Chapter 8
Cancer Immunity and Immune Evasion Mechanisms

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Abstract Understanding the role of the immune system in cancer development and progression is a challenging process. The collective efforts unequivocally show that the immune system is playing a dual role in promoting and inhibiting tumor development. The tumor microenvironment is highly infiltrated by immune cells, which includes innate (macrophages, mast cells, neutrophils, dendritic cells, and myeloid-derived suppressor cells) and adaptive (T and B) cells. This diverse set of cells contributes to the secretion of different pro-inflammatory immune mediators creating a microenvironment that influences cancer growth in a pleiotropic manner. It is the composition of inflammatory mediators and the activation status of different immune cells that interact with the tumor to dictate either tumor regression or tumor progression. CD4+ and CD8+ T cells play a pivotal role in anticancer immunity. The CD4+ T cells are instrumental in eliminating cancer cells by secreting various cytokines and activating and recruiting other cell types such as macrophages and granulocytes. However, CD4+ T cell-mediated activation of CD8+ T cells and subsequent cytotoxic activity of the CD8+ T cells represent the major effector mechanism of antitumor immunity. Here, we review and discuss the current knowledge with respect to the functional role and prognostic significance of individual T cell subsets in various malignancies.

Keywords CD4+ T cells • CD4+ Th cell subsets • CD8+ CTLs • Tregs • GATA3 RORγt • T-bet • FOXP3 • Cytokines • Immunosuppression • Plasticity • Tumor-infiltrating T cells • Prognosis

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Cancer Immunoediting and Tumor Immune Evasion Mechanisms

While the role of the immune system in controlling microbial pathogens is well appreciated, the notion that the immune system can also control tumor development and progression has been a controversy for over a century. In 1909, Paul Ehrlich was the first to suggest that the immune system could protect the host from malignancies [1]. Nearly 50 years later, Thomas and Burnet predicted that adaptive immunity is responsible for preventing tumor formation and progression in an immunocompetent host and proposed the concept of cancer immunosurveillance [2, 3]. However, due to the absence of experimental support, the cancer immunosurveillance concept was abandoned. This was largely due to the lack of mouse models with pure genetic backgrounds available at that time. By the 1990s, with improved genetically modified mouse models available, several seminal works have validated the role of cancer immunosurveillance in both chemically induced and spontaneous tumor models [4]. Multiple components of the immune system have been identified as having central roles in cancer immunosurveillance, such as T cells, B cells, natural killer (NK) cells and INFγ, and perforin [4, 5]. Similarly, several experimental and clinical studies have confirmed the existence of cancer immunosurveillance (T cell-mediated cancer immunosurveillance is described in detail in the following sections) [5]. Collectively, these findings suggest that cancer immunosurveillance can function as a microenvironmental tumor suppressor. However, despite the presence of an active cancer immunosurveillance process, many immunocompetent individuals still develop cancer. This paradox has been explained via seminal mice studies showing that the immune system not only eliminates but also reduces the immunogenicity of the tumor but also has the capability to promote tumor growth [4]. This led to a significant revision of the original cancer immunosurveillance theory wherein Robert Schreiber and colleagues proposed a new concept termed "cancer immunoediting," which emphasized the cancer-promoting and cancer-suppressing role of the immune system during tumor growth [4, 6]. Cancer immunoediting consists of three phases, elimination, equilibrium, and escape, and termed as "three Es of cancer immunoediting" [6]. The elimination phase represents the original concept of cancer immunosurveillance, in which the cooperative actions of innate and adaptive immunity eliminate the tumor before it is clinically manifest. Several studies suggest that the immune component required for the elimination of tumors depends on specific-tumor characteristics such as origin (spontaneous vs. carcinogen-induced), anatomical location, histology, and growth rate. During the elimination phase, rare tumor cell variants may survive and enter into an equilibrium state. In this period, tumor cells undergo antigenicity sculpting by immune cells applying a selective pressure leading to the survival of the fastest growing cells that escape elimination by the immune system. This process induces reduced immunogenicity and acquired resistance to immune effector cells. The equilibrium state is the longest phase, and it extends throughout the life of the host. The end stage of the equilibrium phase results in generation of several tumor clones with the most immunoevasive

Table 8.1 Tumor immune evasion mechanisms

Evasion strategy	Mechanism
Impaired tumor antigen presentation	• Downregulation of tumor antigens or antigen-processing machinery (e.g., lack of LMP and TAP proteins) [8] • Downregulation of MHC genes [9]
Impaired trafficking of immune cells into tumor microenvironment	• Epigenetic silencing of chemokine expression [10] • Lack of endothelial adhesion molecules [11–13] • Physical barrier by stroma [14] • Lack of tumor antigens in lymphoidal organs [15]
Immune cell dysfunction or subversion	• Immune suppression mediated by CD4+ FOXP3+ regulatory T cells (Tregs) and myeloid-derived suppressor cells (MDSCs) [16–20] • Secretion of suppressive cytokines (TGF-β, IL-10, etc.) [21–23] and other soluble immunosuppressive factors (prostaglandins, VEGF, RCAS1, extracellular adenosine, reactive oxygen, nitrogen species, etc.) [24–28] • Expression of IDO in tumor cells leading to secretion of immunosuppressive tryptophan metabolites [29] • Induction of T cell tolerance by expressing cognate ligands for T cell checkpoint inhibitory receptors such as CTLA-4, PD-1, LAG-3, and Tim-3 [30, 31] • Apoptosis of immune cells induced by tumor cell expression of CD95L (FasL) (tumor counterattack) [32] triggering CD95 (Fas)-mediated T cell apoptosis • Immune cell deviation and plasticity [33–36]
Tumor cell resistance to apoptosis	• Abnormal expression of anti-apoptotic molecules (Bcl-2 and IAPs family protein) [37] • Mutations or loss of pro-apoptotic molecules (TRAIL and CD95 receptors) [37] • Interference with granzyme/perforin pathway [38, 39]

mutations and epigenetic instability. These cells ultimately enter the escape phase and develop into visible tumors and successfully avoid immune destruction, which is now considered as an emerging hallmark of cancers as described by Hanahan and Weinberg [7]. Tumor cells evade the protective immunity by several mechanisms as presented in Table 8.1. Currently, targeting one or more of these mechanisms clinically holds the most promising approach to improve antitumor immunity [24].

T Lymphocytes and Cancer Immunity

T cells are generally classified into two lineages: CD4+ T cells and CD8+ T cells. CD4+ T cells are further classified into CD4+ T helper cells (Th) that mediate tumor immunity and CD4 + FOXP3+ regulatory T cells (Tregs) that suppress antitumor immunity (described later). Naïve T cells that express a unique T cell receptor (TCR) on the surface develop through stringent positive and negative selection pathways in the thymus. T cells migrate through tissues and scan for cognate antigen

peptide-MHC complex that activates their TCR, resulting in functional differentiation into a variety of subsets [40]. Here we focus on conventional TCRα/β T cell subsets and their role in tumor immunity.

CD4+ T Cells in Anticancer Immunity

CD4+ Th cells are crucial in orchestrating humoral and cell-mediated immune responses [41]. However, their role in anticancer immunity is complex and reflects the diverse role of various CD4+ Th cell subsets [33]. The CD4+ Th cell TCR recognizes antigenic epitopes in the form of 12–20-residue long peptides, presented by major histocompatibility complex II (MHC-II) expressed on professional antigen-presenting cells (APCs) which include dendritic cells (DCs), macrophages, and B cells [42]. Upon recognition of antigen on the APC surface by the TCR along with appropriate interaction of co-stimulatory receptors such as CD28 on T cells with ligands such as CD80/86 on APCs leads to naïve CD4+ Th cell activation [43], which results in clonal expansion, triggered effector functions, and subsequent memory formation. During this period, the fate and functional specialization of activated CD4+ Th cells are largely dependent on the concentration and source of antigen, the type of APC engaged by CD4+ Th cells, the co-stimulatory receptors expressed by APCs, and, most importantly, the polarizing cytokine milieu of the microenvironment at the time of activation that drives the naïve CD4+ Th cells toward a particular Th cell subtype [40]. Together, these polarizing factors contribute to the specific expression of key subset-defining transcriptional factors and the subsequent secretion of effector cytokines that defines the functional subsets of CD4+ Th cells [40]. The cytokines secreted by CD4+ Th cells then activate and recruit a variety of other immune effector cells that together define the type of immune response [41]. Table 8.2 summarizes the CD4+ Th cell subsets in the human and murine systems, the polarizing cytokines that drive their development, their master transcription factors, and the effector cytokines they secrete.

Table 8.2 CD4 + Th cell subsets: polarizing cytokines, master transcription factors, and effector cytokines

Th subset	Polarizing cytokine	Transcription factor	Effector cytokine
Th1	IL-12, IL-18, INFγ, IL-27	T-bet, STAT4	IL-2, IL-10, INFγ, TNF-α, TNF-β (LT-α), CCL2, CCL3
Th2	IL-4, IL-25, IL-33, TSLP	GATA3, IRF4, STAT6	IL-4, IL-5, IL-9, IL-10, IL-13, IL-21, IL-31, TNF-α
Th17	TGF-β, IL-1β, IL-6, IL-21, IL-23	RORγt, RORα, IRF4 Batf, STAT3	IL-17A, IL-17F, IL-21, IL-22, IL-26 (human), TNF-α, CCL20
Th22	IL-6, IL-13, TNF-α	AHR, Batf, STAT3	IL-10, IL-13, IL-22, IL-21, TNF-α, IL-26 (human)
Th9	TGF-β, IL-4	PU.1, IRF4	IL-9, IL-10
Tfh	IL-6, IL-21	Bcl6, BATF, c-MAF	IL-4, IL-10, IL-12, IL-21, INFγ

Conventional Role of CD4+ T Cells in CD8+ Cytotoxic T Cell (CTL) Responses

CD4+ Th cells play an essential role in priming, activation, and expansion of CTL responses, a concept known as CD4+ T cell help [44–46]. CD4+ T cell help is complex and involves multiple mechanisms broadly classified into direct and indirect help. During the primary immune response to the tumor, the major indirect help from activated CD4+ Th cell comes through CD40/CD40L interaction with APCs that leads to maturation of the APCs [47–49]. This process provides all three necessary signals for CD8+ T cell activation, including antigen-mediated TCR triggering, co-stimulation, and stimulatory cytokines, most notably IL-12, which are all critically important for naïve antigen-specific CD8+ T cells to differentiate into CTLs. Alternatively, CD4+ Th cells can directly activate CTLs through CD40/CD40L [50]. Furthermore, activated CD4+ Th cells also directly help CTLs through secretion of IL-2, which supports growth and expansion [51, 52]. Furthermore, secretion of INFγ by CD4+ Th1 cells upregulates the expression of MHC molecules on the surface of tumor cells leading to a feed-forward loop of enhanced CTL responses as well as CD4+ Th responses [53]. In addition to priming the primary CTL response, CD4+ Th cells also help during the post-priming stage that takes place at the tumor site [54, 55]. Moreover, tumor-specific CD4+ Th cells have been shown to enhance the expansion of both low-avidity [56] and cognate [57] CTLs at the tumor site and enhance tumor rejection. In addition to their support to optimize CTL responses, CD4+ Th cells also play an essential role in generation and maintenance of memory CD8+ T cells during active CTL responses and homeostatic proliferation [58, 59]. Hosts lacking CD4+ Th cells have been shown to have reduced number of CD8+ memory T cells and impaired secondary CD8+ T cell responses [60].

Unconventional Role of CD4+ T Cells in Tumor Immunity

CD4+ Th cell-mediated antitumor immunity is primarily thought to involve activation and maintenance of CTL responses. However, more recent studies have shown that CD4+ Th cells also play independent roles in antitumor immunity. Here we discuss the specific roles of different CD4+ Th cell subsets in antitumor immunity.

CD4+ Th1 Cells in Tumor Immunity

In 1986, Mossman and Coffman demonstrated that antigen-specific mouse CD4+ Th cells can be categorized into two types, Th1 and Th2, based on their pattern of cytokine production [61]. In 1991, Romagnani and colleagues discovered that human CD4+ Th clones specific for intracellular *Mycobacterium tuberculosis* were mostly Th1 cells, whereas the CD4+ Th clones specific for the extracellular helminth *Toxocara canis* were mainly Th2 cells [62]. This firmly established the Th1/Th2 paradigm in both human and mice. The Th1 lineage is controlled by the key

transcription factor T-bet and the key polarizing cytokine IL-12 [40, 63–65]. Th1 cells secrete a set of cytokines that includes IL-2, INFγ, and TNF-α and the chemokines CCL2 and CCL3 that attract macrophages (Table 8.2), and they are best characterized for their role in clearance of intracellular pathogens such as viruses and their role in the pathogenesis of autoimmune conditions [66]. Th1 cells are considered to have potent antitumor activity due to their secretion of INFγ, IL-2, and CD40/CD40L co-stimulation to help initiate CD8+ T cell responses as described earlier [58]. Several human Th1 cells can also mediate antitumor immunity independently of helping CTL responses. INFγ plays a crucial role in antitumor responses and acts directly on tumor cells as well as promoting immune cell responses against tumor cells [67, 68]. Interestingly, an earlier study in mice demonstrated that Th1 cell-mediated INFγ secretion in the tumor microenvironment is essential for inhibiting angiogenesis and regression of tumors that do not express MHC-II [69]. Similarly, a study of mouse B cell cancer suggests that Th1 cell-mediated INFγ secretion in the tumor microenvironment is essential for eliminating MHC-II-negative tumor cells through activation of type 1 macrophages and angiogenic inhibitors like IP-10 [70]. However, their mechanistic relevance in human cancer is yet to be determined. Furthermore, a key function of Th1-derived INFγ in tumor-bearing hosts is to substantially increase the IL-12 secretion by DCs, which serves to further polarize the naïve CD4+ T cells into a Th1 phenotype, thereby contributing to their own development and maintenance [71]. In addition, secretion of cytokines and chemokines by Th1 cells also leads to recruitment and activation of pro-inflammatory type 1 macrophages (M1) and natural killer (NK) cells at the cancer site [68, 72, 73]. The cytotoxic mediators secreted from type 1 macrophages and NK cells have multiple antitumor properties [74, 75]. In line with this, patient studies show that the presence of Th1 cells and increased levels of their associated cytokines correlate with superior antitumor immunity and good clinical outcome in a majority of cancers [76]. Despite their potent antitumor role, Th1 cell functions are efficiently hindered by tumor cells by varying suppressive factors (Table 8.1 and described later), and imbalance or alterations in Th1/Th2 ratio in many human cancers lead to poor clinical outcome [77]. Owing to their importance, Th1 cells are also being utilized in clinical studies. Adoptive transfer of tumor antigen-specific Th1 cells in patients with metastatic melanoma [78] and metastatic cholangiocarcinoma [79] was recently shown to induce regression of the tumor for prolonged periods. In contrast, responses in melanoma patients that received only autologous, in vitro-expanded, tumor-infiltrating CD8+ T lymphocytes (TILs) [80] were found to be suboptimal and suggest the importance of inducing tumor antigen-specific Th1 cells for successful antitumor immunity.

CD4+ Th2 Cells in Tumor Immunity

CD4+ Th2 cells are recognized for their role in the host defense against extracellular parasites and their involvement in allergy and asthma. In both mice and humans, Th2 lineage commitment is controlled by the transcription factor GATA3 and

exposure to the polarizing cytokine IL-4 in the microenvironment of APC-naïve CD4+ Th cells [40, 81, 82]. Th2 cells then produce their signature cytokines such as IL-4, IL-5, IL-13, and IL-10 (Table 8.2). These cytokines mutually antagonize the development of Th1 cells [40, 64]. Th2 cells have been extensively studied for their role in antitumor immunity and in the context of disease progression and disease outcome. Initial studies from murine models and in vitro studies showed that IL-4 secreted from Th2 cells has a direct anti-angiogenic and tumoricidal activity [83–85]. IL-4 and IL-13 are critical for the recruitment of eosinophils and macrophages and in some cases neutrophils and CD8+ T cells to the tumor site and result in regression of tumor [86–90]. Conversely, Th2 cytokines also interfere with antitumor activity, which is largely attributed to Th2 cytokines that antagonize the development of INFγ-secreting Th1 and CTLs at the cancer site. IL-4 and IL-13 have an anti-apoptotic role [91–94], and IL-13 also has a pro-fibrotic role [95, 96] that may affect antitumor activity. Numerous studies indicate that the Th1/Th2 ratio is altered in a variety of cancers [76, 77]. Initial murine studies suggested that both Th1 and Th2 cells contribute to antitumor immunity [73, 97, 98]. However, the increased presence of Th2 cells was found to be pro-carcinogenic in many human cancers [33, 76, 99, 100]. These pro-tumorigenic roles of Th2 cells were proposed to be cancer specific rather than a global effect, as the Th1 response in these patients was not impaired [101, 102]. Multiple tumor-derived factors may favor the development of Th2 cells. Tumor cell-derived IL-10 induces skewing toward Th2 cells and inhibits the maturation of dendritic cells (DCs), which effectively reduces the secretion of INFγ and IL-12 from T cells resulting in impaired antitumor activity [103, 104]. Early reports demonstrated that human renal cell carcinoma and non-small cell lung cancer actively produced Th2-polarizing cytokines [105, 106]. Pancreatic cancer, an aggressive malignancy, is typically infiltrated by Th2 cells [107]. A clinical study from pancreatic cancer patients showed that the skewing toward Th2 was primarily due to the secretion of thymic stromal lymphopoietin from cancer-associated fibroblasts that activate DCs to produce Th2-associated cytokines and polarize T cells toward Th2 cells [108]. A similar mechanism was observed in mouse models of breast cancer [109], and chronic gastritis [110], which is the causative factor for gastric cancer. Similarly, studies in mice have shown that the expression of the human tumor antigen EpCAM strongly promotes Th2 skewing despite of the presence of strong Th1-polarizing conditions [111]. Thus, the involvement of Th2 cells in antitumor immunity is still controversial and that their effect may be context dependent.

CD4+ Th17 Cells in Tumor Immunity

In 2005, a third subset of CD4+ Th cells was identified in mice and named as Th17 cells based on the production of the cytokine IL-17 [112, 113]. Two years later, the existence of Th17 cells was confirmed in the human immune system [114, 115]. The development of Th17 cells are controlled by the master transcription factor RORγt and multiple polarizing cytokines [116–118] (Table 8.2). Owing to their

inflammatory properties, Th17 cells have been studied in a number of diseases both in mice and in humans and found to be important in the host defense against extracellular bacteria and fungi, but pathogenic in many inflammatory and autoimmune diseases [34, 116, 119, 120]. Th17 cells are shown to infiltrate several cancer types in both mice and humans [34]. However, their exact role in antitumor immunity is controversial and still elusive. Contradictory findings with respect to their role in antitumor immunity versus a pro-carcinogenic role may be due to the existence of multiple flavors of Th17 cells that are fostered by different cancerous cell types and mediators in the cancer microenvironment. Furthermore, the use of a variety of mouse tumor models adds complexity to this issue. Evidence for the role of Th17 cells in antitumor immunity came from studies with established models of B16 melanoma [122], and B16/F10 lung metastatic melanoma [123] in mice, in which adoptive transfer of in vitro-expanded, tumor antigen-specific Th17 cells induced regression of the cancer to a larger extent than Th1 cells transferred in a parallel experiment. The transfused Th17 cells were found to promote the infiltration of DCs and enhanced cross-antigen presentation to naïve CD8+ T cells as well as to induce the secretion of CCL20 from cancer-residing lung cells to further recruit CD8+ CTLs into the tumor site [123]. Therefore, the Th17 cells were proposed to have a synergistic function with CD8+ CTLs. In contrast, other tumor models in mice, which included leukemia [124], cervical cancer [125], non-small cell lung cancer [126], lung cancer [127], and colon cancer [128], suggested that Th17 cell-secreted inflammatory cytokines in the tumor microenvironment promoted neutrophil recruitment and secretion of elastase, a pro-tumorigenic factor [129]. They also promoted the secretion of pro-angiogenic factors and pro-inflammatory cytokines from tumor cells, which promote angiogenesis and cancer progression [129]. Recent studies with genetically modified mice with colon cancer [130] and pancreatic cancer [131] showed that the preinvasive epithelial layer expressed large amounts of IL-17R that facilitated the infiltration of Th17 cells further substantiating the above findings. Subsequently, the IL-17A derived from Th17 cells triggered the oncogenic signal through the IL-17R-STAT3 pathway and accelerated the transformation of epithelial cells into invasive neoplasia. Recently, β-catenin signaling was also implicated in the development of Th17 cells in colon cancer [132]. Similar dichotomous findings were observed in human cancer patients where infiltration of Th17 cells was positively associated with CD8+ T cell count and better survival in ovarian cancer [133] and esophageal cancer [134], whereas increasing evidence suggests the opposite in many solid tumors [34, 76].

Th17 cells are also found to be a major fraction of TILs in human cancers, attracted by tumor-derived RANTES and MCP-1 [135, 136]. Human Th17 cells also undergo plasticity (secreting cytokines of other lineages) [117, 120]. Interestingly, in vitro-expanded, tumor antigen-specific Th17 clones from melanoma and breast and colon cancer produced large amounts of polyfunctional cytokines including IL-8 and TNF-α, but not IL-2, IL-4, IL-12, or IL-23 [135]. Furthermore, the same authors also suggested that Th17 cells can be converted into FOXP3-expressing, Treg, cells that produce IL-10 and TGF-β1, indicating a possible regulatory function [137]. In contrast, other studies suggest that in vitro-expanded, tumor antigen-specific Th17 clones from colon cancer and ulcerative colitis mainly produced IL-2, TNF-α, INFγ, and GM-CSF and exhibited plasticity

to convert into FOXP3- and INFγ-expressing cells with suppressive properties [129, 133, 138]. These findings were contrasted by the proposed cytokine signature of freshly isolated Th17 cells from healthy patients [139] and argue that these differences may arise from in vitro induced changes or may reflect their actual function in the cancer microenvironment. The conversion of Th17 cells into Th1 cells is well documented in autoimmune diseases and cancer [117, 120]. However, recent findings have shown that ex vivo-isolated Th17 cells from peripheral blood mononuclear cells (PBMCs) of human pancreatic cancer patients can also produce Th2 and Th17 cytokines [140]. Notably, these findings demonstrate that Th17 cells from human cancers not only correlate with IL-17 secretion but can also acquire Th1- or Th2-associated features. To summarize, Th17 cell-mediated antitumor immunity is due to enhancement of DC and CD8+ CTL function. However, Th17 cells also contribute to cancer-promoting inflammation and angiogenesis. Further, their plasticity-associated complexity in the tumor microenvironment may determine their pro-tumorigenic, suppressive, or anti-tumorigenic role that may influence cancer prognosis.

CD8+ Cytotoxic T Lymphocytes (CTLs) in Cancer Immunity

CTLs recognize their cognate antigen through binding of their TCR to antigen-MHC-I complex expressed on the surface of tumor cells. Th cells also provide help to CTL responses (see the section "Unconventional Role of CD4+ T Cells in Tumor Immunity"). CTLs potentially eliminate the tumor cells and have been shown to correlate with good prognosis in almost every type of human malignancy (Table 8.3). CD8+ T cells use multiple mechanisms to kill tumor cells mediated by granzyme B, perforin, and the triggering of the Fas signaling pathway through Fas ligand (FasL). FasL expressed on CTLs binds to its cognate receptor on the tumor cell surface and induces apoptosis. Similarly, perforin secreted by activated CTLs forms pores on the surface of tumor cells that aid in directed delivery of granzyme B into the tumor cell that subsequently induces apoptosis. In addition, naïve CD8+ T cells also differentiate into different subsets such as Tc1 (Tbet+ Eomes+ INFγ+), Tc2 (GATA3+ IL-4+), and Tc17 (RORγt+ Tbet+ IL-17+) cells, which are driven by master transcription factors and polarizing cytokines similar to those described for Th1, Th2, and Th17 cells (Table 8.2) and also produce key cytokines similar to that of Th subsets (Fig. 8.1). Since type 1-, 2-, and 17-related cytokines are mainly produced by Th subsets rather than Tc subsets in the cancer microenvironment, their functional relevance is not yet clearly known. However, recent studies in mice suggest that T cells secrete INFγ, but not IL-4 and TNF-α, in a directional way (at the immunological synapse) onto the target cell [205]. It is possible that INFγ secreted by tumor-infiltrating Tc1 cells can have direct antitumor activity by enhancing MHC expression on cancer cells, inducing angiostatic effects, and also recruiting macrophages [68]. The role of IL-4-secreting Tc2 cells in the cancer microenvironment is largely unknown, although a study from breast cancer [206] showed their association with cancer progression. In contrast to Tc1 cells, IL-17-secreting Tc17 cells were found

Table 8.3 The association of tumor-infiltrating T cell subsets and prognosis

Cancer type	CD8+ T cells	CD4+ Th1 cells	CD4+ Th2 cells	CD4+ Th17 cells	CD4+ Treg cells
Head and neck cancers	Good [141, 142]				Good [142]
Esophageal cancer	Good [143, 144]	Good [145]		Good [134]	
Lung cancer	Good [146]	Good [146]		Poor [147]	Poor [148]
Pancreatic cancer	Good [149, 150]		Poor [108]	Poor [151]	Poor [151, 152]
Distal bile duct cancer	Good [153a] Poor [153b]				Good [153a]
Breast cancer	Good [154]	Good [155]	Good [156]	Poor [157]	Poor [158, 159] Good [160]
Gastric cancer	Poor [161, 162]	Good [163]	Poor [163]	Good [164] Poor [165]	Good [165] Poor [166]
Hepatocellular carcinoma	Good [167, 168] Poor [168]	Good [169]		Poor [170]	Poor [168, 171]
Colon cancer	Good [172–178]	Good [172–174]	None [173]	Poor [173, 179, 180]	Good [173, 180–192] Poor [183] None [176]
Ovarian cancer	Good [184]	Good [185, 186]	Poor [186]	Good [133]	Good [187, 188] Poor [189]
Renal cell carcinoma	Good [190]	Good [191]			Poor [191]
Prostate cancer	Good [192]				
Urothelial carcinoma	Good [193]				
Endometrial cancer	Good [194]				
Cervical cancer	Good [195] Poor [196]				
Melanoma	Good [197, 198]				None [199] Poor [200, 201]
Follicular and Hodgkin's lymphoma			Good [202]		Good [203, 204] Poor [202]

to be impaired in cytotoxic activity [207, 208]. However, adoptive transfer studies in mouse tumor models have shown that Tc17 cells inhibited tumor growth, which was primarily associated with their plasticity to convert into Tc17/1 cells that produced INFγ along with IL-17A [209]. However, Tc17 cells identified in gastric cancer [161], hepatocellular cancer [210], distal bile duct cancer [153b], cervical cancer [196], breast cancer [206], and endometrial carcinoma [211] were primarily found to be less cytotoxic and promoted cancer. Especially in gastric [161] and cervical cancer [196], Tc17 cells were shown to promote angiogenesis and to recruit suppressor cells, including myeloid-derived suppressor cells (MSDCs) and Tregs. Therefore,

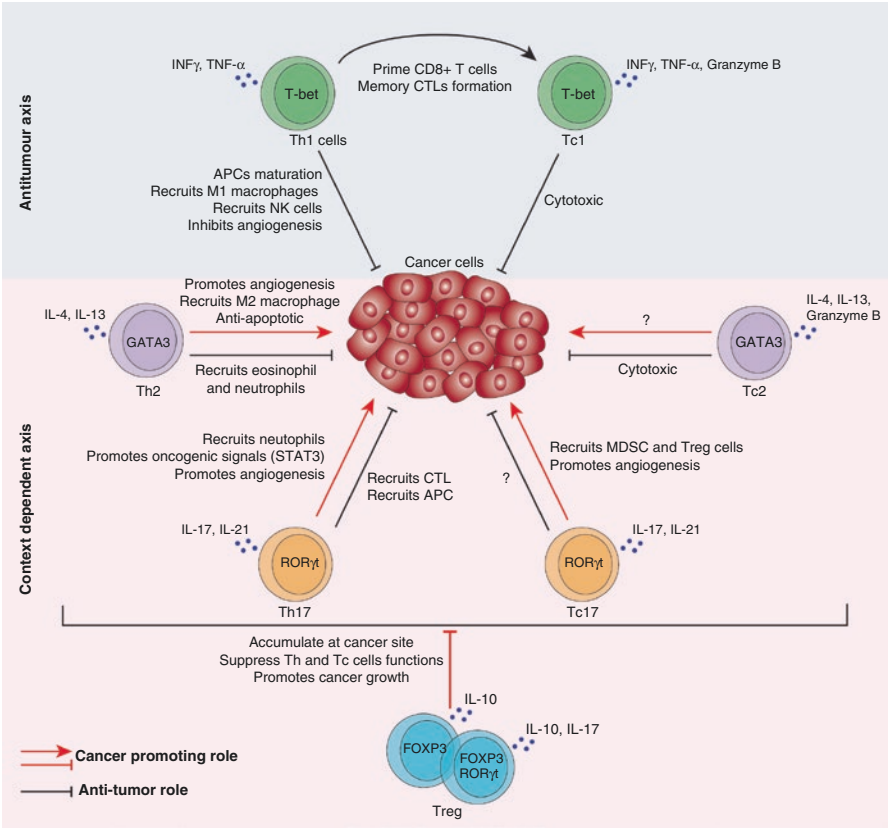

Fig. 8.1 Role of T cell subsets in antitumor immunity. Th1 cells express T-bet, INFγ, and IL-12. Th1 cell is superior in antitumor activity primarily due to activating APC, M1 macrophages, NK cells, and prime CTL (Tc1 cells) response. Both Th1 cells and Tc1 cells associate with good prognosis in many cancers and form a prominent antitumor axis in humans. Th2 cells express GATA3, IL-4, and IL-13 and contribute to cancer regression via recruiting eosinophils and neutrophils. Furthermore, cytokines produced by Th2 cells also contribute to angiogenesis, recruit M2 macrophages, and have an anti-apoptotic role. Their counterpart the Tc2 cells contribute to cancer regression through their cytotoxic activity, but their possible cancer-promoting features are not clearly known. Th17 cells contribute to cancer regression via activating APC and CTL. However, they may also contribute to cancer progression by various mechanisms. Similarly, their counterpart Tc17 also primarily contributes to cancer progression by recruiting suppressor cells into cancer stroma, mainly Tregs and MDSCs. Both Th17 and Tc17 cells contribute to angiogenesis. Tregs contribute to cancer progression by suppressing the effector functions of Th and Tc cell subsets. Tregs also largely accumulate at the cancer site, and their phenotypic heterogeneity and plasticity also contribute to pro-carcinogenic inflammation and cancer progression. Therefore, Th2, Th17, Tc17, and Treg subsets form a context-dependent axis in antitumor immunity in human malignancy

emerging results suggest that the cytotoxic activity of CD8+ T cells is context dependent, and under specific polarizing conditions, they may potentially lose their cytotoxic activity.

Tumor-Infiltrating T Cell Subsets and Their Prognostic Value

Despite the associations described above of various types of Th and Tc subsets with different cancers, the use of phenotyping of tumor-infiltrating T cell subsets as a prognostic marker is a complicated endeavor. In addition to the complex interactions in the tumor microenvironment, CD4+ Th cells in the tumor can be found in different maturation states such as activated, exhausted, or regulatory. Moreover, they may share phenotypic markers with other immune cells adding more complexity to analyses and interpretations of individual patient TIL profiles. Conflicting conclusions with respect to TIL phenotype could also potentially be due to differences in methodologies used, such as immunohistochemistry (IHC), multicolor flow cytometry, and polymerase chain reaction (PCR). Nonetheless, similar conclusions drawn for a particular cancer type by several groups substantiate the need for studying the link between Th cell subsets and prognosis and/or response to therapy. Here we summarize the prognostic value of analyzing the abundance of Th1, Th2, Th17, and CD8+ T cell subsets in several human malignancies (see Table 8.3). Th1 cells and CD8+ T cells are strongly associated with good prognosis in many human cancers including esophageal cancer [143–145], colon cancer [172–178], head and neck cancer [141, 142], lung cancer [146], pancreatic cancer [149, 150], distal bile duct cancer [153a], breast cancer [154, 155], gastric cancer [163], prostate cancer [192], urothelial cancer [193], ovarian cancer [184–186], endometrial cancer [194], cervical cancer [195], hepatocellular carcinoma [167–169], melanoma [197, 198], and renal cell carcinoma [190, 191]. Despite this, the presence of CD8+ T cells has also been reported to associate with poor outcome, particularly in hepatocellular carcinoma, gastric cancer, distal bile duct cancer, and cervical cancer (Table 8.3), which is thought primarily to be due to conversion of CD8+ T cells into Tc17 cells [153b, 161, 162, 196]. In contrast to Th1 cells and CD8+ T cells, Th2 and Th17 cells correlate with either good or poor prognosis (Table 8.3). Th17 cells have been associated with good prognosis in esophageal cancer [134], ovarian cancer [133], and gastric cancer [164] but correlated with poor prognosis in colon cancer [173, 179], lung cancer [147], pancreatic cancer [151], breast cancer [157], gastric cancer [165], and hepatocellular carcinoma [170] (Table 8.3). Whereas the presence of Th2 cells is associated with good prognosis in breast cancer [156] and follicular and Hodgkin's lymphoma [202], their presence associates with poor prognosis in pancreatic cancer [108], gastric cancer [163], and ovarian cancer [186], but does not appear to have an impact on colon cancer prognosis [173] (Table 8.3). Interestingly, in gastric cancer accumulation of Th17 cells have been shown to associate with either good prognosis irrespective of the cancer stage [164] or poor prognosis at early stage of the cancer [165]. These disparities could originate from differences in experimental setup and markers used to define Th17 and Th2 cells. Some of the abovementioned studies used only IL-17 as a predictor, investigating the CD4+ IL-17+ T cells. This may affect the results as other immune cell types including γδ T cells, myeloid cells, and innate lymphoid cells (ILCs) can also produce IL-17 [41, 121]. In addition, as we described earlier (see the section "Unconventional Role of CD4+ T Cells in Tumor Immunity"), Th17 cells also undergo plasticity, and therefore the conflicting observation of Th17 cells and Th2 cells may also reflect the fundamental differences in the inflammatory tumor

microenvironment and stress the importance of well-delineated Th lineage analysis in these patients. In addition, Galon and colleagues earlier proposed a concept termed "immune contexture" in which the location and density of CD8+ T cells and CD4+ Th cells in both the invasive margin and intra-tumoral region predicted a favorable outcome in colorectal cancer patients [172, 212]. Recently, this particular immune contexture has also been demonstrated in other cancer types [153a, 213, 214]. These findings provide a framework to further standardize the studies that involve T cell subset association with prognosis in human cancer.

CD4+ T Cells Suppressing Antitumor Immunity

CD4 + FOXP3+ Regulatory T Cells (Tregs)

Tregs are crucial in the maintenance of peripheral tolerance and prevention of autoimmunity [215]. The transcription factor FOXP3 is essential for the development and function of Tregs [215]. Various CD4+ Treg subsets have been identified in humans that can be broadly divided into thymus-derived (tTregs) and peripherally induced Tregs (pTregs). The essential function of Tregs is to suppress the activation, clonal expansion, and effector functions of various immune cells including CD4+ T cells, CD8+ T cells, natural killer T (NKTs) cells, and antigen-presenting cells (APCs) through a myriad of mechanisms [216, 217]. Accumulating evidence suggests that Tregs are specifically attracted by chemokines secreted by cancer cells [16]. Similarly, the cancer microenvironment provides a niche to strongly expand Tregs [218] where the Tregs contribute to the suppression of antitumor immunity initiated by Th cells, CTLs, and other innate immune cell tumors [17]. The conversion of Th cells into pTreg cells has been suggested to account for the high number of Tregs in tumor tissue. However, recent findings using epigenetic analysis of Tregs from tumor sites from mice and human found that a significant proportion of intra-tumoral Tregs were of nTreg origin and suggested that Th to Treg conversion is only partly contributing to the expansion of the Treg population [219]. Apart from their suppressive function, IL-10 secreted by Tregs can also skew Th subset tumor into a Th2 phenotype, which is associated with poor prognosis in many tumor types (Table 8.3). In addition, recent evidence from many solid tumors especially colon cancer [183, 220, 221], pancreatic cancer [140], and breast cancer [222] suggests that IL-17+ FOXP3+ Tregs retain their suppressive function but also contribute to Th17-associated inflammation, which is associated with poor prognosis in these tumor types (Table 8.3).

Tumor-Infiltrating Tregs and Their Prognostic Value

Tumor-infiltrating Tregs have been extensively studied and the prognostic value of their presence varies in different tumors. Tregs have been reported to correlate with poor outcome in colon cancer [183], lung cancer [148, 223], pancreatic cancer [151, 152], breast cancer [158, 159], gastric cancer [166], ovarian cancer [189], renal cell

carcinoma [191], and hepatocellular carcinoma [168, 171] as well as melanoma and follicular and Hodgkin's lymphoma [202]. In contrast, the presence of Tregs was found to be associated with good prognosis in colon cancer [173, 181, 182], head and neck cancer [142], distal bile duct cancer [153a], gastric cancer [165], ovarian cancer, and breast cancer [160], as well as follicular and Hodgkin's lymphoma [203, 204] (Table 8.3). Interestingly, associations with both good and poor prognosis were observed within the same cancer type for colon, breast, gastric, and ovarian cancer and Hodgkin's lymphoma (Table 8.3). Moreover, some studies have reported that the presence of Tregs has no impact in colon cancer and melanoma (Table 8.3). These discrepancies in prognostic value may arise from the use of different markers to define Tregs. Other factors that may contribute to these discrepancies are the presence of tumor subtypes where the impact of antitumor immunity varies, tumor stage, and the location of the characterized Tregs (within the tumor tissue, at the margin of the tumor or in the inflamed tissue outside the tumor). Finally, the role of Tregs in cancer progression may also be dependent on whether the cancers were preceded, or stimulated, by inflammation. In addition, many of these studies have not reported Treg-suppressive function or their phenotypic plasticity. The positive impact of Tregs in some tumor types may reflect their anti-inflammatory role in suppressing tumor-promoting inflammation. Moreover, discrepancies within the same tumor type such as colon, breast, and gastric cancer may indicate that Tregs may predominantly share other Th lineage phenotypes, such as IL-17+ FOXP3+ Treg, which have been found to be the major Treg pool in colon, breast, and pancreatic cancer patients [140, 183, 222]. Nonetheless, these data suggest that the original view on Tregs in suppressing antitumor immunity is oversimplified and that Tregs may have multiple roles in influencing inflammation and shaping the tumor microenvironment as well as in suppressing antitumor immunity.

Conclusion

Experimental and clinical studies now indicate that T cells play a pivotal, albeit sometimes paradoxical role in shaping antitumor immunity (Fig. 8.1). Nonetheless, the presence of Th1 and CTL cells is strongly associated with favorable outcomes in many tumor types and indicates that active cancer immunosurveillance is an integral part of many human malignancies. However, the potency of CTL function in several malignant tumors is generally compromised. The main factors contributing to tumor immune evasion include reduced MHC-I and MCH-II expression by tumor cells to eliminate the direct detection by CTLs, along with reduced help from CD4+ Th tumor cells. In addition, the differentiation of CD8+ T cells into less cytotoxic and pro-inflammatory subsets under polarizing conditions in the tumor microenvironment together with Treg-mediated immunosuppression at the cancer site contributes to the functional defect in tumor-specific Th1 cells and CTLs that ultimately lead to tumor progression. In addition, Th2, Th17, and Tregs are largely associated with poor outcome in many tumor types. The bifurcation of the pro- and

anti-tumorigenic nature of T cell subsets is too complex to predict, as it largely depends on cytokines secreted in the cancer microenvironment. To add to this complexity, recent reports suggest that T cells share different lineage-specific transcription factors and exhibit heterogeneity and plasticity. This may explain the paradoxical role of Th2, Th17, and Treg subsets observed, as many earlier studies assessed the prognostic value of individual subsets, but did not consider the potential of phenotypic plasticity. It is also inevitable that the location of T cells and the niche they share with other immune cells, cancer cells, and stromal cells along with their complex interactions dictate their functional status. An integrated picture of all these factors will shed more light on the role of T cells in cancer and enable us to better tailor T cell therapies in the future.

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