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POSTER ABSTRACTS

503.CLONAL HEMATOPOIESIS, AGING, AND INFLAMMATION

Distinct Effects of Cigarette Smoke and e-Cigarette Aerosols on Inflammation and Stem Cell Proliferation in Clonal Hematopoiesis

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Clonal hematopoiesis of intermediate potential (CHIP), the outgrowth of mutant hematopoietic stem cell (HSC) clones without blood count abnormalities, increases the risk of hematological malignancies and cardiovascular disease. The most common CHIP driver mutations are in *TET2, DNMT3A, TP53, ASXL1*, and *JAK2*, which are also classically seen in myeloid malignancies. Asxl1 is the CHIP variant most strongly associated with smoking. In a UK Biobank study of >30,000 participants, 69% of people with ASXL1 mutations were past or current smokers, while TET2 and DNMT3A mutations also had a significant but more modest association with smoking status. However, few studies have investigated the impact of the popular cigarette alternative, the electronic cigarette (e-cig). As the popular alternative to smoking, one of the biggest CHIP risk factors, it is crucial to characterize the impact of e-cigarettes on systemic inflammation and hematopoietic function. This study aims to elucidate the mechanisms by which CHIP mutations regulate hematopoiesis and inflammation in response to e-cigarette exposure and antioxidant modulation. N-acetylcysteine (NAC) is a safe and freely available dietary supplement that has anti-inflammatory and antioxidant properties.

To test the impact of cigarettes vs. E-cigarettes on *in vivo* ASXL1 expansion, lethally irradiated CD45.1/2 recipient mice were transplanted with equal numbers of whole bone marrow cells from wild type (WT) (CD45.1) and ASXL1-/- mice (CD45.2). Eight weeks post-transplant, mice were exposed to smoke (n=12) or room air (n=8) using a nose-only inhalation exposure system for 2 hours/day, 4 days/week. Smoke-exposed mice were exposed to traditional cigarette smoke for 3 months, then this cohort was split in two, with one half switching to air (i.e. smoking "cessation") or to e-cigarette aerosol (smoking "substitution"). At regular intervals, peripheral blood was drawn to determine the percentage of WT and ASXL1 cells by flow cytometry. In all groups, the percentage of Asxl1-/- in peripheral blood initially fell from ~60% to 40% and was stable throughout the exposure period. CBCs were mostly normal, with a slight derangement of platelets and monocytes near the end of exposure. At euthanasia, liver and spleen weights were normal in all mice. We measured TNFa production in LPS-stimulated splenocytes via ELISA and found no significant difference at 24hours. However, when plated in methylcellulose, splenocytes from both the smoke-air "cessation" and smoke-e-cigarette "substitution" cohort had a 30% decrease in colony proliferation. Peritoneal macrophages displayed similar rates of phagocytosis of fluorescent Zymosan *S. cerevisiae* particles. This was unexpected, as literature suggests smoke exposure should impair macrophage phagocytosis. Further studies will include use a smaller number of particles to better differentiate limited phagocytosis. In a secondary transplant, transplanted cells from the smoke-e-cigarette "substitution" cohort had reduced engraftment and survival. Together, these results suggest that traditional cigarette smoke and e-cigarette aerosols can both alter hematopoietic stem cell function but potentially by distinct cellular processes.

To explore the mechanisms of e-cigarette inflammation, whole BM from WT or CHIP-mutant mice or peripheral blood mononuclear cells (PBMCs) from MPN patients were incubated overnight with cigarette smoke extract (CSE), e-cigarette aerosol+/-nicotine, and/or NAC and plated in methylcellulose for colony formation assays. In WT and Tet2+/- cells, CSE and e-cigarette liquid significantly reduces colony formation at similar rates. However, the Tet2-/- cells were more resistant to e-cigarette reduction of colony formation. In WT cells, NAC rescued CSE-reduced colony formation, but not e-cigarette-

reduced colony formation. This suggests that the effects of CSE are related to reactive oxygen species that may be mitigated an antioxidant, while the e-cigarette extract is reducing colony formation by a different mechanism unaffected by NAC. Taken together, we can conclude that the impact of e-cigarettes on hematopoietic stem cell function is complex and warrants further characterization to improve safety for the millions of users. The inflammatory nature of smoking provides a context to study the impact of this environment on CHIP-mutant competition.

Disclosures No relevant conflicts of interest to declare.

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