3 Janus or Hydra: The Many Faces of T Helper Cells in the Human Tumour Microenvironment

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Abstract

CD4+ T helper (T_H) cells are key regulators in the tumour immune microenvironment (TIME), mediating the adaptive immunological response towards cancer, mainly through the activation of cytotoxic CD8+ T cells. After antigen recognition and proper co-stimulation, naïve T_H cells are activated, undergo clonal expansion, and release cytokines that will define the differentiation of a specific effector T_H cell subtype. These different subtypes have different functions, which can mediate both anti- and pro-tumour immunological responses. Here, we present the dual role of T_H cells restraining or promoting the tumour, the factors controlling their homing and differentiation in the TIME, their influence on immunotherapy, and their use as prognostic indicators.

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Keywords

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3.1 Introduction

Although most of our knowledge on the adaptive immunological response against cancer relies on cytotoxic CD8+ T cells, T helper (T_H) cells are also key regulators of the tumour immune microenvironment (TIME) [1]. T_H cells each possess the cell cluster of differentiation surface marker CD4 and thus are also known as CD4+ T cells. T_H cells assist other lymphocytes through the activation of other immune cells such as cytotoxic T cells and macrophages. Specific subsets of T_H are also known to contribute to the maturation of B cells into plasma cells and memory B cells. To perform specialized functions such as these, a naïve T_H cell must be activated. For activation to occur, an antigen-presenting cell (APC) presents an antigen on its major histocompatibility complex (MHC) class II molecule and binds with the T-cell receptor (TCR) of the T_H cell. Upon recognition of the antigen-MHC molecule and proper co-stimulation, the naïve T_H cell becomes activated, undergoes clonal expansion, and releases cytokines that programme the cells to differentiate into a specific effector cell type, which have different roles (Fig. 3.1, Table 3.1). T_H1 cells produce interferon-γ (IFN-γ), interleukin 2 (IL-2), and tumour necrosis factor-α (TNF-α) and are mostly involved in immune responses against bacteria and viruses [2]. T_H2 cells are characterized by the expression of IL-4, IL-5, and IL-13 and play a significant role in the immune response against extracellular pathogens, such as parasites [2]. T_H17 cells express IL-17A, IL-17F, and IL-22 and are critical for antifungal and antibacterial responses [3]. Another subset of cells, T follicular helper (T_{FH}) cells, contributes to humoral immunity within germinal centres and characteristically present with CXCR5-positive expression [4]. These cells produce IL-21 and IL-4, which are important for B-cell stimulation, immunoglobulin class switching, and homing of B cells to B cell-rich germinal centre of secondary/tertiary lymphoid organs [5]. Another type of CD4+ cell, the T regulatory (T_{Reg}) cell, expresses CD25; they secrete the cytokine IL-10 and have been shown to carry an immunosuppressive role [6]. The contribution of T_{Regs} in immune evasion observed in cancer is an area of active research, and these cell subsets are targets for cancer immunotherapeutics [7, 8]. Many other T helper cell subsets with well-described functions have been defined, including TH3, TR1, TH9, and TH22. In this chapter, we focus on the major subsets of T_H cells and discuss their roles in the TIME.

In addition to their traditional roles in the immune response against pathogenic microorganisms, accumulating evidence has emerged on the importance of CD4+ T cells and their role in mediating anti-tumour responses [9, 10]. Accumulating evidence suggests that select CD4+ T_H cell subsets may have a more "direct" role in inhibiting tumour growth and progression that are independent of their more "indirect" helper activities [11]. However, recent studies have revealed additional CD4+ T_H cell functions that can not only influence tumour immunity and inhibit growth but, paradoxically, can also promote tumour growth and progression [12, 13].

It is generally accepted that human tumours are immunogenic, meaning that they may provoke an immune response. Tumour immunogenicity varies greatly between types of cancer and between different individuals with the same type of cancer [14]. These responses are mostly mediated by T cells, and their presence is often associated with a more favourable outcome [15]. Immune checkpoint inhibitors are derived from advanced melanoma squamous non-small cell lung cancer [16]. These solid tumour cancers, which can be hard to treat, have shown favourable responses when treated with immunotherapy [17, 18]. The immune cell population is a major factor that influences prevention or encourages initiation, metastasis and invasion, and

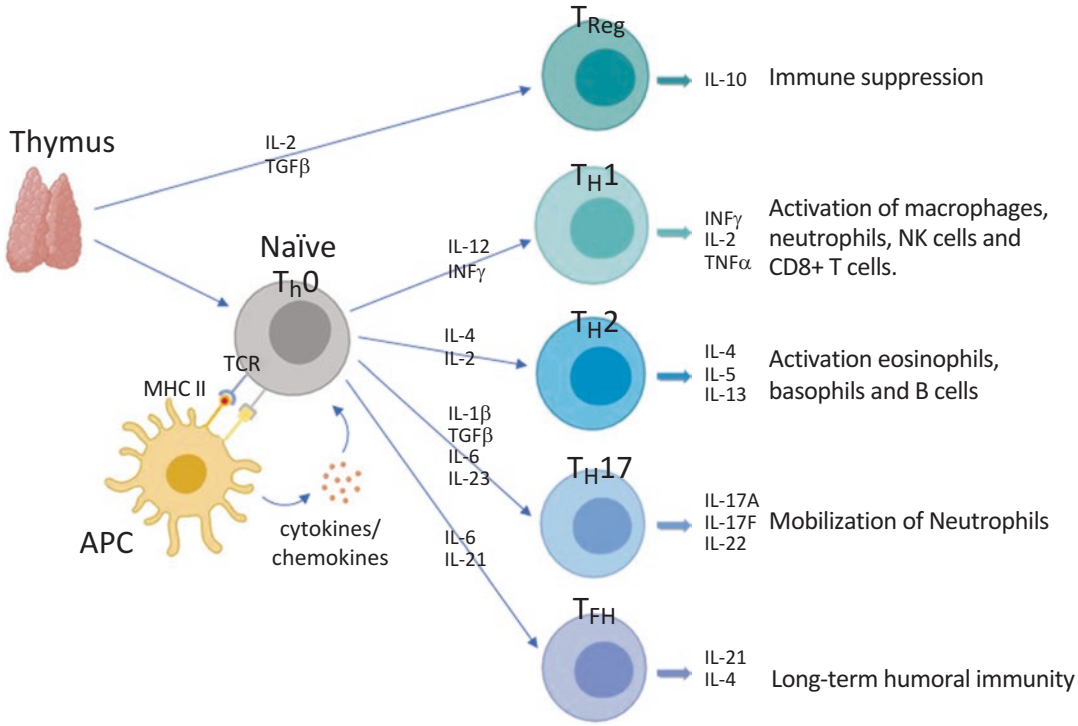

Fig. 3.1 Canonical lineage and differentiation of CD4+ immune cells

Table 3.1 Factors regulating T helper cell differentiation

Cytokines produced by APC programme naïve CD4+ T_H cell differentiation	IL-12 IFN**γ**	IL-4 IL-2	IL-6 IL-21	IL-1β TGFβ IL-6 IL-23	IL-2 TGFβ
CD4+ T helper cells	**T_H1**	**T_H2**	**T_{FH}**	**T_H17**	**T_{Reg}**
Cytokines produced	INF-γ IL-2 TNFα	IL-4 IL-5 IL-13	IL-21 IL-4	IL-17A IL-17F IL-22	IL-10
Key transcription factors	Tbet	GATA-3	BCL6	RORyt	FOXP3
Role in immune defence	Antiviral, antibacterial immunities	Extracellular pathogens	Humoral immunity within germinal centres	Antifungal and host defence against intra- and extracellular bacterial infection	Immunosuppression
Signal transducer	STAT4	STAT5 STAT6	STAT3	STAT3	STAT5

angiogenesis. The composition and characteristics of the TIME differ between different types of cancer or between patients that have the same type of cancer. The TIME is composed of resident stromal cells and non-resident components. It can be classified according to the composition of the immune infiltrate and the nature of the inflammatory response. Currently, three broad classes exist (Fig. 3.2): (1) poorly immunogenic, or "cold", where immune cells (mainly cytotoxic T lymphocytes) are only present along the tumour periphery; (2) infiltrated, inflamed, or immuno-

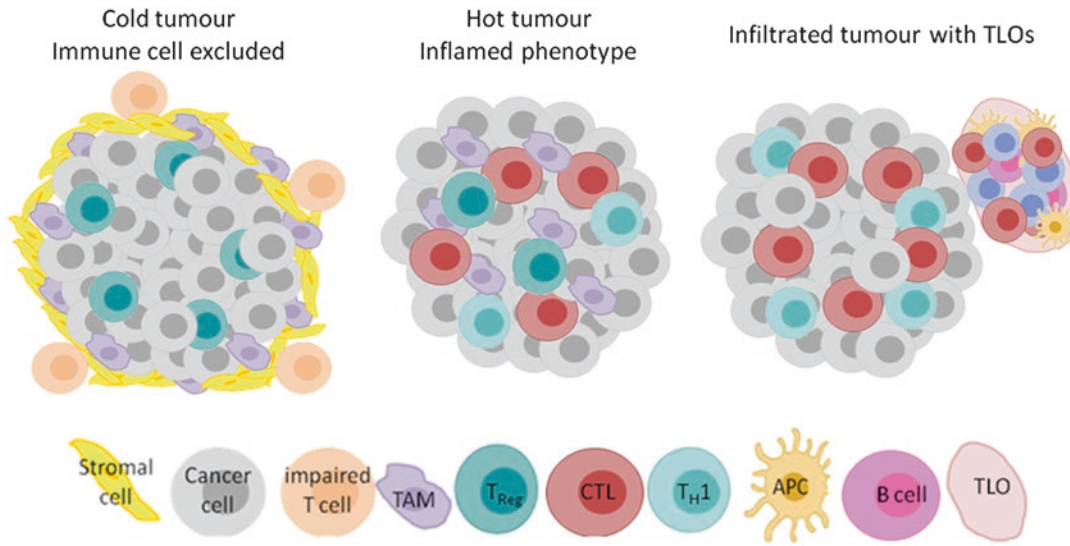

Fig. 3.2 Three phenotypes of the tumour immune microenvironment. T^{Reg} regulatory T cell, *TH* T helper cell, *TFH* T follicular helper cell, *EMT* endothelio-mesecnhymal transition, *NK* natural killer cell, *IFN* interferon, *DC* dendritic cell, *TAM* tumour-associated macrophage, *CTL* cytotoxic T lymphocyte, *APC* antigen-presenting cell, *TLO* tertiary lymphoid organ

logically "hot", with an abundance of programmed death ligand 1 (PD-L1) expression and highly activated cytotoxic T cells; and (3) those with groups of immune cells with constituents similar to those in lymph nodes, including B cells, dendritic cells, and T_{Reg} cells [19]. This later categorization undoubtedly misses key subclasses that require higher-resolution techniques to uncover and characterize heterogeneity in immune cell composition.

Here, we review the dual pro- and anti-tumour functions of T_H cells as well as factors influencing their homing and differentiation in the TIME. Finally, we provide an overview of the interactions between cancer immunotherapy and T_H cells and the prognostic role of T_H cell infiltration.

3.2 Dual Role of T Helper Cells in Tumour Development and Progression

According to the cytokine context of the TIME, naïve CD4+ T cells can differentiate into specific T_H cell subtypes, and other already differentiated subsets are recruited to the area [1]. While CD8+ cytotoxic and interferon-gamma-producing CD4+ T_H1 helper cells are the main players against tumours, other types of CD4+ cells can act in favour of cancer in combination with other cell types, such as myeloid-derived suppressive cells (MDSC) and tumour-associated macrophages (TAM). Pro-tumour functions driven by these cells and their secreted factors are able to inhibit anti-tumour innate and adaptive immune responses [1].

Further, the recruitment of specific T-cell subset to the TIME has been shown to correlate with prognosis and immunotherapeutic efficacy, underlying the importance of tumour infiltration and the role of T-cell homing to and within the tumour [20, 21]. In normal conditions, naïve T cells are produced in the thymus and cycle through complex networks of blood vessels, lymphatic vessels, and lymph nodes until they are signalled to home into specific tissues [22]. Homing of T cells is a very complex and tissue-specific process requiring activation of various patterns of receptors on the surface of the T cells specific to the tissue they will infiltrate [23]. Depending on the specific activation of receptors,

the T cell will express specific chemokines and integrins. Integrin activation causes firm adherence to the vessel, so the T cell can begin transmigration through the endothelial surface of the vessel and into the tissue they are homing toward. This process is the same for T cells destined to home into tumours, but homing T cells to tumours is not always successful because tumours may possess a number of deficits to prevent the process of proper T-cell homing and infiltration [23].

As each major T-cell subset is regulated by different mechanisms of differentiation and recruitment, we discuss the dichotomous roles of each type of CD4+ T cells and their potential use as prognostic markers below.

3.2.1 T Helper Type 1

T helper 1 lymphocytes are important players in modulating immune response against cancer, linking innate and adaptive immunity, since IFN-γ also induces anti-tumour activity from tumour-infiltrating macrophages [24]. It was previously demonstrated that IFN-γ and TNF-α produced specifically by T_H1 cells are necessary for inducing senescence in cancer cells and to turn macrophages cytotoxic to tumour cells [24, 25]. In fact, increased circulating levels of IFN-γ and TNF-α were described as a protective factor for prostate cancer [26]. In addition, many studies reports good patient outcome related to T_H1 cell and related cytokines in the TIME and in the blood of patients in a variety of cancer types [27–31]. Therefore, T_H1 is known to consistently promote immune responses against tumour cells.

3.2.2 T Helper Type 2

T helper type 2 lymphocytes are known to have less effective anti-tumour response than T_H1, presenting dual functions depending on the context [32, 33]. The shift from T_H1 to T_H2 in the TIME has been reported in a variety of cancer types [29, 34–38]. A pan-cancer analysis from The Cancer Genome Atlas consortium (more than 10,000 tumours from 33 cancer types) revealed 6 immune subtypes defined by genetic and immunological features, including the T_H1:T_H2 ratio [39]. Tumours characterized by a T_H2 immune infiltrate bias, the *wound healing* subtype T_H ratio, is enriched in colorectal, lung squamous cell, breast cancer (luminal A molecular subtype), head and neck (classical molecular subtype), and chromosomally unstable gastrointestinal cancer. This immunogenomic subtype was associated with shortened survival [39], agreeing with previous reports of high T_H2/T_H1 ratio as a poor prognostic indicator [29, 37, 40]. The trafficking of T_H1 or T_H2 cells into the TIME is influenced by many factors secreted by tumour cells [41]. In addition, the switch from T_H1 to T_H2 immune response was shown to be influenced by T_{Reg} cells in hepatocellular carcinoma after transarterial chemoembolization treatment [29]. Nonetheless, T_H2 have been described to modulate anti-tumour activity in many cancer types and conditions [32, 42–45], relying on the attraction of innate immune cells to the tumour [46, 47]. Specific T_H2 adoptive cell therapy in mice models has been shown to eliminate myeloma and lymphoma cells, in a process independently of CD8+, natural killer, B cells, and IFN-γ and dependent of M2-type macrophages [45].

3.2.3 Regulatory T Cells

Tumour escape strategies comprise primarily the recruitment of immunosuppressive cells to the TIME [48, 49]. One important inhibitory cell subset thought to contribute to the suppressive immunity associated with cancers is the regulatory T cell (T_{Reg}) [50, 51]. While the existence of "suppressor" T cells was discussed as early as the 1970s, discovery and definition of what is now referred to as T_{Regs} began in earnest in 1995 when autoimmunity was rescued in a mouse model with CD25+ T cells, leading to the first description of a highly immune inhibitory T cell [6, 52]. Since that time, T_{Reg} cell subsets have been further defined to include naturally occurring CD4+CD25^{high} T_{Reg} cells, inducible T_{Reg} cell subsets such as T_r1 and T_H3 cells, and those derived

from the induced expression of CD25 in CD4+CD25− subsets in the periphery, all capable of immunosuppression [53–56]. In addition, the transcription factor forkhead box P3 (FoxP3) has been further identified as a common marker for T_{Reg} cells [57].

In general, CD4+CD25^{high}FoxP3+ T_{Reg} cells are antigen-experienced memory T cells capable of inhibiting a variety of immune cell subsets including CD4+CD25− T cells, CD8+ T cells [58], dendritic cells [59], natural killer cells, natural killer T cells [60], and B cells [61]. T_{Reg} cells represent only between 5 and 10% of the T-cell populations in healthy human conditions [62]. They are present at higher levels in a wide range of human neoplasias [7, 63–68] and support tumour development and progression [69]. Suppression of effector T cells and NK cells by T_{Reg} was found to be cell-cell contact dependent [58], but other demonstrated that the function of T_{Reg} is dependent on the cytokines IL-10, IL-35, and TGFβ [70]. T_{Reg} also depletes immune-inducing cytokines, such as IL-2 [71, 72].

3.2.4 T Helper Type 17

The dual role of T_H17 cells in inflammatory disease and cancer has been widely reported [73, 74]. T_H17 cells were demonstrated to have anti-tumour functions, by inducing the recruitment of dendritic cells in the tumour and in the adjacent lymph nodes promoting tumour-specific cytotoxic T cell responses [75]. In ovarian cancer, it was demonstrated that the presence of T_H17 cells in the TME was correlated with the infiltration of effector T cells in the tumour [76].

Nevertheless, T_H17 cells can also release potent immunosuppressive signals into the TIME, supporting their dichotomous nature [77]. Moreover, IL-17 can result in pro-tumour responses through effects in the tumour cells, myeloid-derived suppressor cells, and other components of the stroma [78]. It was previously demonstrated that IL-17 derived from T_H17 cells promotes migration and invasion and induces stem cell-like features in lung cancer using the STAT3-NF-κB-Notch1 signalling [79]. In colorectal cancer, infiltrating IL-17-producing cells are associated with poor prognosis [30].

3.2.5 T Follicular Helper

Although T_{FH} cells are reported to have pro-tumour functions in haematologic types of cancer [80], in solid tumours, they are generally associated with anti-tumour functions [81–83]. In lung [81] and breast cancer [82], T_{FH} cells are enriched in the germinal centre of tumour-adjacent tertiary lymphoid organs. Moreover, the T_{FH} density positively correlates with the lung cancer mutation burden, indicating their role in the immune response against cancer neoantigens [81].

Conversely, differentiated T_{FH} cells also express high levels of programmed cell death protein 1 (PD-1), suggesting that they can also decrease the activation of T cells [4]. However, PD-1/PD-L1 blockage results in deficient germinal centre formation and cytokine production by the T_{FH} cells [84].

3.2.6 T Helper Type 9

T_H9 cells are thought to have a strong anti-tumour effect in the presence of TGF-β and IL-4 [85, 86]. This anti-tumour property is related to their effects on mast cells, dendritic cell recruitment, and promoting cytotoxic function by CD8+ T cells [86, 87]. Additionally, IL-9 was shown to directly inhibit the proliferation of melanoma cells in mice models, and IL-9 blocking is able to enhance both melanoma and lung cancer growth [86]. Conversely, pro-tumourigenic roles of T_H9/IL-9 in lymphoma and gastric cancer have been described [88–90]. In fact, IL-9 can also induce immunosuppressive responses from T_{Reg} [91]. Thus, the context of activity may dictate the pro- or anti-tumour effect of T_H9 cells.

3.2.7 T Helper Type 22

T_H22 cells and IL-22 have been shown to be tumour-promoting in the TIME and have

therefore been suggested as potential immunotherapy targets [92–95]. When IL-22 is overexpressed in a mouse model of liver cancer, increased proliferation of the cancer cells was observed [92, 93]. Similarly, reduced proliferation in IL-22-deficient mice was demonstrated. In colon cancer, IL-22 can promote proliferation and stem cell-like features through the activation of STAT3 signalling and consequent epigenetic modification on development-related genes [96]. In breast and lung cancer models, it was demonstrated that IL-22 secretion from CD4+ memory T cells to support the tumour cell proliferation can be induced through NLRP3 inflammasome activation and release of IL-1β from immune cells [94]. In patients, T_H22 cells and IL-22 have been found in the primary tumour, serum, and malignant pleural effusion of these patients [97, 98].

3.3 Regulation of T Helper Cell Homing and Differentiation in the TIME

The process of T-cell maturation from a naïve T_H in the thymus involves the destruction of self-reactive lymphocytes, but the existence of autoimmunity demonstrates that while T cells do undergo education to promote central tolerance, self-reactive T cells can escape this process [99]. Indeed, self-reactivity is required for the detection and destruction of cancer cells, a crucial immunological function requiring recognition of self-antigen [100]. A delicate balance is therefore required to ensure that the immune system responds appropriately to altered self-like state observed in cancer but also kept under careful control to prevent self-destruction and autoimmunity. This important function is achieved through the co-recruitment of suppressor cells and inhibitory molecules in addition to effector cells during an immune response.

3.3.1 Homing of Regulatory T Cells

An increase of regulatory T cells has been observed in both the peripheral blood and the tumour microenvironment from a wide variety of both solid tumours and haematological cancers such as breast, prostate, lung, and pancreatic cancers, lymphomas, and leukaemias [65, 68, 101–106]. T_{Regs} are recruited to the TIME upon signals delivered by the tumour, mainly CCL22 [1, 69]. Another source of T_{Regs} in the TIME is the conversion of T effector cells into T_{Regs}, through interaction with DCs in the context of high TGFβ and IDO, which are secreted by tumour cells [107].

3.3.2 Dendritic Cells as Key Regulators of TIME Composition

Interactions between specific DC subsets and immature T cells are crucial for generating and maintaining both effector T cells and T_{Regs} [108, 109]. This duality highlights the versatility and variability of DCs and their capacity to shape the immune response [110]. Infiltrating DCs should activate anti-tumour responses, but tumour cells are capable of suppressing DC function and promoting their activity to induce T_{Regs} [111]. Detailed descriptions of the numerous types of DCs are beyond the scope of this chapter, but the capacity to overcome the suppressive tumour microenvironment, recruit T cells into the tumour bed, and activate effector T cell responses appears to be dependent on the chemokines CXCL9 and CXCL10 produced by CD103+DCs [108]. On the contrary, development of T cells into a regulatory phenotype in the tumour microenvironment appears to be mediated by plasmacytoid DCs and dependent on expression of inducible co-stimulatory ligand (ICOS-L) [112]. Naturally occurring T_{Regs} are similarly induced by DCs in the thymus through interactions with CD80 and CD86 controlled by Hassall's corpuscles [113]. Interestingly, CD8+ T_{Regs} also exist in humans and can be induced through interaction with CD40 ligand on plasmacytoid DCs [114]. While targeting T_{Regs} could improve immunotherapy outcomes, the function and activity of T cells are tightly controlled by DCs making them an attractive target for immunotherapeutic approaches [115, 116].

3.4 T Helper Cells in the Context of Immunotherapy

Immunotherapy using antibodies directed against immune checkpoint inhibitors such as PD-1, PD-L1, and CTLA-4, has emerged as a major treatment modality for metastatic cancer in various malignancies, including melanoma and lung cancer.

In addition to release of CD8+ T-cell inhibition, PD-1 blockade also alters T_H cell function. Since PD-1 signalling induces T_H1 cells to transdifferentiate in T_{Regs}, it was supposed that PD-1 blockade would help in reducing the immunosuppressive role of T_{Regs} [117]. Nevertheless, depending on TIME context, it has been shown to stimulate T_{Reg} suppressive signals [118, 119], to impair germinal centre formation and cytokine production by T_{FH} cells [84], and to promote hyperprogression of cancer [119], a pattern of progression that exists in approximately 10% of patients treated with anti-PD-1/PD-L1 [120].

Disrupting CTLA-4 interaction with CD80 induces T_H cell infiltration into tumours [121, 122], particularly a subset of T_H cells with high expression of ICOS and secretion of IFN-gamma [123].

It has been shown in mice studies that anti-CTLA-4 antibodies induce tumour rejection by selective depletion of T_{Regs} in the tumours [124]. Nevertheless, results from human studies are controversial, and it is not yet clear if the depletion of T_{Regs} plays a major role in the clinical setting [122].

Major predictive factors of treatment outcome in anti-CTLA-4 and anti-PD-1/PD-L1 blockade are cancer type, tumour mutational burden, CD8+ T-cell infiltration, and TCR repertoire diversity [125]. Nonetheless, T_H cells have also been studied as biomarkers to predict response to immune checkpoint inhibition. In metastatic melanoma, a high level of pretreatment infiltrating T_{Regs} and tumour infiltration by $ICOS^{high}$ T_H cells during treatment was associated with response to anti-CTLA-4 therapy [126, 127].

3.5 Prognostic Role of T Helper Cells

The density and cell type characterization of tumour-resident T cells have been described in the prognostication of cancer patients [128, 129]. Improved survival was correlated with the presence of these cells in the TIME [129–131]. The integration of CD4+ and CD8+ quantification can improve the prediction of the patient outcome, and the estimation of CD8+/CD4+ ratios is frequently suggested [132, 133]. However, the dual role of some CD4+ lymphocyte subtypes adds more complexity in the immunology response against cancer and should be considered [130]. In general, the presence of interferon-γ-producing CD4+ T_H1 lymphocytes are related to a favourable prognosis, while the presence of other CD4+ subtypes is cancer type-dependent (Table 3.2) [130, 134]. A high T_H2:T_H1 ratio, representing a T_H2 prevalence trend in the TIME, can predict shortened survival in many cancer types [29, 34–38]. In resected colorectal cancer, a high level of T_H1 infiltration and a low level of T_H17 infiltration are associated with prolonged disease-free survival [30]. Interestingly, the integration of the density and location of these cells (tumour core or invasive margin) resulted in better predictions. In oesophageal squamous carcinoma and non-small cell lung cancer, T_H1 infiltration correlates with a better prognosis [135, 136]. Combining T_H1 infiltration assessment to numeration of CTL resulted in added predictability.

High level of T_{Reg} infiltration is a reliable indicator of more aggressive disease in many cancers [7, 137], such as breast [138], gastric [139], head and neck [63], liver [140], lung [141], pancreas [142], and ovary [68]. The prevalence of T_{Regs} in the lymph nodes of patients with non-small cell lung cancer has prognostic value, with 5-year survival rates significantly lower in patients with higher proportions of T_{Regs} present [143]. Another study found increasing prevalence of T_{Regs} in non-small cell lung cancer patients that correlated with disease stage and tumour burden [144]. A

Table 3.2 T helper cells as prognostic factors in tumours

High abundance	Association with clinical outcome	References
T_H1	Favourable prognosis in RCC, CRC, oesophageal squamous carcinoma	[30, 135, 136, 163]
	Response to neoadjuvant chemotherapy in HER2+ and triple-negative breast cancer	[164]
T_{Reg}	Favourable prognosis in CRC	[147–149]
	Poor prognosis in NSCLC, HCC, HNSCC, RCC, MPM, melanoma, and bladder, gastric, pancreatic, breast, and ovarian cancer	[139, 142, 144, 145, 157, 165–175]
	Response to anti-CTLA-4 in melanoma	[127]
T_{FH}	Favourable prognosis in CRC, NSCLC, and breast cancer	[81–83, 151]
T_H17	Poor prognosis in HNSCC and CRC	[30, 176]
T_H22	Poor prognosis in GI tumours	[92, 156, 177]
PD-1^+LAG3^+TIM3^+	Favourable prognosis in MPM	[157]
$ICOS^{high}$	Response to anti-CTLA-4 in melanoma	[126]
TLO	Favourable prognosis in NSCLC and melanoma	[151, 152]

RCC renal cell carcinoma, *CRC* colorectal carcinoma, *NSCLC* non-small cell carcinoma, *HCC* hepatocellular carcinoma, *MPM* malignant pleural mesothelioma, *HNSCC* head and neck squamous cell carcinoma, *GI* gastrointestinal, *TLO* tertiary lymphoid organ.

meta-analysis comprising over 86,000 lung cancer patients showed that the presence of tumour-infiltrating CD4+ cells is associated with better prognosis; however, FOXP3+ cells are a poor prognostic marker [145]. In addition, the enrichment of migrated CD4+ T and CD8+ T lymphocytes in the pleural effusions from lung adenocarcinoma was reported as a good prognosis indicator [146].

Although enrichment of T_{Reg} cells within tumours of various origins can signify poor prognosis, the opposite may be true for colon cancer. For example, T_{Reg} infiltration is frequently described as a good prognostic marker in colorectal cancer [147–149]. However, colorectal carcinoma can be infiltrated in variable ratios by two different types of T_{Regs}, one suppression-competent and other non-suppressive [150]. In fact, the infiltration by predominantly non-suppressive T_{Regs} is related to improved survival in colorectal cancer patients [150].

T_{FH} cell infiltration, measured through the expression of CXCL13, CXCR5, and IL-21, is associated with longer disease-free survival in CRC and breast cancer [82, 83]. Moreover, deletion or dysfunction of CXCL13 correlates with shorter DFS in CRC. The presence of tertiary lymphoid organs (TLOs), which formation is dependent on T_{FH}, is a good outcome predictor in various malignancies [151, 152].

Although T_H9 has been shown to induce potent anti-tumour responses [86, 153, 154], it delivers survival and proliferation signals in lymphoma cells, where high IL-9 is considered a poor prognostic marker [88, 89]. Increase of T_H22 cells was also reported as related with advanced tumour stages, and higher IL-22 expression in the TIME is associated with shorter survival of hepatocellular carcinoma and gastric cancer [155, 156].

Recently, it was shown that tumour-infiltrating CD4+ lymphocytes upregulating molecules responsible for CD8+ T-cell exhaustion (PD-1, LAG-3, and TIM-3) were associated with shorter overall survival in malignant pleural mesothelioma [157]. Similarly, the enrichment of ineffective CD4+ memory T cells in the TIME of follicular lymphoma, due to the lack of co-stimulatory receptors, was correlated with a shorter survival [158].

In addition to the T-cell subtype, the T-cell receptor (TCR) repertoire is related to the outcome of cancer patients [159–162]. Higher intratumour T-cell receptor (TCR) heterogeneity, which is positively correlated with the neoantigen heterogeneity, is associated with an increased lung cancer recurrence risk [159]. TCR heterogeneity from both CD4+ and CD8+ was associ-

ated with a higher recurrence risk in lung adenocarcinoma [160]. Interestingly, TCR diversity was higher in CD4+ compared to CD8+ cells [160].

3.6 Conclusions

T helper cells are key players of the immune system and modulate the efficiency of anti-tumour immune response. Of these, T_{Regs} are an intensively studied subset since their immunosuppressive role has been well documented in other clinical settings. In the cancer setting, they have been extensively studied in the context of their use as therapeutic targets and prognostic biomarkers in various tumours, including lung cancer. In comparison, there is a relative paucity of data on other T_H cell subsets; however, T_{FH} cells have recently attracted interest as regulators of tertiary lymphoid organ organization and B-cell function. As such they may be leveraged to obtain long-term immune response, an elusive goal for most patients even when treated with combined immunotherapy.

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