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T CELL EXHAUSTION

Two parallel worlds of memory T cells

In contrast with the classical dogma that the pathways generating either memory or 'exhausted' T cells are strictly segregated, data now identify a clonally distinct hybrid memory T cell subpopulation with an exhausted phenotype.

Talyn Chu, Jacqueline Berner and Dietmar Zehn

T cell differentiation correlates with the type of infection that triggers the immune response. If the infection resolves rapidly, a fraction of activated T cells go on to form different types of circulating and resident memory T cells. By contrast, infections that continue with high replication rates induce a T cell differentiation program known as 'T cell exhaustion.' This refers to a hypofunctional effector stage whereby the cells typically produce decreased levels of proinflammatory molecules and upregulate the expression of inhibitory receptors[1,2]. Because chronic infections were shown to preclude the formation of classical memory T cells and acute infections were not considered to generate exhausted T cells, the worlds of normal and exhausted T cells were thought to be strictly segregated. In this issue of *Nature Immunology*, Galletti et al.[3] discover a population of cells within the memory compartment that shows memory features and key elements of exhausted T cell expression patterns. Moreover, they provide compelling evidence for the coexistence of two types of memory T cells and show that they form independently of each other.

Using a combination of unbiased high-dimensional flow cytometry and single-cell RNA sequencing approaches, Galletti et al. assessed the diversity of the healthy human T cell population. By separating the $CD8^+$ memory pool from the remaining cells on the basis of CD95 and CCR7 expression, they identified that healthy humans contain granzyme K (GZMK) positive and negative memory T cell subpopulations. Both populations shared expression of high levels of the memory markers CD27, CD28 and CD127 at the protein level, but the $GZMK^+$ population also expressed significant levels of the key inhibitory receptors PD-1 and TIGIT, which we normally associate with exhausted T cells, and other typical transcription factors linked to T cell exhaustion, such as TOX, TOX2, PD-1, NFATC2, MAF, ZEB2 and BATF[4]. The authors refer to this population as exhausted-like memory T (T_{PEX}) cells, while the other $GZMK^-$ population comprises
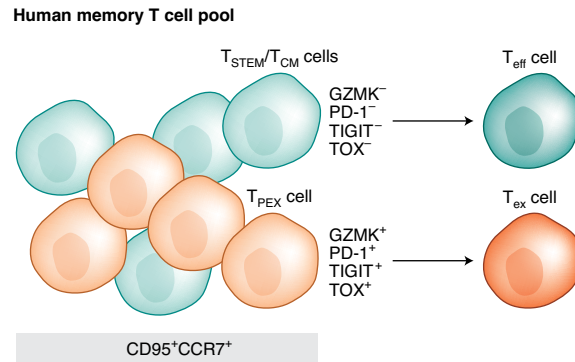
Fig. 1 | Heterogeneity of the human memory T cell pool. Galletti at al. demonstrate that $CCR7^+CD95^+$ T cells are a heterogeneous population consisting of $GZMK^+$ and $GZMK^-$ subsets. $GZMK^+$ memory T (T_{PEX}) cells coexpress markers of T cell exhaustion and are committed to give rise to T cells with an exhausted phenotype. $GZMK^-$ memory T (T_{STEM}/T_{CM}) cells have the potential to form normal, polyfunctional effector T cells. Interestingly, when $GZMK^+$ cells are removed from the $CCR7^+CD95^+$ memory pool, the resulting population is homogeneous, and T_{STEM} and T_{CM} cell subpopulations show almost identical gene expression profiles.

classical, previously described stem-cell-like memory T (T_{STEM}) cells (Fig. 1). Key to understanding the identity of these populations were functional studies in which both T cell populations showed the ability to proliferate and produce inflammatory cytokines, although the T_{STEM} cells were more effective at both tasks. However, both types of T cells showed similar degranulation capacity. Unexpectedly, while the T_{PEX} cells showed lower cytokine production following activation via the T cell receptor (TCR), their cytokine response exceeded that of T_{STEM} cells following TCR-independent mitogen stimulation. This suggests that the effector capacity of T_{PEX} cells is limited at the level of TCR signaling, even though the cells in principle have a much higher effector capacity. Ultimately, all of these data underline that typical features of T cell exhaustion can be found in this newly defined subset of memory T cells (Fig. 1). Of note, these data do not contradict prior conclusions that T cell exhaustion precludes the formation of classical memory T cells. Nonetheless, the report indicates that, under yet to be determined

conditions, a part of the T cell population that has stably acquired an exhausted phenotype transitions to this memory stage. Supporting this is the observation that T_{PEX} cells are transcriptionally, epigenetically and functionally similar to precursor $TCF1^+$ exhausted progenitors found in chronic antigen settings. Several groups recently described $TCF1^+$ progenitors as comprising a reservoir population with high proliferative capacity that continuously regenerates short-lived exhausted effector T cells during chronic infection[3]. Accordingly, the T_{PEX} cells might have formed from the $TCF1^+$ progenitor population in a similar fashion to that of classical memory T cells, which originate from $CD127^+KLRG1^-$ memory precursor cells.

The second, and maybe the largest, surprise is that both types of memory T cells contain non-overlapping T cell receptor repertoires. This indicates that two kinds of memory T cells are generated independently of each other from distinct naive T cell clones and that there is no major transition between the two populations. Moreover, the presence of different receptor repertoires

also implies that both memory forms are induced by different antigens. It is possible that these distinct memory T cells emerge from a specific type of prolonged infection, but their resting phenotype suggests that these infections resolved over time or that their activity and replication rate declined substantially. Alternatively, specific levels of T cell stimulation or activation may generate T_{PEX} cells independently of whether the infection is chronic or acute, as the level of TCR stimulation is highly critical for the formation of exhausted T cells[5]. Maybe T cells with an exhausted phenotype could also be generated in certain types of acute infection after exceptionally strong TCR ligation, which may resemble the level of T cell stimulation found in many chronic infections. In fact, several reports suggest that T cells with exhausted features appear in severe COVID-19[6,7]. A consequence of this is that we may incorrectly link the exhausted phenotype exclusively to chronic infections. However, we also know that exhausted T cells are not found among influenza-specific memory T cells[8,9]. The key challenge that emerges from the present study is, therefore, to identify the antigen specificity of the described population, as this will ultimately answer questions about the type of infection that generates the T_{PEX} cell population. Another impressive finding is the frequency with which T_{PEX} cells are represented in the normal human memory population — a conclusion that is supported by another recent publication[9]. This indicates that this type of differentiation is rather common.

Conceptually, the findings made by Galletti et al. synergize well with several recent reports. They relate to the discovery made by several groups that ongoing chronic infections give rise to a population of proliferation-competent TCF1+ cells, which are needed to maintain the exhausted effector population and for population re-expansion following checkpoint inhibition[4]. These functional T cells also express both signature genes of T cell exhaustion and features of memory T cells or memory precursor cells. Similarly, it was demonstrated that mouse T cells experimentally removed during chronic infection form functional memory T cells and, following their re-activation, regenerate T cells with an exhausted profile[4]. This occurs even after re-expansion induced by

an acute infection, further emphasizing that the exhausted phenotype can access a functional memory compartment. A similar form of differentiation occurs in formerly chronic hepatitis C viral infections that have been resolved pharmacologically, which results in the generation of functional memory T cells that show an exhausted phenotype[10]. All of these observations suggest that exhausted T cell populations may be a separate entity of effector T cells. This conclusion is well supported by the findings of Galletti et al. and also by another report recently published in *Nature Immunology*[11]. In this earlier study, it was shown in mice that the earliest signs of T cell exhaustion could be detected in the TCF1+ progenitor population before the appearance of this exhaustion signature in the effector compartment. This early commitment of the progenitors also fits best to a model whereby the exhausted and non-exhausted T cell populations diverge in the very early infection phase, and the two lines subsequently develop independently of each other[8,11]. In line with this, the transcription factor TOX is needed to stably commit T cells to this lineage[8,12–15].

The observations made by Galletti et al. also have implications beyond the discovery of a memory T cell population with 'exhausted' features, as the authors question the established developmental hierarchy for human antigen-specific CD8+ T cell differentiation. T_{STEM} cells have enhanced stem-like/memory properties as compared to their downstream central memory population. T_{STEM} cells are believed to be the earliest differentiated memory population that gives rise to central and effector memory. The authors identify that PD-1 and TIGIT are expressed on both T_{STEM} and T_{CM} populations, or, more specifically, they discovered a significant contamination of T_{PEX} cells within both T_{STEM} and T_{CM} compartments. After excluding the CCR7+GZMK+ T_{PEX} cell contamination from the CCR7+GZMK– T_{STEM} compartment, Galletti et al. concluded that T_{STEM} and T_{CM} cells were nearly transcriptionally identical and could only be distinguished by eight differentially expressed genes. One of the differentially expressed genes was *HNRNPLL*, which regulates alternative splicing of CD45RO, a phenotypic marker used to differentiate between T_{STEM} and T_{CM} cells. Thus, the presented evidence questions

the existence of T_{STEM} cells, as Galletti and colleagues' data show that differences between T_{STEM} and T_{CM} cells occur predominantly because of different levels of T_{PEX} cell contamination. The absence of T_{PEX} populations in specific-pathogen-free mice could also explain why the T_{STEM} and T_{CM} distinction made between human cells was difficult to reconcile in mice.

Overall, the article is an elegant study that sheds new light on the phenomenon of T cell exhaustion. It points out that the phenotype we normally associate with rare or very specific diseases (certain chronic infections and cancer) is surprisingly prevalent. Furthermore, cells with features of T cell exhaustion seem to comprise a significant fraction of the normal T cell repertoire. This work advances the notion that exhausted T cells are a separate line of highly differentiated effector cells with a tempered effector capacity. This appears to be relevant in persistent infections wherein prolonged T cell effector responses would otherwise cause massive immunopathology. ❐

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Competing interests

The authors declare no competing interests.