Abstract
Research over the past decade has revealed the increasingly complex biologic features of the CD4 T-cell lineage. This T-cell subset, which was originally defined on the basis of helper activity in antibody responses, expresses receptors that recognize peptides that have been processed and presented by specialized antigen-presenting cells. At the core of the adaptive immune response, CD4 T cells display a large degree of plasticity and the ability to differentiate into multiple sublineages in response to developmental and environmental cues. These differentiated sublineages can orchestrate a broad range of effector activities during the initiation, expansion, and memory phase of an immune response. The contribution of CD4 cells to host defense against pathogenic invasion and regulation of autoimmunity is now well established. Emerging evidence suggests that CD4 cells also actively participate in shaping antitumor immunity. Here, we outline the biologic properties of CD4 T-cell subsets with an emphasis on their contribution to the antitumor response. Cancer Immunol Res; 2(2); 91–98. ©2014 AACR.

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No potential conflicts of interest were disclosed.

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Learning Objectives
Upon completion of this activity, the participant should gain a basic knowledge of the biologic properties of CD4 T-cell subsets and their contribution to the antitumor immune response. CD4 T cells are core components of adaptive immunity. In response to developmental and environmental cues, CD4 T cells differentiate into sublineages responsible for effector activities spanning the initiation, expansion, and memory phase of an immune response, including their role in shaping antitumor immunity. A broader understanding of the cross-talk among CD4 T cells that modulate the tumor microenvironment will enhance the effectiveness of immunotherapy in cancer treatment.

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Introduction
Multiple strategies that depend on the immune system’s ability to inhibit tumor growth have been tested both experimentally and in the clinic. A recent report that summarizes three decades of studies suggests that the most successful approaches have entailed passive antibody treatment and adoptive T-cell therapy (ACT; ref. 1). However, curative therapy is rarely achieved, and there is an urgent need to improve and optimize existing immunotherapy. Currently, basic and clinical research in tumor immunology focuses mainly on improving CD8 CTL–mediated cellular immunotherapy. This focus can probably be explained by the ability of CD8 CTL to directly target, engage, and destroy tumor cells expressing MHC class I molecules. Because most tumors do not express MHC class II, the potential antitumor protective role of CD4 T cells, which bind MHC class II molecules on target cells, has been less obvious. Nevertheless, the antitumor activity of these CD4 cells soon became apparent based on their ability to prevent virus-induced sarcomas, to eradicate disseminated leukemias, and to reject transplantable tumors (2–5).

Several aspects of CD4 T-cell biology suggest that this T-cell population can be effectively used for cancer immunotherapy. CD4 T<sub>H</sub> cells orchestrate a broad range of immune responses and are equipped to differentiate into multiple sublineages that can induce and maintain destructive immune responses to self-antigens, including tumor antigens. Animal studies and clinical research have suggested two effector CD4<sup>+</sup> Th subsets—CD4-CTL and T<sub>FH</sub> cells—that may exert particularly...
potent antitumor activity. Further definition of the contribution of these effector CD4+ T-cell subsets may allow development of new and effective approaches to tumor immunotherapy. On the other hand, certain subsets of CD4 T cells [such as regulatory T cells (Treg), or, under some circumstances, T\(_{h}17\) cells] may have tumor-promoting activity, which may need to be curtailed to obtain optimal antitumor responses (Fig. 1).

Here, we summarize the CD4-mediated T-cell responses toward tumors based on their differentiation characteristics and potential application to the development of cell-based immunotherapies. As interest in the function of tumor-associated CD4 cells grows, there is increased need for insight into mechanisms that control their ability to change immunologic environments and to shape protumor and antitumor responses. These studies will provide a solid foundation for the application of CD4 T cells to anticancer immunotherapy in approaches that limit their protumorigenic activity.

### CD4 T Cells Promoting Antitumor Immunity

**T\(_{h}1\) and T\(_{h}2\) cells**

T\(_{h}1\) and T\(_{h}2\) cells are the first-defined and best-characterized T\(_h\) lineages. Their alternate fates are determined by the transcription factors T-bet and GATA-3, respectively (6, 7). T\(_{h}1\) cells are characterized by the secretion of cytokines such as IFN-\(\gamma\), TNF-\(\alpha\), monocyte chemotactic protein-1 (MCP-1 or CCL2), and macrophage inflammatory protein-1\(\alpha\) (MIP1\(\alpha\) or CCL3). They are thought to be mainly responsible for immune responses against intracellular pathogens by either enhancing CD8 T-cell response or by directly activating macrophages to phagocytose intracellular pathogens. T\(_{h}1\) cells are also thought to augment autoimmunity. T\(_{h}2\) cells secrete the signature cytokines interleukin (IL)-4, -5, and -13, and may orchestrate humoral immunity and promote allergic inflammatory responses. Previous studies have suggested that both T\(_{h}1\) cell types mediate antitumor immunity (8–10), although T\(_{h}1\) cells may be more potent. The superior antitumor effects of T\(_{h}1\) cells may reflect the production of large amounts of IFN-\(\gamma\), as well as chemokines that enhance the priming and expansion of CD8 cells. T\(_{h}1\) cells help in recruiting natural killer (NK) cells and type 1 macrophages to tumor sites, which can act in concert toward tumor eradication (9, 11–13; Fig. 1). A pioneering study provided early insight into mechanisms that allow CD4 T\(_h\) cells to promote antitumor responses against cells that do not express MHC class II antigens (14). These observations indicated that CD4 T cells can mediate tumor rejection in an IFN-\(\gamma\)-dependent manner through targeting of tumor stroma and inhibition of angiogenesis.

The contribution of T\(_{h}2\) cells to antitumor immunity has been somewhat contradictory and the effects of this T\(_h\) subset may be context dependent. The IL-4 cytokine may exert antitumor effects (15), reflecting enhanced infiltration of eosinophils and macrophages into the tumor bed, rather than a direct effect of IL-4–secreting lymphocytes (16). The ability of T\(_{h}2\) cells to mobilize innate cells, including eosinophils, may represent a general pathway for their impact on the host antitumor response. Adoptive transfer of T\(_{h}2\) cells and induction of T\(_{h}1\)2 immunity to secreted tumor-specific antigens may induce eosinophil-dependent elimination of metastatic melanoma in pulmonary tissues without histologic evidence of lung damage or inflammation (10). Conversely, the induction of T\(_{h}1\)2 effector cells specific for pancreatic antigens may promote neoplastic transformation and pancreatic tumor growth (17), and the frequency of antigen-specific CD4 cells that produce IL-5 has been correlated with progressive growth of renal cell carcinoma and melanoma (18; Fig. 1).

**T\(_{h}17\) cells**

A binary T\(_h\) polarization model (T\(_{h}1\)–T\(_{h}2\)) has not provided a sufficient framework for understanding the complexity of the CD4 T-cell response. Identification of other T\(_h\) subsets, including T\(_{h}1\) and T\(_{h}17\) cells, has helped in reshaping our understanding of the contribution of CD4+ T\(_h\) cells to autoimmunity, infection, and cancer. The T\(_{h}17\) subset depends on expression of the STAT3 and ROR\(\gamma\)T transcription factors, and is characterized primarily by the production of cytokines IL-17A and IL-17F (19, 20). T\(_{h}17\) cells are induced by a combination of TGF-\(\beta\) and IL-6 cytokines, while stabilization and maintenance is mediated by IL-23. The signature T\(_{h}17\) cytokine, IL-17A, induces the expression of multiple chemokines including CCL2, CCL7, CXCL1, and CCL20 as well as matrix metalloproteinases that promote inflammatory responses. This response affords protection against certain microbial invaders, including fungi, but can also result in the induction of severe inflammation and promote the development of autoimmunity.

Although T\(_{h}17\) cells and IL-17A have been recovered from multiple human tumors, including ovarian, gastric, prostate, renal, and pancreatic cancers, their contribution to tumor progression versus immune protection remains unclear (21–27). Chronic inflammatory responses have been linked to protumorigenic effects. Intratumoral IL-17 expression can promote angiogenesis and tumor growth via the elevation of a variety of proangiogenic factors and increased secretion of proinflammatory cytokines by tumor cells. Induction of T\(_{h}17\)-associated inflammatory processes by IL-23 also results in a tumor-promoting environment for nascent malignancies through the increased angiogenesis and possibly the inhibition of CD8 cell infiltration (Fig. 1). Together, these findings may explain why low level of chronic exposure to T\(_{h}17\)-associated cytokines may facilitate cancer progression (28–30).

Recent studies in mouse models in which tumors were probably exposed to relatively high levels of IL-17 after the transfer of polarized T\(_{h}17\) cells support a more promising role for T\(_{h}17\) cells in antitumor immunity. *In vitro* differentiated tumor antigen-specific T\(_{h}17\) cells possessed superior antitumor activity compared with T\(_{h}1\)-polarized cells against murine B16 melanoma (31, 32). The antitumor effects of transfected T\(_{h}1\)7 cells correlated with the generation of a tumor microenvironment that allowed recruitment of dendritic cells and other leukocytes into the tumor as well as priming of CD8 cells specific for tumor-associated antigens (TAA; ref. 32).

The therapeutic potential of T cells depends in part on their ability to undergo successful engraftment and prolonged survival. Although T\(_{h}17\) cells have been regarded as
short-lived effector cells, recent studies of CD4 cells reactive to an endogenous melanoma antigen suggest that the anti-tumor activity of $T_{H17}$ cells may reflect, in part, genetic programming that endows them with a less differentiated phenotype, similar to stem cell–like memory cells (TSCM; Fig. 1). This attribute of $T_{H17}$ cells may contribute to their long lifespan, plasticity, and ability to self-renew as IL-17A producers, in contrast to $T_{H1}$ cells, which display a gene signature consistent with terminal differentiation and senescence (33, 34). This stem cell–like trait of murine $T_{H17}$ cells has also been observed for human $T_{H17}$ cells that express high levels of hypoxia-inducible factor 1a (HIF-1a) and transcription factor BCL2 (35). Indeed, analysis of spontaneously arising tumor antigen-specific $T_{H17}$ cells in patients with lung cancer has suggested that some $T_{H17}$ cells can differentiate further into IFN-γ–secreting effector cells (36). These considerations support the development of new immunotherapeutic approaches that exploit the differentiative potential and plasticity of human $T_{H17}$ cells.

CD4 CTL
The subpopulation of CD4 T cells that acquires cytolytic capacity has clear-cut antitumor activity. Transfer of small numbers of tumor-reactive CD4 cells into lymphopenic hosts followed by radiation and anti-CTLA-4 antibody treatment results in the expansion and expression of IFN-γ and granzyme B and is associated with regression of established tumors (37). The cytotoxic activity of CD4 CTL depends on the recognition of MHC class II expressed by tumor cells (upregulated by B16 melanoma after radiotherapy and anti-CTLA-4 antibody) and is independent of FAS–FASL interactions or TRAIL-mediated killing. Special conditioning of the host seems to be critical for the generation of cytotoxic CD4 cells. Recent studies have shown that the costimulatory activity of OX-40 and 4-1BB can
promote the generation of cytotoxic CD4 cells in the context of chemotherapy-induced lymphopenia or FVAX (Flt3-ligand expressing B16 cells) immunization, respectively (38, 39; Fig. 1). Acquisition of cytolytic activity by CD4 cells also seems to be associated with the expression of the eomesoderm (Eomes) transcription factor.OX-40 engagement may induce upregulation of Eomes, leading to the acquisition of cytolytic activity by tumor-reactive CD4 cells and secretion of multiple cytokines, including IFN-γ, TNF-α, IL-4, and IL-5 (38). Eomes may also contribute to the induction of cytotoxic CD4 cells after the administration of 4-IBB agonist antibodies (39). These observations highlight the clinical potential of therapeutic approaches to human cancers that depend on the activation of CD4 CTL. Earlier studies demonstrating the cytotoxic activity after in vitro expansion of human CD4 clones may provide important guidelines for the generation and use of cytotoxic CD4 cells in the context of ACT (40). In vivo expansion of cytotoxic CD4 cells in combination with blockade of inhibitory molecules, such as CTLA-4, represents an attractive approach to tumor therapy.

Follicular helper T cells

The designation of follicular helper T (TFH) cells comes from their ability to migrate to follicles in secondary lymphoid organs and interact with B cells to promote their differentiation into antibody-secreting cells. Surface expression of CXC chemokine receptor 5 (CXCR5 or CD185), inducible T-cell costimulator (ICOS), and programmed cell-death protein 1 (PD-1) along with the secretion of IL-21 are hallmarks of TFH cells, and the expression of their canonical transcription factor Bcl-6 is required for the acquisition of genetic programming associated with specialized TFH cell function. Activated TFH cells are essential for the generation of neutralizing antibody responses to viral infections. However, tight regulation of self-reactive TFH cells is also essential to prevent the production of autoantibodies and the consequent autoimmune tissue damage.

The contribution of TFH cells to antitumor immune responses has been inferred from studies that evaluate the potential contribution of B cells and antibodies to antitumor immunity. Although some of these studies have associated humoral responses with promotion of tumor growth (41–44), the contribution of TFH cells to these responses was not defined.

Chronic inflammation associated with tumor progression can be associated with the formation of ectopic germinal centers in mouse tumor models and in biopsies of human tumors, including renal cell carcinoma and ductal breast carcinoma (45, 46). Recent studies of colorectal and breast cancer have indicated that tumor-infiltrating TFH cells play a key role in immune cell recruitment to the tumor and in the formation of intratumoral follicular structures, which correlate with a positive prognosis (47, 48).

A recent analysis of the immune cell types that infiltrate human colorectal cancers during early- and late-stage tumor growth indicates that TFH and B cells are the central players in long-term protection against tumor growth and strongly correlate with patient survival (49). The intrinsically activated phenotype of TFH cells and their ability to promote formation of ectopic follicular structures that can serve as the nidus for durable antitumor immune responses may underlie the contribution of this TFH subset to protective immunity against tumor growth.

Although tight control of TFH cells is critical to prevent development of autoimmune disease, TFH cell expansion may allow production of effector cytokine and tumor-specific antibody responses. The therapeutic potential of TFH cells in cancer is suggested by a recent study showing that genetic disruption of the inhibitory interaction between CD8 Tregs and Qa-1+ TFH cells results in the induction of robust autoimmune antitumor responses that reflect enhanced TFH cell helper activity and increased therapeutic antibody production by B cells (50; Fig. 1). The impact of this newly defined sublineage of CD4 TFH cells to antitumor responses remains a critical issue that deserves further study.

CD4 T Cells Supressing Antitumor Immunity

**FoxP3+ CD4 Tregs**

CD4+ Tregs are characterized by the expression of the FoxP3 transcription factor. The regulatory activity of this CD4 subset is critically important for the maintenance of immunologic homeostasis and self-tolerance, and its contribution to the inhibition of autoimmune disease and prevention of excessive immune responses to pathogens is well recognized. CD4 Tregs also have an important impact on antitumor immune responses. There is increasing evidence that tumor-derived factors can promote FoxP3+ Treg recruitment and expansion. Although the proportion of FoxP3+ Tregs in peripheral lymphoid tissues ranges from 5% to 10% of the CD4 T-cell compartment, their frequency in tumor environments increases from 20% to 30%, depending on the type of tumor (51). These intratumoral FoxP3+ Tregs impede effective immunity against cancer and high Treg–CD8 ratios in tumor infiltrates correlate with poor patient survival (52–54; Fig. 1).

Conversion of T effector cells (Teff) into Tregs through the induction of FoxP3 expression has been suggested to account for the high percentage of intratumoral Tregs (55, 56). However, T-cell receptor (TCR) sequence analyses of T cells from mice bearing transplanted or chemically induced tumors suggest that tumor Teffs and Tregs display distinct TCR repertoires. Tregs derived from draining lymph nodes and from tumor sites display overlapping TCR sequences. These findings suggest that a Teff → Treg conversion process is unlikely to account for the accumulation of Tregs in tumor tissues (57, 58). A recent study by Malchow and colleagues may provide a clue to the origin of tumor-infiltrating Tregs (59). Using transgenic mice that express a TCR derived from FoxP3+ Tregs in a mouse model of prostate cancer (TRAMP model), these findings strongly suggest that tumor-infiltrating FoxP3+ Tregs do not arise from de novo conversion of tumor-specific Teffs, but are recruited from preexisting thymic-derived Tregs reactive to self-antigens associated with the tissue origin of the tumor (59, 60).

Regardless of origin (thymic vs. peripheral, Treg vs. pTreg, respectively), a central feature of intratumoral Tregs that may contribute to their enrichment at this site is their high proliferative capacity compared with intratumoral Teffs. This may reflect the modulation of antigen-presenting cells (APC)
toward a Treg-promoting state by tumor-derived factors (61, 62). The limited availability of IL-2 within tumors may also favor the expansion of Tregs over Teffs, in view of the increased competitive efficiency of Tregs in response to this cytokine (63). The intratumoral microenvironment may also promote CD4 Treg activation leading to increased inhibition of antitumor responses. For example, expression of ICOS-L by melanoma cells can costimulate ICOS+CD4 Tregs resulting in increased expression of Foxp3, CD25, and ICOS. In some cases, tumor cells that directly present MHC class II and self-antigens may provide costimulation through the expression of ICOS-L (64).

Tregs migrate into tumors in response to chemokines produced locally within the tumor microenvironment. In human ovarian cancer, for example, inflammatory cytokine CCL2 produced by tumor cells and intratumoral macrophages induces Treg migration into the tumor that is associated with poor survival (65). Secretion of the CCL21 chemokine by melanoma cells and the consequent induction of a lymphoid stromal network by this chemokine also promotes accumulation of Foxp3+ Tregs that is associated with robust tumor growth (66). Tumor-derived VEGF can also contribute to the recruitment of Foxp3+ Tregs via an interaction with neuropilin-1, which is highly expressed by at least some Tregs (60, 67). These findings suggest that the antitumor effects observed in some studies of anti-VEGF might reflect, in part, reduced recruitment of Foxp3+CD4 Tregs (68, 69). A recent study showed that selective targeting of CCL5/CCR5 signaling can reduce Foxp3+ Treg infiltration into tumors and retard tumor growth, suggesting the feasibility of approaches that target chemokine-based Treg migration (70). However, the redundant role of chemokines that attract both Tregs and Teffs may complicate this approach.

Many cancer immunotherapy strategies have targeted Foxp3+CD4 Tregs. Early observations that the administration of an anti-CD4 antibody elicited strong antitumor immunity suggested that CD4 T cells might include a suppressive subset that dampened efficient antitumor immune responses (71, 72). However, the depletion of Tregs using CD25-targeting immunotoxins (LMB-2, denileukin diftitox) that resulted in enhanced antigen-specific CD8 T-cell responses failed to display clinical effectiveness (73, 74). Moreover, although depletion of CD25+ cells promoted strong systemic antitumor immunity against established tumors, it did not protect against tumor outgrowth due to inefficient tumor infiltration of CD8 T effector cells associated with a lack of intercellular adhesion molecule (ICAM) and vascular cell adhesion molecule (VCAM) expression by the tumor vasculature (75). These findings suggest that therapeutic depletion of Tregs may need to be combined with an efficient activation of the tumor vasculature to allow tumor-specific effector T-cell infiltration and rejection of the tumor. Indeed, remodeling of tumor vasculature has been associated with increased tumor rejection by tumor-infiltrating CD8 cells after CD25 depletion, after irradiation and GVAX/α-CTLA-4 and CpG-ODN treatment, and after Treg depletion in methylcholanthrene (MCA)-induced tumor-bearing mice (58, 73, 76). These observations suggest the need for efforts that combine modulation of the tumor microenvironment with strengthening of tumor-specific CD4 or CD8 effector cell responses.

Most approaches to “checkpoint blockade” have been based on anti-CTLA-4 antibody therapy, and recent studies have shed light on mechanisms that underpin this approach. Anti-CTLA-4 therapy resulted in the depletion of intratumoral Tregs through FcγR-dependent uptake by tumor-infiltrating macrophages (77, 78). Confinement of Treg-depleting effects to the tumor site after anti-CTLA-4 treatment is especially attractive as it may reduce systemic side effects during immunotherapy. Another immune-checkpoint receptor, PD-1, is emerging as a potential target, as preclinical and clinical studies have demonstrated that blockade of the PD-1/PD-L pathway enhances antitumor responses, in part, by increasing CD4+ T effector–Treg ratio within tumors (79–81). In contrast to CTLA-4, which is induced at the time of initial T-cell response to antigen, PD-1 plays a more prominent role in modulating T-cell activity in peripheral tissues through its interaction with its ligands PD-L1 and PD-L2 (80, 82, 83). This biologic feature of PD-1 expression and upregulation of PD-1 ligands by many tumor cells suggest that anti-PD-1 treatment may have reduced toxicity compared with anti-CTLA-4 treatment. In any case, clinical strategies targeting costimulatory or inhibitory molecules that are preferentially expressed by Tregs will need to be calibrated to exploit the phenotype of Tregs within the tumor microenvironment to yield optimal efficacy and reduced side effects.

Concluding Remarks

By virtue of their inherent ability to orchestrate a wide range of immune responses, and their flexible differentiation into different effector lineages, including Th1 and CD4 CTL, enhancement of the quantity and quality of tumor-associated effector CD4 cells has become highly relevant to tumor immunotherapy. In some tumor models, there is evidence that the antitumor response of CD4 T cells can be more potent than that of CD8 T cells (84). The majority of tumors do not express MHC class II and CD4-mediated tumor rejection has been associated with the activation of tumor-infiltrating APCs or the modulation of tumor stroma (12, 14). This suggests a requirement for strategies that enhance the uptake and presentation of antigen by tumor-infiltrating APC to promote optimal CD4-mediated antitumor responses. Although there is an obvious gap in our knowledge pertaining to antigen recognition and antigen specificity of tumor-associated CD4 cells, a number of studies have described MHC class II–restricted epitopes of TAAs that may help to develop ACT with CD4 T cells. A recent study has validated this concept by showing that a single infusion of a clonal population of CD4 cells with specificity for a TAA (NY-ESO-1) resulted in complete regression of the tumor. The antitumor response induced by the infused CD4 cells was durable in the host and was associated with broadening of endogenous responses against melanoma antigens through antigen-spreading (85). Possibly, maximizing the ability of endogenous CD4 cells to induce antitumor
responses via modulation of chemokine pathways and blockade of inhibitory interactions may allow us to bypass the cumbersome procedures required for ACT. Rapid progress in the areas of biomaterials and drug delivery systems will also have a positive impact on approaches to efficiently promote effector CD4+ cell responses in situ (86, 87).

Summary

Tumor immunity is a sum of complex interactions between cells in the tumor microenvironment. There is still much to learn about the intricate cross-talk among CD4+ cells that modulate the tumor microenvironment. Nevertheless, basic insight into these interactions has allowed immunotherapy to become one of the mainstays of cancer treatment. We expect that further insight into the unique capacity of CD4+ cells to modulate tumor responses will allow more robust application of immunotherapy to cancer treatment.

Authors’ Contributions

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CD4 T Cells and Antitumor Immunity


CD4 T-cell Subsets and Tumor Immunity: The Helpful and the Not-so-Helpful

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