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ORAL ABSTRACTS

503.CLONAL HEMATOPOIESIS, AGING AND INFLAMMATION

Clonal Derivation and Dynamics in Rhesus Macaques Following Hematopoietic Stem Cell Transplantation and the Impact of Clonal Hematopoiesis

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Microglia (MG) are the resident macrophages of the central nervous system (CNS). Under steady-state conditions, MG play essential roles in immune surveillance; phagocytosis and processing of pathogens, dying cells and protein aggregates; and synaptic pruning to maintain neuronal health. MG dysfunction has been linked to various CNS disorders, including neurodegenerative disorders such as Alzheimer's disease, with multiple pathways implicated, including increased and prolonged activation with excessive release of pro-inflammatory mediators. Lineage tracing studies in mice have demonstrated that yolk sac macrophages form the CNS MG compartment during development, with little ongoing replacement from circulating monocyte/macrophages at steady state or even following hematopoietic stem/progenitor cell (HSPC) transplantation. However, improvements in disease outcomes in human patients with CNS disorders such as adrenoleukodystrophy and metachromatic leukodystrophy following HSPC gene therapies have suggested that adult HSPC-derived cells can contribute to MG function. Recently, Bouzid et al reported that individuals with somatic HPSC mutations in hematopoietic expansion driver genes (clonal hematopoiesis/CH) had a significantly lower risk of Alzheimer's disease (AD). The same study utilized nuclear chromatin profiling of a small set of postmortem brain samples to suggest substantial contribution of CH-derived HSPC to the MG compartment. The authors suggested that enhanced function of CH-derived MG enables them to better clear AD plaques.

To more directly investigate the replacement and clonal source of adult CNS resident MG/myeloid cells by HSPC-derived cells and to and assess the impact of CH mutations on MG replacement, we utilized clinically-relevant rhesus macaque (RM) models. Fresh MG and other tissue-resident myeloid cells were purified from the brains of RM that had undergone autologous transplantation 2- 9 years previously with HSPCs transduced with a barcoded lentiviral vector expressing GFP following ablative total body irradiation (TBI, n=6) (Koelle et al, Blood, 2017) or busulfan (n=1, vector without GFP)(Abraham et al, MTMCD, 2023); or HSPCs engineered to carry *TET2* mutations using CRISPR/Cas9 (n=2)(Shin et al, Blood, 2022). In addition, we studied

an aged RM with natural CH (*RUNX1* mutation) in order to investigate MG replacement in the absence of conditioning and transplantation.

We optimized RM MG (CD45+CD11b+CX3CR1+) and CNS myeloid cell (CD45+CD11B+CX3CR1-) purification. To ensure separation of bona fide tissue-resident CNS cells away from perivascular cells or contaminating blood, we incorporated CD45-specific antibody serial intravascular staining (SIVS) (Mortlock et al., 2022) and sorted SIVS- parenchymal MG, SIVS- perivascular MG, and SIVS+ myeloid cells. In all 6 TBI-conditioned lentiviral-barcoded animals, at most 2% of tissue-resident SIVS- MG cells were GFP+ (Fig 1A), with a mean ratio of <0.01 compared to the level of GFP+ circulating blood monocytes in each animal (Fig 1B). Barcode analyses demonstrated similar clonal derivation of GFP+ MG cells to peripheral blood (PB) monocytes, representing output from stable long-term multipotent HSPC clones. A higher MG repopulation level was found in the busulfan-conditioned animal by vector copy number. MG replacement with CH-derived cells was no higher absolutely or relative to blood monocytes in the two engineered *TET2*-CH animals nor in the aged animal with natural CH (Fig 1B). These results demonstrate only low-level replacement of MG from HSPCs even many years following ablative conditioning and autologous transplantation, and no evidence for enhanced repopulation in the setting of engineered or natural clonal hematopoiesis. The clonal ontogeny of the HSPC-derived MG cells matched that of blood myeloid cells.

Further characterization of MG derived from transplanted HSPCs versus control MG is ongoing, as is functional characterization of CH versus unmutated macrophages, asking whether mutated cells are hyper-functional. Our clinically-relevant macaque model provides insights into MG ontogeny, and information relevant to improving CNS-directed HSPC gene therapies, as well as information relevant to interpreting a potential protective impact of CH on human AD.

Disclosures Vassiliou: *AstraZeneca:* Other: Educational Grant; *STRM.BIO:* Consultancy.

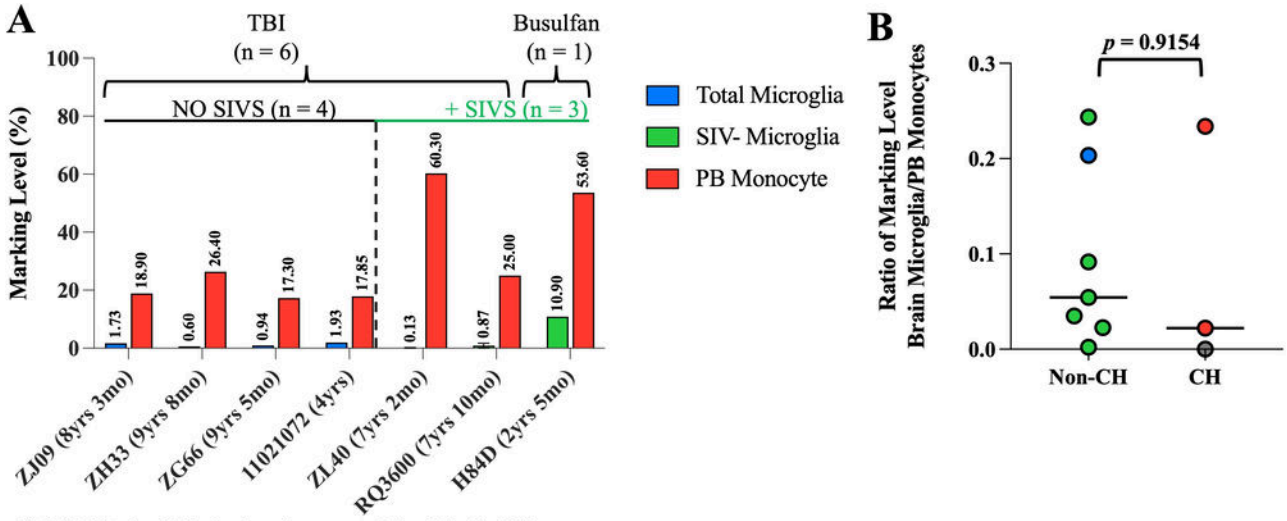

Fig 1. Marking level in brain microglia versus peripheral blood in RMs.
(A) The bone marrow HSPC associated marking level in brain MG is compared with peripheral blood monocyte in 7 rhesus macaques that had undergone autologous transplantation 2-9 years previously with HSPCs transduced with a barcoded lentiviral vector expressing GFP following ablative TBI (n=6) or busulfan (n=1, vector without GFP). ZL40, R3600 and H84D received SIVS labeling prior to MG isolation. Marking level (%) is assessed by GFP in TBI RMs, or by vector copy number in the busulfan animal. Marking levels in total MG ($CD45^{int}/CD11b^{high}/CX3CR1+$) or SIVS- MG ($CD45^{int}/CD11b^{high}/CX3CR1+/SIVS-$) are compared with contemporaneous peripheral blood monocytes (CD14+/CD163-). The time post-transplant is indicated for each animal. The marking level is labeled above each bar. **(B)** Comparison of the ratio of bone marrow HSPC-associated marking in microglia/PB monocytes in non-CH RMs (n = 7) and CH RMs (n = 3). The marking level in TBI- or busulfan-conditioned non-CH RMs is assessed by GFP or vector copy number while marking level in TBI-conditioned CH RMs is assessed by mutation variant allele frequency (VAF). The ratio of microglia/PB monocyte is calculated by dividing the marking (%) in brain microglia over the marking (%) in PB monocytes. Green dots indicate TBI conditioned RMs with lenvitral vector transduction while the blue dot indicates busulfan conditioning with lentiviral transduction in non-CH RMs. Red dots indicate TBI-conditioned RMs with CRISPR/Cas9 engineered *TET2* mutations, while the grey dot indicates a RM with naturally developed *RUNX1* mutation. The p-value is calculated using T-Test.

Figure 1

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