

Introduction

Electronic cigarettes have surged in popularity and are often promoted as safer alternatives to traditional cigarette smoking, yet the cardiovascular consequences of nicotine exposure remain incompletely understood. Nicotine is a key driver of atherosclerotic cardiovascular disease, a direct precursor to myocardial infarction and stroke, and has been shown to promote pathological angiogenesis within atherosclerotic lesions.¹ Within these lesions, newly formed neovessels are often structurally immature and prone to rupture, leading to intraplaque hemorrhage (IPH). IPH is a critical driver of plaque instability and acute cardiovascular events, and whether nicotine-driven angiogenesis specifically generates these hemorrhage-prone vessels remains unclear.²

Recent work from our laboratory identified TNF- α as a driver of LRG1 expression in atherosclerotic plaques, promoting pathological angiogenesis and intraplaque hemorrhage in the context of cancer-associated cardiovascular disease.³ Notably, nicotine has independently been shown to elevate TNF- α ,⁴ raising the hypothesis and proposed mechanism we set out to investigate here.

Hypothesis

Given nicotine's capacity to elevate TNF- α , we hypothesize that nicotine exposure promotes intraplaque hemorrhage in murine models through TNF- α /LRG1 signaling and pathological angiogenesis.

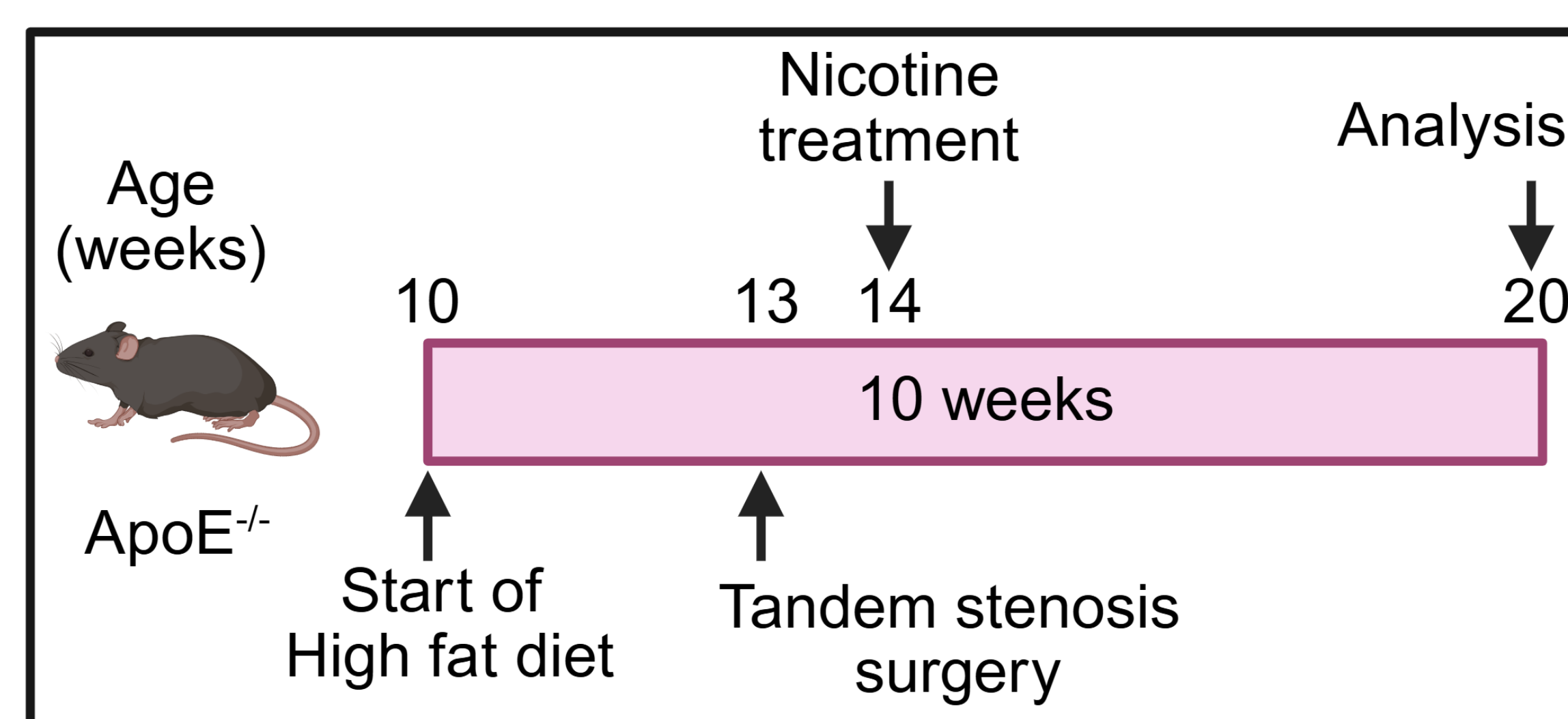
Methods

We transitioned ApoE^{-/-} mice to a high-fat diet at 10 weeks of age. After three weeks, we performed tandem stenosis surgery on the right common carotid artery to promote atherosclerotic plaque development and intraplaque hemorrhage. One week later, we implanted osmotic minipumps subcutaneously to deliver nicotine at 3 mg/kg/day in the nicotine group (n=4) and 0.9% saline in the control group (n=4). Mice were harvested six weeks after pump implantation and carotid artery plaque samples were collected for analysis.

We first assessed IPH with gross morphological evaluation of harvested arterial plaque samples. We plan to perform histological and immunofluorescence analyses of plaque sections to precisely identify intraplaque features, including:

- Plaque volume and intraplaque hemorrhage (TER-119 and CD31)
- Macrophage accumulation (CD68) and polarization (Ly6C^{high/low})
- Inflammatory markers (IL-6 and TNF- α)

Finally, we will measure circulating TNF- α by ELISA to evaluate the TNF- α /LRG1 angiogenic axis as a candidate mechanism.



Results

1. Gross Visualization of Intraplaque Hemorrhage

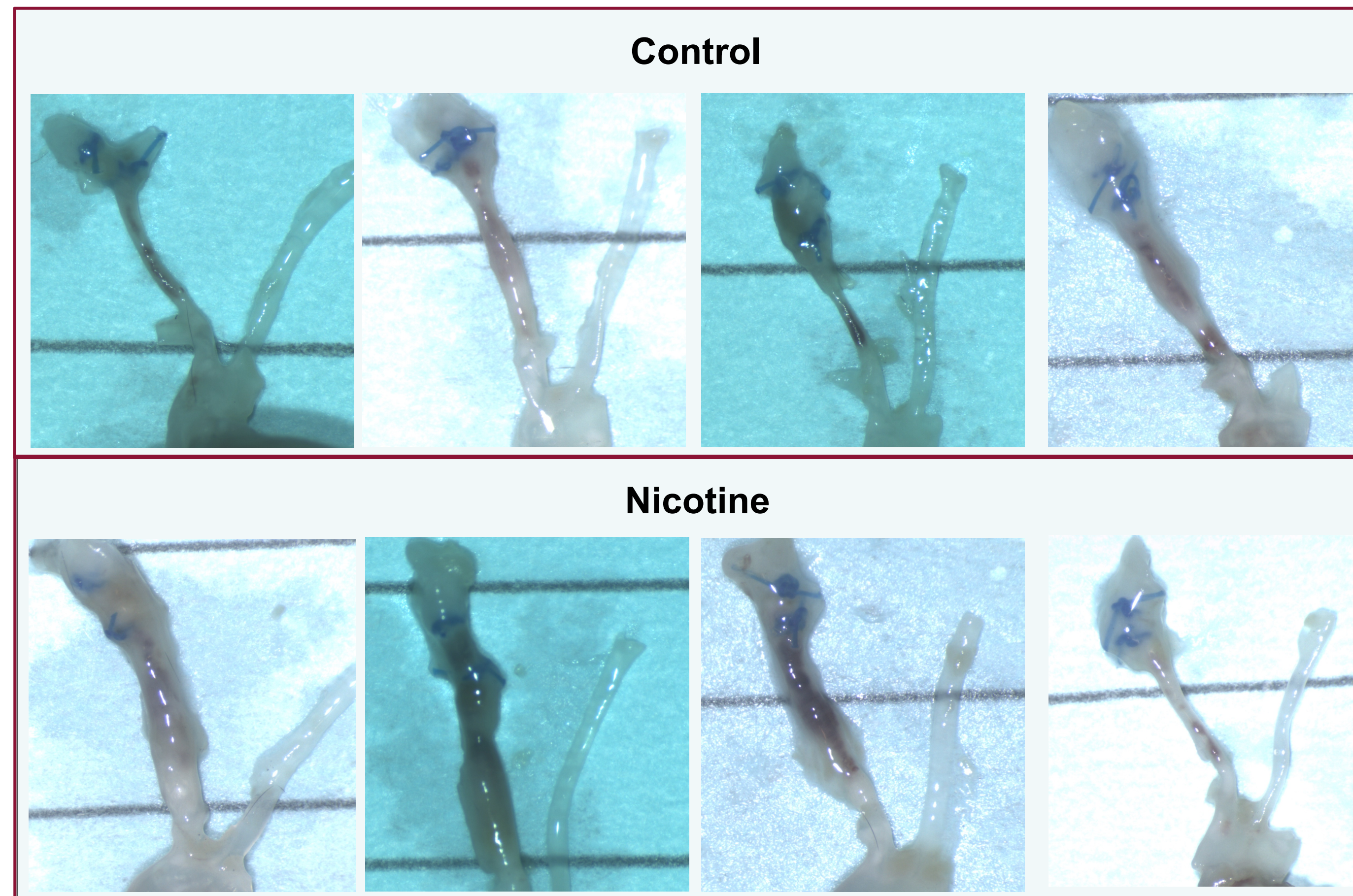


Figure 1. Gross Visualization of Intraplaque Hemorrhage. Images of carotid artery samples taken with the Leica microscope camera under the same magnification prior to histological analysis.

2. Proposed Mechanism of Nicotine-Induced Intraplaque Hemorrhage

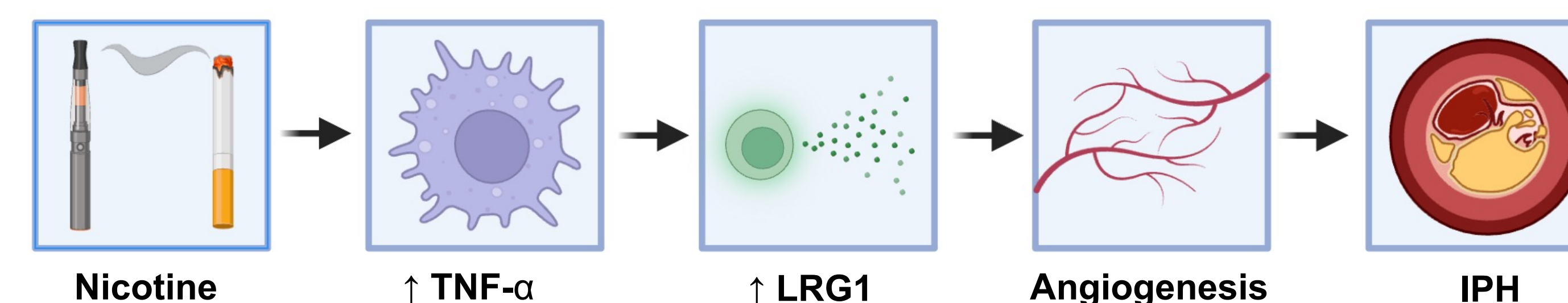


Figure 2. Proposed Mechanism of Nicotine-Induced IPH. Nicotine increases circulating TNF- α , promoting LRG1 expression and pathological angiogenesis.

3. Flow Cytometry of Blood from Nicotine-Exposed C57 Mice

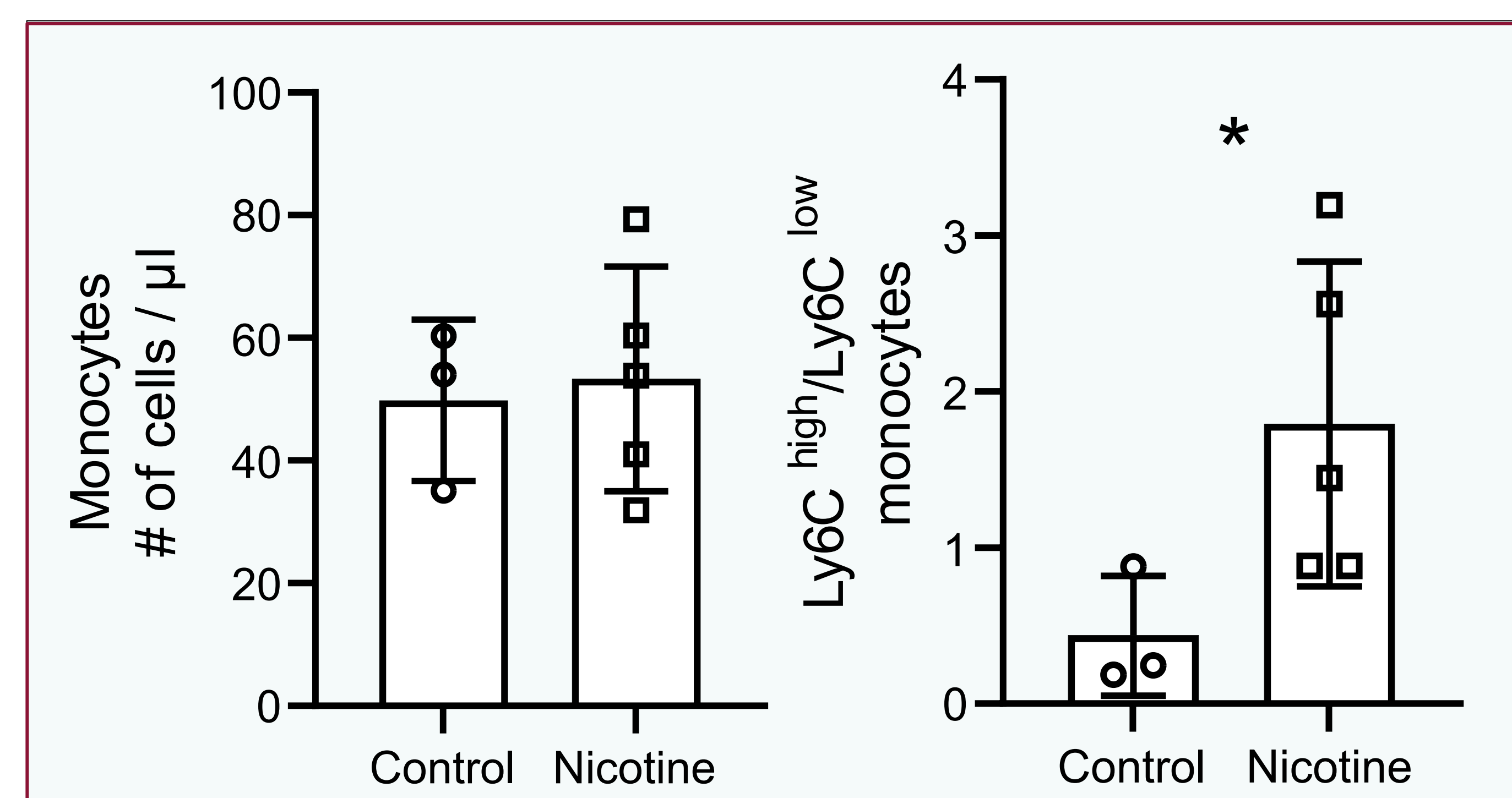


Figure 3. Flow Cytometry of Blood from Nicotine-Exposed C57 Mice. Previously in our lab, nicotine was delivered through drinking water for two weeks, followed by flow cytometry to evaluate macrophage burden and polarization.

Conclusions

Preliminary gross morphological evaluation of harvested carotid artery samples revealed potentially increased intraplaque hemorrhage in three of four nicotine-exposed mice compared to vehicle-treated controls. This provides initial observational support for our hypothesis that nicotine promotes intraplaque hemorrhage, and we intend to increase our sample size before performing quantitative analysis.

These observations are consistent with prior evidence that nicotine promotes plaque vulnerability and provide the scaffold for our upcoming histological analysis of IPH, neovascularization, inflammation, macrophage accumulation, and components of the TNF- α /LRG1 angiogenic axis. Confirmation of nicotine-induced IPH and its underlying mechanisms will require detailed histological analysis.

If validated, **these results support the hypothesis that nicotine promotes intraplaque hemorrhage through pathological angiogenesis and impaired vessel formation.** This mechanism may parallel the TNF- α /LRG1 axis established by our research in cancer-driven cardiovascular disease (CVD). Elucidating the detailed mechanisms of nicotine's harmful effects on plaque vulnerability would reveal novel therapeutic targets for nicotine-associated CVD – a significant opportunity to reduce morbidity and mortality.

Future Directions

- Analyze human cardiovascular databases including AtheroExpress and UK Biobank to validate these molecular mechanisms in human plaques.
- Evaluate the role of pericyte recruitment and blood vessel quality in nicotine-induced pathological angiogenesis and intraplaque hemorrhage.
- Conduct in vitro experiments with nicotine in human endothelial cells to trigger TNF- α /LRG1 and potential rescue by TNF- α blockade (Etanercept).

References

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