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Adoptive immunotherapy of cancer using CD4⁺ T cells

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Abstract

CD4⁺ T cells are central to the function of the immune system but their role in tumor immunity remains underappreciated. It is becoming clear that there is an enormous diversity of CD4⁺ T cell polarization patterns including Th1, Th2, Th17, and regulatory T cells (Tregs). These functionally divergent T cell subsets can have opposing effects — they can trigger tumor rejection or inhibit treatment after adoptive cell transfer. Some polarized CD4⁺ cells have plasticity, and their phenotypes and functions can evolve *in vivo*. Recent advances in understanding of polarization and differentiation of lymphocytes, as well as some intriguing developments in the clinic, indicate that the use of CD4⁺ T cell subsets in the immunotherapy of cancer has unrealized potential.

Introduction

CD4⁺ T (Th) cells are crucial for the activation and regulation of most aspects of the host defense against infections and for adequate function of cytotoxic CD8⁺ lymphocytes. Th cells are also central to the pathogenesis of many autoimmune diseases. The rejection of cancer in certain aspects resembles intentionally induced autoimmune disease that eventually destroys a particular organ or tissue. Curiously, the role of Th cells in cancer immunity remains largely ignored, under-investigated, and controversial [1-4]. This review will focus on potential use of tumor-specific CD4⁺ T cells for the adoptive immunotherapy of cancer.

Much of the research in tumor immunology has been focused on naturally occurring autologous tumor-specific T cells that are predominantly CD8⁺ and can be isolated from melanoma and some other tumors. These cells can be expanded *in vitro* and reinfused, resulting in objective responses and long-term survival in some patients whose disease is refractory to other interventions [5,6]. Objective response rates reached 72% upon addition of aggressive lymphodepleting conditioning with cyclophosphamide, fludarabine, and fractionated 1200 cGy total body irradiation, although not all of these cases resulted in complete and durable remissions [7••]. Tumor regression was also achieved upon transfer of EBV-specific T cells in post-transplant lymphoproliferative disease, nasopharyngeal carcinoma, and Hodgkin lymphoma [8,9]. Trials that target various cancer-associated antigens and markers with either TCR-based or chimeric receptor-based gene therapy are currently under way. Thus, there is an extensive clinical experience using CD8⁺ cytotoxic T cells and the approach seems clearly feasible, but multiple findings underscore the need to improve these therapies in order to more consistently achieve complete tumor rejection. CD4⁺ T lymphocytes used as alternative effector cells might be part of the solution.

Traditionally, CD8⁺ T cytotoxic lymphocytes have been considered the only component necessary for the elimination of malignant tissue, while Th cells were thought of as mere providers of additional stimuli [10]. Indeed, CD8⁺ lymphocytes can fail to maintain

functionality *in vivo* in large part because of the absence of CD4⁺ T cell help [11-14]. *In vitro* CD8⁺ cells release large quantities of interferon- γ (IFN- γ) upon exposure to MHC-compatible cell lines and lyse autologous antigen-positive and MHC class I-positive tumors. However, the genetic instability of tumor cells frequently leads to the losses in the ability to process and present endogenous antigens rendering tumors inherently unreliable targets for CD8⁺ T cells [15,16]. Furthermore, CD8⁺ T cells appear to lack the intrinsic ability to orchestrate a broad antitumor response that seems inherent in some CD4⁺ T cell subsets [17].

Although Th cells would be an attractive addition to the available immunotherapy armamentarium, there are significant barriers in working with naturally occurring tumor-specific CD4⁺ cells. The genetic diversity of the class II HLA in any given population of patients is much more complex than in the case of class I, making identification of epitopes and TCRs more problematic [18]. Moreover, they expand *in vitro* less well than CD8⁺ lymphocytes [19] and the culture conditions significantly affect their characteristics. Finally, there is a paucity of realistic animal models based on tumor-specific CD4⁺ cells. These factors have limited translational research using CD4⁺ T cells and limited their clinical use.

Th cells in cancer immunity

Some very early reports by Fujiwara *et al.* and Greenberg *et al.* clearly showed that CD4⁺ T cells alone were capable of protecting experimental animals against inoculation with hematologic or solid tumors without the participation of a CD8⁺ population [20,21]. In a more recent publication, Matzinger and coworkers compared the responses against HY antigen expressing tumors mediated by T cells derived from a CD8⁺ TCR transgenic mouse with cells derived from two different CD4⁺ TCR transgenic mice [22•]. The authors concluded that Th cells were capable of rejecting the tumors more effectively than CD8⁺ cells with no requirement for exogenous cytokines or vaccination. Given the differences in antigenic epitopes and TCR avidities, this sort of direct comparison between the relative therapeutic capabilities of cells derived from just three transgenic mice is limited, but the authors' observations remain provocative.

Why might CD4⁺ T cells have an advantage over their CD8⁺ counterparts? The crucial difference between CD8⁺ and CD4⁺ T cells is the way they recognize antigens via two separate antigen processing pathways (for a recent review see Ref. [23]). CD8⁺ cells predominantly (with many exceptions) recognize endogenous, intracellular antigens that are presented by MHC class I molecules, which are ubiquitously expressed by the majority of normal and cancerous tissues. Direct recognition of the antigens in the context of MHC class I by the CD8⁺ cytotoxic cells seems intuitively appealing. Unfortunately, cancerous cells are notoriously unreliable as antigen presenters and as targets for immune-mediated killing (Figure 1). They lack activating costimulatory molecules, produce immunosuppressive soluble factors, and they have unstable genomes that are prone to losing key molecules required for antigen processing and presentation [15,16].

CD4⁺ T cells recognize antigens presented by MHC class II molecules, whose expression is generally restricted to the professional antigen presenting cells (APCs) derived from the hematopoietic stem cells. These specialized cells are capable of providing appropriate costimulatory and activating signals. They are present in lymph nodes and can infiltrate neoplastic tissues. They take up exogenous/extracellular tumor antigens, process them in phagosomes, and display them on the surface to CD4⁺ helper cells. Th cells in turn license APCs to provide costimulation of CD8⁺ cells and other immune cell components, augmenting tumor destruction. In addition, MHC class II-restricted peptides can also be processed endogenously via autophagy and displayed on the surface of the tumor cells [24]. The majority of solid cancers constitutively do not have MHC class II at significant levels and cannot be

directly recognized or killed *in vitro* by CD4⁺ lymphocytes, but many human (and murine) tumor cells can upregulate the MHC class II upon exposure to IFN- γ . Thus, CD4⁺ T cells are also capable of direct cytotoxicity [25].

Cancers that are devoid of MHC class II can still be recognized and eradicated by Th cells via indirect recognition of tumor-associated antigens on professional APC, which in turn recruit various arms of innate and adaptive immunity [26]. This process was demonstrated in a mouse model using TCR-transgenic Th cells specific for the immunoglobulin idiotype of multiple myeloma, where CD4⁺ T cells activated tumoricidal macrophages to secrete IFN- γ [27,28]. In a system targeting the HY antigen, CD4⁺ T cells activated NK cells to reject MHC class II-negative tumor [22•]. Therefore, CD4⁺ lymphocytes can circumvent a serious limitation that renders CD8⁺ cells ineffective.

Th cells as the master regulators of the immunity against cancer

Th cells can be categorized into multiple subsets, capable of eliciting a variety of contradictory effects sometimes, leading either to the immune rejection of cancer or to suppression of the immune responses and tumor growth (Table 1). As helpers, they provide cytokines and costimulation to the CD8⁺ populations [29•], augmenting the priming, persistence, and trafficking [30] of cytotoxic effectors. In the pmel-1 CD8⁺ TCR transgenic model [31], the addition of purified naïve CD25^{low}CD4⁺ T helper cells augmented the regression of established tumor [32]. In sharp contrast, CD25^{high} Foxp3⁺ CD4⁺ regulatory (Treg) cells inhibited the activities of tumor-specific CD8⁺ T lymphocytes. Interestingly, bulk populations of unseparated CD4⁺ cells were also detrimental to tumor treatment, implying that the net impact of open repertoire CD4⁺ T cells was negative in the antitumor setting [32].

Classically, the effector CD4⁺ cells were thought to differentiate into Th1 and Th2 cells [33, 34]. Both of these subtypes were shown to mediate anticancer function in early experiments [35,36], but IFN- γ -secreting Th1 cells appeared to be more effective in this role [37]. Recently a distinct subset producing IL-17 and designated Th17 was shown to be generated under the influence of TGF- β and IL-6 via STAT-3-dependent signaling [38] and expanded with IL-23 [39]. Physiologically, Th17 cells are responsible for protection against certain pathogens and are involved in inflammation and recruitment of innate immunity. Th17 responses are also linked to autoimmune tissue destruction previously attributed to Th1 activity [40,41]. Induction of an autoimmune-like process that targets tumor might be an appealing strategy.

We recently compared the treatment efficacy of various subsets of TCR-transgenic CD4⁺ T cell recognizing self/melanoma antigen TRP-1 in a realistic mouse model of advanced disease [42••]. We found that Th17-polarized cells were more effective than Th0 (unpolarized) or Th1 cells in mediating rejection of large B16 melanomas. Why were Th17-polarized cells better than Th1-skewed effectors? Th17 and Th1 differ in many biological aspects that might contribute to treatment success (Table 1 and Figure 2), including polarization-defining cytokines, chemokines, adhesion molecules, matrix metalloproteinases, rate of proliferation, and the ability to persist.

The importance of an inflammatory milieu in the initiation of cancer progression has been well described [43••] but the role of IL-17 and other cytokines is unclear in the setting of advanced disease. Indeed, tumors often arise in proinflammatory conditions, fueled by infections (*H. pylori*), environmental irritants (chemicals and tobacco), or autoimmunity (inflammatory bowel disease and rheumatoid arthritis). The IL-6/IL-23/IL-17 axis of inflammation might contribute to carcinogenesis [44••,45]. IL-6 and IL-23 via STAT3 signaling might deliver prosurvival stimuli to some malignancies in an autocrine or paracrine mechanism, as the animals deficient in IL-23 were protected from cancer progression [46,47]. TGF- β is also produced in various tissues and tumors, perhaps contributing to the induction of either

regulatory FOXP3⁺ or Th17 phenotype [48,49]. Naturally occurring Th17 cells have been found in the tumor microenvironment in animal models and in patients, but their specificity and function remain unclear [50•,51]. IL-17 might promote tumor-associated neovascularization [52,53] and possibly attract myeloid suppressor cells into the inflammatory environment, potentiating tumor growth. On the other hand, systemic overexpression of IL-23 results in increased antitumor activity of T cells, involving both IFN- γ and IL-17 secretion [54•,55•].

In our model, the treatment with tumor-specific Th17 was critically dependent on IFN- γ indicating that these cells were plastic and capable of acquiring Th1 characteristics after adoptive cell transfer. We and others have subsequently found that even highly polarized Th17 populations can be metastable under certain conditions, gradually evolving into more Th1-like cells secreting less IL-17A and more IFN- γ (Muranski, unpublished). In a model of autoimmune uveitis, Th17-polarized T cells produced significantly more tissue damage and persisted better than Th1 cells. However, the Th17 cells gradually acquired a Th1-like phenotype [56•]. Th17 cells in a diabetes model remained stable in nonlymphopenic conditions only causing insulinitis, but progression to full-blown diabetes mellitus required additional host lymphodepletion that promoted conversion of the transferred Th17-skewed population into Th1-like cells [57•]. Recently, the late plasticity of Th17-polarized population has been shown to depend on IL-12 and IL-23 induction of STAT4 and t-bet, key factors associated with the development of Th1 responses [58••]. This may explain the potential duality of responses observed in some autoimmune disease models, where Th17 cells initiate the disease, but the elimination of t-bet confers resistance to autoimmunity [59,60]. Finally, epigenetic studies have provided a molecular foundation for plasticity in the expression of key transcriptional factors that enable the evolution of the Th17 lineage, particularly toward the activation of t-bet [61••].

T-bet-expressing Th1 cells might represent a terminally differentiated phenotype that is more prone to senescence and/or apoptosis than their Th17 counterparts [62••]. The Th17-defining transcription factor ROR- γ t might have antiapoptotic and antiproliferative activity, analogous to its well-described role during the thymocyte development [63-66]. The early acquisition of t-bet might impair the ability of T cells to persist, but t-bet deficient Th17-polarized cells, which are not capable of producing IFN- γ [67,68], are ineffective in treating tumors (Muranski, unpublished), thus the ability of the transferred population to evolve *in vivo* is crucial for the therapeutic outcome. The dualistic nature of Th17 cells might provide the strong initial tissue-damaging response via innate immunity, whereas IFN- γ secreted by the cells evolving from the original Th17-skewed population may confer a direct cytolytic effect [58••]. In contrast, high concentrations of IFN- γ produced by Th1-polarized effectors might act in a negative feedback loop and prevent overactivation [47]. Additionally, a significant proportion of Th1 cells acquire the ability to produce IL-10, which has a tissue-protective role in certain infectious models [69-71].

Our understanding of the biology of Th cell and their various differentiation subsets has been advancing rapidly. Moreover, multiple intermediate polarization states are possible. IL-10 secreting Th17 cells may elicit protective rather than tissue-damaging effect. Novel follicular helper cells (Th_f) have been described recently which produce IL-21 and are involved in B cell activation [72,73]. Induced FOXP3⁺ T cells (iTreg) can become Th17 cells and vice versa [74]. It appears that CD4⁺ effector cells form a complex functional array that can evolve, adapt, and self-regulate. The roles of these new Th cell subsets in cancer immunology are still unclear.

Clinical experience with Th cells

There is limited experience with adoptive transfer of anticancer CD4⁺ cells, because of the lack of well-established tumor-associated targets or technical difficulties in obtaining adequate numbers of specific Th cells. Clinical trials have been restricted to inducing Th immunity by priming with putative class II epitopes [75] or by administering CD4⁺ cells as an undefined component of bulk antitumor populations [76]. The efficacy of donor lymphocyte infusions in the setting of allogeneic stem cell transplant in hematological malignancy has been attributed in part to the activity of CD4⁺ T cells [77] because they recognize MHC-class II, associated with the majority of the bone marrow derived cells, including leukemias.

Hunder *et al.* reported a single case of a dramatic response in a patient treated with *ex vivo* generated autologous CD4⁺ T cell clones recognizing tumor-associated antigen NY-ESO-1 [78•]. The selected clone was expanded 3000-5000-fold and reinfused. The patient had not received any lymphodepleting conditioning that is usually administered before adoptive immunotherapy with the intention of augmenting homeostatic proliferation and persistence of effector cells [7•,79,80]. Moreover, IL-2 was not administered after the transfer. Nevertheless, the patient experienced a complete response and remains disease-free two years later with persistence of the transferred cells and induction of *de novo* CD8⁺ T cell reactivities against melanoma antigens. Unfortunately, a press release by the university revealed that eight other patients failed to respond in the same clinical trial. Our group's experiences with adoptive transfer of cloned cancer-specific T cells were highly disappointing [81], as shown not only by the poor clinical responses, but also in a frustrating lack of persistence of the transferred clones, which likely had been exhausted by prolonged *in vitro* culturing [82•]. Moreover, the NY-ESO-1 antigen is highly expressed in only about 10% of melanomas (Robbins, unpublished data and [83]) and its distribution is not uniform in the tumors, if present. Despite these caveats, the observations made by Hunder *et al.* remain intriguing.

Conclusions

There has been significant progress in the field of cell-based immunotherapy of cancer [79, 84-86]. We can insert highly avid TCRs recognizing cancer-associated antigens from autologous, allogeneic, or xenogeneic humanized sources [6,87•]. Some MHC class I-restricted receptors have already been tested in human trials [88]. These genetically engineered cells can elicit antigen-specific effects upon adoptive transfer, including the potential for severe (but specific) toxicities, thus target selection will become critical [89•]. Clinical-grade vectors encoding MHC class II-restricted TCRs have not yet been generated or tested.

CD4⁺ T cells play a central role in virtually every aspect of immunity, including the antitumor response and they might be a key missing component in current cancer immunotherapies. The addition of antigen-specific helper cells to CD8⁺ effectors could increase the rate of clinical success of adoptive immunotherapies. CD4⁺ T cells can be directly cytotoxic to MHC class II-expressing target cells. Moreover, Th cells recognize antigens that are efficiently processed by professional APCs, leading to activation of immunity against MHC-negative targets.

The activity of Th effectors depends not only on the specificity of their TCR, but also on the degree of their differentiation and the qualities imprinted on them by stimulation and expansion conditions. Commonly used *in vitro* culturing conditions, using IL-2 alone as a growth factor, might be detrimental to the *in vivo* activity after adoptive transfer, as might be the case with CD8⁺ T cells [90]. Clearly, the appropriate *in vitro* polarization of CD4⁺ T helper subsets is critical for the optimal function of anticancer lymphocytes [42•]. Th17-polarized populations might be very effective in mediating tumor rejection, but the ability to evolve *in vivo* and generate a type 1 response may be important. This underscores the parallel between tissue

destruction during the autoimmune process and rejection of the established tumor. Thus, we have at our disposal a vast arsenal of novel methods and technologies but the challenge is now to apply them effectively.

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Figure 1.

Recognition of poorly immunogenic tumor via endogenous and exogenous pathways. Relying on presentation of antigens by a genetically unstable tumor can result in unreliable and suboptimal responses. Defects in the antigen processing via the endogenous pathway, including β_2 -microglobulin, TAP1, and TAP2 deficiencies and loss of MHC chains can affect a tumor cell's ability to process and present MHC class I-restricted antigens. These defects can render CD8⁺ cytotoxic cells unable to eliminate tumors cells, leading to preferential survival of escape variants. Direct recognition of tumors via MHC class II is equally dependant on the unstable autophagy and antigen processing by cancer cells. Normal professional antigen presenting cells (APCs) infiltrating the tumor or present in the draining lymph nodes are less subject to derangements of their antigen processing and presenting machinery. APC continually monitor the environment, take up tumor-derived material and efficiently present it to CD4⁺ T cells even if the tumor cells lost the endogenous ability to process antigens.

Figure 2.

A proposed model for Th cell subset evolution in antitumor immunity. Th cells can play a central role in tumor rejection by recognizing and killing malignant cells directly. Indirect recognition of tumor-associated antigens can result in the activation of antigen presenting cells (APCs), which in turn are capable of activating cytotoxic CD8⁺ T cells and natural killer (NK) cells. *In vitro* polarized tumor-specific Th17 T cells express the transcription factor ROR- γ t and secrete an array of cytokines, including proinflammatory IL-17A and IL17F. Th17-polarized cells are plastic and can evolve into t-bet-expressing Th1-like population secreting IFN- γ that can eliminate tumor after adoptive transfer. Acquisition of t-bet might represent the maturation into a more terminally differentiated phenotype that is closer to senescence and/or apoptosis. Thus, early (*in vitro*) expression of t-bet during Th1 polarization might be less

desirable as it generates cells less likely to survive upon adoptive transfer. Th17-polarized cells are closely related to induced-FOXP3⁺ regulatory cells (iTreg), which might negatively influence antitumor immunity. There is a considerable plasticity between iTreg and Th17 cells.

Table 1

Subsets of Th cells in cancer immunology

Th subset	Transcription factor	Induction	Cytokines produced	Function	Role in cancer immunity
Th1	t-bet (tbx21)	IL-12, IFN- γ , anti-IL-4	IFN- γ , TNF- α , IL-2, IL-10, MCP-1, MIP1 α	Virus defense, responses against intracellular pathogens, augmenting CD8 + T cell function, autoimmunity	Direct elimination of tumor cells via cytotoxicity, indirect effect via activation of macrophages
Th2	GATA3	IL-4, anti-IFN- γ	IL-4, IL-5, IL-6, IL-10, IL-25, IL-33	Defense against parasites, allergy, asthma, activation of eosinophiles and B cell responses	Indirect elimination of cancer cells via activation of eosinophiles
Th17	ROR- γ t, ROR α	TGF- β , IL-6, IL-21, IL-1, IL-23, anti-IL-2, anti-IFN- γ , anti-IL-4	IL-17A, IL-17F, IL-2, IL-9, IL-10, IL-21, TNF- α , CCL-20 (MIP3 α)	Defense against extracellular pathogens, autoimmunity, recruitment of neutrophils into the site of inflammation	Promotion of early cancer growth in the inflammatory environment? Cancer elimination via autoimmune-like process, conversion to Th1-like cells
iTreg	FOXP3	TGF- β , anti-IFN- γ	TGF- β , IL-10	Regulation, immunosuppression, may convert into other Th subsets (Th1 or Th17)	Suppression of immune surveillance and anticancer responses, possibly convert into Th1 or Th17 effectors
nTreg	FOXP3	Generated in the thymus	TGF- β , IL-10	Regulation, immunosuppression	Suppression of immune surveillance and anticancer responses