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The Role of CD4 T Cell Help in CD8 T Cell Differentiation and Function During Chronic Infection and Cancer

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Conflict of Interest
The authors declare no potential conflicts of interest.

Abbreviations
ACT, adoptive cell therapy; BATF, basic leucine zipper transcription factor, ATF-like; CAR, chimeric Ag receptor; cDC1, type 1 conventional dendritic cell; DC, dendritic

ABSTRACT

CD4 and CD8 T cells are key players in the immune response against both pathogenic infections and cancer. CD4 T cells provide help to CD8 T cells via multiple mechanisms, including licensing dendritic cells (DCs), co-stimulation, and cytokine production. During acute infection and vaccination, CD4 T cell help is important for the development of CD8 T cell memory. However, during chronic viral infection and cancer, CD4 helper T cells are critical for the sustained effector CD8 T cell response, through a variety of mechanisms. In this review, we focus on T cell responses in conditions of chronic Ag stimulation, such as chronic viral infection and cancer. In particular, we address the significant role of CD4 T cell help in promoting effector CD8 T cell responses, emerging techniques that can be utilized to further our understanding of how these interactions may take place in the context of tertiary lymphoid structures, and how this key information can be harnessed for therapeutic utility against cancer.

Keywords: CD4 T cells; CD8 T cells; Chronic infection; Cancer; Immunotherapy

INTRODUCTION

CD4 and CD8 T cells are key players in the immune response against both pathogenic infections and cancer. Oftentimes CD4 T cells are considered to take on a more supportive or "helper" function in CD8 T cell-mediated, as well as humoral, Ab-mediated, immunity. During acute infection and vaccination, CD4 T cell help is important for the development of CD8 T cell memory. However, during chronic viral infection, CD4 T helper cells are critical for the sustained effector CD8 T cell response, through a variety of mechanisms. In this review, we focus on T cell responses in conditions of chronic Ag stimulation, such as chronic viral infection and cancer. In particular, we address the significant role of CD4 T cell help in promoting effector CD8 T cell responses, emerging techniques that can be utilized to further our understanding of how these interactions may take place in the context of tertiary lymphoid structures (TLSs), and how this key information can be harnessed for therapeutic utility against cancer.

cell; GC, germinal center; HPV, human papillomavirus; ICB, immune checkpoint blockade; ICOS, inducible costimulatory; LCMV, lymphocytic choriomeningitis virus; PDAC, pancreatic ductal adenocarcinoma; Tfh, T follicular helper; TIL, tumor-infiltrating lymphocyte; TLS, tertiary lymphoid structure; TME, tumor microenvironment.

Author Contributions

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CONCEPTS OF CD4 T CELL HELP

Although it is known that CD4 T cells help CD8 T cells, precisely when, where and by what mechanisms this "help" takes place, continues to be of great scientific interest (1). CD4 T cells provide help via multiple mechanisms that guide the CD8 T cell response during chronic viral infection and cancer. Such mechanisms may include CD4 T cell licensing of dendritic cells (DCs), co-stimulation, and cytokine production.

CD4 T cell help during acute infection and vaccination

One of the critical mechanisms by which CD4 T cells provide help to CD8 T cells is via DC licensing and cross-presentation. Priming of Ag-specific CD8 T cells appears to take place in a two-step process (2). *In vivo* imaging found that upon infection or immunization, CD4 and CD8 T cells undergo their first priming steps independently and in different regions of lymphoid tissues (2-5). During the second step of priming, both Ag-specific CD4 and CD8 T cells interact with the same cross-presenting (6), lymph node-resident XCR1$^+$ type 1 conventional dendritic cells (cDC1) (7,8). Cross-presentation takes place when the same Ag-presenting dendritic cell that presents endocytosed Ags on MHC class II surface molecules presents endocytosed Ags via MHC class I molecules, as well (9). Interestingly, a recent study found that CD4 T cells license cDC1 cells, which are necessary for priming both CD8 and CD4 T cells (10). Consistently, deficiency of XCR1$^+$ cDC1s results in aberrant memory CD8 T cell formation following viral infection (8). Interaction between CD40L, expressed on CD4 T cells, and CD40 on cDC1 cells signals DCs (11,12) to enhance Ag presentation and expression of costimulatory molecules (13), revealing that CD4 T cells deliver help to CD8 T cells via interaction with DCs (14). Additionally, recent pre-clinical studies have shown the utility of CD40 agonist Abs in combination with immune checkpoint blockade (ICB) as a cancer therapeutic (15-17). In addition to co-stimulation, during this second step of priming, the production of key cytokines such as type 1 IFNs, IL-12, IL-15, and IL-2 by CD4 T cells and cDC1s is also important for driving effector and memory CD8 T cell differentiation and survival (2,18,19).

In the absence of CD4 T cell help, CD8 T cells have a cell-intrinsic deficiency for secondary expansion; thus, help from CD4 T cells is important in generating an optimal CD8 T cell memory response (20-26). In addition, an immunization model found that, without CD4 T cell help, CD8 T cells increase their expression of inhibitory molecules, as a result, developing a transcriptional profile that resembles exhausted CD8 T cells found in chronic infection (27-29). Collectively, these findings substantiate the significant role of helper T cells in supporting and enhancing the CD8 T cell response during immunization or viral infection.

CD4 T cell help during chronic infection

CD4 T cells play a significant role in helping maintain CD8 T cells and their response in many models of chronic infection (30-34). Early studies using genetic knockout or depletion of CD4 T cells during chronic lymphocytic choriomeningitis virus (LCMV) infection suggested that CD4 T cells are critical in sustaining the CD8 T cell response (35,36). Additionally, in the absence of CD4 T cell "help," CD8 T cells enter a dysfunctional state, losing their cytotoxic capacity for viral control (30,35,36).

The secretion of IL-21 by CD4 T cells was found to be one of the major mechanisms by which CD4 T cells help CD8 T cells maintain their functionality and facilitate viral control during chronic infection (37-39). Consistently, in patients with chronic viral infections, such as HIV and hepatitis C virus, CD4 T cell production of IL-21 often positively correlates with CD8 T

cell function and improved viral control (40-43), suggesting that the IL-21 pathway may hold therapeutic potential.

During viral infection, there are several subsets of CD4 T cells that can produce IL-21, including T follicular helper (Tfh) cells and Th1 cells (25,44,45). Persistent viral infection may promote CD4 T cell differentiation towards Tfh cells (46,47). This differentiation of CD4 T cells towards Tfh cells may be due to their critical role in the germinal center (GC) response (48,49), because resolution of the viremic phase of LCMV Cl13 chronic viral infection critically depends on Ab production (46,47,50,51). In addition to helping CD8 T cells, IL-21 produced by CD4 T cells, specifically Tfh cells, is necessary for GC responses; the absence of IL-21 results in impaired GC maintenance, reduced affinity maturation by B cells, and isotype class switching (52-54). Therefore, CD4 T cell help during chronic infection is critical for both the CD8 T cell-mediated response, as well as the humoral response to control persistent viral infection.

CD4 T cell help affects CD8 T cell heterogeneity

Under chronic Ag stimulation, such as chronic viral infection and cancer, CD8 T cells were suspected to progressively differentiate towards an exhausted state, which is counterproductive in fighting viral infections or preventing tumor growth (55). Due to advancements in biotechnology, identification of CD8 T cell heterogeneity in response to chronic viral infection was defined at single-cell resolution. At least three phenotypically, functionally, and epigenetically distinct CD8 T cell subsets have been identified in response to chronic viral infection. Progenitor Ly108^{hi} TCF-1^{hi} CD8 T cells are a precursor population with self-renewing abilities (56-61). During early infection, progenitor CD8 T cells undergo a bifurcation pathway that can give rise to either terminally differentiated "exhausted" PD-1^{hi} CD8 T cells or, with the help of IL-21 produced by CD4 T cells (62), progenitor cells can give rise to effector CX$_3$CR1^{hi} CD8 T cells, which maintain their functional capacity to control viral infection (62-68). In particular, the effector CX$_3$CR1^{hi} CD8 T cell subset exhibits augmented cytolytic function and expression of effector molecules, such as granzyme B (**Fig. 1**) (62,63,69).

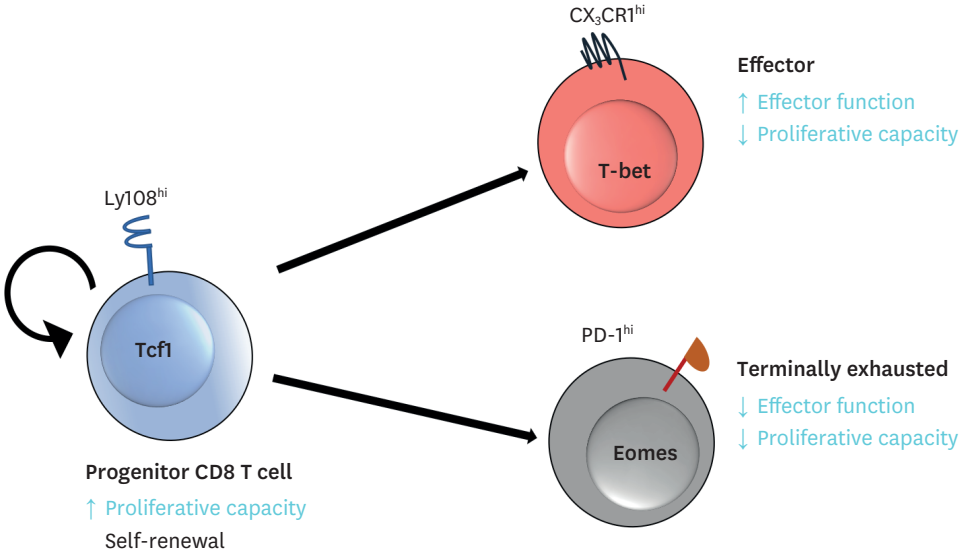

Figure 1. Model of CD8 T cell heterogeneity during chronic antigen stimulation. Progenitor Ly108^{hi} TCF-1^{hi} CD8 T cells maintain a proliferative capacity for self-renewal, while also giving rise to effector CX$_3$CR1^{hi} CD8 T cells or "exhausted" PD-1^{hi} CD8 T cells.

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On the other hand, during cancer, the tumor microenvironment (TME) can be immunosuppressive, with Tregs taking up a significant proportion of CD4 T cells found in some tumors (70-73). Interestingly, in a preclinical melanoma model, CD8 T cells were found to primarily exist in two differentiation states within the TME, either terminally exhausted or progenitor-like (66), which resemble an immunological state similar to "unhelped" CD8 T cells during chronic LCMV infection (62,65). In addition, a recent study of CD8 T cells in human papillomavirus (HPV)-positive head and neck cancer also identified $PD1^+$ stem-like CD8 T cells in the TME that resembles progenitor-like cells that contribute to the immune response against tumor cells (74). Our recently published work has shown that when CD4 T cell help is removed via CD4 depletion, the development of CX_3CR1^+ effector CD8 T cells during chronic LCMV infection is abrogated (62), thus mimicking the TME, where CD4 T cell help is insufficient.

CD4 T cells during cancer

In cancer, CD4 T cell help has been found to promote antitumor CD8 T cell responses, whereas Tregs suppress such responses (71,75,76). Interestingly, expression of MHC class II on the surface of tumor cells, may be associated with improved progression-free survival and overall survival following immunotherapy (77-79). In addition, MHC class II expression on tumor cells was also associated with increased numbers of CD4 and CD8 tumor-infiltrating lymphocytes (TILs), increased TLS formation, and higher expression of IFN-γ, IL-2 and IL-12, and Th1-associated cytokines (80). Some CD4 T cells may even have a direct ability to recognize and kill target cells (81-85). Thus, CD4 T cells may play an important role, both directly and indirectly, in the antitumor response.

Meanwhile, Tregs make up a major component of immune cells found in the immunosuppressive TME (70,71). Tregs may inhibit antitumor activity and are associated with poor prognosis in cancer patients (86,87). Importantly, Tregs maintain their suppressive activity through the production of various immunosuppressive cytokines (IL-10, TGF-β, and IL-35), as well as their surface expression of immunosuppressive receptors (lymphocyte activation gene-3, T-cell immunoglobulin and ITIM domain (TIGIT), CTLA4, and PD-1), which can inhibit effector T cells (88-92). In addition, intra-tumoral Tregs may also interact with dendritic cells to suppress CD80 and CD86 expression, which enhances expression of inhibitory receptor, thus pushing CD8 T cells towards a dysfunctional state (75). Unlike Tregs, helper CD4 T cells are important in maintaining CD8 T cell recruitment, proliferation, and effector function in some models of cancer (93-96). Thus, harnessing the appropriate helper CD4 T cell support may be important in preventing CD8 T cell dysfunction. The following section describes potential applications of CD4 T cell help in enhancing and sustaining effector CD8 T cells.

APPLICATIONS OF CD4 T CELLS

Cancer immunotherapy has made great strides in improving patient outcomes, especially with hematologic malignancies. However, cancer immunotherapy efficacy in treating solid tumors remains limited. A major reason for the setback in treating solid tumors is that TILs often differentiate into dysfunctional states, resembling exhausted T cells that arise during chronic viral infections (97-99). In addition to upregulation of inhibitory molecules such as PD-1, the dysfunctional state of exhaustion in T cells is characterized by diminished effector function, namely decreased cytotoxic activity and reduced secretion of effector molecules, such as

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granzyme B and IFN-γ (28,97), resulting in reduced antitumor activity. These dysfunctional TILs are ineffective at killing tumor cells, in part due to epigenetic imprinting that maintains them in an exhausted state of differentiation (100-102). Thus, it is important to understand the cellular and molecular mechanisms that regulate CD8 T cell differentiation in the setting of cancer. Addressing this critical knowledge gap will be essential in developing novel strategies that enhance and restore CD8 T cell effector function within the TME. Applying our current knowledge of CD4 T cell help in generating robust CD8 T cell responses, we will discuss the immunotherapeutic potential of applying these mechanisms of CD4 T cell help against cancer.

ICB

One of the major advances in cancer treatment has been the use of monoclonal Abs to block immune regulatory checkpoints, such as PD-1 and CTLA4 (34,103,104). ICB therapy targets inhibitory receptors, such as PD-1, which are upregulated on the surface of dysfunction tumor-infiltrating T cells (105) in an effort to maintain the T cell response against cancer. Studies have shown that chronic antigenic exposure of T cells results in continuous PD-1 signaling, which epigenetically programs T cell exhaustion (106,107). However, it was recently observed that PD-1 therapy preferentially supports the proliferative burst of a specialized subset of PD-1$^+$CXCR5$^+$TCF1$^+$ CD8 T cells, in a chronic LCMV infection model (60). More recent studies have recapitulated these findings in cancer models, showing that these stem-like, or progenitor, TCF1$^+$PD-1$^+$ CD8 T cells are preferentially targeted in response to checkpoint blockade immunotherapy (66,108,109). Taken together, these findings suggest that PD-1 therapy supports progenitor CD8 T cells in the tumor, allowing them to continue proliferating and give rise to more effector-like cells in order to control tumors. However, not all patients respond to ICB therapy, and about a third of them relapse (103). Therefore, there has been increased interest in using combination therapies to boost responsiveness to cancer immunotherapies.

Recent studies have shown that the antitumor response to ICB therapy requires the response of both tumor Ag-specific CD8 and CD4 T cells (110). In particular, MHC-II neoantigens are crucial for activating CD4$^+$ T cells, which are important for generating functional CD8 T cells in response to checkpoint blockade immunotherapy (110). Interestingly, a study found that enhanced CD4 T cell responses are one of the underlying mechanisms of anti-CTLA-4 blockade (104). Additionally, clinical studies have shown that CD4 T cell populations may determine responsiveness to ICB (111-113). Thus, methods of enhancing the tumor-specific CD4 T helper cell population may provide insight into improving ICB therapy responsiveness.

Adoptive cell therapy (ACT)

ACT entails using tumor-specific cells, typically TILs or genetically engineered chimeric Ag receptor (CAR)-expressing T cells, expanding them *ex vivo*, and then infusing them back into a lymphodepleted patient (114). As previously reported, ACT along with administration of IL-2 can lead to prolonged eradication of tumors in cancer patients that have exhausted other treatment options (115-119). Adoptively transferred TILs typically consist of a mixture of CD4 and CD8 T cells, however most studies in the field of cancer immunotherapy focus on CD8 T cells (120). In a previous study, mutation-specific CD4 T cells from a patient were expanded and adoptively transferred back, resulting in tumor regression (121), which warrants further exploration into the mechanisms of the CD4 T cell response against cancer.

More recently, adoptive cell transfer of autologous, genetically engineered T cells to target tumor Ags has provided a breakthrough treatment for hematological malignancies (122). CAR-T cells are genetically engineered T cell receptors that reprogram T cells to target tumor-

associated Ags (123). Although this cutting-edge technology is efficacious for some patients, 30%–60% of patients relapse after CAR treatment (124). Evidence shows that the cellular composition and immunophenotypes of CAR-T cells is instrumental for therapeutic efficacy (125). In particular, the ratio of CD4 to CD8 T cells may play a role in the antitumor response of CAR-T cells (126). A 2016 clinical trial reported that a 1:1 ratio of CD4 to CD8 T cells during CAR-T manufacturing resulted in high remission rates among B-cell acute lymphoblastic leukemia patients undergoing CAR-T cell therapy (126). This is congruent with preclinical CAR-T therapy studies that found CD4 T cell help induces CD8 T cell memory function, which plays a role in antitumor responses (127). In addition, different subsets of CD4 helper T cells may also affect the effector response of CAR-T therapy. Interestingly, CAR containing the inducible costimulatory (ICOS) intracellular domain redirected CD4 T cells towards a Th17 phenotype with augmented effector function and persistence of CAR-T cells in the circulation (128). Furthermore, CD4 T helper cells play a clinically important role in CAR-T therapy, as observed in a recent study which showed that CD4 CAR-T cells persist in decade-long leukemia, and continue to exhibit functional characteristics of activation, proliferation, and cytotoxicity (129). Thus, CAR CD4 T cells not only facilitate CD8 T cell effector functions but may have the potential for direct cytotoxicity against tumor cells.

CD4 T cells are widely known to assist cytotoxic CD8 T cells (71,130) and help in conferring a cytotoxic T cell effector program (2,131). In addition, ACT of CD4 helper T cells has shown promise in multiple cancer immunotherapy preclinical models (84,132-134), where cells may function as a 'living drug,' by providing costimulatory signals and continued cytokine production (120). Notably, Th17 and Th9 cells may be more effective than Th1 cells in limiting tumor progression (135). Interestingly, IL-21 is a commonly produced cytokine by both Th9 and Th17 T helper cell subsets, which may play an important role in their response to cancer (62,136,137). However, the precise mechanisms by which helper CD4 T cells mediate anti-tumor responses remains under investigation.

MECHANISMS OF CD4 T CELL HELP

IL-21-mediated CD4 T cell help

CD4 T cells are one of the predominant producers of IL-21 (138). Accumulating data reveals that IL-21 signaling on CD8 T cells is vital for their sustained function and control of chronic viral infection (37-39). Additionally, IL-21 treatment of both human and mouse tumor-specific CD8 T cells, resulted in enhanced longevity and anti-tumor activity of CD8 T cells *in vivo* (139-141), supporting the notion that CD4 T cell help via their production of IL-21 may enhance effector CD8 T cell responses. Notably, our pre-clinical studies have shown that adoptive transfer of IL-21-producing CD4 T cell help increases the intra-tumoral effector CX_3CR1^+ CD8 T cell population, subsequently correlating with reduced tumor burden (62). Thus, harnessing and enhancing CD4 T cell help may hold the answer to combating T cell exhaustion during chronic viral infection and cancer.

Our laboratory has previously shown that IL-21 signaling, through STAT3 induces basic leucine zipper transcription factor, ATF-like (BATF) activation in $CD8^+$ T cells resulting in sustained $CD8^+$ T cell survival and effector function during chronic viral infection (142), further corroborating the importance of this finding in a preclinical melanoma model (143). BATF cooperatively binds to other transcription factors, which remodel the chromatin landscape to produce changes in chromatin accessibility (144). Through these changes in

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the chromatin landscape, BATF promotes the differentiation and function of several types of immune cells, including $CD8^+$ T cells (145-152). Most recently, our lab found that BATF is required in maintaining a permissive chromatin structure, which may allow for the transition from progenitor $TCF\text{-}1^+$ $CD8^+$ T cells to effector CX_3CR1^+ $CD8^+$ T cells in a chronic viral infection model (153). Additionally, another study in a preclinical tumor model also showed that BATF may improve CAR T cell antitumor responses by skewing their transcriptional profiles towards a effector phenotype (154). Taken together, these findings support our current knowledge of IL-21-producing CD4 T cell help, via the IL-21-BATF pathway, as being critical for the progenitor to effector differentiation of CD8 T cells, and suggest the potential utility of $IL\text{-}21^+$ CD4 T cell ACT as a cancer immunotherapeutic.

Cellular localization in chronic infection and cancer

Multiple subsets of CD4 T cells are capable of producing IL-21, however it remained unknown until recently, whether a specific subset may be the primary "helper" of these effector CD8 T cell responses (45). We found that Tfh cells may be the main IL-21 producers critical for sustaining these effector CD8 T cell responses during chronic infection (45,155). Likewise, a recent study by Cui et al. (156) found a correlation of Tfh cell and GC B cell transcriptional signatures in tumors of lung adenocarcinoma patients, which also positively correlated with prolonged survival. Meanwhile, in their preclinical lung adenocarcinoma model, which expresses neoantigens for both T and B cells, they showed that interactions between tumor-specific GC B cells and Tfh cells, along with IL-21 produced by Tfh cells, are necessary for effector CD8 T cell function and tumor control (156). Another study also showed that MHC II-expressing cells may provide niches for maintaining the progenitor subset of CD8 T cells (157). Additionally, the preferential localization of the progenitor subset of CD8 T cells in lymphoid-like stromal areas has also been observed in head and neck cancer (74). Interestingly, our own recent findings, using spatial transcriptomics, support the potential colocalization of B cells, IL-21-producing Tfh cells, and progenitor CD8 T cells, which are the likely responders to IL-21-mediated help (**Fig. 2**) (155). Taken together, these studies suggest that the organized

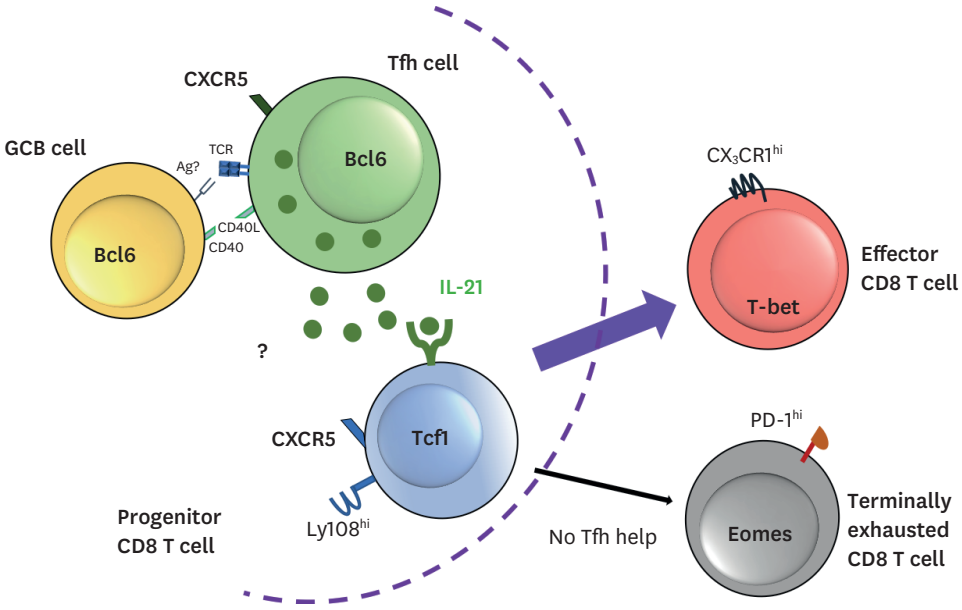

Figure 2. Hypothetical model depicting colocalization of GC B, Tfh, and progenitor CD8 T cells within organized lymphoid structures. GC B cells, and IL-21 producing T follicular helper cell colocalization with progenitor CD8 T cells help facilitate progenitor to effector CD8 T cell differentiation. Importantly, this colocalization may take place within TLSs.

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interactions of B cells and Tfh cells with CD8 T cells may be a conserved feature across chronic inflammatory states, such as cancer and chronic infection/inflammation, during which the formation of TLSs may arise (158). In the remainder of this section, we will further discuss TLS in the context of cancer and its correlation with therapeutic efficacy.

It has been known that Tfh cells provide essential help signals for B cells to facilitate GC reactions, isotype class switching, production of high-affinity Abs, and generation of memory B cells and long-lived plasma cells in the context of infection and vaccination (48,49). However, it has become increasingly clear that the presence of Tfh cells (159-161) and B cells (162-170) is correlated with prolonged survival, as well as positive therapeutic responsiveness in patients with a wide variety of cancers (164,165,168). These correlations are particularly strong when B cells and Tfh cells are found in TLSs, which may be important in facilitating their interactions (171-173). A recent study found that in epidermal growth factor receptor-mutant lung cancer patients who have an unfavorable response to anti-PD-1 therapy, there is a disruption in the cooperative interactions between Tfh, B cells, and resident-memory CD8 T cells. This dysregulation may contribute to the reduced effectiveness of immunotherapy in this specific subset of lung cancer patients (174).

TLSs are postnatal ectopic lymphoid formations, consisting of organized aggregates of immune cells that arise in chronically inflamed disease states, such as cancer (158), chronic inflammation/infection (175,176), autoimmune diseases (177-179), and in other immune responses. The presence of TLSs has been reported in multiple types of cancers, including but not limited to breast cancer (161,162), non-small cell lung cancer (163,180,181), head and neck squamous cell carcinoma (160,170,171), colorectal cancer (182,183), gastric cancer (166), ovarian cancer (167,169), and melanoma (164,184). Interestingly, conventional therapies to treat cancer may include corticosteroids, which are often administered alongside chemotherapy or immunotherapy to reduce side effects, resulting in a dampening of the immune response. Corticosteroid administration in cancer patients has been shown to impair TLS formation and maturation, resulting in GC loss (185,186). These findings should be further explored, as steroids, which induce immunosuppression, may play a detrimental role in dampening TLS formation and, in turn, therapeutic efficacy.

Some studies using ICB immunotherapy to treat patients with non-small cell lung cancer (187), or urothelial cancer (185), found an increase in TLSs in responsive or regressing lesions, indicating that TLSs may harbor immune cells that are responsive to ICB therapy. It is important to note that dysfunctional or "exhausted" PD-1^{high} CD8 T cells may also localize within TLSs, near Tfh cells and B cells, and could help predict responsiveness to PD-1 blockade therapy (188). As previously observed, in a chronic LCMV infection model (60) and recapitulated in cancer (66,108,109), blockade of PD-1 may support the proliferative burst of PD-1^{+}CXCR5^{+}TCF1^{+} CD8 T cells, also recognized as progenitor CD8 T cells. Taken together, this suggests that TLSs may facilitate the environment necessary for progenitor CD8 T cells to respond to ICB therapy by proliferating and giving rise to effector CD8 T cells.

Future studies should be conducted in cancer models, including patient tumor samples, to determine if progenitor CD8 T cells and Tfh cells are found colocalizing within intra-tumoral TLSs, and whether such colocalizations may be associated with enhanced responsiveness of patients to ICB therapy. Furthermore, as TLS presence is associated with patient responsiveness to certain therapeutics, attempts should be made at elucidating methods to initiate or facilitate TLS formation within tumors.

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TLSs as prognostic and therapeutic targets

Recent studies have shown evidence of therapeutically-induced TLSs that are associated with tumor control in preclinical models of neuroendocrine pancreatic cancer and breast cancer (158,189,190). In these studies, PD-L1 blockade was used in combination with anti-angiogenic therapies, which resulted in tumor blood vessel transformation into high endothelial venules, TLS formation, enhanced CD8 T cell infiltration and activity, as well as tumor destruction (158,189,190). Additionally, in a preclinical model of colorectal cancer used immunogenic intestinal bacteria to induce an immune response and found that Tfh cells drove TLS formation and tumor control (191). In another study, pancreatic ductal adenocarcinoma (PDAC) patients were therapeutically vaccinated with GVAX, an irradiated allogeneic GM-CSF-secreting PDAC tumor vaccine, and cyclophosphamide (to deplete regulatory T cells), which resulted in a conversion of "nonimmunogenic" PDAC neoplasms, into "immunogenic" neoplasm, with increased TLS formation in the majority of patients (192). These studies suggest potential mechanisms that can be harnessed to induce TLSs in cancer, which may augment ICB and ACT to target "non-responders."

CONCLUSION

CD4 T cells assist in CD8 T cell effector responses during states of chronic Ag stimulation, such as chronic infection and cancer. Specifically, CD4 T cell help via IL-21 secretion, is critical to facilitating effective CD8 T cell responses in such conditions. Our recent studies discovered Tfh cells as the primary IL-21-producing CD4 T cells that provide this necessary support to CD8 T cells during chronic infection. Additionally, others have further corroborated the importance of IL-21-producing Tfh cells in the cancer model, as well as the necessity of Tfh and GC B cell interactions in effector CD8 T cell responses resulting in tumor control. Interestingly, the presence of TLSs during chronic inflammatory states, including cancer, further suggests the important interplay of these key lymphocytes. Furthermore, there is often a strong, positive correlation between the presence of TLS and anti-cancer therapeutic efficacy, especially when Tfh and B cells are found in TLSs. Therefore, understanding and enhancing CD4 T cell helper mechanisms may harness powerful therapeutic potential against cancer.

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