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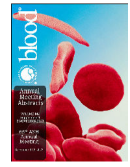

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ORAL ABSTRACTS

503.CLONAL HEMATOPOIESIS, AGING AND INFLAMMATION

Chronic TNF in the Aging Microenvironment Exacerbates *TET2*-loss-of-Function Myeloid Expansion

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Introduction: Age-associated *TET2* somatic mutations impart an intrinsic hematopoietic stem cell (HSC) advantage and contribute to the phenomenon of clonal hematopoiesis of indeterminate potential (CHIP). Individuals with *TET2*-mutantCHIP have a higher risk of developing myeloid neoplasms and other age-related conditions, including heart, lung, liver, kidney and infectious disease, and have increased risk of all-cause mortality. Despite its role in unhealthy aging, the extrinsic mechanisms driving *TET2*-mutant CHIP clonal expansion remain unclear. We previously showed an environment containing TNF favours *TET2*-mutant HSC expansion *in vitro*. We therefore postulated that age-related increases in TNF also provide an advantage to HSC and progeny with *TET2*-mutations *in vivo*.

Methods: All human and mouse studies met ethics approval. C57Bl/6 background wildtype (WT), TNF-α knockout (*TNF-/-*), Tet2 hematopoietic knockout (Vav1-iCre-mediated; *Tet2-/-*) and floxed control mice (*Tet2f/f*) mice were obtained from the Jackson Laboratory. Young (6-mo [n=9]) and old (18-22 mo [n=9]) WT mice, and old *TNF-/-* mice (n=7) were subjected to nonirradiative myeloablation with busulfan, and retro-orbital injections of $8x10^{6}$ cells/ml T-cell-depleted bone marrow (BM), equally harvested from 4-mo-old WT CD45.1 (n=5) and *Tet2-/-* CD45.2 donor mice (n=5). Flow cytometry was used to confirm and monitor engraftment. Mice were harvested 8 weeks post-transplant for analysis of HSC, progenitor, monocyte and neutrophil populations, and cytokine analyses. Consenting human research participants diagnosed with rheumatoid arthritis (RA) were recruited from the Greater Hamilton Area (Ontario, Canada) from 2016-2018. Blood draws occurred prior to any immunomodulatory treatment (baseline), and at 3- and 6-months following treatment with Adalimumab (Humira®), an anti-TNF agent. CHIP status was determined with our successful 48-gene, targeted, Ion-Torrent based sequencing approach to isolated genomic DNA from PBMC. Confirmation of calls and increased sensitivity to detect clones with VAF < 0.02 employed our established single molecule molecular inversion probes (smMIP)-targeted genomic capture technique and high-depth (47,500X avg. coverage) paired-end sequencing, employing high-stringency filters for error-suppression. Statistical analyses were performed in GraphPad Prism V9.2 or R 4.1.2.

Results: 1. Mixed BM chimeric mice of WT and $TNF^{-/-}$ genotypes reconstituted with WT CD45.1 $^{+}$ and $Tet2^{-/-}$ CD45.2 $^{+}$ HSC showed that age-associated increases in TNF significantly increased by 2- to 2.5-fold the proportions of HSC in old recipient mice, with myeloid lineage skewing (2- to 2.5-fold more granulocyte-monocyte, monocyte-dendritic and common monocyte progenitor cells in BM, and dramatic expansion of *Tet2-/-* monocytes and neutrophils in blood). This aberrant myelomonocytic advantage was mitigated in old $TNF^{-/-}$ recipient mice, suggesting that TNF signalling in the BM is essential for *Tet2*-mutant myeloid expansion. 2. Age-associated TNF predisposed $Tet2^{-/-}$ HSC to the development of $Ly6C^{high}$ inflammatory monocytes, including increased intracellular and serum TNF levels, further exacerbating an inflammatory environment in favor of *Tet2*-mutantexpansion. 3. Examination of human RA patients (n=7; avg. age 57y) with serial PBMC sampling revealed one patient with CHIP driven by *CBL* and *PPM1D* variants, with a 32% reduction in clone size between 3 and 6 months of anti-TNF therapy (adalimumab). Two additional variants were detected at baseline in this RA patient, *TP53* R196* (VAF = 1.34%) and *ASXL1* P689L (VAF = 3.13%), but these were not detected at 3- and 6-months post-treatment even with high-depth, error suppression sequencing. Two additional patients had detectible low-level CH clones prior to anti-TNF treatment - one with a *STAG2*

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R1033Q variant at baseline (VAF = 1.65%) and the other with a *TET2* baseline E798K variant (VAF = 1.68%) - but not detected during anti-TNF treatment.
Conclusions: We present novel findings that TNF in the aging BM environment has a causal role in driving *TET2*-mutant CHIP *in vivo*, and the first examination of the impact of TNF blockade on CH dynamics in humans, suggesting promise for reducing clonal burden. Larger, prospective studies are required to confirm these findings and to determine if inflammatory cytokine blockade may improve CHIP-comorbid conditions and myeloid cancer risk.

Disclosures No relevant conflicts of interest to declare.

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