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POSTER ABSTRACTS

506.BONE MARROW MICROENVIRONMENT

Leukaemia Exposure Alters the Transcriptional Profile and Function of Macrophages in the Bone Marrow Niche

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Macrophages are fundamental cells of the innate immune system. Bone marrow (BM) resident macrophages are involved in antigen presentation, phagocytosis, efferocytosis (clearing of apoptotic cells) and play a critical role in regulating haematopoietic stem cell (HSC) function. In chronic myeloid leukaemia (CML), the disease-initiating leukaemic stem cells (LSCs) modulate the BM microenvironment to support leukaemia maintenance and progression. However, whether the CML BM niche alters the function of macrophages remains unknown.

Using single-cell RNA sequencing (scRNA-seq) of human BM mononuclear cells, we demonstrate heterogeneity of tissue resident macrophages, isolated from CML patients at diagnosis, which cluster separately from BM macrophages deriving from healthy individuals. As most BM macrophages at diagnosis express BCR::ABL1 and are sensitive to TKI treatment, we further investigated if Philadelphia chromosome negative (Ph⁻) macrophages play a major role in CML development. We applied a chimeric CML mouse model (SCLtTA/BCR::ABL1) and reveal that temporal depletion of macrophages prior to leukaemia induction (using CSF1R antagonist antibody), increases leukaemia burden and decreases survival of chimeric mice following BCR::ABL1 induction ($p < 0.05$). Comparison of CML exposed Ph⁻ BM macrophages to control counterparts by scRNA-seq demonstrates that the CML niche drives two unique subpopulations of immature and anti-inflammatory macrophages. Functionally, we discover that Ph⁻ BM macrophages exposed to a CML microenvironment display reduced phagocytic and efferocytic capacity, which correlates with reduction in mitochondrial respiration ($p < 0.05$). Mechanistically, we show that diminished efferocytosis is mediated by reduced expression of the plasma membrane fatty acid translocase CD36. Additionally, treatment of primary mouse and human BM derived macrophages (BMDM) with the irreversible CD36 inhibitor sulfo succinimidyl oleate (SSO), or genetic deletion of CD36 in THP-1 derived macrophages, results in reduced clearance of apoptotic cells *in vitro*. Furthermore, CD36 deficient macrophages display decreased mitochondrial respiration, similarly to CML exposed Ph⁻ BMDM.

Through untargeted liquid chromatography-mass spectrometry approach we identified the immunomodulatory protein lactotransferrin (LTF) as differentially secreted protein by leukaemic mouse stem/progenitor cells, when compared with secretome of normal counterparts ($p < 0.05$). Treatment with LTF replicates the effect of CML exposure on BMDM, including decreased phagocytic function of mouse BMDM and THP-1 derived macrophages ($p < 0.05$). Furthermore, mouse/human BMDM display reduced clearance of CML apoptotic cells following LTF exposure. Notably, LTF treatment reduces CD36 transcript and protein levels on BMDM and/or THP-1 derived macrophages, which correlates with reduced oxygen consumption rate and a 40% decrease in expression of the monocytic marker CD11b during macrophage differentiation, suggesting that secreted LTF is a major contributor to the immature phenotype of CML exposed macrophages.

Overall, we present novel findings that dysregulated CML microenvironment modifies BM macrophage function, including their phagocytic and efferocytic capacity. This is at least partially contributed by secretion of LTF from CML stem/progenitor cells and modulation of CD36 expression on altered BM macrophages.

Disclosures Copland: *Incyte*: Honoraria, Membership on an entity's Board of Directors or advisory committees, Research Funding, Speakers Bureau; *Servier*: Honoraria, Membership on an entity's Board of Directors or advisory committees; *Novartis*: Honoraria, Membership on an entity's Board of Directors or advisory committees, Speakers Bureau; *Pfizer*: Honoraria, Speakers Bureau; *Astellas*: Honoraria, Speakers Bureau; *Jazz*: Honoraria, Membership on an entity's Board of Directors or advisory committees.

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