No Time to Die, Born to Be Killers: Survival Is Accompanied by Exhaustion after an Endless Battle

After intense combat, defending killer T cells surrender to chronic disease pathogens or cancer by capitulating into an exhausted, dysfunctional cell type, T_{Ex} . Is their surrender definitive?

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<https://doi.org/10.14348/molcells.2021.0263>

www.molcells.org

From protective fighters to resting spectators: Changes within CD8⁺ T cells in chronic diseases and cancer. Naïve CD8⁺ T cells differentiate to effector T cells (T_{Eff}) and memory T cells (T_{Mem}) upon contact with foreign intruders during acute infections. However, chronic stimulation causes T cells to become exhausted and differentiate into progenitor exhausted T cells (T_{Ex}^{prog1} and T_{Ex}^{prog2}), intermediate T_{Ex} (T_{Ex}^{int}), and irreversible terminally exhausted T_{Ex} (T_{Ex}^{term}). Only progenitor T_{Ex} respond to PD-1/PD-L1 checkpoint inhibition. The attempt to reinvigorate T_{Ex} cells is only possible for T_{Ex}^{prog} , resulting in an intermediate phenotype, between T_{Mem} and T_{Ex}^{prog} , characterized by “epigenetic scars”. A high expression level is indicated by a blue bar, an intermediate expression by a green bar, and a low expression by a red bar.

Received 11 November, 2021; revised 25 November, 2021; accepted 25 November, 2021; published online 24 December, 2021

eISSN: 0219-1032

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T_{Eff}, T_{Mem}, AND T_{Ex} ARE THREE DISTINCT SUBSETS OF CD8⁺ T CELLS, DERIVING FROM THE SAME ANCESTOR

The immune system is well-equipped to eliminate foreign or dangerous entities, including naïve CD8⁺ T cells capable of differentiating into effector T cells (T_{Eff} or cytotoxic T lymphocytes) and memory T cells (T_{Mem}) (Yao et al., 2019). Persistent antigen interactions in chronic infections or cancer drive T cells toward a dysfunctional, named “exhausted” phenotype without antigen-independent self-renewal (Abdel-Hakeem et al., 2021; Khan et al., 2019; Yao et al., 2019). Exhausted T cells (T_{Ex}) cells characterize malignancies, such as hepatitis C virus or human immunodeficiency virus infection, and lung cancer (Khan et al., 2019).

T_{Ex} cells are a distinct subset with a unique transcriptional and epigenetic landscape from T_{Eff} and T_{Mem} (Abdel-Hakeem et al., 2021; Khan et al., 2019). Their epigenetic program differs similarly to epigenetic differences between distinct hematopoietic lineages (Beltra et al., 2020; Khan et al., 2019). Exhaustion is characterized by a reduced metabolic capacity and loss of cytokine production (Khan et al., 2019). This dysfunctional state induces a potential leakage of memory formation and T cell function, reduced proliferation, and impaired survival (Khan et al., 2019; Yao et al., 2019). T_{Ex} cells alter effector functions, express inhibitory receptors (IR), and reduce immunoreactivity. Programmed cell death protein-1 (PD-1), lymphocyte activation gene 3 (LAG-3), T cell immunoglobulin mucin-domain containing-3 (TIM-3), and cytotoxic T-lymphocyte-associated protein 4 (CTLA-4) are examples of IRs (Abdel-Hakeem et al., 2021; Khan et al., 2019; Kim et al., 2021; Yao et al., 2019). T_{Ex} cells are distinguished by their heterogeneity, allowing classification into four different subsets (Beltra et al., 2020).

HETEROGENEITY WITHIN EXHAUSTED T CELLS

While the progenitor and terminally exhausted T_{Ex} subtypes have already been described (Alfei et al., 2019; Khan et al., 2019; Kim et al., 2021; Yao et al., 2019), Beltra et al. (2020) recently identified four distinct T_{Ex} stages of exhaustion. As summarized in the figure, T_{Ex} cells can be classified as progenitor, intermediate, or terminally exhausted T cells based on the expression of Ly108 (or signaling lymphocyte activation molecule family member 6 [SLAMF6]), CD69, and Ki67. T cell factor-1 (TCF-1), thymocyte selection-associated high mobility group (HGM) box protein (Tox), T-box expressed in T cells (T-bet), and eomesodermin (Eomes) are transcription factors that determine T_{Ex} subsets. CD69 expression indicates a resident and anti-proliferative state, associated with Eomes and Tox but not with T-bet. These markers partially antagonize each other, Tox antagonizes T-bet, T-bet represses PD-1, and TCF-1⁺ cells are gradually lost when Ki67 positivity increases. As all T_{Ex} subsets express Tox, it is considered the master transcription factor of exhaustion (Abdel-Hakeem et al., 2021; Beltra et al., 2020).

Progenitor T_{Ex} 1 (T_{Ex}^{prog1}) express all previous markers but present low T-bet expression. Their Ki67 level is low, indicating a reduced proliferative potential. The key characteristics

of this subset are quiescence, residency in the white pulp of the spleen, and inability to enter the bloodstream. However, the progenitor T_{Ex} 2 subset (T_{Ex}^{prog2}) expresses Ly108, but not CD69, and all other previously mentioned markers are only intermediately expressed. This subset proliferates, presents intermediate Ki67 expression levels, and circulates in the blood and red pulp of the spleen. Despite their anatomical, transcriptional, phenotypic, and functional differences, both progenitors share the potential to exchange through conversion and re-localization through the TCF-1 and Tox transcriptional checkpoints. The maintenance of progenitor cells depends on TCF-1, a key transcription factor of progenitor T_{Ex} (Abdel-Hakeem et al., 2021; Beltra et al., 2020). Under the epigenetic and transcriptional control of T-bet, the initial proliferation of the T_{Ex}^{prog2} subset results in the appearance of the downstream intermediate T_{Ex} (T_{Ex}^{int}) subset, which is still considered irreversible.

This intermediate subset expresses high T-bet, low Tox, and low Eomes but no Ly108, CD69, or TCF-1. Additionally, these cells express a high Ki67 level and are highly proliferative. T_{Ex}^{int} circulate in both the blood and the red pulp of the spleen. This subset is weakly cytotoxic and resembles circulating effector-like cells but with a distinct epigenetic makeup compared with T_{Eff}. Furthermore, these three subsets express CD44 with intermediate PD-1 levels.

The epigenetic and transcriptional checkpoint Tox regulates the final and irreversible transition. In contrast to T_{Ex}^{int} cells, this terminally exhausted T_{Ex} (T_{Ex}^{term}) subset loses T-bet but gains Eomes expression and presents the highest Tox activity. These cells exit the cell cycle permanently, and CD69 expression is restored. T_{Ex}^{term} is resident in peripheral tissues, blood, and the red pulp of the spleen. Considering their low Ki67 levels, the proliferation of these cells is unlikely. The high PD-1 expression and intermediate CD44 levels further underline their terminal exhaustion. To the best of our understanding, this T_{Ex} classification is preserved across species and presents the conserved core developmental mechanisms.

EPIGENETIC SCARRING HINDERS REPROGRAMMING OF EXHAUSTED T CELLS

The corresponding epigenetic mechanisms were recently described (Abdel-Hakeem et al., 2021). Exhausted T cells were rescued from terminal exhaustion by an early antigen removal, with a four-week recovery after transferring them to healthy mice. These reinvigorated T_{Ex} cells were called recovered T_{Ex} (REC-T_{Ex}) because they switched from an exhausted to a memory-like phenotype. Nevertheless, the REC-T_{Ex} did not fully recover memory functions. Interestingly, Tox expression levels remained high after 500 days, potentially limiting reinvigoration. By eliminating the antigen, some transcriptional changes occurred compared to the originally transferred T_{Ex} cells. The gene expression profile associated with exhaustion, including Tox, Pdcd1 (encodes PD-1), and LAG-3, was decreased in REC-T_{Ex}, resulting in an intermediate transcriptional profile between T_{Ex} and T_{Mem}. In contrast, memory cell-specific genes, including IL-7 receptor (*Il7r*) and TCF-7 (encodes TCF-1), were upregulated. Tox remained high in REC-T_{Ex}, although TCF-7 was lower than in T_{Mem}. By com-

paring REC- T_{Ex} with different subtypes established by Beltra et al. (2020), Abdel-Hakeem et al. (2021) concluded that REC- T_{Ex} arises from TCF-1-expressing progenitor T_{Ex} . These recovered cells also expressed higher PD-1 but lower killer cell lectin-like receptor subfamily G member (KLGR)1 level, representing a more exhausted and less effector-like phenotype. Furthermore, upon rechallenge, the cytotoxicity potential of REC- T_{Ex} was inferior to T_{Eff} or T_{Mem} , presenting higher PD-1 and Tox expression levels. Compared with T_{Ex} , we observed only a slight improvement of effector functions. Overall, REC- T_{Ex} resembled T_{Ex} more than T_{Mem} . Investigating chromatin accessibility revealed consistent differences between REC- T_{Ex} and T_{Mem} open chromatin regions called “epigenetic scars” of exhaustion. Altogether, the recovery after antigen removal did not improve REC- T_{Ex} effector functions. This incomplete functional recovery reveals a lack of epigenetic plasticity and failure to reestablish the transcriptional programs of T_{Eff} and T_{Mem} . Nevertheless, these reinvigorated T_{Ex} cells could become a target for future immune checkpoint blockade (ICB) therapies that already targeted T_{Ex} without groundbreaking success.

ICB AND OTHER THERAPIES TARGET T CELL EXHAUSTION

Because ICB offered significant therapeutic improvement, a better characterization of the function and regulation of exhausted T cells is critical, as T_{Ex} cells are major cell types responding to anti-PD-1/PD-L1 biologics (Beltra et al., 2020). Among the different T_{Ex} subsets, only PD-1^{int}CD44^{hi} progenitor T_{Ex} responded efficiently to anti-PD-1/PD-L1 treatment compared to PD-1^{hi}CD44^{int} terminally exhausted T_{Ex} (Abdel-Hakeem et al., 2021; Beltra et al., 2020; Kim et al., 2021). After ICB, the circulatory subsets T_{Ex}^{prog2} and T-bet high T_{Ex}^{int} expanded preferentially, and differentiation into different T_{Ex} subsets is most likely affected by anti-PD-1/PD-L1 blockade (Beltra et al., 2020). Hence, after PD-1/PD-L1 blockade, the chromatin landscape stabilized, and some characteristics of exhaustion were reversed. However, after ICB, the effector properties were only temporarily and partially restored, and the open chromatin landscape remained mostly unchanged (Abdel-Hakeem et al., 2021; Beltra et al., 2020). In REC- T_{Ex} , ICB with anti-PD-1/PD-L1 generated minor effects without causing significant proliferation (Abdel-Hakeem et al., 2021).

In the future, therapies specifically targeting selected exhaustion subtypes will play significant roles in immune cancer therapies, chronic, and autoimmune diseases. Following T_{Ex} recovery, the IL-7R-TCF-1 axis could target the poor accessibility of the chromatin regions. The upregulation of both CD127 and *Il7r* genes increased chromatin accessibility. In the TCF-1⁺ REC- T_{Ex} subset, this reinforced accessibility could create a survival mechanism with therapeutic potential for this restored cell subset. This suggests a combination of epigenetic and immunologic therapies to ensure durable and persistent memory formation and prevent widespread relapses (Abdel-Hakeem et al., 2021). In addition, targeting the TCF-1-T-bet axis is crucial for determining the progression of T_{Ex}^{prog2} to T_{Ex}^{int} . T_{Ex}^{int} cells regain some effector-like functions and express high T-bet levels; by reinforcing this subtype, terminal

exhaustion could be prevented. T-bet is a crucial transcription factor for different subsets because it regulates and balances different T_{Ex} subsets (Beltra et al., 2020). Even though exhausted cells remain rigid after therapy, Tox manipulation may have therapeutic value in the future (Khan et al., 2019).

CD4⁺ T-helper cells are another cell type to consider for future therapies, as Th17 immunity contributes to tumor-resistant CD8⁺ T cell exhaustion. The depletion of CD4⁺ T cells promotes T_{Ex}^{term} formation and increases PD-1 expression. Regardless of tumor type, injection route, or CD4 depletion, IL-17-producing cells cause terminal exhaustion within the tumor microenvironment. Inhibiting the PD-1/PD-L1 pathway increases the population of terminally exhausted CD8⁺ T cells in mice. Future investigation will show to what extent the inhibition of the retinoic acid receptor-related orphan nuclear receptor (Ror)γt pathway contributes to exhaustion caused by PD-1/PD-L1 ICB or CD4⁺ depletion. Recent discoveries point to novel anti-tumor immunity approaches by inhibiting IL-17 immunity or Rorγt pathways combined with other immunotherapies (Kim et al., 2021).

Overall, the research progress made by identifying different T_{Ex} subsets and associated therapeutic approaches is quite promising. These findings may indicate a crucial target for future therapies to combat chronic diseases and cancer.

ACKNOWLEDGMENTS

This work was supported by the research grants: Global Research Laboratory Program (2017K1A1A2004511 [Y.C.]).

The paper was written during an internship (S.D.) at the College of Pharmacy of the Seoul National University as a part of the master's program “Molecular and Cellular Biology” of the University of Munich.

AUTHOR CONTRIBUTIONS

S.D. and Y.C. wrote the paper.

CONFLICT OF INTEREST

The authors have no potential conflicts of interest to disclose.

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