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T cell exhaustion due to persistent antigen: Quantity not quality?

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Abstract

T cell exhaustion is characterized by failure to respond to a persistent antigen and is a hallmark of chronic infections and cancer. In this issue, *Ritcher et al.* examine the importance of the amount of persistent antigen *versus* the cell type presenting it to induce CD8 T cell exhaustion and the consequences for host survival during chronic viral infection.

T cell exhaustion is a central consequence of chronic infection with an actively replicating virus such as human immunodeficiency virus (HIV), hepatitis B virus (HBV) and hepatitis C virus (HCV) in humans and lymphocytic choriomeningitis virus (LCMV) in mice [1]. CD8 T cells sequentially lose their function in a hierarchical manner with IL-2 secretion, proliferation and killing capacity lost first, followed by reduction in TNF- α secretion and (in some cases) impairment of IFN- γ production [2]. In extreme cases there is also a loss of virus-specific CD8 T cells via apoptosis. CD8 T cell exhaustion appears to be the result of multiple cumulative pathways that negatively regulate T cell responses and the relative contribution of each may vary depending on the infectious setting (i.e. virus, time post infection, host). Nonetheless, there is clear conservation of many of the negative T cell regulators during distinct persistent viral infections in different hosts. Indeed, T cell expression of multiple inhibitory receptors such as PD-1, LAG3, and TIM3, along with the activity of the suppressive cytokines IL-10 and TGF- β , are seen during multiple chronic viral infections, and targeted blockade of these pathways can improve CD8 T cell function and/or numbers [1, 2]. Similarly, compromised CD4 T cell help and increased numbers of regulatory T cells (Treg) have been linked to CD8 T cell exhaustion in mice and humans [1, 2]. Notably, CD8 T cell exhaustion and many of the underlying mechanisms mentioned above have also been described in chronic parasite and bacterial infections as well as cancers [2, 3].

The appearance of T cell exhaustion is highly associated with pathogen burden of the host, with latent viruses such as herpes simplex viruses inducing functional T cell memory, low level replicating infections such as human cytomegalovirus causing a partial loss of function, while HIV, HCV, HBV and LCMV, which continuously replicate, induce a severe loss of T cell numbers and/or function [1]. There is, in addition, a clear association between the ability of a virus (i.e. LCMV or simian immunodeficiency virus) to infect a large frequency of cells in relation to the emerging virus-specific CD8 T cells and the induction of T cell exhaustion [4]. Furthermore, within the same chronically infected host, CD8 T cell specific for abundant epitopes become more exhausted (or deleted) than those recognizing antigens (Ag) with low expression [5]. Consistently, reduction in the level of specific antigen due to treatment with antiretroviral drug or emergence of escape mutants results in

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improved CD8 T cell function in HIV infected patients [6, 7]. Similarly CD8 T cells specific for a viral epitope that mutates early after persistent LCMV infection remain functional, indicating that sustained antigen presentation is necessary to promote exhaustion [8]. Importantly, in this same infection setting, ablation of MHC-I expression in irradiation resistant cells (which are important targets of LCMV replication) results in functional CD8 T cell responses, demonstrating that antigen presentation by non-bone marrow (BM) derived cells is essential for CD8 T cell exhaustion [9]. However, whether direct antigen presentation by other cell types could drive CD8 T cell exhaustion or whether this is an exclusive attribute of irradiation resistant cells remained unclear. Understanding this question is particularly important for chronic viral infections with viruses that mainly replicate in hematopoietic cells, such as HIV. In this issue of *European Journal of Immunology* Richter *et al*, shed light on this matter by exploring the relevance of the cellular source *versus* amount of Ag for CD8 T cell numbers and function during chronic LCMV infection in mice [10].

Upon initial infection, T cells are primed by specialized antigen presenting cells (APCs) known as dendritic cells (DCs) [11]. DCs provide high levels of all three signals necessary for optimal T cells responses in the form of MHC-TCR interactions, surface receptor-ligand co-stimulation and cytokine signaling (e.g. interleukin-12 or type I interferon-IFN-I-). Thus, DCs are essential for priming of T cell responses during encounters with most pathogens including LCMV infection in mice [12]. At later timepoints after infection a number of hematopoietic cell types, such as macrophages and B cells, along with multiple directly infected cells, can also participate in antigen presentation [13]. To examine the role of cell specific antigen presentation during chronic viral infection Richter *et al*. took advantage of DC-MHC-I mice in which MHC-I expression is restricted to CD11c expressing cells (mainly DCs), keratinocytes (which are not known to be infected with LCMV and therefore are not expected to present antigen) and cortical thymic epithelial cells (which allow positive selection of CD8 T cells) [14]. In agreement with an essential role for antigen presentation by non-hematopoietic cells in CD8 T cell exhaustion [9], the current study shows that restriction of MHC-I presentation to CD11c expressing cells results in increased numbers and functions of virus-specific CD8 T cells during chronic LCMV infection. However, incremental re-introduction of MHC-I expressing hematopoietic cells results in staggered decrease in CD8 T cell responses, suggesting an almost linear relationship between antigen dose and T cell exhaustion. Furthermore, the authors showed that despite the essential need for DCs in priming [12], DCs are also capable of exhausting T cell responses as CD8 T cell inactivity could be induced in DC-MHCI mice by increasing the numbers of antigen presenting DCs, similar to data seen for increasing amounts of Ag⁺ hematopoietic cells. These results indicate that BM derived cells (including DCs) as well as irradiation resistant cells are capable of inducing CD8 T cell exhaustion provided that they are presenting persistent antigen in high doses. This supports an emerging model where abundant persistent antigen dampens CD8 T cell responses regardless of the BM or non-BM origin of the APC and that the frequency of cells infected is critical in driving the immunosuppression and pathogen persistence (Figure 1).

The relative contribution of one cell type *versus* another to CD8 T cell exhaustion may be dictated in great part by the pathogen tropism that influences the degree of antigen presentation in a particular cell population. For example, hematopoietic cells may play a more relevant role in driving CD8 T cell dysfunction during HIV infection, in which CD4 T cells represent the most abundant target cell. In contrast, during HCV or HBV infection where hepatocytes are the most numerous infected cell population, non-hematopoietic cells may contribute most significantly to sustained MHC-I antigen presentation and play a central role in CD8 T cell exhaustion [15]. In the case of persistent LCMV, which infects numerous non-hematopoietic cells [9, 16] and less abundant hematopoietic cells (i.e. DCs

and macrophages), antigen presentation by irradiation resistant cells is essential for CD8 T cell exhaustion [9, 10]. In contrast, during this same infection, antigen presentation by DCs is insufficient and dispensable for impaired CD8 T cell responses as indicated by the aforementioned lack of CD8 T cell exhaustion in DC-MHCI mice [10] and a recent study demonstrating that selective depletion of CD11c⁺ cells after priming does not affect CD8 T cell function [17]. On the other hand, a strong association between DC infection, CD8 T cell exhaustion and LCMV persistence has been reported [18, 19] and this could result from the inability of infected DCs to provide stimulatory signals that are necessary to maintain functional CD8 T cell responses during natural LCMV infection. In addition, limited IFN-I production in response to intrinsically replicating virus in conventional (c) and plasmacytoid (p) DCs [20] combined with sustained pDC dysfunction [21] may limit direct IFN-I signalling, which is essential for optimal CD8 T cell responses during LCMV infection [22]. Consistently, early IFN-I administration prevents CD8 T cell exhaustion and enables viral clearance during an otherwise chronic LCMV infection [23].

It should be noted that the study by Richter *et al* does not exclude the possibility that certain cell types may have distinct capacity to promote/prevent CD8 T cell exhaustion. This could be influenced by their intrinsic ability to process and present antigens associated with MHC-I as well as their expression of co-stimulatory and/or inhibitory molecules. In this regard, DCs are a highly heterogeneous population and whether different subsets of DCs could differentially influence CD8 T cell exhaustion remain unknown. Richter *et al.* show that increased generation of GM-CSF derived DCs (predominantly CD11b⁺ cDCs) lead to a greater loss of CD8 T cell responses than increased Flt3L derived DCs (a mixture of CD11b⁺ cDCs, CD8⁺ cDCs and pDCs), especially with regards to virus specific T cell numbers. While this could be explained by the higher frequency of DCs in the GM-CSF *versus* Flt3L conditions, it could also reflect differential ability of distinct DC subsets to present MHC-I associated antigens and/or exhaust T cells. We have recently shown that, in contrast to cDCs, pDCs exhibit a negligible ability to present antigen to T cells early after persistent LCMV infection [20]. Thus, the reduced CD8 T cell exhaustion in Flt3L *versus* GM-CSF conditions could be in part due to a higher pDC:cDC ratio that result in lower numbers of antigen presenting cells under the Flt3L conditions.

The precise molecular mechanism used by different cell types to induce CD8 T cell exhaustion is an important area of investigation. While persistent antigen presentation causes continuous TCR stimulation that could directly induce CD8 T cell exhaustion and up-regulation of inhibitory receptors, it may also prolong intimate cell contacts that may facilitate delivery of negative signals. Interestingly, PDL-1 expression in hematopoietic cells is essential for CD8 T cell dysfunction and deletion while its expression in non-hematopoietic cells is irrelevant for CD8 T cell exhaustion [24]. Similarly, hematopoietic cells are the major source of IL-10 during chronic LCMV infection and a subset of cDCs, along with other APCs, acquire a unique suppressive phenotype during persistent LCMV infection, expressing high levels of IL-10, PD-L1 and PD-L2 molecules [17]. In addition, sustained cell-intrinsic TGF β signalling contributes to CD8 T cell deletion during chronic LCMV infection [25]. Notably, TGF β is secreted in a latent form and its activation can be mediated by α v β 8 and/or α v β 6 integrins [26, 27], which are expressed in DCs, fibroblasts and epithelial cells [26-28]. Since these cells are infected by persistent LCMV, it is conceivable that virus-specific CD8 T cells become continuously exposed to bioactive TGF β when binding MHC-I-viral peptide complexes on α v expressing hematopoietic and/or non-hematopoietic APCs during chronic infection.

Functional exhaustion due to sustained antigen may not be restricted to T cells during chronic infections. In this regard, pDCs also become unable to produce IFN-I during viral persistence in mice and humans [21, 29, 30]. This “exhausted” pDC phenotype appears to

correlate with viral burden, suggesting the intriguing possibility that certain innate cells, along with T cells, are in some way silenced in an antigen level dependent fashion. Exhaustion of innate cells may not only facilitate CD8 T cell dysfunction and viral persistence but may be of particular importance in response to secondary illnesses (i.e. opportunistic infections and cancers). Indeed, individuals with chronic HIV are more susceptible to opportunistic infections and this is the eventual cause of death in HIV⁺ patients [31]

The study by Richter *et al.* highlights the therapeutic potential of temporally blocking antigen-presentation and/or reducing viral load as a way to promote anti-viral CD8 T cell responses. However, while early reduction/discontinuation of antigen presentation is able to prevent/revert CD8 T cell suppression [8-10], exhausted CD8 T cells remain dysfunctional when transfer into an Ag free environment at later stages of infection [32]. Furthermore, there are risks involved in limiting antigen presentation indicated by the fatal immunopathology occurring in DC-MHCI mice infected with persistent LCMV [10] (Figure 1). This is not an un-common finding with chronic LCMV infection, mice lacking the PD-1/PD-L1 pathway or mice depleted of NK cells, which exhibit enhanced CD4 T cell help, show more powerful anti-viral immune responses but greatly reduced survival [33, 34]. Although the precise mechanism of immunopathology remains unknown, these findings have far reaching implications for attempts to therapeutically eradicate chronic viral infection in humans. It is possible that successful therapies may require complex, multi-faceted approaches tailored to an individual patient's viral load and immunological function. Fortunately our knowledge of the unique infectious environment created by chronic infection is expanding at a rapid pace. Recent advances have not only identified inhibitory pathways but also uncovered positive factors key to promoting viral control such as IL-6, IL-21, IFN- γ and TLR7 [23, 35-39]. While a great deal of investigation is required before this knowledge is converted into a clinical setting, understanding mechanisms such as antigen driven T cell deletion and/or dysfunction are clearly critical steps for success.

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Figure 1. Antigen dose dictates T cell function during chronic infection

This cartoon depicts the major role of antigen in driving CD8⁺ T cell function and exhaustion. During **priming** antigen is presented to naïve CD8⁺ T cells by dendritic cells leading to their activation and proliferation. At later stages of chronic infection **exhaustion** occurs as a result of excessive antigen presentation provided by both directly infected cells or antigen bearing professional APCs (DCs, B cells etc). This is characterized by reduced expression of functional molecules such as IL-2, TNFα and IFN-γ and increased expression of inhibitory molecules such as PD-1. Reduction of antigen burden, in the presence of ongoing infection, can result in CD8⁺ T cell mediated **immunopathology**, where function is restored to the CD8⁺ T cells without rapid resolution of infection. **Clearance** can be seen when MHC:TCR interactions reduce to a level that does not result in T cell exhaustion and pathogen load can be successfully resolved by the anti-viral CD8 T cell response.