Investigating the Determinants of Clinical Phenotypes of Peripheral Artery Disease Using Mouse Models of Limb Ischemia

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Introduction

Peripheral artery disease (PAD) affects more than 200 million people worldwide and is associated with a 15-20% amputation rate at 1 year and 50% mortality at 5 years despite existing therapies. Anatomic lesions do not always correlate with clinical phenotype; thus, patients with the same vascular lesion and similar limb perfusion on angiography and ankle-brachial index may develop chronic limb-threatening ischemia (CLTI) or intermittent claudication, which is exertional pain without tissue necrosis. This can be observed in mice where different inbred strains of mice (BALB/c and C57BL/6) respond differently to the same ischemic insult. Understanding the influence of genetics on skeletal muscle tissue regeneration and characterizing the cellular heterogeneity and intercellular signaling that occur in the ischemic limb in murine models may provide insights into the development of novel regenerative therapies for PAD.

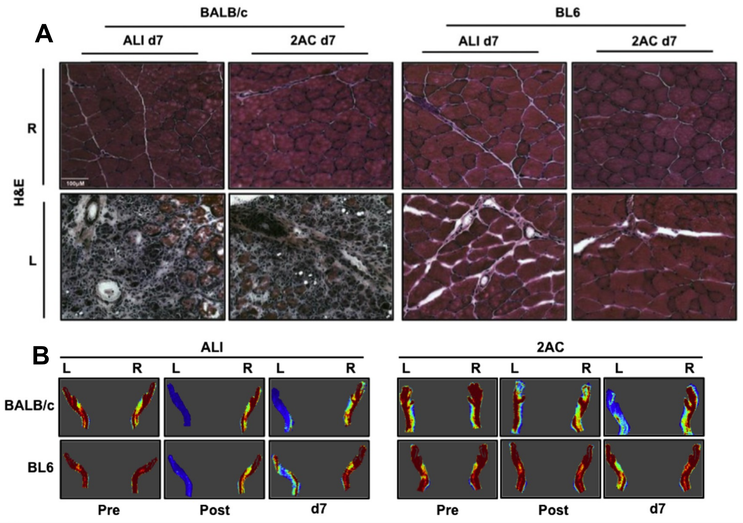
Methods

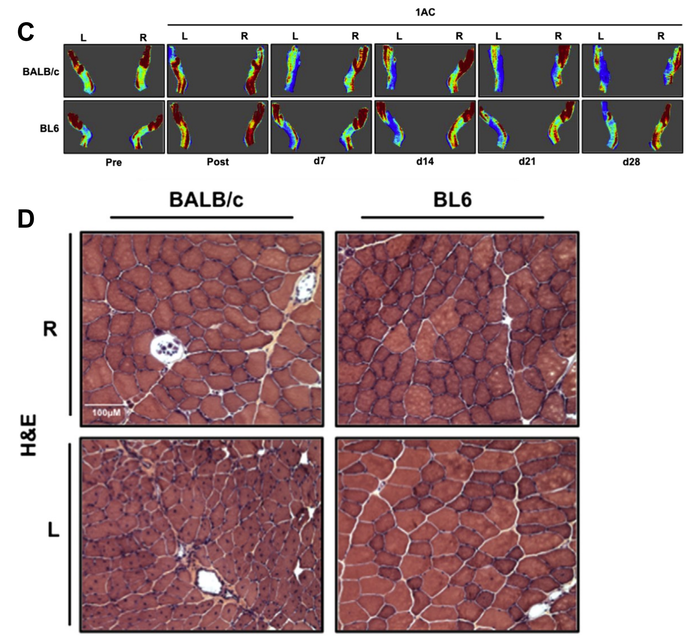
This poster presentation provides an overview of three separate publications:

1. Experiments were conducted on adult C57BL/6 (BL6) (n=51) and BALB/c (n=58) mice. Acute limb ischemia (ALI) was induced by ligation and resection of the femoral artery. Subacute limb ischemia (SLI) was induced by placement of either one or two ameroid constrictors. Muscle samples were stained with hematoxylin and eosin and quantification of blood flow was done with laser Doppler perfusion image (LDPI) scanning.
2. Mice were treated with either adeno-associated viruses encoding a control (green fluorescent protein) or the Met81 or Ile81 variants of BAG3. The mice were then subjected to hind-limb ischemia (HLI) surgery.
3. Single-cell RNA sequencing was applied to samples obtained from BL6 and BALB/c mice after HLI surgery. Cell-cell signaling interactions were obtained via CellChat analysis and macrophage differential gene expression results were analyzed using bulk RNA sequencing of macrophages. Immunostaining of ischemic and non-ischemic lower extremity skeletal muscle was done on patients undergoing amputation for CLTI.

Results

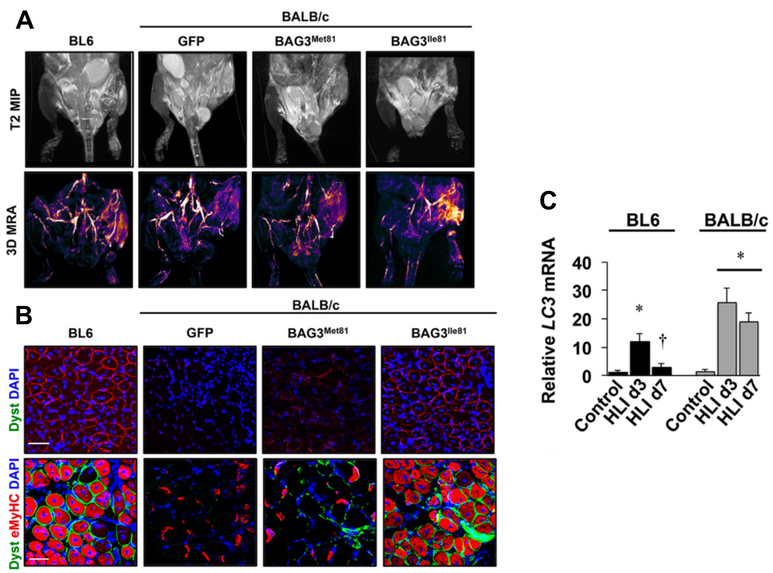
1. Subacute limb ischemia leads to significant tissue necrosis and limb perfusion deficits in genetically susceptible BALB/c but not BL6 mice independent of vascular density.





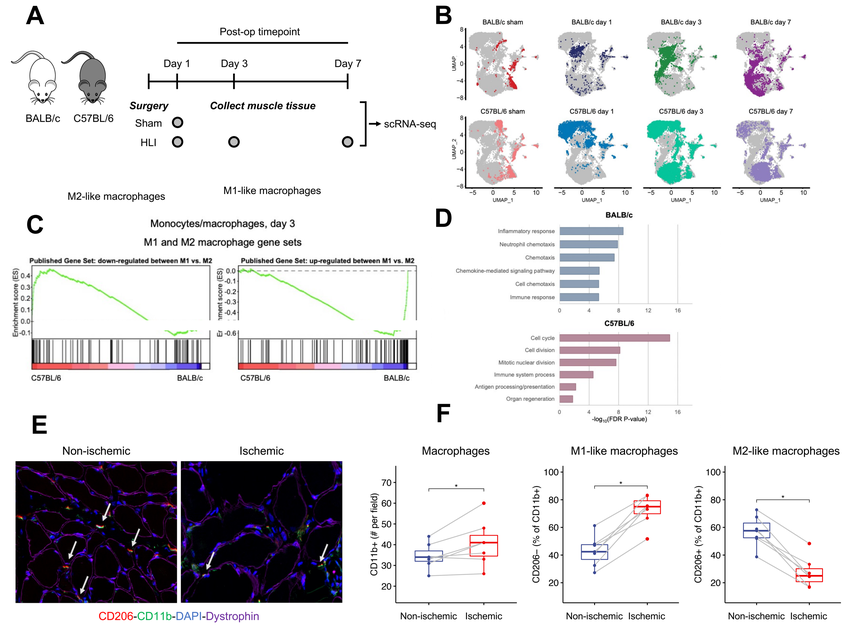
(A) H&E of TA morphology 7 days after ALI and 2AC surgery. **(B)** LDPI before and after ALI or 2 AC placement up to 7 days post-op **(C)** LDPI before and after 1AC placement up to 28 days post-op. **(D)** H&E staining of TA morphology 28 days after 1AC placement.

2. BAG3 Ile81Met variant in BL6 mice leads to enhanced binding to the small heat shock protein (HspB8) in ischemic skeletal muscle cells and augmented ischemic muscle autophagic flux.



(A) MR T2-weighted and MR angiography images. **(B)** Immunofluorescence images of TA muscle labeled with antibodies against embryonic myosin heavy chain (eMyHC) and dystrophin. **(C)** BL6 and BALB/c mice were subjected to HLI for 3 or 7 days and the gastrocnemius LC3 mRNA expression was determined by qRT-PCR.

3. Altered macrophage response is associated with impaired muscle regeneration.



(A) Hindlimb skeletal muscle cells were collected for scRNA-seq at the indicated timepoints following sham or hindlimb ischemia (HLI) surgery. **(B)** Monocytes/macrophages with cells colored by mouse strain and time point following HLI. **(C)** Gene set enrichment analysis showing enrichment of genes associated with M2 and M1 polarization in BL6 and BALB/c, respectively. **(D)** Gene ontology analysis reveals pro-inflammatory gene expression in BALB/c macrophages. **(E)** Immunofluorescence staining of ischemic and non-ischemic lower extremity skeletal muscle from a representative CLTI patient. Arrows mark CD206+CD11b+ M2-like macrophages. **(F)** Quantification of results from 7 CLTI patients.

References

Publication 1

Publication 2

Publication 3

Conclusions

The sequential findings of these three publications are that (1) a critical determinant of susceptibility to ischemia following ALI or SLI may be the genetically determined muscle cell autonomous response that is at least partly independent of muscle blood flow and vascular density, (2) the BAG3 Ile81 allele in BL6 mice confers protection to ischemic tissue necrosis and (3) there is a correlation between the persistence of pro-inflammatory macrophages and impaired skeletal muscle regeneration in the BALB/c strain. Obtaining an understanding of the genetics and cellular and transcriptional dynamics in skeletal muscle regeneration in the setting of limb ischemia is an exciting area of vascular research and offers potential opportunity for the development of novel treatments.

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