vivo* clonal expansion of tumor-reactive T cells during concomitant IL-2 treatment, with several patients developing significant lymphocytosis. Unlike the previous clinical experience with TIL therapy, the non-myeloablative preoperative regimen facilitated sustained high precursor frequency of transferred T cells with the capacity to traffic to tumor and retention of reactivity to specific melanoma antigens. Partial response was observed in 6 of 13 patients, with an additional 4 patients exhibiting mixed response. In addition, 4 of the responding patients developed vitiligo, as has been previously observed in some melanoma patients responding to immunotherapy. The ability to develop dominant T cell clones with sustained *in vivo* function in patients with metastatic melanoma represents a considerable therapeutic advance that awaits confirmation in additional clinical studies.

Adoptive Transfer of Donor T Cells Following Stem Cell Transplant

One form of adoptive immunotherapy, donor leukocyte infusion, involves passive transfer of unselected lymphocytes from a tumor-free donor to the tumor-bearing recipient in the context of a hematopoietic stem cell transplant^{55, 75}. This form of therapy seems to lend itself well to malignancies that have a high intrinsic antigen-presenting capacity such as chronic myeloid leukemia. Attempts to recapitulate this therapy in solid tumors produced impressive clinical responses; however, these were short lived and subjected many patients to significant graft-versus-host disease as a side effect^{14, 15}. A theoretical feature of using donor T cells is that they can be acquired from a host that does not suffer from tumor-induced or pharmacologic immunosuppression. A logical approach would be to induce a vigorous immune response to specific tumor antigens in the donor before harvest and adoptive transfer to the recipient. The development of vitiligo in some melanoma patients experiencing an effective anti-tumor response raises concern about iatrogenic autoimmunity in an otherwise normal donor. Obviously, this ethical concern would need to be examined before this approach could be tested clinically.

Conclusions

The goal of effective, sustained T cell immunotherapy for cancer patients remains unrealized despite encouraging anecdotal instances of dramatic regression of metastatic disease without toxicity. The paradigm of using tumor vaccines comprised of single peptide antigens has considerable appeal from the standpoint of ease of production, low cost, and applicability in a broad range of outpatient settings. Unfortunately, the results of numerous clinical trials employing single peptide epitopes indicate that more effective strategies to induce a broadened immune response through intermolecular epitope spreading may be required to generate clinically relevant immune effects. The critical role of DCs in the process of antigen presentation and development of T cell responses has been appreciated in recent years and has led to their incorporation into a new generation of active immunotherapy trials. The need to enhance stable presentation of antigenic peptides has spurred innovative strategies to introduce single tumor-associated genes with broad applicability. Alternatively, sophisticated strategies to incorporate the entire complement of tumor antigens into DC through total tumor mRNA transfection, lysate loading, or fusion have been developed. These strategies are still in very early stages of clinical development and it will be several years before their efficacy and definition of the relevant parameters for improvement are defined. Adoptive transfer of *ex vivo* generated tumor-reactive T cells currently remains the most potent method to cure bulky metastatic disease in preclinical models. The recent development of reliable methods to generate cloned T cells of defined reactivity in therapeutically relevant numbers lends considerable excitement to this approach. Development of strategies to augment cognate CD4 help is likely to promote the clinical efficacy of these CD8 clones. Perhaps the most striking recent development for T cell immunotherapy of cancer is the recent observation of the potency of combining host immunosuppression with adoptive transfer of TIL. This has achieved the goal of producing extremely high frequencies of tumor-reactive T cells and induced regression of bulky metastatic disease in a substantial percentage of patients with malignant melanoma. The fact that this exciting clinical development occurred with a polyclonal population of TIL that was naturally sensitized in the tumor-bearing host demonstrates how much remains to be learned about the intricacies of the T cell anti-tumor response.

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