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Features of Responding T cells in Cancer and Chronic Infection

Peter S. Kim and Rafi Ahmed

Emory Vaccine Center, Emory University School of Medicine, Atlanta, GA 30322, USA

Peter S. Kim: pskim@emory.edu; Rafi Ahmed: rahmed@emory.edu

Summary

Ever since T cell exhaustion was initially characterized and thoroughly analyzed in the murine LCMV model, such a functional impairment has been validated in other chronic viral infections such as HIV, HCV, and HBV. In tumor immunology, it has always been postulated that tumor-reactive T cells could also become functionally exhausted due to the high tumor-antigen load and accompanying inhibitory mechanisms. However, the empirical evidences for this hypothesis have not been as extensive as in chronic infection perhaps because much of the focus on T cell dysfunction in tumor immunology has been, and appropriately so, on breaking or bypassing immune tolerance and anergy to tumor/self antigens. Based on recent reports, it is becoming clear that T cell exhaustion also plays a critical role in the impairment of antitumor immunity. In this review, we will comparatively evaluate the T cell responses in cancer and chronic infection, and the therapeutic strategies and interventions for both diseases.

Introduction

The immune system is evolutionarily programmed to respond to a variety of foreign pathogens. Therefore it is not surprising that a significant part of our current understanding of T cell immunity comes from acute and chronic viral infections. Analyses using acute viral models have led to the elucidation of immunological T cell memory, a cardinal property of adaptive immunity, as re-exposure to the same pathogen results in more rapid and robust T cell responses [1-6]. On the other hand, in chronic infections, the persistence of viral antigens results in dysfunctional T cell responses. Therefore, therapeutic vaccines have been designed in hopes of boosting the overall immune response against chronic viral infections, such as HIV [7-9], HBV [10,11], and HCV [12-14]. However, the results were not as promising as initially envisioned, indicating that during chronic viral infections, there exists an intricate network of regulatory mechanisms that are suppressing the necessary immune responses required for pathogen clearance.

Because the important discoveries on immunological memory and functional exhaustion of T cells have been made in acute and chronic viral models [1-6,15-19], they serve as practical models for studying T cell responses in cancer. Tumor immunology has made significant progresses in the past decade, and various modalities of cancer immunotherapy have been used

Corresponding Author: Rafi Ahmed Emory Vaccine Center, Emory University School of Medicine, 1510 Clifton Road, G211 Rollins Research Bldg. Atlanta, GA 30322, USA.

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to determine the extent to which anti-tumor responses, in particular the T cell effector function, could be generated. However, the tumor microenvironment, like the immunological milieu of chronic infection, contains a multitude of suppressive mechanisms that allow tumors to escape immune surveillance. Consequently, various treatment methods in tumor immunotherapy have been met with outcomes similar to those seen in chronic infections.

This is a brief review of the features of responding T cells in cancer and chronic viral infection. We will look at the extent to which responding tumor-reactive and chronic viral-specific T cells are similar to and different from each other. In addition, we will discuss current immunotherapeutic treatments for chronic infection and cancer, and future treatment strategies to perhaps overcome immunological barriers that limit the success of tumor and antiviral immunotherapy.

Responding T cells in Chronic Viral Infection

In chronic viral infection, where antigen and/or inflammation persist, virus-specific T cells exhibit various levels of exhaustion. CD8⁺ T cell exhaustion was first analyzed in chronic LCMV infection of mice [16] and could be described in several stages: partial exhaustion I & II, full exhaustion, and deletion [15,20,21], in which the hierarchical loss of effector cytokines, IL-2, TNF- α , and IFN- γ , and *ex vivo* cytotoxicity were well-demonstrated. Antigen-independent proliferation was also diminished in exhausted CD8⁺ T cells, as they were poorly responsive to IL-7 and IL-15 [5]. As for virus-specific CD4⁺ T cells in chronic LCMV infection, these cells, like their CD8⁺ T cell brethren, lost the capacity to produce IL-2 and TNF- α immediately post-infection and were unresponsive to rechallenge with antigen [22]. In addition, they exhibited increased production of immunosuppressive IL-10 in the spleen and more significantly in the liver [22]. It has been well-documented that CD4⁺ T cell help is important for maintaining the functionality of CD8⁺ T cells during chronic infections [23, 24]. Interestingly, there does not seem to be deletion of virus-specific CD4⁺ T cells, albeit inactivated, during chronic LCMV infection [22], hence a potential for therapeutic restoration of their helper function, which may then increase the CTL response. Last but not the least, T regulatory cells during chronic infection minimize tissue damage, but at the same time, aid the establishment of viral persistence [25].

An extensive genome-wide array analysis has been performed on exhausted viral-specific CD8⁺ T cells in chronic LCMV infection, compared to effector and memory CD8⁺ T cells in acute LCMV infection [18]. One of the more pronounced results from the analysis was the overexpression of mRNA for inhibitory cell-surface molecules. It had been determined that PD-1 was highly expressed during chronic LCMV infection and capable of regulating CD8⁺ T cell exhaustion [19]. This array analysis also showed that PD-1 was one of the most over-expressed inhibitory receptors by exhausted CD8⁺ T cells. Other highly-expressed inhibitory receptors mentioned in this array analysis were 2B4, CTLA-4, and LAG-3. Neither CTLA-4 nor LAG-3 blockade *in vivo* improved virus-specific T cell responses in chronic LCMV infection [26], but LAG-3, and not CTLA4, blockade, showed synergy with PD-1 blockade [26]. Furthermore, when compared to genome profiles of memory cells, exhausted CD8⁺ T cells exhibited decreased expression of cytokine receptors, IL-4R α , IL-7R α , and IL-2R β , and their unresponsiveness to IL-7 and IL-15 may be explained by deficiencies in cytokine signaling molecules, Jak1 and Stat5b [18]. The gene array analysis also showed that exhausted CD8⁺ T cells expressed a distinct set of transcription factors, exhibited altered gene expression for chemotaxis, adhesion and migration, and displayed dramatic deficiencies in metabolism and energy [18]. Finally, certain anergy-associated genes, such as Egr-2, Egr-3, and grail, were not selectively expressed in exhausted CD8⁺ T cells, suggesting that anergy and exhaustion were distinct processes in chronic LCMV infection [18].

Functional exhaustion of T cells was not only observed in chronic LCMV infection but also has been confirmed in other chronic mouse models and human chronic infections. In HIV infection, persistent antigen load has shown to be a major cause for impairment of the ability of HIV viral-specific CD8⁺ T cells to generate multiple effector cytokines and upregulation of PD-1 [27-29]. *In vitro* blockade of the PD-1/PD-L1 pathway has shown to improve the effector function of not only HIV [27-29]- but also HCV [30-32]- and HBV [33]-specific CD8⁺ T cells, which also had upregulated levels of PD-1 during infection. Furthermore, IL-10 production was all increased in HIV [34,35], HCV [36-38], and HBV [39] infections, indicating that like chronic LCMV infection, the IL-10/IL-10R pathway plays a key regulatory role in viral persistence. Another inhibitory molecule that has been garnering attention is the Tim-3 receptor, a member of the T cell Ig and mucin family of proteins with galectin-9 as its ligand. In CD8⁺ and CD4⁺ T cells of HIV-infected individuals, Tim-3 was significantly elevated in both T cell types [40]. Similar to PD-1⁺ CD8⁺ T cells, Tim-3⁺ CD8⁺ T cells correlated positively with viral load and inversely with the number of CD4⁺ T cells during progressive HIV infection [40]. More interestingly, in HIV infection, Tim-3⁺ T cells were identified as a functionally exhausted population distinct from PD-1⁺ T cells, and Tim-3 blockade restored T cell effector function [40]. Subsequent findings of Tim-3 as a regulator of T cell exhaustion have also been made very recently in HCV [41] and HBV [42].

Responding T cells in Cancer

Cancer and chronic infection have been often paired together due to their ability to establish high antigen and immunosuppressive environment. However, a fundamental difference between the two pathogeneses is that viral antigens in general are exogenous and quite immunogenic since no central tolerance is involved, whereas tumor antigens are self-molecules that are weakly immunogenic due to the deletion of high avidity T cells during the thymic selection process. Moreover, high avidity cells that have escaped are inactivated by peripheral tolerance mechanisms. Because of the poor immunogenicity of tumor antigens and the low functional frequency of tumor-reactive T cells, one of the initial methods to overcome these hurdles has been to adoptively transfer *in vitro* stimulated and expanded tumor-reactive T cells and observe their antitumor responses. It has been shown that tumor-reactive CD8⁺ cells with central memory qualities confer better antitumor immunity than their effector memory counterparts [43]. In addition, IL-2 treated tumor-reactive CD8⁺ T cells, albeit highly cytolytic, were shorter lived and were less efficacious *in vivo* than their IL-15-treated counterparts, partly due to their lack of terminal effector differentiation [44]. Interestingly, induction of Wnt- β -catenin signaling prevented tumor-reactive CD8⁺ T cells from differentiating into effector cells, but rather promoted the development of self-renewing multipotent CD8⁺ memory stem cells [45], which exhibited superior proliferative and antitumor properties than both central and effector memory T cells.

Tumor-reactive T cells in high tumor antigen load have shown to respond in an analogous fashion as viral-specific T cells in chronic infection. First, their phenotypic (upregulation of inhibitory molecules and downregulation of cytokine receptors) and functional (loss of production of effector cytokines) profiles resemble those of exhausted T cells from chronic infection. For instance, in a retroviral-induced murine CML model, CML-specific CD8⁺ T cells displayed upregulation of PD-1 and decreased production of IFN- γ , TNF- α , and IL-2 [46]. Tumor infiltrate lymphocytes (TIL) from human metastatic-melanoma lesions also exhibited similar phenotypic expression and functional impairment. Both CD8⁺, in particular MART-1-specific, and CD4⁺ TILs had significantly higher expression levels of PD-1 than peripheral blood T cells and those from normal tissues [46,47]. Phenotypic analysis revealed that compared to T cells from normal tissues and blood, a large proportion of CD8⁺ TILs were CTLA-4⁺, which was mainly expressed by PD1⁺ CD8⁺ TILs [47]. Furthermore, CD25 and IL-7R α were lacking in PD1⁺ CD8⁺ TILs, indicating that these cells were unable to proliferate,

produce effector cytokines, and differentiate into memory cells [47]. CD4⁺ PD-1 TILs also shared similar phenotypic expression, as they upregulated CTLA-4 and lacked CD25 [47]. Lastly, the impairment of effector function of PD-1⁺ CD8⁺ TILs was evident by significant reduction of IFN- γ -production, compared to that of PD-1⁻ CD8 TILs [47]. Another study involving human metastatic melanoma has shown that PD-1 was highly expressed in NY-ESO-1-specific CD8⁺ TILs, and that PD-1 blockade enhanced the frequency of cytokine-producing cells [48]. Besides PD-1 and CTLA-4, LAG-3 has shown to be expressed in a substantial number of CD8⁺ TILs in cancer patients and tumor-bearing mice [49,50]. As mentioned previously, Tim-3 has been shown to be upregulated on exhausted T cells in several chronic infections. In tumor settings, it has yet to be determined the extent to which Tim-3 is expressed in TILs and regulates the T cell effector function.

In chronic viral infection, high antigen load is the major driving force in T cell dysfunction through functional exhaustion, but in cancer, anergy also influences the impairment of T cell function (Figure 1). First, tumor cells themselves are poor APCs as they are incapable of expressing costimulatory molecules to provide the second signal, rendering TILs anergic. Immature myeloid-derived dendritic cells (MDC) [51], plasmacytoid DCs (PDC) [52], myeloid-derived suppressor cells (MDSC) [53] and tumor-associated macrophages (TAM) [54] have also shown to be potent inducers of T-cell anergy. It has been suggested that induction of antigen-specific T cell anergy is an early event in the course of tumor progression and significantly occurs before the immunosuppression generally seen in advanced tumor burdens [55]. On the other hand, another study has demonstrated that highly immunogenic tumor growth created antigen overload, causing functional exhaustion and rapid elimination of tumor-reactive T cells [56]. Therefore, from a temporal standpoint, T cell anergy may be dominant early on, but T cell exhaustion likely plays critical roles in the later stages of tumor progression (Figure 2). As stated previously, the gene expression profile of T cell exhaustion has shown to be distinct from anergy in chronic LCMV infection [18]. Thus, a similar analysis at different time points of tumor progression will reveal the extent to which TILs are anergized and/or exhausted at each pathogenic stage, and the results may have important therapeutic implications. For example, if TILs predominantly show the molecular signature of functional exhaustion in advanced tumor burdens, immunotherapeutic modalities that have shown success in chronic viral infections could provide similar therapeutic efficacy in cancer patients particularly in the later phases of their illnesses.

Therapeutic Interventions for Cancer and Chronic Viral Infection

For chronic viral infection, therapeutic interventions aim to counter the effects of the immunosuppressive environment and high antigen load. One approach for boosting T cell responses during chronic infection is therapeutic vaccination (e.g., recombinant vaccinia vaccine, DNA vaccine, peptide vaccine, DC vaccines, etc...), which is to modulate host immune responses in an antigen specific manner by providing a better stimulus for virus-specific T cells. For the most part, the effectiveness of therapeutic vaccines for HIV, HBV, and HCV, as stated previously, has not been as strong as initially expected. Therefore, therapeutic vaccination in combination another immune-based modality may prove to be a more effective strategy to achieve additive or synergistic efficacy. For instance, the combination of LCMV GP33-encoding vaccinia vaccine and anti-PD-L1 blocking antibody significantly improved viral-specific CD8⁺ T cell immunity and consequently decreased viral load in chronic LCMV infection, compared to either modality alone [57]. Similar enhancement of antiviral T cell responses was seen upon neutralization of IL-10, followed by administration of DNA vaccine encoding LCMV antigen [58].

Just as in chronic infection, therapeutic vaccines have been developed against cancer to increase the effector function of endogenous tumor-reactive T cells. To increase the activation of these

T cells, cancer vaccines, in numerous tumor-bearing hosts, have been paired with modalities that break intrinsic inhibitory elements and/or counter the immunosuppressive tumor microenvironment. For instance, a combinatorial treatment, using HER-2/*neu*-targeted vaccine cells that are retrovirally-transduced to secrete GM-CSF (GVAX) for enhancing DC recruitment/cross-priming and cyclophosphamide to deplete T regulatory cells, has resulted in increased activation of high avidity CD8⁺ T cells [59]. Similarly, GVAX using irradiated tumor cells combined with CTLA-4 [60] or PD-1 [61] blockade significantly potentiated tumor-reactive T cells compared to either the vaccine or the antibody treatment alone. Besides GM-CSF, Fms-like tyrosine kinase 3 ligand (Flt3L), which supports the survival, proliferation, and differentiation of hematopoietic progenitors, and induces and chemoattracts DCs, has also exhibited similar synergy with anti-CTLA-4 antibody when it was retrovirally-transduced into tumor cells used for vaccination [62]. Other cancer vaccines utilizing vaccinia virus, peptides, DNA, and dendritic cells, all of which have been used for chronic viral infection, have also shown promise in enhancing antitumor immunity and generating better T cell responses especially when some were combined with CTLA-4 blockade [63,64] or 4-1BB stimulation [65].

Much of combination tumor immunotherapy have centered on cancer vaccines plus one of the following modalities of blocking inhibitory receptors, activating costimulatory receptors or depleting Tregs. This type of combination treatments arose out of necessity to unleash endogenous tumor/self-reactive T cells from the regulatory checkpoints, thereby potentiating the efficacy of the vaccines. It is interesting that such combination pairings have not been fully explored for adoptive T cell therapy because the addition of an immunomodulating agent may significantly improve its efficacy. One example is the augmented therapeutic efficacy of adoptive T cell therapy when combined with agonistic anti-4-1BB mAb in a dose dependent manner [66]. In light of the negative correlation between prolonged *in vitro* culture of tumor-reactive T cells and their *in vivo* function [67,68], the evidence of superior antitumor immunity generated by adoptively-transferred effector cells derived from naïve rather than central memory CD8⁺ T cells [68], and the need to investigate *in vivo* priming of adoptively transferred naïve T cells, perhaps now is the time to explore various combination treatments centered around adoptive T cell therapy.

Even though numerous studies on CTLA-4 blockade as a therapeutic modality for cancer has shown promise in enhancing T cell responses, it has had mixed results in chronic infection. In *in vitro* settings, blockade of the CTLA-4 inhibitory pathway augmented HIV-specific CD4⁺ T cell function [69] and exhibited synergy with PD-1 blockade in restoring intrahepatic HCV-specific CD8⁺ T cell exhaustion [70]. However, in chronic LCMV-infected mice, anti-CTLA-4 treatment had no effect on T cell function and viral control *in vivo*, whereas PD-1 blockade rescued T cells from functional exhaustion and reduced the virus load [19]. Furthermore, in the LCMV murine model, CTLA-4 deficient mice following virus infection showed no significant alteration in regulating viral-specific immunity [71]. In the SIV macaque model, which is a closer *in vivo* reflection of human HIV disease than murine LCMV infection, CTLA-4 blockade not only was unable to improve viral-specific T cell responses, but also increased viral replication at mucosal sites [72], whereas the treatment with partially humanized mouse anti-human PD-1 Ab enhanced SIV-specific immunity and showed reductions in the viral load [73]. This disparate *in vivo* efficacy between CTLA-4 and PD-1 blockade in chronic infections compared to cancer possibly suggests that the inductive mechanisms of T cell dysfunction differ between chronic infection and cancer.

Conclusions

The phenotypic, functional, and molecular changes that occur in T cell exhaustion have been extensively analyzed in chronic viral infections, which have served as a practical model for T

cell dysfunction during tumor growth and pathogenesis. Some of the key features of exhausted T cells in chronic infection are exhibited in TILs, in particular upregulation of the inhibitory receptor PD-1 and loss of production of effector cytokines. The initial aim of tumor immunotherapy has been to break immunetolerance and anergy, but the efficacy of this strategy may now be limited by T cell exhaustion (Figure 2). The discovery of PD-1 as a major regulator and its blockade as a potent rejuvenator of T cell exhaustion has translated into clinical cancer trials. Recently, a phase I/II clinical trial using anti-PD-1 human mAb MDX-1106 has been conducted in patients with various solid tumors, and the antibody treatment induced clinical responses against renal cell carcinoma and melanoma with well-tolerable side effects [JR Brahmer *et al.*, abstract in ASCO Annual Meeting 2009, No. 3018]. Thus, understanding T cell responses in chronic infections has helped to broaden our view on the functional dynamics of T cells in the tumor microenvironment, and will continue to play a major role in therapeutic advancement of antiviral and tumor immunotherapy.

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Chronic Infection **Cancer** **Figure 1.**

Comparison of T cell dysfunction between chronic infection and cancer. In chronic infection, T cell dysfunction mainly occurs through functional exhaustion driven by high antigen load. In addition, there is an increased level of IL-10- and Treg-mediated immunosuppression of T cells. In cancer, functional exhaustion and immunosuppressive environment also negatively influence antitumor T cell responses, but there are additional factors that contribute to T cell dysfunction. Since most tumor antigens are endogenous, tumor-reactive T cells are inherently influenced by central and peripheral tolerance mechanisms. Anergy also plays a major part in T cell impairment in cancer. For example, tumor cells lack costimulatory molecules and are unable to provide the second signal to TILs during direct priming, and various antigen presenting cells in the tumor microenvironment have shown to induce T cell anergy.

Figure 2.

Comparison of T cell dynamics between chronic infection and cancer. In chronic infection, antigen load primarily drives T cells to hierarchical exhaustion and ultimately deletion. In cancer, tumor/self-reactive T cells are initially kept in check by central and peripheral tolerance. Anergy is believed to occur immediately in tumor pathogenesis perhaps as early as in *in situ* cancer, whereas exhaustion/deletion most likely affects T cell function in more invasive cancer stages. One of the main purposes of tumor immunotherapy is to break immune tolerance and anergy. Treg depletion and CTLA-4 blockade can unleash tumor-reactive T cells for a potent antitumor response, but exhaustion/deletion may ultimately limit the treatment efficacy. Therefore, the therapeutic strategies used in chronic infection to rescue T cells from exhaustion, such as PD-1 or PD-1 plus LAG-3 blockade, also should be considered in tumor immunotherapy.