Fragility and Plasticity of CCR8^{+}Tregs: Providing New Directions for Cancer Treatment

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Abstract. Regulatory T cells (Tregs) are crucial for maintaining immune homeostasis. However, in the tumor microenvironment, tumor cells induce Tregs to exert immunosuppressive effects, leading to the failure of anti-tumor immunity. Traditional Treg clearance strategies, due to their lack of selectivity, damage peripheral normal Tregs during treatment, causing severe autoimmune side effects. This article analyzes the biological characteristics, fragility, and plasticity of CCR8^{+}Tregs and their molecular mechanisms. The results show that CCR8^{+}Tregs are specifically highly expressed on tumor-infiltrating Tregs, and after binding to the C-C Motif Chemokine Ligand 1 (CCL1), they maintain the stability of the Foxp3 transcriptional complex through the PI3K/AKT signaling axis. Blocking CCR8 signaling can trigger FOXO1 nuclear export and inhibit c-MAF expression, inducing IFN-γ-dependent fragility in Tregs. This fragility is the molecular basis of its functional plasticity, which can drive Tregs to reprogram to a Th1-like pro-inflammatory phenotype, thereby reshaping the TME from inhibition to activation. In summary, targeting the fragility and plasticity of CCR8^{+}Tregs holds promise for achieving precise immune regulation, providing new insights for optimizing combined immunotherapy for tumors.

Keywords: Regulatory T cells (Tregs); CCR8; fragility; plasticity; tumor immunotherapy.

1. Introduction

The clinical challenges of tumor immunotherapy largely stem from the complex local immune tolerance mechanisms within the tumor microenvironment (TME). Tregs have established themselves as a core node of tumor immune tolerance by consuming key cytokines, secreting inhibitory mediators, and directly blocking antigen presentation [1]. Early broad-spectrum depletion strategies aimed at breaking this barrier (such as targeting CD25) have been thwarted by causing severe systemic autoimmune toxicity [2]. Therefore, precise targeting targets with tumor local infiltration specificity has become an inevitable trend.

Among many molecules, chemokine receptor 8 (CCR8) stands out: CCR8 is a G protein-coupled receptor belonging to the chemokine receptor subfamily. It is expressed at very low levels in normal peripheral tissues but shows significant upregulation on the surface of Tregs infiltrating solid tumors [3]. Based on this extremely high spatiotemporal specificity, the core logic of targeting CCR8 has evolved from the traditional extensive "absolute exhaustion of macroscopic cells" to inducing Tregs to become fragility by interfering with the signaling network at the receptor's base layer and ultimately stimulating the plastic transformation of its anti-tumor function. In recent years, the development of monoclonal antibodies targeting CCR8 has moved beyond the preclinical proof-of-concept stage and entered the early clinical evaluation phase. Current intervention strategies are shifting from simply relying on immune cells to "physically clear" CCR8^{+}Tregs to "remodeling" cellular function by blocking their receptor signaling. This mechanism points to a new direction for clinical application: in particular, the combined use of CCR8 antibody with other immunotherapies such as PD-1/PD-L1 is expected to reconstruct the tumor immune microenvironment and overcome compensatory drug resistance [4]. However, clinical translation in this field still faces three major challenges: First, the TME is highly complex, and Tregs in a fragile state are at risk of malignant transformation into pro-tumor phenotypes. Second, the immune systems of animal models differ from those of humans, often resulting in reduced efficacy of drugs in humans. Third, there is currently a lack of precise biomarkers, making it difficult to identify the patient population most likely to benefit.

Therefore, a thorough understanding of the regulatory mechanism of CCR8 is of significant practical importance for optimizing future combination immunotherapy. Based on these challenges, this article systematically reviews the biological characteristics of CCR8^{+}Tregs in the TME and analyzes the core molecular network that facilitates the transformation from fragility to plasticity, providing a solid theoretical basis for innovative immunotherapies targeting CCR8 and clinical combination drug strategies.

2. CCR8 Specificity and Chemotactic Mechanism

2.1. Specificity of CCR8

Before exploring the targeting mechanism of CCR8, it is crucial to clarify its spatial expression map on Tregs. Unlike pan-Treg markers such as CD25 and CTLA-4, the expression of CCR8 exhibits extreme tissue heterogeneity. In peripheral blood mononuclear cells and healthy lymphoid organs, the transcriptional and protein levels of CCR8 on the surface of normal Tregs in a resting state are extremely low, even below the detection threshold [3]. However, driven by the hypoxic state in the TME, chronic T cell receptor stimulation, and a specific chemokine network, CCR8 is specifically and significantly upregulated on the surface of Tregs infiltrated by various solid tumors (such as breast cancer, colorectal cancer, and melanoma) [3]. Multiple studies based on single-cell RNA sequencing and multicolor flow cytometry have consistently shown that CCR8 is not uniformly distributed across all Tregs but is highly enriched on effector Treg subsets with terminal differentiation characteristics and strong immunosuppressive activity. This difference in expression gradient, characterized by "extremely low levels in the periphery and high enrichment in the tumor," constitutes the core phenotypic feature that distinguishes tumor-infiltrating Tregs from peripheral circulating Tregs. It is this almost discontinuous difference in expression levels that provides a key molecular biological basis for accurately detaching from local immunosuppression and avoiding systemic autoimmune toxicity in clinical practice [3].

2.2. CCR8 Chemotaxis Mechanism

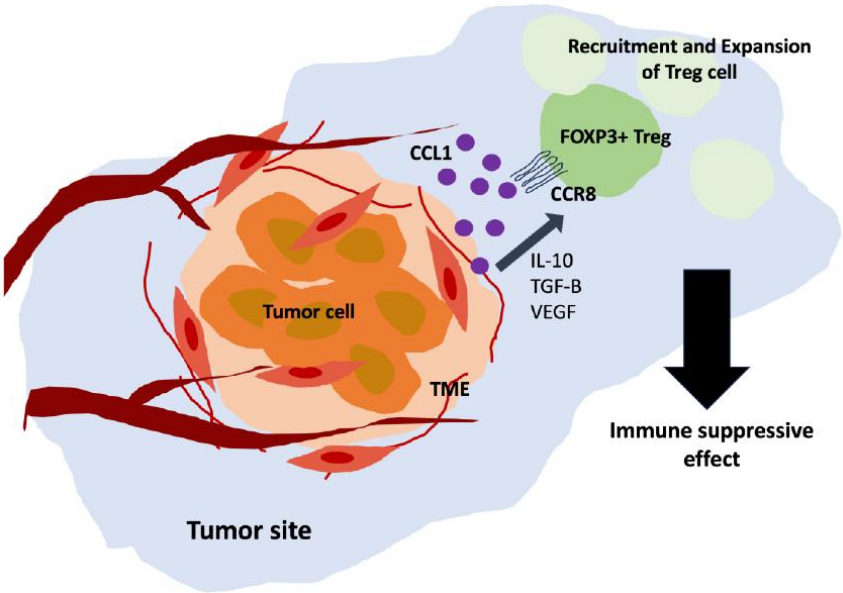

Fig. 1 Chemotaxis of CCL1-CCR8 [3].

To explore the biological significance of the specific upregulation of CCR8 on Tregs, it is necessary to trace its pathological causes. Studies have shown that the highly heterogeneous network within tumor tissue determines the distribution of immune cells, especially tumor-associated macrophages (TAMs) which can continuously secrete C-C Motif Chemokine Ligand 1 (CCL1), a high-affinity ligand of CCR8[5]. The CCL1-CCR8 axis forms a concentration gradient within the

TME, which directionally recruits peripheral Tregs to the tumor lesion and produces a strong immunosuppressive effect by releasing factors such as Interleukin (IL-10), Transforming Growth Factor-β (TGF-β), etc. (Figure 1). More importantly, $CCR8^+$ Tregs show a clear spatial aggregation tendency in their microscopic distribution. They are specifically anchored at the peripheral edge of the tertiary lymphoid structure (TLS) and in areas with dense antigen-presenting cells [6]. This occupancy allows Tregs to exert a strong spatial and biochemical blockade on the interaction between dendritic cells (DCs) and $CD8^+$ T cells at the upstream of antigen presentation [1,7].

3. Fragility of Tregs

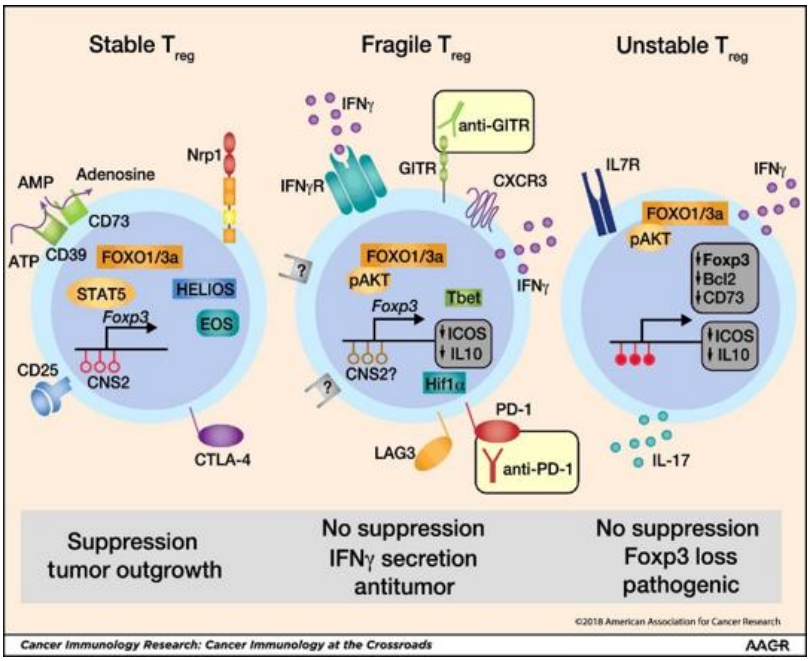

Fig. 2 Functional fragility induction of Tregs [8].

Intervention targeting CCR8 signaling aims primarily to deprive Tregs of the survival and functional maintenance signals in extreme microenvironments, inducing their transcriptional and metabolic degradation, i.e., entering a fragile state. In immunology, fragility does not refer to direct cell necrosis or apoptosis, but rather to the loss of core immunosuppressive functions by Tregs without the complete loss of the lineage-determining factor Foxp3 [4,8]. Under undisturbed homeostasis, Tregs mainly rely on the CD39/CD73 metabolic pathway and highly expressed molecules such as CTLA-4 on their surface to maintain the immunosuppressive barrier that promotes tumor growth [8].

Specific blocking of CCR8 signaling is highly dependent on the disintegration of the core transcriptional network within Tregs, particularly the PI3K/AKT-FOXO1-c-MAF signaling cascade. Under physiological conditions, CCL1-CCR8 signaling fine-tunes the PI3K/AKT pathway, ensuring that the transcription factor FOXO1 remains dephosphorylated and stably resides in the nucleus, thereby maintaining high-throughput transcription of Foxp3 and other repressive genes; simultaneously, it upregulates c-MAF expression to maintain lineage stability. When an antagonist is introduced to block CCR8 signaling input, the PI3K/AKT pathway experiences compensatory abnormal fluctuations, leading to FOXO1 hyperphosphorylation and its expulsion from the nucleus into the proteasome degradation pathway. Accompanying the degradation of FOXO1, Foxp3 promoter activity decreases significantly, and c-MAF expression is greatly reduced [9]. The collapse of this internal transcriptional network directly leads to a significant downregulation of inhibitory molecules such as ICOS, IL-10, and CD73, resulting in the loss of their inhibitory function. Intracellular T-bet and HIF-1α are activated, and IFN-γ is secreted. Subsequently, the cell enters a deeply fragile state, the local immunosuppressive field of the tumor is disintegrated, and Tregs begin

to exert anti-tumor effects [9]. They become fragile Tregs that still contain Foxp3 but have lost their function, no longer inhibiting but instead promoting inflammation and fighting tumors; however, if the stimulation is too strong, pAKT continues to rise, leading to the complete loss of Foxp3, becoming pathogenic unstable Tregs [8] (Figure 2).

4. Plasticity of Tregs

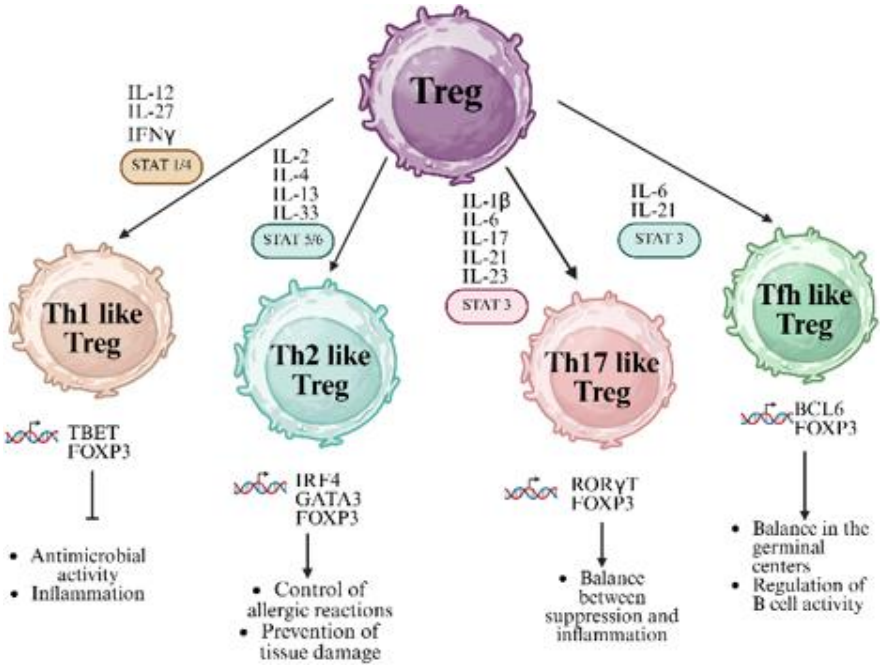

Fig. 3 Phenotypic plasticity of Tregs and transcriptional reprogramming networks [10].

Fragility signifies the loss of the original homeostasis, while plasticity represents the remodeling of cell function. Driven by TME factors, Tregs in the fragile phase exhibit high plasticity across the subpopulation barrier, not only completely losing their inhibitory function, but also actively transforming into effector-like cells with anti-tumor activity [11] (Figure 3).

4.1. Th1-like Phenotypic Polarization

Blocking CCR8 not only alters the transcriptional network but also reshapes the cell's metabolic preferences, prompting Tregs to shift from oxidative phosphorylation to glycolysis. The significant upregulation of glycolytic flux stabilizes hypoxia-inducible factor 1α (HIF-1α). HIF-1α accelerates the positive cycle of glycolysis on the one hand, and on the other hand, it synergizes with T-bet induced by residual IL-12 in the microenvironment and directly binds to the promoter region of the IFNG gene. Ultimately, these Tregs, which were initially recruited by the tumor, undergo phenotypic reversal, synthesize large amounts of the anti-tumor cytokine IFN-γ, and achieve functional transition to Th1-like cells [9].

4.2. Immune Cascade Activation

The transformation of Tregs to the Th1-like phenotype initiates a wide range of environmental network effects. The high concentration of IFN-γ released by reprogrammed Tregs directly acts on DCs around TLS, causing a high upregulation of MHC class I/II molecules on their surface, a rapid increase in antigen cross-presentation efficiency, and prompting DCs to release IL-12. Under this dual synergistic effect, the previously suppressed $CD8^+$ T cells and NK cells quickly recover from the exhaustion phenotype, regain their proliferative capacity, and release large amounts of perforin and granzyme [7]. Targeted intervention activates the synergy of various levels of the immune system by promoting the plasticity transformation of Tregs, thereby achieving an exponential amplification of the anti-tumor response [7,12].

5. Current Clinical Status

Currently, CCR8 targeting strategies are mainly divided into two complementary pathways: physical clearance and functional remodeling. The former focuses on enhancing ADCC/ADCP effects through Fc region. For example, Bayer's BAY 3375968 has entered Phase I clinical trials for advanced solid tumors (NCT05537740). In a CT26 colon cancer model (n=10), Roider et al. used a 10 mg/kg twice-weekly regimen to significantly reduce tumor volume (T/C ratio decreased to 0.02); In the MC38 colon cancer model, after combination therapy with anti-PD-L1 monoclonal antibody, the survival rate of mice increased to 80% and induced durable immune memory [13]. Analysis shows that although this strategy can rapidly reduce the absolute burden of Tregs to relieve spatial blockage, the efficacy is highly dependent on the infiltration of NK cells and macrophages in the core area. Once the core area penetration is insufficient, physical depletion alone is still difficult to completely dismantle the intrinsic chemotactic network of the deep hypoxic area. The other pathway induces phenotypic remodeling by blocking receptor signals. The antagonistic monoclonal antibody IPG0521m aims to intervene in the underlying transcriptional homeostasis of Tregs without reducing the total number of Tregs. Tian et al. demonstrated in an H22 hepatocellular carcinoma model (n=40, randomly divided into 4 groups, administered for 19 days) that a dose ≥3 mg/kg could completely inhibit tumor growth. scRNA-seq profiles showed that the total local Tregs did not decrease statistically after administration, but a large-scale shift from high-inhibitory subsets to low-inhibitory subsets occurred, thereby relieving the microenvironmental inhibitory field and highly activating the cytotoxicity of $CD8^+$ T cells and NK cells [9]. This further indicates that non-depleting blockade directly targets the transcriptional network that maintains Treg homeostasis, breaking away from the heavy dependence on local infiltration of host innate effector cells, and establishing a mechanistic reference for the next generation of highly selective combination therapies.

6. Clinical Challenges and Biomarker Development

The TME of patients is rich in high concentrations of IL-6 and TGF-β. Tregs in a fragile state are very likely to deviate from the Th1-like trajectory and undergo "malignant plasticity" transformation - being reprogrammed into Th17 cell lineages with high pro-tumor properties [10]. Abnormally activated transcription factors drive the secretion of large amounts of IL-17, which not only induces dense tumor neovascularization networks, but also recruits myeloid-derived suppressor cells (MDSCs) to take over immunosuppression, leading to fatal distant metastasis and compensatory drug resistance [6]. In the face of this risk, it is crucial to develop a precise biomarker system for predicting efficacy. In the future, clinical practice needs to accurately assess the expression abundance and spatial distribution of CCL1 in tumor specimens, and quantify the dynamic ratio of local pro-inflammatory factors and immunosuppressive factors (such as the IL-12/IL-6 ratio). Combining digital pathology to assess TLS density and the spatial aggregation of $CCR8^+$Tregs may help to accurately screen high-benefit populations and avoid the risk of malignant phenotype deviation in advance by combining strategies such as IL-6 receptor antagonists [6].

7. Conclusion

This article addresses the translational medicine challenge of traditional Treg clearance strategies easily inducing systemic immunotoxicity, systematically reviewing the spatial infiltration characteristics and targeted intervention mechanisms of $CCR8^+$ Tregs in tumor sites. The clinical value of targeting the CCL1-CCR8 chemokine axis has now surpassed that of early simple physical exhaustion. Its core logic lies in intervening in underlying transcriptional networks such as PI3K/AKT-FOXO1, causing Tregs to escape immunosuppressive homeostasis and transform into a fragile state that secretes IFN-γ, even undergoing phenotypic reversal. This intervention strategy based on cell plasticity transforms the local, singular release of inhibition into a systemic anti-tumor cascade amplification, providing a crucial theoretical basis for overcoming immune checkpoint

monotherapy resistance and designing next-generation combination therapy regimens. Of course, clinical translation in this field still faces certain limitations. The heterogeneity between existing animal models and the real human immune microenvironment often limits the direct application of efficacy data; simultaneously, fragile Tregs, within complex local inflammatory cytokine networks, still harbor the risk of malignant shift towards the pathogenic Th17 lineage. Based on this, future research should focus on developing dual-effect targeting molecules that balance receptor antagonism and moderate depletion; more importantly, it is also necessary to construct a precise biomarker prediction system to truly achieve personalized and precise breakthroughs while avoiding the risk of immune deviation.

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