Topic: 13. Myeloma and other monoclonal gammopathies - Biology & translational research

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Age-Associated Macrophage Polarization to an M2 Phenotype Drives Myeloma Proliferation in the Aged Bone Marrow by Loss of Tumor-Associated Phagocytosis

Type: Poster Presentation

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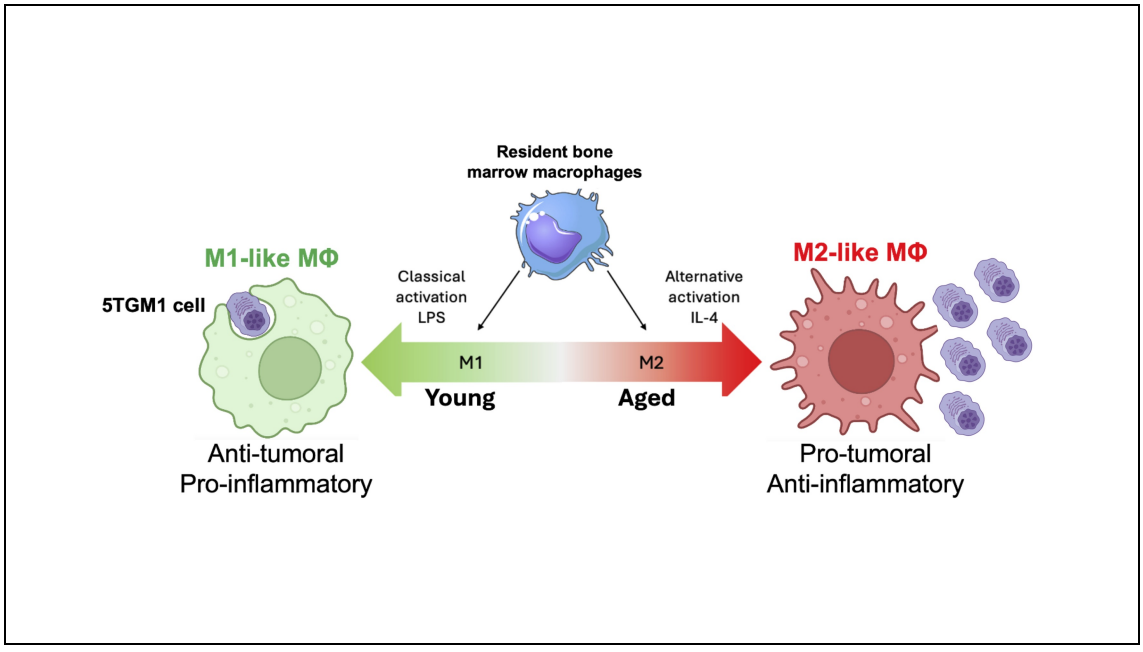
Background

Multiple myeloma (MM) is an age-related disease which hijacks cellular interactions in the bone marrow microenvironment (BMM) to promote its survival and progression. M2-like macrophages reportedly exhibit a pro-tumoral phenotype that supports immune BM remodelling in MM (4,5). Further, the senescence-associated secretory phenotype (SASP) promotes a pro-tumoral BMM with increasing age.



Aims

Using *in vitro* and *in vivo* modelling, we aim to determine if ageing drives changes in BM macrophage phenotype and function that may promote MM.

Methods

5TGM1 myeloma cells were established in the ageing KaLwRij mouse model. Harvested cells were injected intravenously into young (3-4 months), middle-aged (12-14 months), and aged (18-22 months) C57BL/6 mice. After 30 days, BM and spleen were harvested for flow cytometry. BM from non-injected young and aged C57BL/6 mice was analysed by flow cytometry for macrophage populations and phagocytosis (pHrodo^{TM} assay). To explore the effect of M1 and M2 macrophages on 5TGM1 viability, bone marrow-derived macrophages were cultured and stimulated to M1 or M2-polarisation state. 5TGM1 cells were co-cultured with M1 and M2 macrophages for 24hr to assess 5TGM1 cell viability. Using publicly available 10x scRNA-seq datasets, we analysed the metabolic profile of M1 and M2 macrophages in the BM of myeloma (n=24) vs healthy patients (n=12) by differential expression analysis. Lastly, aged C57BL/6 and KaLwRij mice were both engrafted with 5TGM1 and macrophages isolated after 30 days to assess expression of NAD+ biosynthesis genes by qPCR.

Results

We and others have shown that 5TGM1 cells do not engraft in young C57BL/6 mice but can engraft in obesity-induced C57BL/6 mice. Here, we show that the ageing C57BL/6 BM is permissive to myeloma as 5TGM1 cells engrafted in all aged mice (7-34% BM GFP^{+}, 3-26% splenic GFP^{+}), while only 2/9 middle-aged mice had minimal engraftment (<2% BM GFP^{+}) and no young mice engrafted. To investigate the underlying mechanism permitting 5TGM1 engraftment in the aged C57BL/6 BMM, flow cytometry identified an elevated total macrophage count in aged mice compared to young, concomitant with an overall elevated M2/M1 ratio in the aged mice. Additionally, 5TGM1 viability was reduced in M1 co-culture vs co-culture with M2 or 5TGM1 cells alone, which correlated with an impaired phagocytic capacity in the aged BM vs young. Furthermore, scRNA-seq analysis identified upregulation of M2 but not M1 gene sets in myeloma patients from four independent datasets vs healthy patients, together with upregulated expression of nicotinamide phosphoribosyltransferase (NAMPT), though its expression was heterogenous across the macrophage cluster. Finally, we verify that NAMPT is upregulated alongside other NAD+ biosynthesis genes in macrophages from aged C57BL/6 and KaLwRij engrafted mice.

Summary/Conclusion

Here, we show that aged C57BL/6 mice are permissive of 5TGM1 cells. We show that this may in part be mediated by an elevated M2/M1 ratio in the aged BMM and demonstrate that macrophages in the aged BM have a reduced phagocytic capacity. Taken together, these data suggest that a loss of M1-like phenotype or a shift toward the M2-like phenotype in the aged BMM may confer a permissive, anti-inflammatory state for MM development. We also identify NAMPT as a potential metabolic target in myeloma-associated (M2) macrophages – future work will assess the effect of NAMPT inhibition on macrophage phenotype and function using our existing models of MM.

Keywords: Aging, Phagocytosis, Macrophage, Multiple myeloma