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POSTER ABSTRACTS

654.MGUS, AMYLOIDOSIS AND OTHER NON-MYELOMA PLASMA CELL DYSCRASIAS: CLINICAL AND EPIDEMIOLOGICAL

Single-Cell RNA Sequencing Reveals Hypo-Responsiveness of T and NK Cells to Interferon Stimulation As an Immune Hallmark in Asymptomatic Waldenstrom's Macroglobulinemia

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Introduction

Waldenstrom macroglobulinemia (WM) is a rare, indolent non-Hodgkin lymphoplasmacytic lymphoma of the bone marrow (BM), characterized by monoclonal IgM protein production. WM is preceded by IgM monoclonal gammopathy of undetermined significance (IgM MGUS) and smoldering WM (SWM), which are clinically detectable but asymptomatic precursor phases (collectively referred to as asymptomatic WM; AWM). Recent work has revealed significant changes in the immune microenvironment of patients with overt WM, however, less is known about immune dysregulation in patients with early-stage disease.

Methods

We performed 5' single-cell RNA sequencing (scRNAseq) on BM immune cells from patients with AWM (n=28), healthy donors (HD, n=23), and patients with smoldering multiple myeloma (SMM) (n=27). We also performed an *in vitro* stimulation experiment using universal Type I interferon (IFN) and subsequent scRNAseq on bone marrow mononuclear cells (BMMCs) and peripheral blood mononuclear cells (PBMCs) from AWM (n=3) and HD (n=3).

Results

Overall, we annotated 210,019 immune cells, excluding normal B cells. Despite their early stage, AWM patients showed significantly altered BM immune cell composition, including increased proportions of CD56dim NK cells, CD8+ effector memory cells, and CD16$^+$ Monocytes and decreased proportions of pro-inflammatory CD14$^+$ monocytes and activated and interferon (IFN)-stimulated T and NK cells ($q<0.2$). Low-risk AWM presented similar changes in immune cell composition as intermediate- and high-risk AWM, implying that compositional alterations may be established early in the disease course. Notably, however, the proportion of Tregs was significantly increased in patients with SWM compared to IgM MGUS ($p=0.04$), suggesting that Tregs may play a role in disease progression.

Next, we compared BM immune cell composition between patients with AWM and SMM. In both tumor types, patients showed significantly higher proportions of CD56dim NK cells and CD16$^+$ monocytes compared to healthy donors. However, strikingly, a significant decrease in activated and IFN-stimulated T and NK cells was only observed in AWM, suggesting it may be a hallmark of disease. Comparison of BMMCs and PBMCs from patients with AWM following *in vitro* culture revealed that the lack of IFN-stimulated cells was systemic-in both BMMCs and PBMCs-although the effect was significantly more pronounced in BMMCs than PBMCs. Interestingly, while *in vitro* stimulation with Type I IFN resulted in the generation of IFN-stimulated T and NK cells in both HD and AWM, the levels of IFN stimulation did not entirely normalize, reproducing the gradient observed in the control samples without *in vitro* stimulation: AWM BM < AWM PB < HD BM. To test whether the observed defect was due to a lack of IFN in AWM, we next compared the gene expression profile of monocytes from AWM and HD, which revealed significantly higher activity of the IFN signature in monocytes from AWM compared to HD (p

$< 10^{-5}$). This suggests that IFN levels in patients are at least not reduced. Collectively, these results indicate that T and NK cells from AWM are hypo-responsive to IFN stimulation, despite the presence of normal or possibly increased IFN in the BM microenvironment.

Conclusion

Our results indicate that despite the absence of symptoms, patients with AWM already have immune dysregulation in the BM, including the absence of IFN-stimulated T and NK cells, which may provide a rationale for therapeutic intervention. Previous studies have demonstrated clinical responses to therapeutic IFN administration, suggesting that novel formulations with improved therapeutic windows may have a role in treating patients with WM in the future.

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