

Concurrent Degranulation and NETosis from PMN-MDSC Suppress CD8⁺ T Cell Function in Waldenström Macroglobulinemia

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WM relapse is almost inevitable, even after remissions exceeding 15 years, due in part to a tumor-associated ineffective immune response. Our previous work showed symptomatic WM TME is enriched for PMN-MDSCs, suppressed T cells function. We hypothesize that PMN-MDSC-mediated suppression contributes to the inhibited immune response and operates through two concurrent mechanisms - degranulation and a regulated effector mechanism in which decondensed web-like chromatin called neutrophil extracellular traps (NETs) are released extracellularly - NETosis.

PMN-MDSCs and neutrophils were sorted from newly diagnosed WM BM aspirates. Surface expression of degranulation receptors namely, GPCRs, Fcγ and complement receptors, were profiled by flow cytometry. Degranulation was assessed across a stimulus panel including C5a, G-CSF, TNF-α, and CD25+/CD8⁺ T cell co-culture. NETosis was induced with the same stimuli, with NET formation assessed by live and confocal imaging analysis. PMN-MDSCs and CD8⁺ co-culture assays resulted in physical entrapment and inhibition of infiltrating CD8⁺ T-cells.

CITEseq analysis (n=5) identified a marked enrichment of degranulation / NETosis-associated proteins in the PMN-MDSCs. Canonical neutrophil granules, including MPO, neutrophil elastase, and AZU1 were upregulated in PMN-MDSCs. The differential gene expression analysis between PMN and M-MDSCs showed upregulated genes of histones H1-5 and H4C3. PAD4, the histone citrullination enzyme, was also overexpressed in PMN-MDSCs.

Confocal imaging and flow cytometry confirmed the presence of azurophilic, specific, and gelatinase granules in PMN-MDSCs, indicating that PMN-MDSCs exhibit components for regulated exocytosis with corresponding degranulation receptors, GPCRs, Fcγ, and C5a.

Stimulus panel treated PMN-MDSCs degranulated (0–2 hours), measured by surface mobilization of CD63 and CD66b. Notably, release of azurophilic granules was restricted to C5a and active CD8⁺ T cells. C5a treatment induced a rapid and sustained increase in CD63 (fold change 1.3, 15 min, p ≤ 0.01; 1.2 at 30 min,

$p \leq 0.01$; 1.2 at 60 min, $p \leq 0.05$), while CD8⁺ T cell co-culture drove a sharper, transient response (fold change 1.47 at 15 min, $p \leq 0.05$; 1.28 at 30 min, $p \leq 0.001$). This indicates that PMN-MDSCs reserve potent granule compartment for controlled, T cell-triggered granule release.

Further, a comparative flow cytometric analysis demonstrated a marked increase in CitH3-positive PMN-MDSC phenotype (89.95%) relative to neutrophils (57.89%, $p = 0.003$). Time-lapse imaging showed that PMN-MDSCs exhibit a concurrent program, consisting of "vital" and "suicidal" NETosis, occurring within the same time frame as degranulation. Both *vital* and *suicidal* NETosis involve chromatin release, where *vital* maintains cell viability and functionality. Stimulus panel triggered robust NET formation, resulting in NETs physically ensnaring T cells. All treatment groups exhibited measurable NETosis, where C5a and CD8 elicited the greatest response 71.33% ($p = 0.00856$) and 93% ($p = 0.000157$), while G-CSF and TNF showed comparatively lower values of 54% and 58% NETosis.

Our data shows that PMN-MDSCs are not passive bystanders but receptor-equipped effectors that deploy both selective granule exocytosis and NETosis to physically and functionally restrain CD8⁺ T cell responses and positions degranulation and NETosis as interconnected therapeutic targets with potential to restore T-cell function and improve drug efficacy in WM.

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Special Instructions: The study has two co-authors Dr. Vaishali Bhardwaj and Dr. Prithviraj Mukherjee, who contributed equally for the research.

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