Review Article

A variety of 'exhausted' T cells in the tumor microenvironment

Joji Nagasaki[1-3] and Yosuke Togashi[1,3]

[1] Department of Tumor Microenvironment, Okayama University, Graduate School of Medicine Dentistry and Pharmaceutical Sciences, Okayama 700-8558, Japan
[2] Department of Hematology, Graduate School of Medicine, Osaka City University, Osaka 545-8585, Japan
[3] Chiba Cancer Center, Research Institute, Chiba 260-8717, Japan

Correspondence to: Y. Togashi
Department of Tumor Microenvironment, Okayama University, Graduate School of Medicine Dentistry and Pharmaceutical Sciences, 2-5-1 Shikata-cho, Kita-ku, Okayama 700-8558, Japan
E-mail: ytogashi1584@gmail.com; Tel: +81-86-235-7390; Fax: +81-86-235-7392

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Abstract

In T cell biology, 'exhaustion' was initially described as a hyporesponsive state in $CD8^+$ T cells during chronic infections. Recently, exhaustion has been recognized as a T-cell dysfunctional state in the tumor microenvironment (TME). The term 'exhaustion' is used mainly to refer to effector T cells with a reduced capacity to secrete cytokines and increased expression of inhibitory receptors. The upregulation of exhaustion-related inhibitory receptors, including programmed cell death protein 1 (PD-1), in such T cells has been associated with the development of tumors, prompting the development of immune checkpoint inhibitors. In addition to $CD8^+$ T cells, $CD4^+$ T cells, including the regulatory T (Treg) cell subset, perform a wide variety of functions within the adaptive immune system. Upregulation of the same inhibitory receptors that are associated with $CD8^+$ T-cell exhaustion has also been identified in $CD4^+$ T cells in chronic infections and cancers, suggesting a similar $CD4^+$ T-cell exhaustion phenotype. For instance, high expression of PD-1 has been observed in Treg cells in the TME, and such Treg cells can play an important role in the resistance to PD-1 blockade therapies. Furthermore, recent progress in single-cell RNA sequencing has shown that $CD4^+$ T cells with cytotoxic activity are also vulnerable to exhaustion. In this review, we will discuss novel insights into various exhausted T-cell subsets, which could reveal novel therapeutic targets and strategies to induce a robust antitumor immune response.

Keywords: $CD4^+$ T cell, cytotoxic $CD4^+$ T cell, regulatory T cell, T-cell exhaustion

Introduction

T-cell dysfunction can be strongly related to physiological and pathological states. Recently, 'exhaustion' has been observed as a state of T-cell dysfunction. Exhausted T cells are characterized by progressive loss of effector functions, high and sustained inhibitory receptor expression, metabolic dysregulation, poor memory recall and homeostatic self-renewal, and distinct transcriptional and epigenetic programs (1). Exhaustion was initially described as a hyporesponsive state in $CD8^+$ T cells during chronic lymphocytic choriomeningitis viral (LCMV) infections (2-4). Mice exposed to chronic infection with LCMV Clone 13 had dysfunctional $CD8^+$ T cells, called exhausted cells, because of persistent antigen exposure (5,6). Although early studies focused on exhaustion during chronic LCMV infection, many studies have shown that exhaustion occurs in many other chronic viral infections, including human immunodeficiency virus (HIV), hepatitis B virus (HBV), hepatitis C virus (HCV), and other persisting infections, as well as autoimmune disorders (1,7).

Recently, exhaustion has become recognized as a mode of T-cell dysfunction in cancer as well (8,9). Immune checkpoints are molecules that enhance (co-stimulate) or suppress (co-inhibit) other signals in immune cells. Importantly, the upregulation of exhaustion-related inhibitory immune checkpoint molecules expressed in such T cells has been associated with the development of tumors (10). Notably, exhausted T cells exhibit high expression of programmed cell death protein 1 (PD-1), primarily limiting T-cell function under chronic T-cell receptor (TCR) stimulation (5,6). This feature of exhausted $CD8^+$ T cells prompted the development of immune checkpoint inhibitors (ICIs) targeting PD-1 and one of its ligands, PD-L1 (11,12). Antibody-mediated blockade of PD-1–PD-L1 interactions restores the cytotoxic functions of $CD8^+$ T cells in chronic infections and tumor models and is associated with improved control of viral and tumor loads (13-15). Clinically, ICIs, including anti-PD-1 or anti-PD-L1 monoclonal antibodies (mAbs), have been proven to be effective in various cancer types

(16-20).

In addition to $CD8^+$ T cells, $CD4^+$ T-cell function is relevant in a number of disease states, including cancer. $CD4^+$ T cells perform a wide variety of functions within the adaptive immune system and are well known for their role as T helper (Th) cell subsets, including Th1, Th2 and Th17 cells, as well as the immune suppressive regulatory T (Treg) cell subset. Furthermore, it appears that certain $CD4^+$ T cells are able to directly lyse tumor cells (21-24), and adoptive transfer of tumor-specific $CD4^+$ T cells alone has demonstrated dramatic efficacy in some studies (25). Direct tumor cell recognition and killing by $CD4^+$ T cells requires major histocompatibility complex (MHC) class II (MHC-II), and overexpression of MHC-II transactivator (CIITA) in tumor cells increased interferon-gamma (IFN-γ) and granzyme B production in $CD4^+$ T cells and restricted tumor growth (23,24).

Recent progress in single-cell RNA sequencing (scRNA-seq) has impacted broad areas of cancer research and improved our understanding of the tumor microenvironment (TME) (26,27), showing that such $CD4^+$ T cells can also be liable to suffer exhaustion. Here, this review summarizes a variety of exhausted T-cell subsets in addition to general $CD8^+$ T-cell exhaustion from these findings.

General $CD8^+$ T-cell exhaustion

When antigen clearance fails and exposure is maintained, as observed in chronic infection or cancer, exhaustion in T cells may occur. Although exhausted $CD8^+$ T cells retain the ability to recognize antigens through their TCRs, antigen exposure fails to elicit a robust, meaningful cytotoxic response (28). A primary feature of exhausted $CD8^+$ T cells is the sustained coexpression of multiple inhibitory immune checkpoint molecules, leading to a dysfunctional state. The inhibitory immune checkpoint molecules include PD-1, T-cell immunoglobulin and mucin-domain containing-3 (TIM-3), lymphocyte-activation gene 3

(LAG-3), cytotoxic T lymphocyte–associated protein 4 (CTLA-4), B- and T-lymphocyte attenuator (BTLA), T-cell immunoreceptor with immunoglobulin and ITIM domains (TIGIT), and SLAM family member 6 (SLAMF6) (29,30). These inhibitory receptors are known to be expressed in exhausted $CD8^+$ T cells, with mounting checkpoint expression associated with more severe phenotypes (6). In addition to high expression of immune checkpoint molecules, exhaustion is also characterized by a common transcriptional, epigenetic, and metabolic program, leading to progressive loss of effector function (1).

The typical characteristics of $CD8^+$ T-cell exhaustion include antigen load-dependent and temporally progressive loss of effector activity (28,31), loss of proliferative capacity (32,33), altered expression of transcription factors (34), loss of antigen-independent homeostatic proliferation (35), and modified epigenetic landscapes (36,37) and metabolic requirements (38). In turn, PD-1 blockade has demonstrated the capacity to reverse features of the exhausted phenotype and restore T-cell proliferative and effector function (13). Recent evidence suggests that the exhausted phenotype in $CD8^+$ T cells is not homogeneous and includes lineage-spanning, stage-like "progenitor" (PD-1^{int}TIM-$3^{lo}$$CXCR5^+$$SLAMF6^+$) and "terminally differentiated" (PD-1^{hi}TIM-$3^{hi}$$CXCR5^-$$SLAMF6^-$) subtypes with varied capacities for effector function and proliferation among the subgroups (29,33).

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 reportedly capable of responding to PD-1 blockade with transcription factor T cell factor 1 (TCF1) expression, which has been linked to the preservation of effector functions (29,33). Loss of TCF1 with concomitant upregulation of multiple inhibitory immune checkpoint molecules is associated with the terminally differentiated exhaustion phenotype and a further decline in effector functions (29,33).

There are several other T-cell dysfunction states, such as anergy and senescence. T-cell anergy is generally described as an induced hyporesponsive state with low IL-2 production. T cells that are presented antigens along with suboptimal CD28 costimulation and/or high coinhibition result in anergic phenotypes, as characterized by their low IL-2 production and cell cycle arrest at the G1/S phase (39,40). It has been proposed that T-cell anergy serves to induce tolerance in the periphery and protect the host from developing autoimmune diseases (39,40). However, the downstream molecular mechanisms involved in the anergic state remain unclear. In addition, the lack of surface marker(s) to define anergic T cells makes it difficult to understand this dysfunctional state.

Senescent T cells are characterized by telomere shortening, phenotypic change (loss of CD28 expression), and cell cycle arrest (41). Telomere shortening is an inherent byproduct of cellular division, which affects cellular function and leads to cell senescence (42). Cell cycle-controlling proteins p16, p21, and p53 normally inhibit cell cycle progression and have been shown to accumulate in senescent cells. Furthermore, senescent T cells manifest defective killing abilities and the development of negative regulatory functions (41). In addition to low expression of CD28, high expression of TIM-3, CD57, and killer cell lectin-like receptor subfamily G, member 1 (KLRG-1) is thought to be associated with T-cell senescence (43). Compelling evidence demonstrates the coexistence of T-cell exhaustion, anergy, and senescence related to various diseases. Thus, further clarification of their pathogenic mechanisms is warranted.

Differences in exhausted $CD8^+$ T cells among diseases or species

$CD8^+$ T-cell exhaustion was first described in mouse chronic LCMV infection, whereas exhaustion occurs in many other human chronic viral infections, including HIV, HBV, and HCV, as well as both mouse and human cancers. Several differences in exhausted $CD8^+$ T cells

among diseases or species have also been reported (7).

In mouse chronic infection, a progenitor exhausted $CD8^+$ T-cell subset was identified as a PD-1^{int}TIM-$3^{lo}$$CXCR5^+$$SLAMF6^+$ subset with high TCF1 expression, and a terminally differentiated exhausted subset was identified as a PD-1^{hi}TIM-$3^{hi}$$CXCR5^-$$SLAMF6^-$ subset with low TCF1 expression (44). Such a progenitor exhausted-like subset of human memory $CD8^+$ T cells has recently been identified, and key molecules are TCF1 and CD127 in human chronic infection (45). In contrast, terminally differentiated exhausted $CD8^+$ T cells in human chronic infection reportedly have high expression of CD39 and TIGIT (46). In murine tumors, TCF1 and SLAMF6 are reportedly key molecules in a progenitor subset, and TIM-3 increases in a terminally differentiated subset. TCF1 is also highly expressed in human tumor-infiltrating progenitor exhausted $CD8^+$ T cells, and CD39, TIM-3, LAG-3, and TIGIT reportedly increase in human terminally differentiated exhausted $CD8^+$ T cells in the TME (29,47). While common features are reported, including high TCF1 expression in a progenitor subset, there seem to be several differences (**Table 1**). Many factors, such as antigen quality and/or quantity, metabolic microenvironment, and interaction with other cells, can cause these differences.

Which T cells attack tumor cells directly in the TME?

Although tumor-infiltrating $CD8^+$ T cells reportedly play a crucial role in antitumor immunity (19,20,48,49), not all tumor-infiltrating $CD8^+$ T cells attack tumor cells, and they frequently contain nonspecific bystander T cells (50-52). Through their TCRs, T cells recognize cancer antigens presented on the MHC of the tumor cell (53,54). Thus, TCR analysis can be used to identify tumor-specific T cells. Indeed, a previous study demonstrated that tumor-infiltrating PD-$1^+$$CD8^+$ T cells possess clonal TCR repertoires that respond to tumor cells, whereas tumor-infiltrating PD-$1^-$$CD8^+$ T cells rarely possess (55). Another study showed that T cells in tumor samples obtained from patients who respond to PD-1 blockade therapies

exhibit a highly skewed clonal TCR repertoire (49,56-60). Therefore, skewed T-cell clonotypes in the TME may represent tumor-specific T cells that directly attack tumor cells.

Recent progress in scRNA-seq has made it possible to integrate transcriptome and TCR sequencing at the single-cell level (scTCR-seq) (26,27). Several recent studies using this technology have revealed that skewed $CD8^+$ T-cell clonotypes bear high expression of exhaustion-related immune checkpoint molecules such as PD-1, TIM-3, and LAG-3 and that such exhausted clonotypes are expected to be tumor-specific (60-62).

We used both scRNA-seq and scTCR-seq to analyze tumor-infiltrating T cells from melanoma patients treated with an anti-PD-1 mAb (63). Considerable amounts of clonally skewed T cells were observed in the TME. However, these skewed clonotypes included both exhausted $CD8^+$ T-cell clusters and other clusters. The sensitive tumor samples had skewed clonotypes, especially in the exhausted $CD8^+$ T-cell cluster. In contrast, T cells in the resistant sample had highly diverse clonotypes compared with those in the sensitive samples. Among skewed $CD8^+$ T-cell clonotypes, exhausted clonotypes, but not non-exhausted clonotypes, responded to autologous tumor cell lines (63). In addition, following PD-1 blockade, tumor-specific exhausted $CD8^+$ T cells in the TME increased in a super-responder (63). These findings are consistent with recent studies (52,56,57). Hence, not all skewed $CD8^+$ T-cell clonotypes — only exhausted ones — are tumor specific in this patient group, and PD-1 blockade activates and increases such exhausted $CD8^+$ T cells, resulting in PD-1 blockade-mediated antitumor immunity.

While a progenitor exhausted subset was first described in LCMV infection with improved persistence and increased expansion in response to PD-1 blockade, this subset has also been identified in the TME, as mentioned above. Particularly, a progenitor subset with capacities of coproducing multiple cytokines, proliferation, and responding to PD-1 blockade rather than a terminally differentiated exhausted subset among tumor-attacking exhausted

$CD8^+$ T cells in the TME is reportedly related to the response to PD-1 blockade therapies (60).

However, there are several conflicting reports, especially from human clinical samples, warranting further basic and clinical research (64). Indeed, both progenitor and terminally differentiated exhausted subsets seemed to increase after treatment with PD-1 blockade in our previous study (63). In addition to exhausted $CD8^+$ T cells, follicular helper T cells and Treg cells in the TME, which express PD-1, also increased (63). From these findings, not only exhausted $CD8^+$ T cells but also such other subsets in the TME should be considered under treatment with PD-1 blockade therapies.

Exhausted $CD4^+$ T cells

$CD4^+$ T cells are well known for their helper functions, including Th1, Th2 and Th17 cells. A similar exhausted state to $CD8^+$ T-cell exhaustion in chronic LCMV infection can exist in these $CD4^+$ T-cell subsets. Compared with acute infections, chronic LCMV infections induce higher expression of immune checkpoint molecules in $CD4^+$ T cells, and high expression of such molecules is observed in exhausted $CD8^+$ T cells (65,66). Upregulation of these inhibitory receptors has also been identified in $CD4^+$ T cells in other chronic infections. These results suggest a similar $CD4^+$ T-cell exhaustion phenotype to $CD8^+$ T-cell exhaustion (67,68). In addition to upregulation of co-inhibitory molecules, $CD4^+$ T-cell differentiation in the presence of persistent antigen resulted in reduction of the antigen-specific population, reduced cytokine production, decreased motility of T cells, and poor responses upon a secondary challenge (65,69,70). As a result, $CD4^+$ T-cell-mediated helper functions, such as licensing dendritic cells to prime $CD8^+$ T cells, promotion of antibody class switching, activation of bacterial phagocytosis, recruitment of neutrophils, and induction of angiogenesis, are suppressed. After treatment with an anti-PD-1 mAb, such functions were recovered, although the recovery of function was inconsistent (65,69,70). These findings suggest the

existence of $CD4^+$ T-cell exhaustion (**Fig. 1**). However, upregulation of inhibitory markers is not sufficient to call a cell exhausted because T-cell activation generally causes induction of inhibitory receptors.

Similar to chronic infection, the expression of exhaustion-related immune checkpoint molecules (PD-1, CTLA-4, LAG-3, TIM-3 and TIGIT) has been observed in tumor-infiltrating $CD4^+$ T cells of various solid tumors and hematologic malignancies (71,72). To further investigate the development of $CD4^+$ T-cell exhaustion and differentiation states, several studies evaluated the transition from progenitor exhaustion ($SLAMF6^+TIM\text{-}3^-$) to terminal exhaustion ($SLAMF6^-TIM\text{-}3^+$) among $CD4^+$ T cells using murine models. Downregulation of TCF1 and SLAMF6 in tumor-infiltrating $CD4^+$ T cells compared with $CD4^+$ T cells in the spleen indicated a tendency to differentiate into the terminally exhausted state in the TME, as was observed in $CD8^+$ T cells. Furthermore, after treatment with PD-1 blockade, TCF1 increased and TIM-3 and LAG-3 decreased in tumor-infiltrating $CD4^+$ T cells, indicating induction of the progenitor exhausted subset (73).

Another study has shown that terminal exhaustion in $CD4^+$ T cells is represented by the expression of CD39 rather than TIM-3 (74). High expression of CD39 is reportedly a feature of $CD8^+$ T-cell exhaustion in the TME (50). $CD39^+$ T cells had higher PD-1 expression and produced fewer cytokines and/or a single cytokine than multiple cytokines. In addition, similar to $CD8^+$ T-cell exhaustion, $CD39^+CD4^+$ T cells expressed the highest level of thymocyte selection-associated high mobility group box (TOX) and lost the expression of TCF1 (75). TOX, which is reportedly highly expressed in exhausted $CD8^+$ T cells, can play an important role in $CD4^+$ T-cell exhaustion because TOX has recently been found to initiate the epigenetic changes associated with the exhaustion phenotype (76). Epigenetic changes are hallmarks of $CD8^+$ T-cell exhaustion, and these similar findings to $CD8^+$ T-cell exhaustion promote further investigation into the epigenetic landscape of $CD4^+$ T-cell exhaustion.

Several studies using tumor cells overexpressing CIITA, which permit $CD4^+$ T-cell recognition of MHC-II-expressing tumors, have shown cytotoxic $CD4^+$ T-cell subsets that directly attack tumor cells (23,24). In these models, upregulation of exhaustion-related immune checkpoint molecules in tumor-infiltrating $CD4^+$ T cells was observed in addition to cytotoxicity, highlighting the role of exhaustion in the cytotoxic $CD4^+$ T-cell subset (23,24). PD-1 blockade activated cytotoxicity mediated by such exhausted $CD4^+$ T cells (23,24). Recent single-cell sequencing studies have shown cytotoxic $CD4^+$ T cells in the TME from human MHC-II-expressing tumors (77). This subset expressed exhaustion-related immune checkpoint molecules, as was observed in tumor-infiltrating exhausted $CD8^+$ T cells. We also identified a subset of cytotoxic $CD4^+$ T cells with upregulation of exhaustion-related immune checkpoint molecules in the TME from human MHC-II-expressing tumors and validated this subset using mouse models (J. Nagasaki, Y. Togashi, unpublished data).

Exhaustion-like Treg cells

$FOXP3^+CD4^+$ Treg cells, a highly immune suppressive subset of $CD4^+$ T cells, control many facets of the immune response, ranging from autoimmune diseases to cancer, and serve to maintain immune homeostasis (78,79). Given that Treg cells promote tumor progression in malignancies by suppressing antitumor immunity (79-81), the control of Treg cells by targeting factors that are specifically expressed by Treg cells and that influence Treg cell homeostasis and function has fundamental implications for understanding disease pathogenesis and developing therapeutic opportunities. Thus, manipulating Treg cells is a new cancer therapeutic strategy.

Interestingly, high expression of exhaustion-related immune checkpoint molecules has also been observed in Treg cells in the TME (79,82-84). While 'exhaustion' is the intrinsic term used mainly to refer to effector T cells (1), these findings suggest that the exhaustion-like state

among $CD4^+$ T cells may not be limited solely to conventional $FOXP3^-CD4^+$ T cells. When antigens for Treg cells are not cleared and their exposure is maintained, an exhaustion-like state may occur in Treg cells as well as other T-cell subsets. PD-1 inhibits excessive activation of T cells because it suppresses signals from the TCR and CD28 (a co-stimulator molecule) and renders them dysfunctional (85-87). Given that Treg cells in the TME exhibit high expression levels of PD-1, PD-1 blockade may activate the immune suppressive function of Treg cells. In line with this hypothesis, PD-1-deficient Treg cells possess noticeably strong immune suppressive activity to rescue the autoimmune phenotype (88).

We have recently found that an anti-PD-1 mAb augmented Treg cell-mediated immune suppressive activity in a proportion of patients, which contributed to hyperprogression during PD-1 blockade therapy (84). In addition, PD-1^+ Treg cells in the TME play an important role in resistance to PD-1 blockade therapies (89) (**Fig. 2**). Indeed, transcriptional profiling of tumor-infiltrating Treg cells has revealed that several genes were coexpressed with PD-1 and associated with IFN-γ production and exhaustion as well as enrichment in exhaustion signatures compared with circulating Treg cells (82). Thus, tumor-infiltrating PD-1^+ Treg cells are dysfunctional and are so-called exhaustion-like Treg cells.

Recently, we showed that Treg cells gained high PD-1 expression in highly glycolytic tumors, such as *MYC* high tumors and liver tumors (90). Treg cells actively absorbed lactic acid through monocarboxylate transporter 1, promoting translocation of the transcription factor NFAT1 into the nucleus, thereby enhancing the expression of PD-1, whereas PD-1 expression by effector T cells was dampened. PD-1 blockade invigorated PD-1^+ Treg cells, resulting in treatment failure, and lactic acid in the highly glycolytic TME can be an active checkpoint for the function of Treg cells in the TME via upregulation of PD-1 expression, suggesting novel therapeutic targets (90).

Future directions

$CD8^+$ T cells in the TME play a crucial role in antitumor immunity, and exhausted $CD8^+$ T cells reportedly directly attack tumor cells (63). Thus, many studies on T-cell exhaustion in antitumor immunity have focused on $CD8^+$ T cells in the TME. Compared with $CD8^+$ T cells, the impact of exhaustion in other T-cell subsets during cancer and other disease states remains relatively unclear. Current studies have provided mostly phenotypic data, necessitating investigations into additional criteria established for $CD8^+$ T-cell exhaustion, such as metabolic profiles and epigenetic landscapes. As a result, we can determine whether other exhaustion-like states comprise a distinct and progressing T-cell differentiation state. Further evaluation of specific factors is warranted to obtain more detailed biological insights into various exhausted T cells in the TME.

Although we have proposed that exhaustion-like Treg cells occur in the TME, which can be related to resistance to PD-1 blockade therapies, it remains unclear whether the processes underlying $CD4^+$ T-cell exhaustion are similar across different diseases and $CD4^+$ T-cell subsets or whether different transcriptional and/or epigenetic programs occur. Furthermore, to understand the relative contribution of each T-cell subset to antitumor immunity, we should investigate the potentially differential susceptibility of various T-cell subsets to exhaustion.

Finally, examination of the dynamics of the initiation and progression of various types of exhaustion and assessment of the role of tumor cells and the TME will be particularly valuable to understand similarities and differences among a variety of exhaustion. These insights will help us to understand exhaustion and lead to therapeutic implications.

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Figures and figure legends

Fig. 1. $CD4^+$ T-cell exhaustion. Exhaustion in $CD4^+$ T cells reportedly has negative effects on proliferation, cytokine production, cytotoxicity, B-cell help and $CD8^+$ T-cell effector functions. $CD4^+$ T cells with reduced effector functions upregulate exhaustion-related immune checkpoint molecules, such as PD-1 and TIM-3, paralleling phenotypes observed in exhausted $CD8^+$ T cells. APC, antigen-presenting cell.

Fig. 2. Exhaustion-like Treg cells and PD-1 blockade. PD-1 expression inhibits TCR and CD28 signals in Treg cells and thereby attenuates Treg cell-mediated immune suppression (left). PD-1 blockade increases TCR and CD28 signaling in Treg cells and thereby enhances their proliferation and suppressive activity. Strong immune suppression by such expanded and activated Treg cells hampers the activation of $CD8^+$ T cells (right). APC, antigen-presenting cell.

Table 1. Features of exhausted $CD8^+$ T cells in chronic infection and cancer

	Chronic infection		Cancer	
	Mouse (LCMV clone 13)	**Human (HIV, HBV, HCV)**	**Mouse**	**Human**
Antigen	Viral antigen	Viral antigen	OVA, neoantigen, and self-antigen	Neoantigen and self-antigen
Key molecules	Progenitor: $TCF1^+$, $SLAMF6^+$, $CXCR5^+$ Terminal: $TIM\text{-}3^+$	Progenitor: $TCF1^+$, $CD127^+$ Terminal: $TCF1^-$, $CD127^-$	Progenitor: $TCF1^+$, $SLAMF6^+$ Terminal: $TIM\text{-}3^+$	Progenitor: $TCF1^+$, $CCR7^+$, $CXCR5^+$ Terminal: $TIM\text{-}3^+$, $CD39^+$
Inhibitory receptors	Progenitor: $PD\text{-}1^{int}$, $TIM\text{-}3^{lo}$, $LAG\text{-}3^{lo}$, $TIGIT^{lo}$ Terminal: $PD\text{-}1^{hi}$, $TIM\text{-}3^{hi}$, $LAG\text{-}3^{hi}$, $TIGIT^{hi}$	Terminal: $PD\text{-}1^{hi}$, $CD39^{hi}$, $TIGIT^{hi}$	Progenitor: $PD\text{-}1^{int}$, $TIM\text{-}3^{lo}$ Terminal: $PD\text{-}1^{hi}$, $TIM\text{-}3^{hi}$, $CD39^{hi}$, $TIGIT^{hi}$, $CTLA\text{-}4^{hi}$	Progenitor: $PD\text{-}1^{int}$, $TIM\text{-}3^{lo}$ Terminal: $PD\text{-}1^{hi}$, $TIM\text{-}3^{hi}$, $LAG\text{-}3^{hi}$, $CD39^{hi}$, $TIGIT^{hi}$, $CTLA\text{-}4^{hi}$