CD4 T-Cell Exhaustion: Does It Exist and What Are Its Roles in Cancer?

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ABSTRACT

In chronic infections and in cancer, persistent antigen stimulation under suboptimal conditions can lead to the induction of T-cell exhaustion. Exhausted T cells are characterized by an increased expression of inhibitory markers and a progressive and hierarchical loss of function. Although cancer-induced exhaustion in CD8 T cells has been well-characterized and identified as a therapeutic target (i.e., via checkpoint inhibition), in-depth analyses of exhaustion in other immune cell types, including CD4 T cells, is wanting. While perhaps attributable to the contextual discovery of exhaustion amidst chronic viral infection, the lack of thorough inquiry into CD4 T-cell exhaustion is particularly surprising given their important role in orchestrating immune responses through T-helper and direct cytotoxic functions. Current work suggests that CD4 T-cell exhaustion may indeed be prevalent, and as CD4 T cells have been implicated in various disease pathologies, such exhaustion is likely to be clinically relevant. Defining phenotypic exhaustion in the various CD4 T-cell subsets and how it influences immune responses and disease severity will be crucial to understanding collective immune dysfunction in a variety of pathologies. In this review, we will discuss mechanistic and clinical evidence for CD4 T-cell exhaustion in cancer. Further insight into the derivation and manifestation of exhaustive processes in CD4 T cells could reveal novel therapeutic targets to abrogate CD4 T-cell exhaustion in cancer and induce a robust antitumor immune response.

Introduction

T-cell dysfunction can strongly impact both physiologic and pathologic states. Among the known modes of T-cell dysfunction, exhaustion has garnered an increasing degree of recent attention. As exhaustion was initially described as a hyporesponsive T-cell state in chronic lymphocytic choriomeningitis viral (LCMV) infections (1–3), significant effort was initially aimed at characterizing exhaustion in virus-combating CD8 T cells, specifically. A hallmark of mice exposed to chronic infection with LCMV Clone-13, exhaustion has come to encompass a broad state of CD8 T-cell dysfunction resulting from persistent antigen exposure under suboptimal conditions (4), including inadequate CD4 T-cell help (5–7). It has evolved as a transcriptionally programmed and host-adaptive state designed to limit collateral immunologic damage in conditions of failed pathogen clearance and continued antigen exposure, establishing a "stalemate" of sorts between host and pathogen.

More recently, exhaustion has become an acknowledged mode of T-cell dysfunction in cancer as well (8, 9). Importantly, the upregulation of exhaustion-demarcating immune checkpoints by T cells has been associated with the development of tumor resistance to checkpoint blockade therapies (10). Although restoration of exhausted CD8 T-cell function is a primary goal for checkpoint inhibition, CD4 T cells are also liable to suffer exhaustion and contribute to rejuvenation of the antitumor immune response after checkpoint blockade. However, thorough investigations into the definition, prevalence, and mechanisms of CD4 exhaustion remain lacking. This presents a gap in our understanding of the summative immune dysfunction characterizing a number of disease states where CD4 T-cell function is relevant, including cancer.

CD4 T cells perform a wide variety of functions within the adaptive immune system and are best known for their role as T helper (Th) cells, including Th1, Th2, Th17, and regulatory T-cell (T_{reg}) subsets. Importantly, CD4 T cells license dendritic cells (DC) to allow optimal priming of CD8 T cells, provide key signals for antibody class switching, promote bactericidal activity of phagocytes, recruit neutrophils, influence angiogenesis, and secrete cytokines, in addition to perhaps possessing direct cytotoxic functions (**Fig. 1**; refs. 11–13). Likewise, CD4 T cells appear to possess significant plasticity, allowing subsets to transition between one another, broadening their functional impact (14).

CD4 T cells are strongly implicated in the development of antitumor responses (**Table 1**), as they can enhance tumoricidal activity of other antitumor effector cells, such as CD8 T cells and macrophages (6, 15, 16). Some CD4 subsets, particularly Th2 and T_{regs}, are known to negatively affect the antitumor response by decreasing antigen presentation and dampening T-cell effector functions, respectively. Furthermore, certain CD4 T cells appear able to directly lyse tumor cells (11, 12), and adoptive transfer of tumor-specific CD4 T cells alone has demonstrated impressive efficacy in some studies (17). Direct tumor cell recognition and killing by CD4 T cells requires class II major histocompatibility complex (MHC), and overexpression of class II MHC transactivator (CIITA) on murine mammary adenocarcinoma cells increased interferon-gamma (IFNγ) and granzyme B production in CD4 T cells and restricted tumor growth (18). These studies remain controversial, however, as many tumor cells will not have the antigen presentation machinery required to properly load

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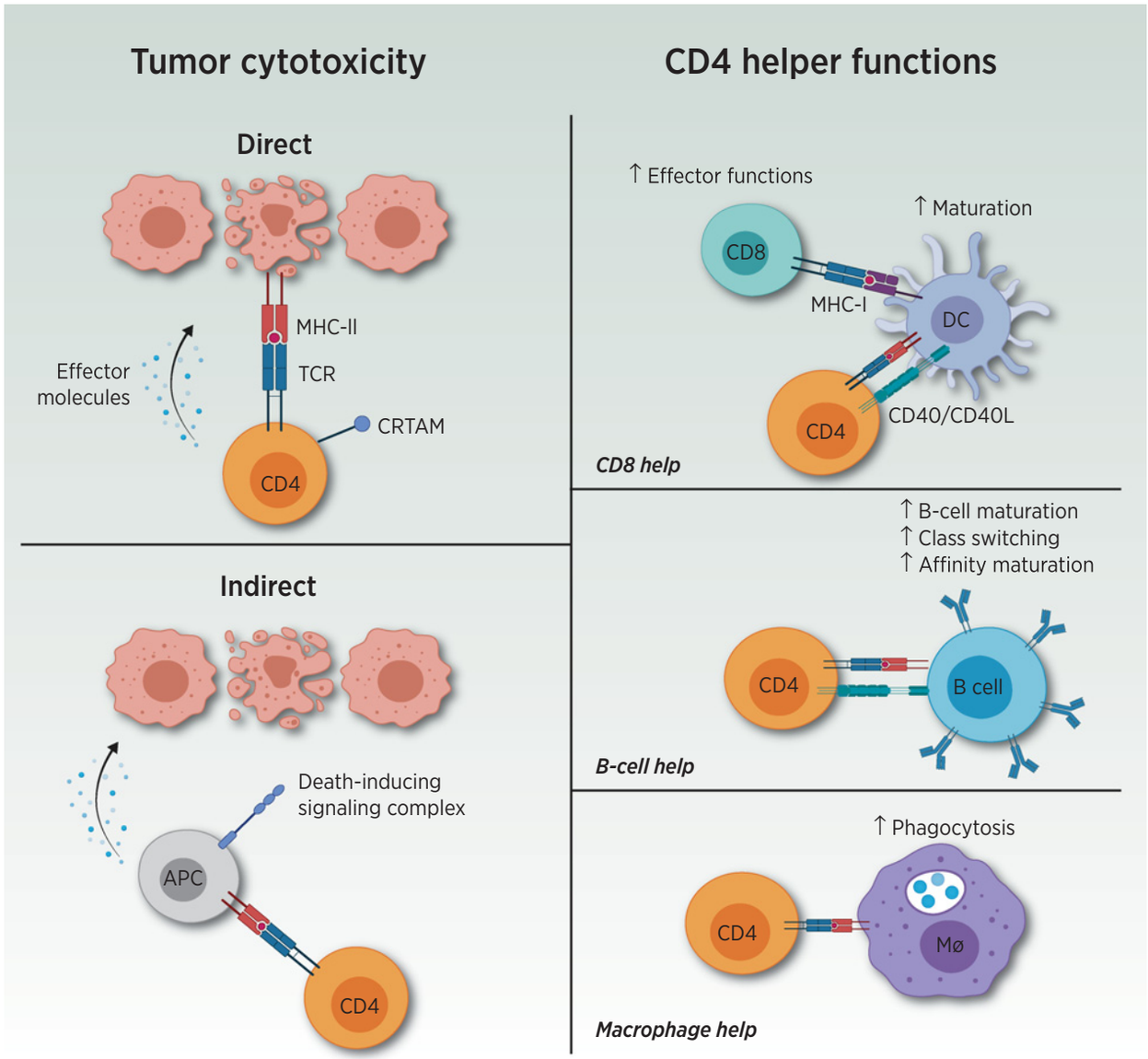

Figure 1.

Overview of CD4 T-cell functions. CD4 T cells are most well known for their Th cell functions (displayed on the right). Through recognition of the TCR of the peptide-MHC complex, CD4 T cells mediate increased maturation and activation of DCs. This process allows augmented CD8 T-cell effectors upon interaction with the activated DCs. Furthermore, CD4 T cells increase B-cell maturation, antibody class switching, and affinity maturation, and enhance phagocytosis within macrophages (Mϕ). Aside from helper functions, CD4 T cells possess both direct and indirect tumor cytotoxicity capacities (displayed on the left). Direct cytotoxicity was demonstrated by cytotoxic CD4 T expressing class I–restricted T-cell–associated molecule (CRTAM). Indirect cytotoxicity could also be guided by CD4 T cells through interaction with antigen-presenting cells (APC) or natural killer cells. Adapted from an image created with BioRender.com.

peptides onto MHC-II. Nevertheless, CD4 T cells with a cytotoxic transcriptional profile have been found enriched in patients responding to immune checkpoint blockade (19).

Given the diverse repertoire of CD4 T-cell capacities, dysfunction in this compartment is assuredly relevant. In this review, we will discuss the current best evidence for the delineation and significance of CD4 T-cell exhaustion in cancer. A brief overview of CD4 T-cell exhaustion in chronic infections, transplantation, and autoimmune diseases will also provide context across pertinent pathologies. Understanding these processes is anticipated to aid in identifying novel therapeutic targets and considerations for improving the antitumor responses.

Overview of Exhaustion

The framework for our current understanding of CD4 T-cell exhaustion is generated from the more extensively studied CD8 T-cell exhaustion, elegantly reviewed by McLane and colleagues (20). When antigen clearance fails and exposure is maintained, as in the setting of chronic infection or cancer, an exhausted T-cell phenotype may

Table 1. Overview of CD4 populations and their contributions to tumor immunity.

T-cell subset	Master regulator	Cytokine	Functions within the tumor
Th1	Tbet	IFNγ	• Activate macrophages to phagocytose • Promote recruitment of antigen-presenting cells • Enhance CD8 effector function • Inhibit angiogenesis
Th2	GATA3	IL4, IL5, IL13	• Recruit eosinophils • Inhibit antigen processing by dendritic cells
Th17	RORγt	IL17	• Promote angiogenesis • Recruit neutrophils
Regulatory T-cell	FoxP3	IL10, TGFβ	• Decrease effector functions of tumor-infiltrating T cells
Cytotoxic CD4 T cells	Runx3	Perforin, granzymes	• Direct tumor cytotoxicity

Abbreviations: FoxP3, Forkhead Box P3; GATA3, GATA binding protein 3; IFNγ, interferon gamma; IL, interleukin; RORγt, retinoic acid receptor–related orphan nuclear receptor gamma; Runx3, RUNX Family Transcription Factor 3; Tbet, T-box expressed in T cells; TGFβ, transforming growth factor beta.

emerge. A primary feature of exhausted T cells is the sustained coexpression of multiple inhibitory surface receptors, referred to commonly as immune checkpoints. The function of these checkpoints is to permit protective curbing of T-cell activity following immune activation. The "classical" immune checkpoints include cytotoxic T lymphocyte–associated protein 4 (CTLA4) and programmed cell death protein 1 (PD-1). Newer "alternative" checkpoints include molecules such as T-cell immunoglobulin and mucin-domain containing-3 (TIM3); lymphocyte-activation gene 3 (LAG3); B- and T-lymphocyte attenuator (BTLA); 2B4; T-cell immunoreceptor with Ig and ITIM domains (TIGIT); and SLAM Family Member 6 (SLAMF6; refs. 21, 22). These inhibitory receptors (checkpoints) are known to be expressed on exhausted T cells, with mounting checkpoint expression associated with more severe phenotypes (8, 10). The typical characteristics of CD8 T-cell exhaustion include antigen load–dependent and temporally progressive loss of effector activity (8, 23, 24), loss of proliferative capacity (25, 26), altered expression of transcription factors (27, 28), loss of antigen-independent homeostatic proliferation (29), and modified epigenetic landscapes (30–32) and metabolic requirements (28, 33, 34). In turn, disruption of the PD-1/programmed death ligand 1 (PD-L1) pathway, in particular, has demonstrated the capacity to reverse features of the exhausted phenotype and restore T-cell proliferative and effector function (35).

Recent evidence suggests that the exhausted phenotype in CD8 T cells is not homogeneous and includes lineage spanning, stage-like "progenitor" ($SLAMF6^{+}TIM3^{-}$) and "terminally-differentiated" ($SLAMF6^{-}TIM3^{+}$) subtypes (21, 26), with varied capacities for effector function and proliferation dispersed among the subgroups. Terminally exhausted CD8 T cells are further characterized by higher levels of PD-1 on their surface. Whereas progenitor exhausted CD8 T cells remain capable of co-producing multiple cytokines and can proliferate *in vivo*, terminally exhausted CD8 T cells are limited to single cytokine production and upregulation of granzyme B. Furthermore, only progenitor exhausted subsets are capable of responding to anti–PD-1 treatment (21, 26). SLAMF6-positive CD8 T cells express the transcription factor T-cell factor 1 (TCF1) (21), which has been linked to the preservation of effector functions (36). Loss of TCF1 with concomitant upregulation of multiple coinhibitory receptors is associated with the terminally differentiated exhaustion phenotype and a further decline in effector functions (21) and/or adoption of immunoregulatory function (37). While exhausted CD8 T cells retain the ability to recognize antigen through their T-cell receptor (TCR), antigen exposure fails to elicit a robust, meaningful cytotoxic response (23).

Our current grasp of CD4 T-cell exhaustion is decidedly anemic when compared with the above understanding we have acquired for CD8s. To begin, an accepted definition of CD4 T-cell exhaustion has not yet been established, limiting the capacity to properly assign the term definitively. Much of the research on CD4 T-cell exhaustion to date has focused merely on the expression versus absence of coinhibitory receptors and/or cytokine production, with data being suggestive of an exhausted state (**Fig. 2**). Further research is required to determine whether additional criteria delineating CD8 T-cell exhaustion, including loss of antigen-independent homeostatic proliferation (29, 38, 39), alterations in metabolic profiles (28, 33, 34), and unique epigenetic features (30–32), also apply to exhausted CD4 T cells. Likewise, it will be crucial to understand whether CD4 exhaustion evolves in a similar stage- and lineage-dependent manner to CD8 T cells (30, 31), and whether or not there are differing relative susceptibilities to and impacts for exhaustion in the various CD4 subsets.

Evidence for CD4 T-Cell Exhaustion

Original evidence: chronic infections

As CD8 T-cell exhaustion was first defined in chronic LCMV infection, this remains a logical place to begin when analyzing the evidence for a similar exhausted state among CD4 T cells. Compared with acute infections, chronic LCMV infections induce markedly greater expression of exhaustion-suggestive immune checkpoints on CD4 T cells (40, 41). Upregulation of these same inhibitory receptors typical of CD8 exhaustion has also been identified on CD4 T cells in other chronic and recurrent infections, suggesting an analogous CD4 T-cell exhaustion phenotype (42–44). Similar to what is seen with CD8 T cells, antigen-specific CD4 T cells (45–49) and CD4 T cells from infected tissues (50, 51) express higher levels of the relevant coinhibitory receptors, drawing a parallel role for antigen exposure in the induction of the seemingly matched CD4 exhausted state. Accordingly, increased coinhibitory receptor expression is generally associated with more advanced disease (52–56), while successful disease treatment correlates in turn with reduced expression of the same markers (56–58). However, upregulation of inhibitory markers is not sufficient to call a cell exhausted, as some coinhibitory receptors are also activation markers (59).

Importantly, then, functional deficits are also observed amidst the phenotypically exhausted CD4 T-cell compartment following chronic infection (45, 60–63). In LCMV in particular, CD4 T-cell differentiation in the presence of persistent antigen resulted in upregulation of coinhibitory molecules (62), premature contraction of the

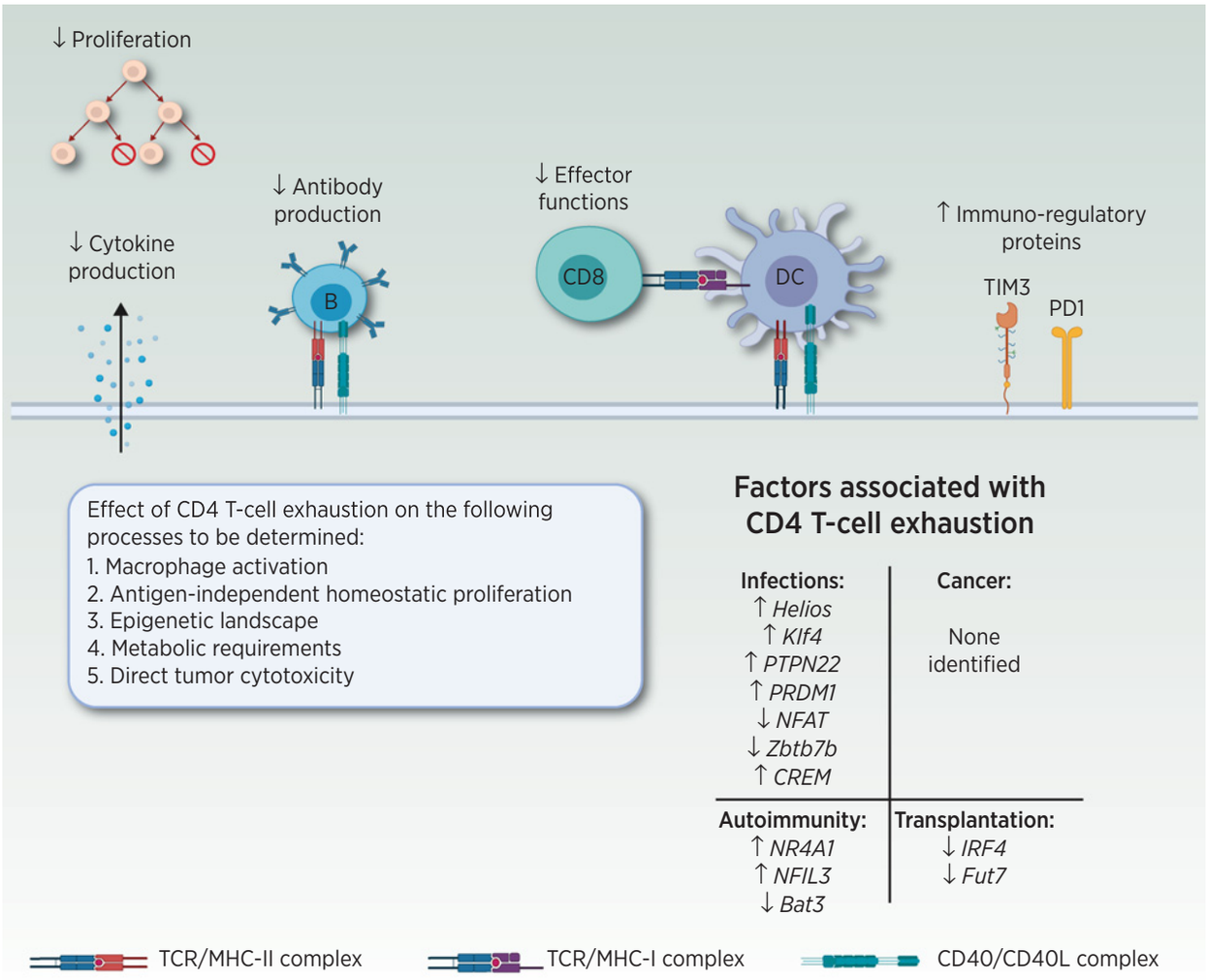

Figure 2.

Consequences of CD4 T-cell exhaustion on CD4 Th functions. Although the details of CD4 T-cell exhaustion remain to be deciphered, negative effects on proliferation, cytokine production, B-cell help, and CD8 effector functions have been reported. In addition, CD4 T cells with reduced effector functions upregulate immune-regulatory proteins, such as T-cell immunoglobulin and mucin domain-3 (TIM3) and PD-1, paralleling phenotypes observed in exhausted CD8 T cells. Whether CD4 T-cell exhaustion negatively impacts macrophage activation and direct tumor cytotoxicity remains to be determined. Further research is required to determine whether loss of antigen independent homeostatic proliferation and alterations in epigenetic and metabolic profiles are features of exhausted CD4 T cells, similar to exhausted CD8 T cells. Abbreviations: Bat3: human leukocyte antigen B (HLA-B)–associated transcript 3; CREM: CAMP responsive element modulator; Fut7: fucosyltransferase 7; IRF4: interferon regulatory factor 4; Klf4: Krüppel-like factor 4; NFAT: nuclear factor of activated T cells; NFIL3: nuclear factor, interleukin 3 regulated; NR4A1: nuclear receptor subfamily 4 group A member 1; PRDM1: PR/SET domain 1 (encodes Blimp1); PTPN22: protein tyrosine phosphatase, non-receptor type 22; Zbtb7b: zinc finger and BTB domain containing 7B (encodes ThPOK). Adapted from an image created with BioRender.com.

antigen-specific immune population (41, 61), reduced cytokine production (41, 61), decreased splenic motility (62), and poor recall responses upon a secondary challenge (61). Reduced CD4 effector functions and increased expression of inhibitory molecules could be induced upon exposure of CD4 T cells to TNF (64) and fibrinogen-like 2 (FGL2; ref. 65). A link between decreased performance of CD4 T cells and exhaustion was established through increased motility and cytokine production following anti–PD-1 treatment (41, 62), although recovery of function was inconsistent (46, 59, 63, 66–68).

Exhaustion is also characterized (and confirmed) by prescribed and stereotyped transcriptional programs. Various studies examining transcriptional programs within CD4 T cells have provided mechanistic insight into factors that may be involved in the establishment of CD4 T-cell exhaustion during LCMV or other chronic infection response. Such studies suggest roles for upregulation of *IKZF2* (encoding Helios; ref. 42), *Klf4* (42), protein tyrosine phosphatase, nonreceptor type 22 (*PTPN22*; ref. 69), cAMP-responsive element modulator (*CREM*; ref. 69), and PR/SET domain 1 (*PRDM1*, encodes Blimp1; ref. 45). Likewise, exhaustion in CD4 T cells has been observed with loss or downregulation of ThPOK (70) and nuclear factor of activated T cells (NFAT; ref. 71).

Not all effector functions of CD4 T cells are necessarily compromised during chronic infections: perforin (72) and granzyme B (73) production in CD4 T cells is increased in patients with HIV when

Table 2. Overview of coinhibitory and costimulatory markers assessed in various primary tumors. Markers were either assessed as single positive or double positive.

Primary tumor	Coinhibitory markers	Costimulatory marker	Main conclusions
Melanoma	PD1, CTLA4, LAG3, TIGIT, TIM3	2B4	• Continuous antigen stimulation decreases antitumor activity (101, 102) • Long-term remission in murine models after αLAG3 + αPDL1 (112, 113)
Lung cancer	PD1, CTLA4, LAG3, BTLA, CD69, TIM3	2B4	• Patients with a high CD4 PD-1+ frequency had decreased overall and progression-free survival, independent of clinical characteristics (145)
GBM	PD1, TIM3, LAG3, CTLA4		• Majority of PD-1+ CD4 T cells were IL7Rα negative (104) • Increased proliferation upon αPD1 observed only in the presence of PD1- CD4 T cells (95) • PD1hi T_{regs} exhibit decreased suppressive functions and increased IFNγ production (114) • Clonal expansion was observed among tumor-infiltrating CD4 T cells (8)
Breast	PD1, TIM3		• αPD-1 treatment increased TCR signaling (97), proliferation (146), cytokine production (98) • Overexpression of MHC-II on tumor cells reduces exhaustion in CD4 T cells and increases tumor control (18)
Head and neck	TIGIT, LAG3, TIM3, PD1, CD69		• αTIGIT delayed tumor progression, although direct effect on CD4 T cells not assessed (103)
Gastrointestinal	PD1, CTLA4, TIM3, LAG3	ICOS	• Checkpoint inhibitors decreased suppressive ability of Tregs (147) and partially restored effector functions (84, 94) • $TIM3^+$ cells expressed increased cycle-dependent kinase inhibitors, preventing cell-cycle entry. αTIM3 restored cell proliferation (100)
AML	PD1, CD57, CD69, CTLA4, TIM3, LAG3	ICOS	• αCD86 and ICOS-ligand prevented the emergence of exhausted CD4 T cells (148)
CLL	PD1, TIM3, TIGIT	2B4, CD226	• αTIGIT impaired IFNγ and IL10 production in the presence of tumor cells the TIGIT ligand (CD155; ref. 111)
Multiple myeloma	PD1, TIM3, CTLA4	CD40L	• Gradual decrease in CD40L expression with more advanced disease (149)

compared with healthy controls, for instance. Interestingly, such regain of cytotoxic function has also been described for "terminally" exhausted CD8 T cells (21). Therefore, the acquisition or retention of these functions might simply indicate varying exhaustion stages, again eliciting similarities with observations made amidst CD8 T-cell exhaustion.

Evidence and significance in cancer

Cytotoxic CD8 T cells promote antitumor immunity that can be correspondingly restricted by their tumor-induced diversion down a pathway toward exhaustion (8, 9, 74–76). Yet, a successful antitumor immune response requires the coordination of a variety of non–T cells constituting the tumor microenvironment (TME), including macrophages, DCs, B cells, and others. Given the role CD4 T cells play in orchestrating the responses by each of these cell types, the potential impact that exhaustion might have amidst the tumor-infiltrating or even systemic CD4 population is substantial. Likewise, the direct cytotoxic role that CD4 T cells can have in mediating antitumor immunity (11–13) makes them a particularly germane population in cancer. As an extension, restoration of exhausted CD4 T-cell function by checkpoint blockade, if feasible, may contribute significant clinical benefit in tumors, either by improving direct CD4 antitumor activity or increasing CD4 helper functions.

Canonical and alternative inhibitory receptors suggestive of exhaustion on CD4 T cells (PD1, CTLA4, LAG3, TIM3, TIGIT) have been identified in multiple solid tumors and hematologic malignancies (77–82) in both humans and mice (**Table 2**). In many cases, similar to that seen with chronic infection, the expression of such checkpoints has been associated with more advanced disease states and diminished progression-free survival (83–85). Furthermore, successful anticancer therapies have been associated with reductions in the level of these markers on the surface of CD4 T cells (86–88), while failure to achieve complete remission and/or disease relapse has positively correlated with their persistent or enhanced expression (86, 89, 90–92).

As with CD8 exhaustion, a number of coinhibitory receptors may also serve to denote T-cell activation (59). Therefore, their expression alone is not sufficient to signal the true emergence of exhaustion. Functional and transcriptomic correlates are needed. Ultimately, it is a balance between costimulatory and coinhibitory signals that provide a gain adjustment on the immune response. Currently, however, such phenotypic and functional assessments of tumor-infiltrating CD4 T cells remain somewhat lacking. Functional deficits in CD4 proliferation, cytokine production, signaling, and provision of B-cell help have varied according to tumor type and source of CD4 T cells (93–99). Nonetheless, current studies in a variety of cancers indicate a correlative relationship between typical markers of T-cell exhaustion on CD4 T cells and the degree of disease severity. Likewise, at least partial restoration of CD4 effector functions has been observed after treatment with checkpoint inhibitors (**Table 2**; refs. 84, 93, 97, 100).

To evaluate whether apparent CD4 T-cell exhaustion parallels the development of CD8 T-cell exhaustion, Rausch and colleagues (101) and Malandro and colleagues (102) investigated the role of antigen stimulation on CD4 T-cell function using murine melanoma models. Persistently increased antigen availability reduced CD4 proliferation, cytokine production, and antitumor responses and increased checkpoint expression on CD4 T cells. Checkpoint inhibition induced only a variable recovery of CD4 effector functions in their hands. Furthermore, clinical observations demonstrate that tumor-infiltrating CD4 T cells express higher levels of coinhibitory markers compared with circulatory (98, 103, 104) or adjacent tissue-infiltrating CD4 T cells (100, 105, 106). These data indicate that perpetual antigen

encounters can induce a severe, and potentially irreversible, exhaustion phenotype that mimics terminal exhaustion in CD8 T cells (21).

To further investigate parallels between the development of CD4 T-cell and CD8 T-cell exhaustion and differentiation states, Fu and colleagues examined the transition from progenitor exhaustion ($SLAMF6^{+}TIM3^{-}$) to terminal exhaustion ($SLAMF6^{-}TIM3^{+}$; ref. 21) occurring among CD4 T cells within a murine melanoma model. They observed a downregulation of TCF1 and SLAMF6 on tumor-infiltrating CD4 T cells compared with CD4 T cells in the spleen, indicating more prevalent differentiation into the terminally exhausted state within tumors. Furthermore, treatment with anti–PD-L1 resulted in an increase in TCF1 and a decrease in TIM3 and LAG3 on CD4 T cells, indicating maintenance of the progenitor exhausted subset (107). These data were corroborated by experiments performed in human samples of head and neck, ovarian, and cervical tumors (108). In contrast to terminally exhausted CD8 T cells, terminal exhaustion in CD4 T cells was represented by the expression of CD39, rather than TIM3. $CD39^{+}$ cells were found to have higher levels of PD-1, produce fewer cytokines, and were more likely to produce a single cytokine (predominantly IFNγ) rather than coproduce multiple cytokines. Treatment with anti–PD-1 increased cytokine production, upregulated CD40 ligand (CD40L), and increased DC maturation and CD8 proliferation, indicating increased CD4 helper functions (108). Paralleling CD8 T-cell exhaustion, $CD39^{+}$ CD4 T cells expressed the highest level of thymocyte selection-associated high mobility group box (TOX; ref. 109) and lost expression of TCF1 (21). The parallel of increased TOX expression with that described in CD8 exhaustion is of particular interest, as TOX has recently been found to initiate the epigenetic changes associated with the exhausted phenotype (110). Epigenetic changes are hallmarks of CD8 T-cell exhaustion (30–32), and this similarity should drive further investigation into the epigenetic landscape of CD4 T-cell exhaustion.

Drawing additional similarities to terminally exhausted CD8 T cells, studies suggest that exhausted CD4 T cells may actually gain certain additional functionality, as the acquisition of noncanonical T-cell function was observed among putatively exhausted CD4 T cells in solid tumors. For instance, C-X-C Motif Chemokine ligand 13 (CXCL13) was found to be exclusively produced by $PD1^{hi}$ CD4 T cells in non–small cell lung cancer (106), suggesting a skew toward effector function in what otherwise resembled exhausted CD4 T cells. Conversely, increased effector function might not translate to increased tumoricidal activity. For instance, enhanced IFNγ production was observed in CD4 T cells positive for the inhibitory marker TIGIT in patients with chronic lymphocytic leukemia. In spite of the improved secretion of proinflammatory cytokines, TIGIT expression on CD4 T cells was also associated with more advanced disease, and TIGIT blockade hindered tumor cell viability *in vitro*, despite also decreasing IFNγ production (111).

Mouse tumor models have been utilized to better evaluate the impact and relevance of CD4 T cells and CD4 T-cell exhaustion for antitumor immunity. Adoptive transfer of melanoma-specific CD4 T cells into a RAG1 knockout recipient resulted in tumor regression. However, a subset of mice presented with tumor relapse. CD4 T cells taken from the recurrent tumors expressed fewer cytokines, increased levels of coinhibitory receptors (112), and were unable to induce tumor regression when transplanted into a secondary tumor-bearing host, suggesting the emergence of response-limiting exhaustion (113). Meanwhile, combination therapy with anti–PD-L1 and anti-LAG3 decreased checkpoint expression, increased CD4 effector functions, and resulted in durable tumor control (112, 113). The lack of CD8 T cells in this model indicates a significant role for CD4 T-cell exhaustion in facilitating tumor escape. Furthermore, these data suggest that checkpoint blockade strategies aimed at CD4 T cells could very well improve tumor control.

Tumor models have likewise been utilized to shed light on important considerations for CD4 T-cell exhaustion, including the relative frequency of exhaustion within the various CD4 subsets. For instance, examining the directly cytotoxic CD4 T-cell subset, we return to an aforementioned study in which tumor cells overexpressing CIITA were generated to permit CD4 recognition of class II MHC-expressing tumors (18). Despite increased cytotoxic CD4 T-cell–mediated tumor control of these tumors, outgrowth eventually occurred, with associated upregulation of coinhibitory markers on tumor-infiltrating CD4 T cells. This expansion was reversed with anti-CTLA4 treatment, highlighting the role of CD4 T-cell exhaustion in tumor progression, as well as the capacity for cytotoxic CD4 T cells, specifically, to undergo an exhaustion program (18).

Interestingly, exhaustion-indicative expression of immune checkpoints has also been observed on the T_{reg} subset of CD4 T cells in the tumors of patients with glioblastoma multiforme (GBM; ref. 114) and hepatocellular carcinoma (115). This suggests that exhaustion among CD4 T cells may not be limited solely to effector CD4 T cells. PD1-expressing T_{regs} in patients with GBM demonstrate enrichment of exhaustion-related genes and decreased suppressive capacities (114), indicating that suppressive functions may be exhaustion-susceptible as well. Given the substantial role T_{regs} play limiting cellular immunity in GBM (and other cancers; refs. 116, 117), selective strategies for reversing exhaustion in cytotoxic and Th1-type CD4 T cells while maintaining or enhancing it in T_{regs} may represent challenging but worthwhile future directions. Accordingly, understanding whether exhaustion-inducing mechanisms are the same in each of these CD4 subsets becomes an important endeavor.

In addition to exhaustion, anergy (118, 119) and senescence (120) have been recognized as modes of T-cell dysfunction that negatively influence antitumor immunity (121, 122). Although these states overlap with regard to various functional and/or phenotypic elements, the mechanisms eliciting the phenotypes are distinct. In contrast to the relatively insidious development of T-cell exhaustion following continuous antigen stimulation, for instance, T-cell senescence is associated with cell-cycle arrest due to shortening of telomeric ends or danger signals, such as oxidative stress (123, 124). Likewise, anergy develops at priming, subsequent to excessive stimulation of the TCR without proper costimulatory signals. Although inhibitory receptors such as PD1, CTLA4, TIM3, and LAG3 are more commonly associated with T-cell exhaustion, other markers, such as CD57 and killer cell lectin-like receptor subfamily G member 1 (KLRG1), are more commonly associated with senescence (124). Unlike senescence T cells, however, progenitor exhausted T cells are capable of responding to checkpoint blockade (21), allowing restoration of function. Further explorations will be required to further delineate these hypo- or unresponsive states and unravel the relative contributions that each of these modes of CD4 dysfunction makes to hindering antitumor immunity.

Ultimately, the data reviewed above begin to establish CD4 T-cell exhaustion as a unique differentiation state impacting antitumor immunity, paralleling exhaustion in CD8 T cells. Further comparisons, including epigenetic and metabolic profiles between exhausted and nonexhausted CD4 T-cell states, will be essential. These experiments will increase our understanding of the complex immune responses generated to cancer and persistent infections and could provide novel therapeutic targets. Additional insights should be gathered by evaluating other pathologies where CD4 exhaustion has been identified. In

addition to cancer and chronic infections, these can include transplantation and autoimmune diseases.

Salient studies in transplantation and autoimmune diseases

Studies into transplantation and autoimmune diseases can help shed light on the possibility of modulating CD4 T-cell exhaustion as a therapeutic strategy. In contrast to cancer and chronic infections, CD4 T cells in transplantation and autoimmunity are a source of undesirable activity and collateral host tissue damage. In the case of hematopoietic stem cell (HSC) and solid organ transplantation, CD4 T cells have been demonstrated to play a significant role in allograft rejection (125, 126) by providing help to the two major cell subsets responsible for tissue damage: cytotoxic donor-specific CD8 T cells and B cells (127). Therefore, inducing specific CD4 T-cell tolerance (128–130) or exhaustion could potentially mitigate the need for systemic immunosuppression by selectively restraining graft-specific effector cells while leaving the remainder of the immune system capable of responding to foreign antigens, reducing the risk of infections and malignancy.

Upregulation of coinhibitory molecules on CD4 T cells is observed following HSC (131, 132) and solid organ transplantation (133). Transgenic overexpression of TIM3 on CD4 T cells resulted in decreased proinflammatory cytokine production and prevented immune-mediated graft pathology (134), indicating a direct role for TIM3 on CD4 T cells in the prevention of rejection. Mechanistically, loss of IFN regulatory factor 4 (IRF4) or Fucosyltransferase 7 (Fut7) in CD4 T cells induced graft tolerance through the establishment of exhaustion in these cells. Graft rejection could be initiated in the early phases after transplant upon treatment with monoclonal antibodies interfering with the PD-1–PD-L1 pathway (135, 136). However, irreversible dysfunction was established in IRF4 knockout (KO) CD4 T cells if anti–PD-1 treatment was delayed until 30 days posttransplant (135).

Much like transplantation, the management of autoimmune diseases frequently involves systemic immunosuppression, and the role of CD4 T cells in autoimmune pathology has long been recognized (137, 138). Conversely, a negative correlation between CD4 inhibitory receptor expression and disease severity has been observed in a rheumatoid arthritis population (139), although this has not been a consistent finding (140). Insights into the role of CD4 T-cell exhaustion and its influence on disease severity in autoimmunity comes from mechanistic studies evaluating Nuclear Receptor Subfamily 4 Group A Member 1 (NR4A1), Nuclear Factor Interleukin 3 Regulation (NFIL3), and human leukocyte antigen B (HLA-B)–associated transcript 3 (Bat3). Upregulation of NR4A1 and NFIL3 and downregulation of Bat3 increased expression of exhaustion markers on CD4 T cells and decreased cytokine production and disease severity (141–143). In addition, persistent stimulation of CD4 T cells with endogenous peptides resulted in loss of cytokine production and proliferation, upregulation of inhibitory markers, and delayed onset of autoimmune diabetes (144).

Conclusion and Future Directions

Compared with CD8 T-cell exhaustion, the impact of CD4 T-cell exhaustion in cancer and other disease states has remained relatively underappreciated. Current studies have provided mostly phenotypic data, and investigations into additional criteria established for CD8 T-cell exhaustion, such as metabolic profiles and epigenetic landscapes, will be required to determine whether CD4 exhaustion likewise comprises a distinct and progressing T-cell differentiation state. Our current mechanistic understanding of factors involved in CD4 T-cell exhaustion is summarized in **Fig. 2**. Further evaluation of CD4 T-cell–specific factors will be essential to increase our understanding of the mechanistic derivations of exhaustion in this population. Analysis of factors associated with CD4 T-cell exhaustion in other pathologies should be extended into tumor models to evaluate similarities and differences in mechanistic determinants. It remains to be seen whether the processes underlying CD4 T-cell exhaustion are similar across different disease pathologies and CD4 subsets, or whether different convergent transcriptional programs happen to result in the same terminally differentiated fate. Furthermore, in-depth assessment of the potentially differential susceptibility of various CD4 T-cell subsets to exhaustion will be required to increase our understanding of its consequences, as well as the relative contribution of each CD4 T-cell subset to antitumor immunity. Finally, examination of the dynamics of the initiation and progression of CD4 T-cell exhaustion and assessment of the role of tumor cells and the TME in the process will be invaluable to understanding parallels and differences between CD4 and CD8 T-cell exhaustion. Crystallizing these insights will be vital to increase our understanding of CD4 T-cell exhaustion and its therapeutic implications.

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