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Combating chronic T-cell exhaustion

“Strategies to overcome exhaustion effectively, as a means to improve immunotherapy for chronic infection or cancer, will likely need to include both blockade of extrinsic cellular and molecular signals as well as intrinsic programming of T-cell exhaustion.”

KEYWORDS: cancer ■ cytokines ■ exhaustion ■ immunotherapy ■ infection ■ inhibition ■ T cell ■ tolerance

T cells are currently being employed for the treatment of patients with persistent viral infections or cancer in many immunotherapeutic approaches, including provision of cytokines, therapeutic vaccines or adoptive transfer of virus- or tumor-specific T cells [1–6]. Despite the different strategies used to enhance T-cell responses to persisting pathogens or malignant cells, a common limitation is tolerization of T cells due to prolonged antigen stimulation. Such exhaustion of CD4⁺ or CD8⁺ T-cell responses has been observed in a number of clinical and experimental infections in humans, nonhuman primates and mice, including in HIV, hepatitis C virus and *Plasmodium* infection, as well as in melanoma, Epstein–Barr virus-induced tumors and ovarian cancer. This suggests that exhaustion is a conserved mechanism for limiting T-cell immunopathology during pathogen persistence [7–13]. In order to enhance the effectiveness of T-cell-based immunotherapeutic strategies, it is necessary to define and overcome the molecular mechanisms that promote and maintain T-cell exhaustion.

The spectrum of exhaustion

T-cell exhaustion is not an all-or-nothing phenomenon; during chronic infection, T cells progressively lose effector functions, with the degree of dysfunction directly correlating with the levels of virus or antigen present [14]. Initially, T cells lose the ability to proliferate in response to antigen stimulation, concomitant with loss of production of the T-cell growth factor IL-2. Subsequently, T cells lose the ability to secrete inflammatory cytokines such as TNF. The highest levels of T-cell exhaustion correlate with impaired killing of infected target cells and reduced production of antiviral cytokines including IFN- γ [15]. It is

now clear that the varying levels of T-cell exhaustion are regulated both by extrinsic mechanisms such as inhibition by Treg subsets and signaling via inhibitory cytokines, as well as by intrinsic mechanisms including loss of expression of cytokines/cytokine receptors and increased expression of surface inhibitory receptors [16,17].

Qui tacet consentit versus the Red Queen hypothesis

T-cell exhaustion, defined by an inability to control the infection or tumor cells and lack of effector function in *in vitro* assays, does not inherently differentiate between a scenario in which T cells are continuously exerting their effector function *in vivo* but have not had sufficient time to recover prior to analysis, versus one in which programmed T-cell tolerance results in lack of *in vivo* effector function. In the former case, the Red Queen hypothesis (“It takes all the running you can do, to keep in the same place”) would describe exhaustion as the result of continuous exertion of effector functions *in vivo*, and implies that the ‘exhausted’ T cells maintain residual functions and continue to exert some selective pressure on the infection or tumor [18]. This is supported by selection of T-cell escape viruses during the chronic phase of infection in HIV patients, despite exhaustion of virus-specific T cells [19]. In addition, regulation of virus-specific T-cell function in these settings by extrinsic factors such as Tregs, IL-10 and TGF- β would suggest that these are required to suppress ongoing T-cell effector responses [20–22].

Conversely, exhaustion is also probably due to intrinsic programming of tolerance in T cells [23,24]. In this case, exhausted T cells do not retain function *in vivo* and their silence

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implies consent (Latin: *qui tacet consentit*) to the infection or tumor in order to limit immunopathology. Exhausted T cells do exhibit unique gene expression patterns compared with either effector or memory T cells, suggesting that intrinsic regulation of T-cell exhaustion is an important mechanism for maintaining T-cell tolerance [25]. Recently, we have helped to show that T cells are programmed to return to their exhausted state even after transient rescue of function and in the absence of continued antigen recognition [23].

Overcoming extrinsic regulation of exhaustion

Both Tregs and cytokines, including IL-10 and TGF- β , have been shown to suppress T-cell function and contribute to T-cell exhaustion during chronic viral infections and tumor persistence [17]. Depletion of Foxp3⁺CD4⁺ Tregs in mouse models of chronic viral infection can increase the functional activity of the antiviral CD8⁺ T cells and result in better control of the virus [22]. Cytokines produced by Tregs, and potentially by other cells, in the setting of prolonged antigen-specific T-cell responses can also attenuate T-cell function either indirectly by suppressing production of proinflammatory cytokines or directly by inhibition of CD4⁺ and CD8⁺ T-cell proliferation [20]. Antibody blockade of IL-10 signaling during chronic viral infection has been shown to result in improved T-cell function and enhanced clearance of disseminated virus in mouse models of infection [20,26,27]. Similarly, expression of TGF- β is increased during chronic viral infection and abrogation or blockade of TGF- β signals *in vivo* during virus infection or tumor growth results in improved retention of T-cell function and accelerated clearance of virus or tumor [21,28].

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Overcoming suppression of T-cell function by extrinsic factors would appear to be relatively straightforward, involving the application of strategies similar to those utilized in experimental models that employ depleting Treg subsets or antibody/drug blockade of cytokine signals in order to promote T-cell function [20,22,26]. However, one concern with regard to using such strategies to modulate T-cell exhaustion is the induction of autoimmunity to self-antigens, as these pathways are also important for limiting

auto-reactive responses [29]. Thus, more work needs to be done to address the relative importance of these regulatory mechanisms after establishment of exhaustion and to test whether the primary role of these extrinsic factors is to suppress new T-cell responses.

Overcoming intrinsic regulation of exhaustion

The differential gene-expression profile in exhausted cells, compared with that of naive, effector or memory CD8⁺ T-cell populations [25], results in decreased expression of proteins that may be important for maintaining continued cell function, including cytokines and cytokine receptors [16], as well as increased expression of inhibitory receptors on the surface of exhausted T cells. It is now clear that the loss of T-cell effector function correlates with increased expression of multiple cell surface inhibitory proteins including PD-1, LAG-3, Tim-3, CD160, 2B4 and CTLA-4 [17,30]. PD-1 is highly expressed on activated T cells, particularly during chronic infection, and antibody blockade of PD-1 can significantly enhance T-cell function during chronic virus infection in both humans and mice [7,31–33]. LAG-3 is a negative regulator of T cells, expressed on both activated CD8⁺ and CD4⁺ T cells, which regulates the ability of these cells to proliferate in response to antigens [9,34]. Although it is still not clear which effector functions the different inhibitory proteins regulate, it is likely that strategies blocking multiple inhibitory pathways will be more efficacious in the treatment of chronic infection or cancer due to enhancement of multiple T-cell effector functions. For example, targeting both the PD-1/PD-L1 pathway and LAG-3 or Tim-3 can further induce recovery of T-cell function during chronic infection or cancer [9,13,35]. Similarly, blockade of PD-1/PD-L1 while simultaneously blocking IL-10 signals can promote viral clearance in experimental mouse models [26].

T-cell programming

Exhausted T cells undergo slow proliferation *in vivo*. Therefore, daughter T cells must either retain the exhaustion 'program' or quickly re-establish exhaustion after division. In light of recent experiments showing that exhausted cells are programmed to return to their exhausted state after transient rescue, even in the absence of continued antigen stimulation, it seems highly likely that epigenetic regulation of exhaustion is an important strategy for maintaining exhaustion [23]. This may be due to inheritance of

specific transcription factors or miRNAs that control the transcriptional profile observed in exhausted T cells. For example, miR-181a was found to be highly expressed in tolerant cells but expressed at lower levels in rescued cells [23]. In addition, different patterns of DNA methylation at specific gene loci in exhausted versus effector T cells may contribute to the exhaustion program. For example, demethylation of the *Pdcd1* gene regulatory region may help to maintain expression of PD-1 in exhausted cells [24].

Conversely, lymphopenia-induced proliferation has been shown to bypass the molecular program of tolerance and at least temporarily rescue the function of exhausted T cells [23]. Similarly, provision of exogenous cytokines such as IL-2, IL-7 and/or IL-15 to promote T-cell proliferation can overcome some types of T-cell exhaustion [16,36–38]. What is even more striking is that T cells can be rescued multiple times from exhaustion. This suggests either that some exhaustion mechanisms are not programmed into cells but rather are acquired shortly after division, or that extensive division is able to overcome epigenetic regulation of T-cell exhaustion. However, once the proliferative signal is removed, the T cells regain tolerance and lose functionality, even in the absence of antigens. While there are many drawbacks to employing lymphopenia-induced proliferation to enhance

T-cell immunotherapeutic approaches, this strategy has been used in cancer patients, resulting in extensive expansion of infused tumor-infiltrating lymphocytes [39].

Conclusion & future perspective

Multiple extrinsic and intrinsic molecular mechanisms contribute to T-cell exhaustion and likely regulate different functional properties of T cells. Strategies to overcome exhaustion effectively, as a means to improve immunotherapy for chronic infection or cancer, will likely need to include both blockade of extrinsic cellular and molecular signals as well as intrinsic programming of T-cell exhaustion. Moreover, rescue from exhaustion has so far been transient, and it is likely that T-cell-based immunotherapies for infection or cancer will require multiple interventions in order to maintain T-cell functionality.

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