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RESEARCH HIGHLIGHT

Pyrimidine nucleotide starvation induces a decrease in the number of effector T cells but not memory T cells

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Scherer et al. investigated the influence of de novo pyrimidine nucleotide synthesis on T-cell responses after antigen stimulation, particularly on effector and memory T-cell development [1]. Their work focused on the effect of leflunomide (trade name Avara®), which is a direct inhibitor of dihydroorotate dehydrogenase (DHODH; a key enzyme in the de novo pyrimidine nucleotide pathway and located on the inner mitochondrial membrane). In this pathway, DHODH converts dihydroorotate to orotate and has been implicated in the reduction of ubiquinol to ubiquinone mediated by Complex III in the electron transport chain. The metabolic effects of DHODH inhibition are unclear [2, 3]. However, it is known to block S-phase progression by activating P53. Leflunomide was originally approved for the treatment of rheumatoid arthritis (RA) in 1998; the mechanism involves the induction of S-phase cell cycle arrest and apoptosis, inhibiting cell proliferation and self-renewal. Preclinical studies have demonstrated the therapeutic potential of leflunomide for cancer [4]. Despite the efficacy of leflunomide, the mechanism by which it targets immune cells in the treatment of RA was unknown; however, the work of Scherer and colleagues has shed new light on this mechanism.

After antigen stimulation, $CD8^+$ T cells proliferate and differentiate, leading to an increase in cell numbers until peak expansion is realized and acute infection is resolved. These cells can be classified into two subsets: memory precursor effector cells (MPECs) and short-lived effector cells (SLECs). MPECs undergo an intermediate stage during memory $CD8^+$ T-cell formation, whereas SLECs constitute a subset of $CD8^+$ T cells that rapidly differentiate and expand during an immune response. During the contraction phase, the number of SLECs decreases markedly, whereas MPECs differentiate into memory $CD8^+$ T cells, and their number remains stable [5] (Fig. 1).

To explore the effects of pyrimidine starvation by leflunomide on the proliferation and differentiation of effector $CD8^+$ T cells, OT-1 T cells carrying the T-cell receptor $CD8^+$ were transferred into naïve mice that were subsequently treated or not with leflunomide before infection. An analysis revealed that, compared to the control group mice, the mice treated with leflunomide exhibited a substantial reduction in the number of antigen-specific $CD8^+$ T cells during the effector phase, a response that remained constant over time. The findings also suggested that the cells in the treated mice lacked a prominent contraction phase. Notwithstanding the limited expansion of $CD8^+$ T cells in leflunomide-treated mice during infection, the memory $CD8^+$ T cells that emerged after the initial challenge were found to be functional in a secondary challenge, and their effects were comparable to those of the control group (no leflunomide treatment). The effect of leflunomide on effector $CD8^+$ T-cell development impaired SLEC generation, whereas the absolute number of MPECs was unaffected. Furthermore, Scherer et al. showed that patients with RA who received leflunomide and were vaccinated against severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2) developed memory $CD4^+$ T cells against peptide pools that included the SARS-CoV-2 spike protein.

During the effector phase, $CD8^+$ T cells with inhibited pyrimidine synthesis showed higher levels of markers and transcription profiles clearly associated with MPECs, whereas markers for SLECs were more prevalent in the control group. Additionally, enumeration of cells during the early exponential phase (Days 1.5–5) revealed significant cell proliferation on Days 3 and 4. Further investigations on Day 4, which is the first day in which $KLRG1^{high}$ effector $CD8^+$ T cells could be measured during LCMV Armstrong infection [5], were aimed to identify key cell differentiation-related gene sets influenced by leflunomide treatment (Fig. 1). A single-cell RNA sequencing analysis performed on Day 4 revealed that cells differentiating into MPECs showed upregulated DHODH expression, and the expression of genes encoding components of the de novo pyrimidine synthesis pathway was also upregulated. A subsequent trajectory analysis, which is used to summarize transcriptome profiles in different cells, confirmed that increased pyrimidine synthesis was required for MPEC development. Lin et al. discussed the effect of TCF1 expression during cell proliferation and differentiation: TCF1 was highly expressed during the response to TCR stimulation, i.e., naïve $CD8^+$ T cells differentiated into MPECs (Fig. 1). Asymmetric cell division resulted in different TCF1 levels between daughter and parent cells, which determined the fate of effector-prone cells and quiescent memory cells [6]. The findings indicated that inhibition of DHODH expression resulted in an increased frequency of $TCF1^+$ $CD8^+$ T cells because cell division was delayed, which led to the generation of MPECs.

The ability of leflunomide to inhibit cell cycle progression has led to speculation that it can be used to enhance the effectiveness of immunotherapy and vaccination. In the context of persistent

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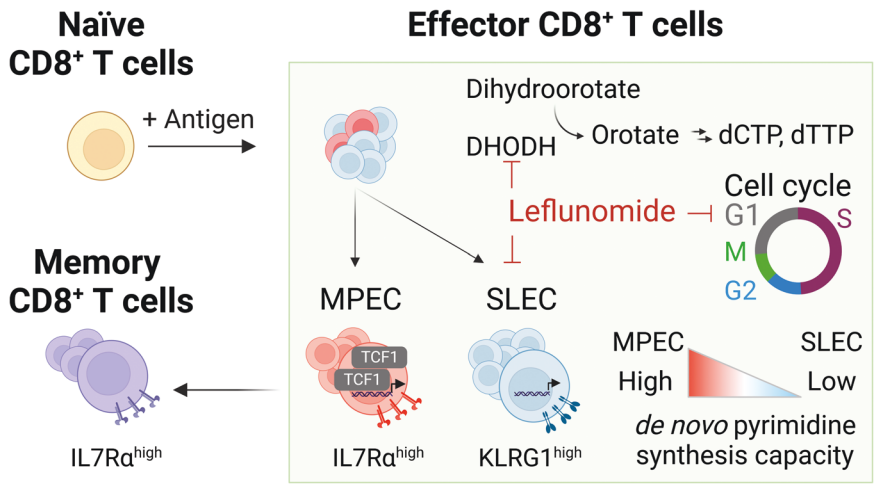

Fig. 1 Mechanism by which leflunomide influences the differentiation of $CD8^+$ T cells. Upon antigen stimulation, $CD8^+$ T cells undergo proliferation and differentiation into two types of effector cells: short-lived effector cells (SLECs) and memory precursor effector cells (MPECs). SLECs show a limited ability to synthesize pyrimidines de novo, which is regulated by dihydroorotate dehydrogenase (DHODH). MPECs show a greater ability to synthesize pyrimidines. Leflunomide inhibits DHODH, which arrests the SLEC cell cycle in the G1-S phase, whereas the cell cycle of MPECs is unaffected. Thus, MPECs continue proliferating and maintain their role as memory $CD8^+$ T cells. The illustration was created using BioRender.com

antigen stimulation during LCMV Clone 13 infection, the number of progenitor cells ($TCF1^+$ $TIM3^-$) increased, whereas the number of terminally exhausted cells ($TCF1^-$ $TIM3^+$) decreased on Days 7 and 41. The stemness of activated $CD8^+$ T cells, as indicated by TCF1 expression level, indicated a proliferative burst of $TCF1^+$ $PD\text{-}1^+$ $CD8^+$ T cells that differentiated into $TCF1^-$ $PD\text{-}1^+$ $CD8^+$ T cells following therapeutic vaccination [7] or anti-PD-1 therapy [8].

In summary, Scherer et al. demonstrated that pyrimidine nucleotide starvation mediated by leflunomide impaired the generation of short-lived effector $CD8^+$ T cells while maintaining the number of memory precursor effector $CD8^+$ T cells. Inhibition of DHODH resulted in an increased number of $TCF1^+$ $CD8^+$ T cells, which differentiate into memory $CD8^+$ T cells. The work of Scherer and colleagues provides insight into the mechanism through which leflunomide acts on immune cells. The findings suggest that leflunomide can improve the efficacy of immunotherapy and vaccination.

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COMPETING INTERESTS

The authors declare no competing interests.

ADDITIONAL INFORMATION

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