Basic Science in Transplantation

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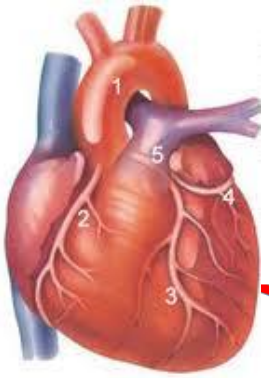
Dalhousie/CDHA

Objectives

- Review Transplant Vasculopathy
- Summarize relevant work from our group from the past ten years concerning AV
- Explore with you current avenues of exploration



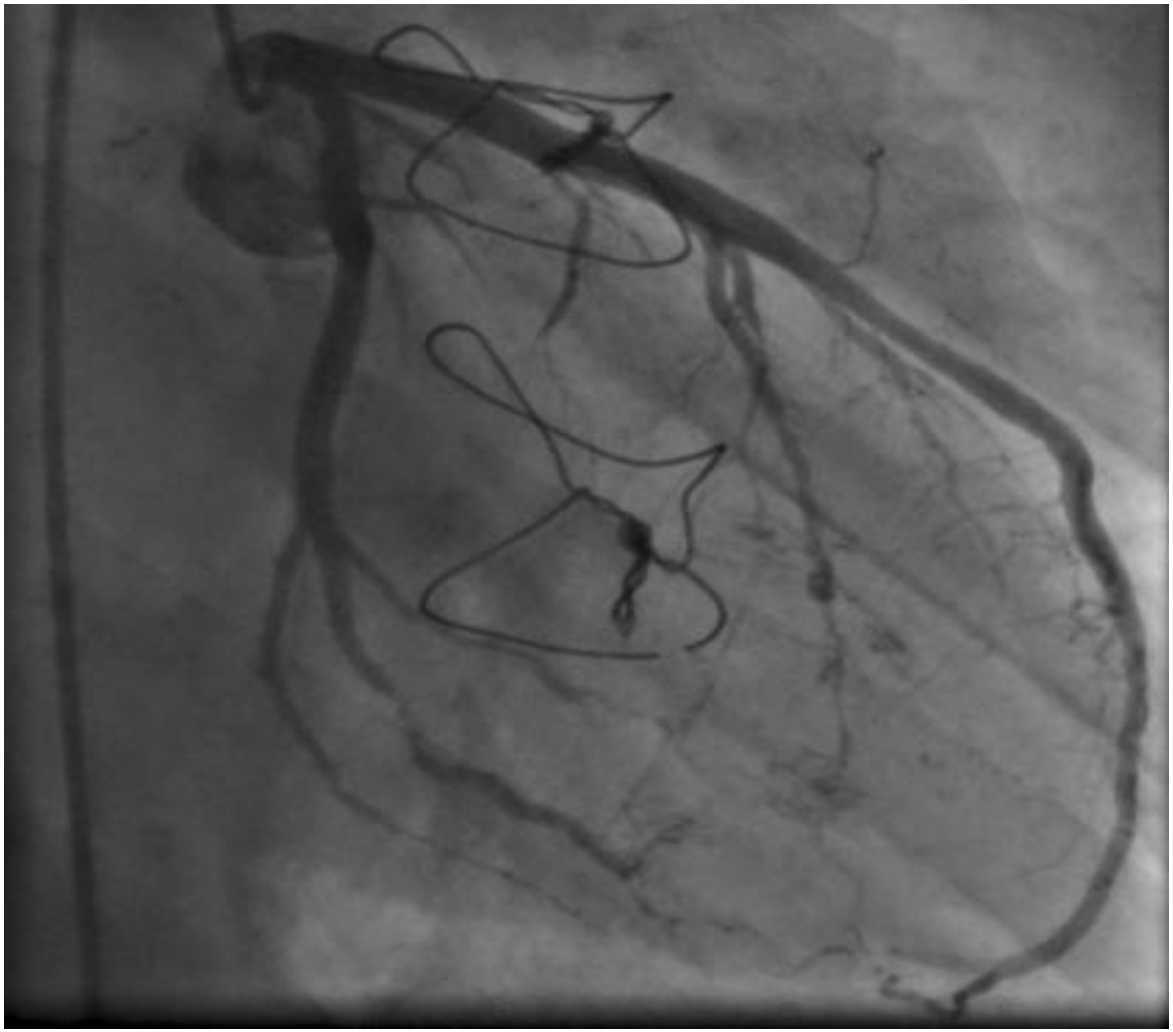
Long Term Cardiac Transplant Survival Remains Poor

(graph adjusted for 1yr survival)

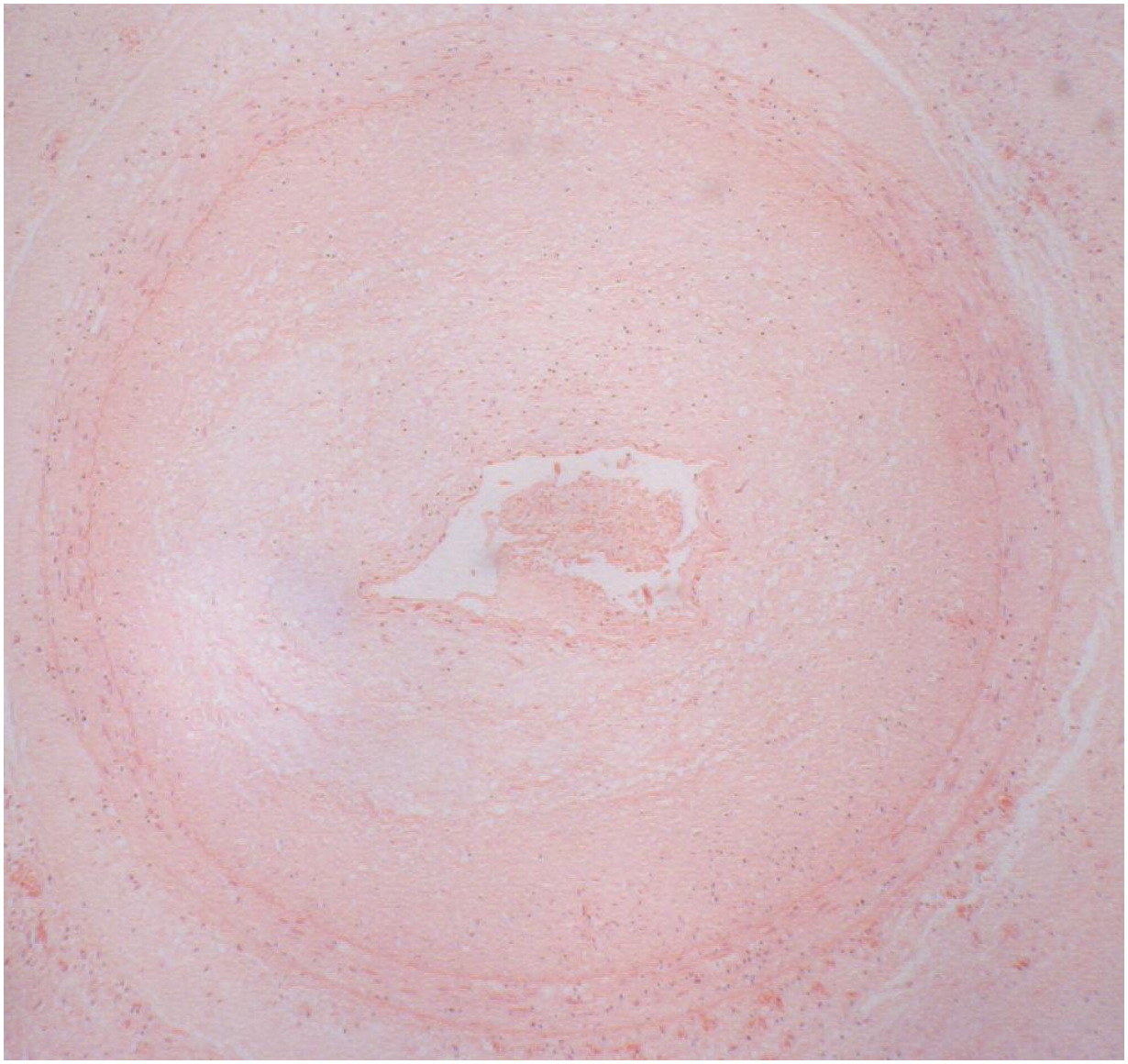
ISHLT 2009

Allograft Vasculopathy (AV)
"The Achilles heel of cardiac transplantation"

- A pathological vascular remodeling process of the coronary arteries after transplantation
- Leads to
  - loss of medial smooth muscle cells (SMCs)
  - Formation of a neointimal lesion
- Intimal proliferative lesion leads to occlusion of the vessel and failure of the graft due to ischemia
- Leading cause of late graft loss



Allograft vasculopathy in a coronary artery



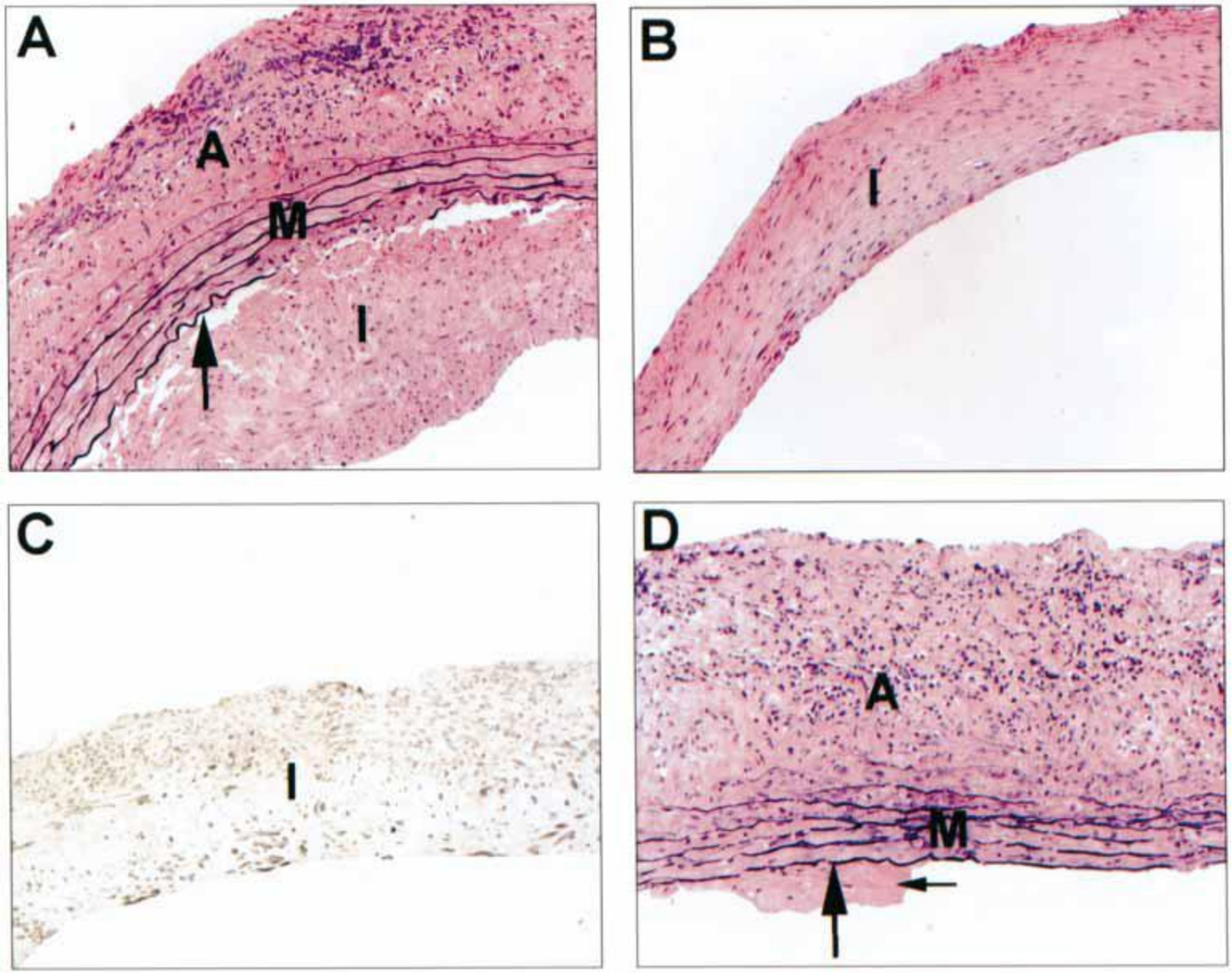
Origin of the neointimal lesion cells

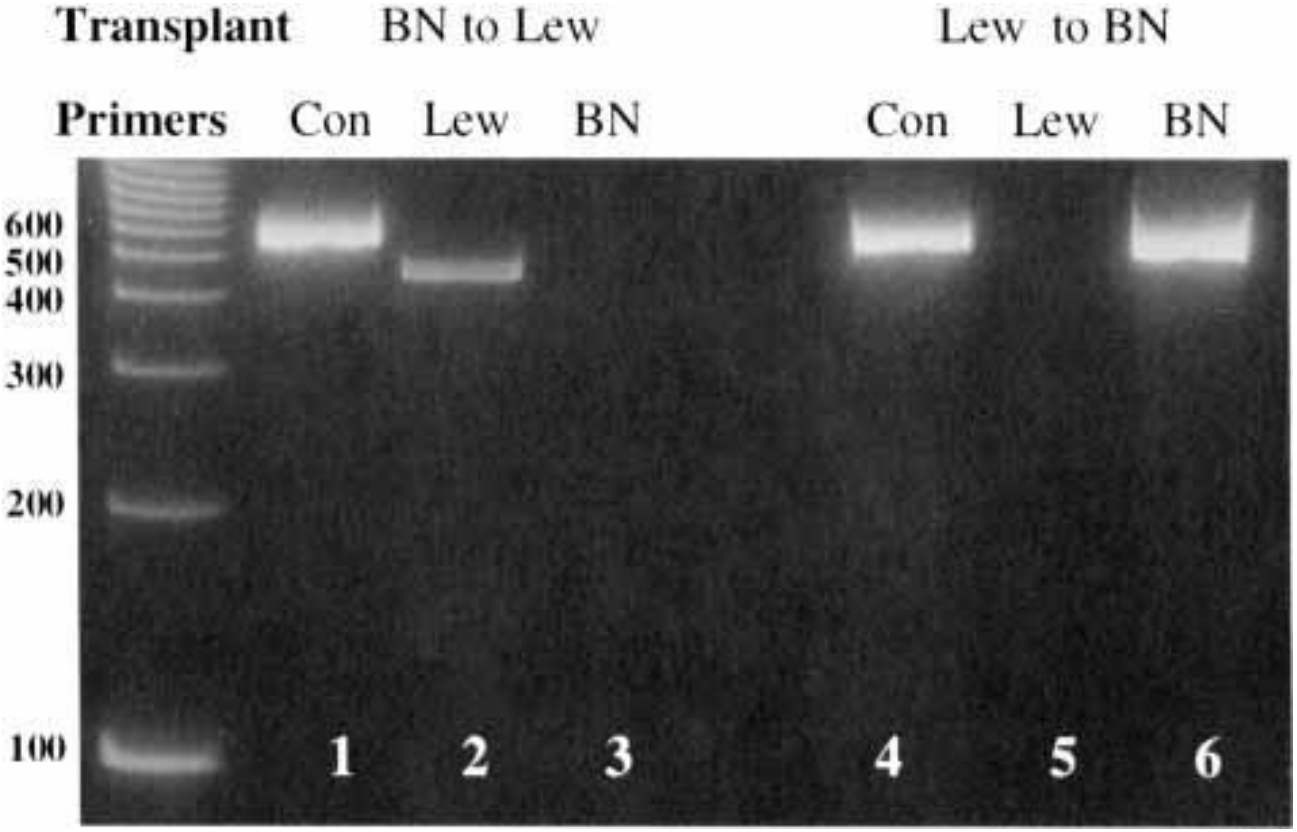
- Human data conflicting w/ preponderance of evidence showing a chimeric arrangment of donor and recipient derived myofibroblasts.
- Hypothesis: The intimal lesion is a repair mechanism populated by recipient derived cells (myofibroblasts).

Origin of the neointimal lesion cells

- Methods
  - Aortic interposition graft transplant between Lewis donor and BN recipient.
  - CyA immunosuppression to ablate acute rejection
  - Allow for robust lesion formation
  - Probe neointima for MHC-I Ag

Experiment 1 RAT





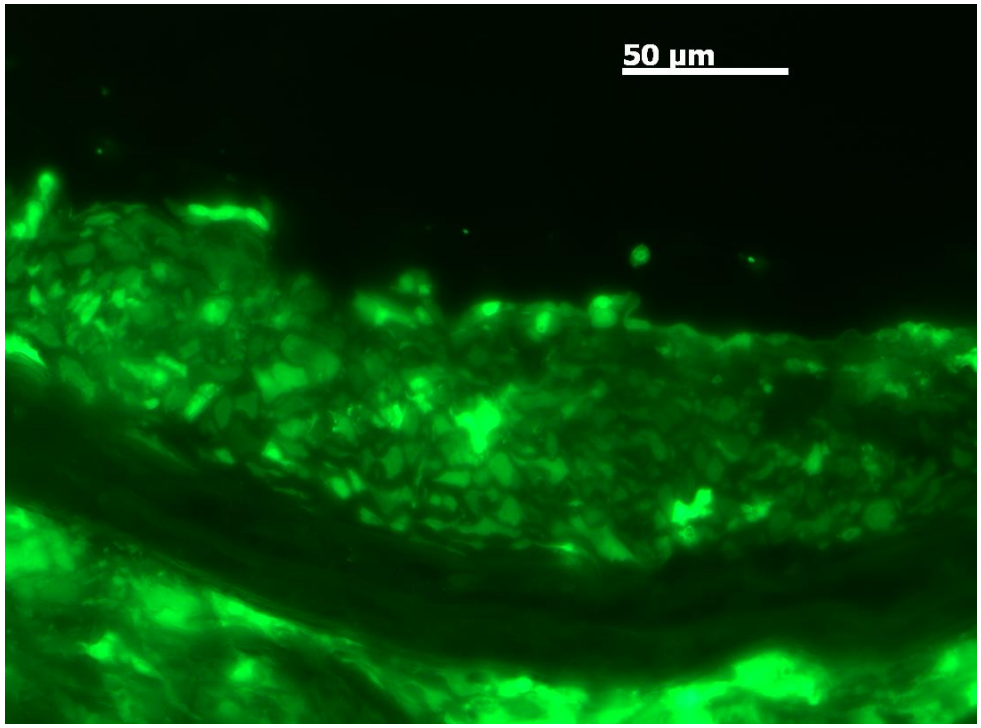
Determination of neointimal cell origin using rat strain-specific PCR. PCR analysis was performed using DNA extracted from isolated neointimal tissue from transplant aortic segments. Primer sets (Consensus, Lew-specific and BN-specific) and transplant type are indicated above the lanes.

Origin of the neointimal lesion cells

Experiment:

- Aortic interposition graft transplant between C3H donor and B6GFP recipient
- CyA immunosuppression to ablate acute rejection
- Allow for robust lesion formation
- Harvest at 8 wk and visualize GFP

Experiment 2 MOUSE



The AV neointimal lesion is recipient in origin in the presence of calcineurin inhibition. To determine the origin of the neointimal lesion under CyA immunosuppression we transplanted WT C3H grafts into B6 GFP mice. The neointimal lesion is almost completely GFP (recipient) positive. The underlying media is GFP negative. The adventitia is heavily populated with GFP -positive infiltrating leukocytes.

Summary

In rodent and murine systems the neointimal lesion is formed exclusively of recipient cells.

Mechanism of lesion formation

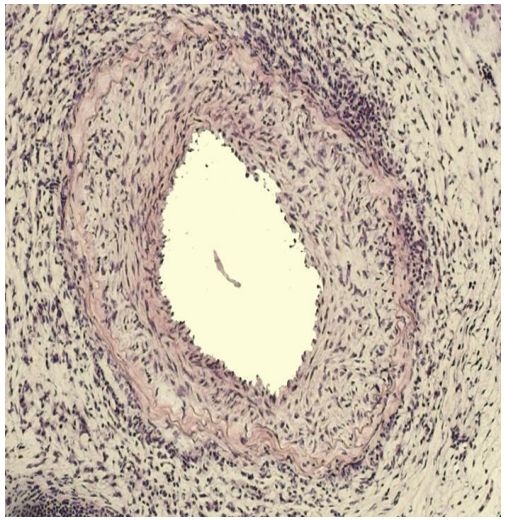
Work to date: AV is dependent upon CD-4+ T Cells

- Work done in minimal mismatch transplants without immunosuppression

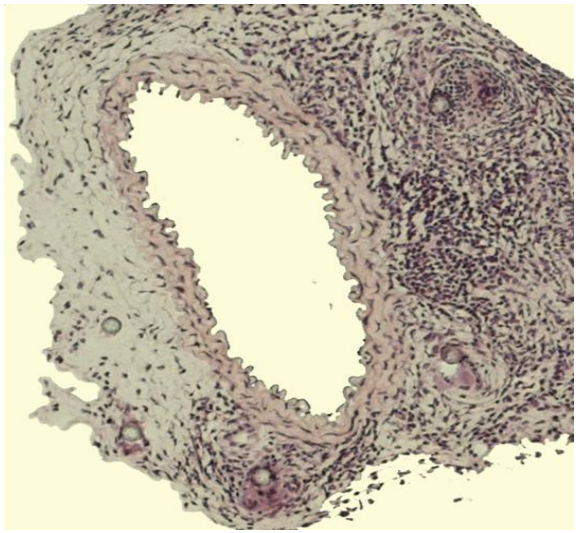
Hypothesis: In the presence of calcineurin inhibitor immunosuppression AV may have different requirements.

Model 1:C3H to B6 CD8+ T cell Knockout

Wildtype



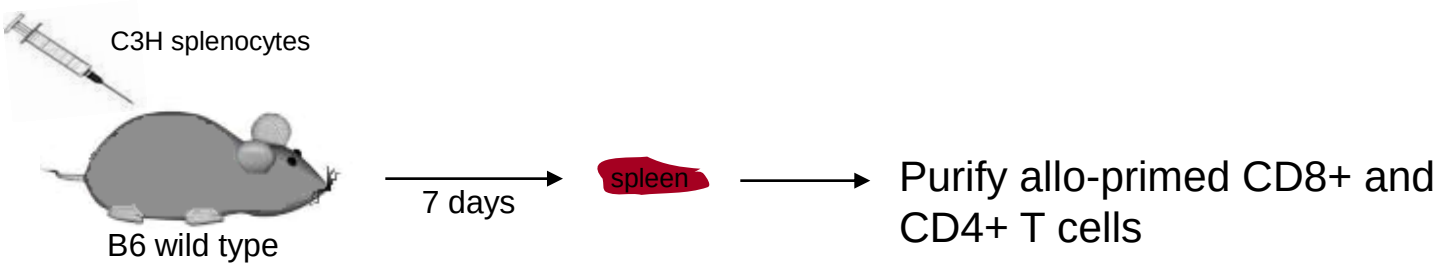
$CD8^{-/-}$



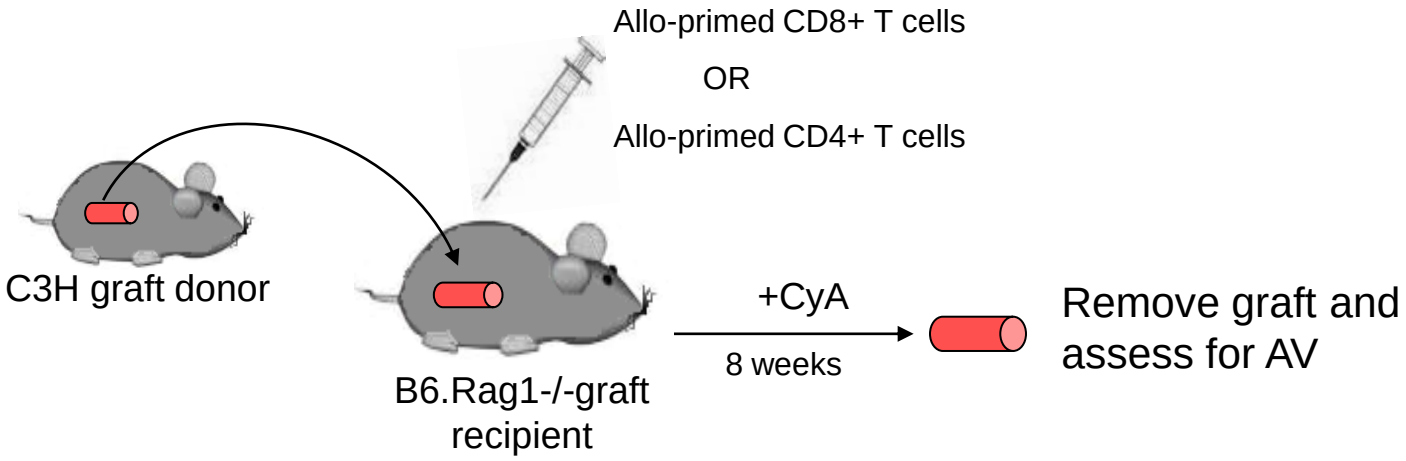
In the presence of CNI immunosuppression $CD8^{+}$ T cells are required for AV

Model 2: Adoptive Transfer Model

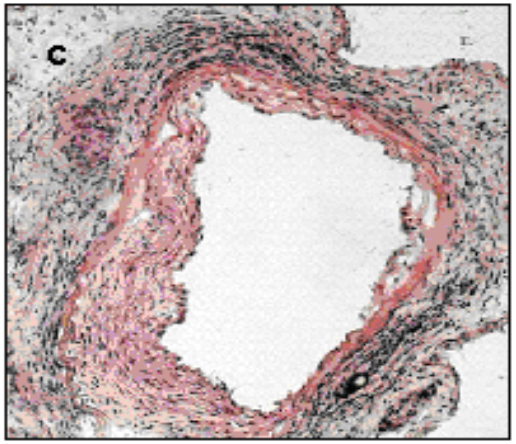
A) Priming : Generation of alloprimed T8 cells +/- CyA



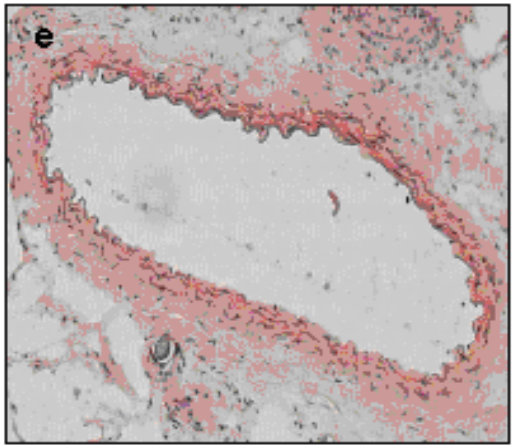
B) Adoptive cell Transfer



Primed pure T8 into RAG



Primed pure T4 into RAG

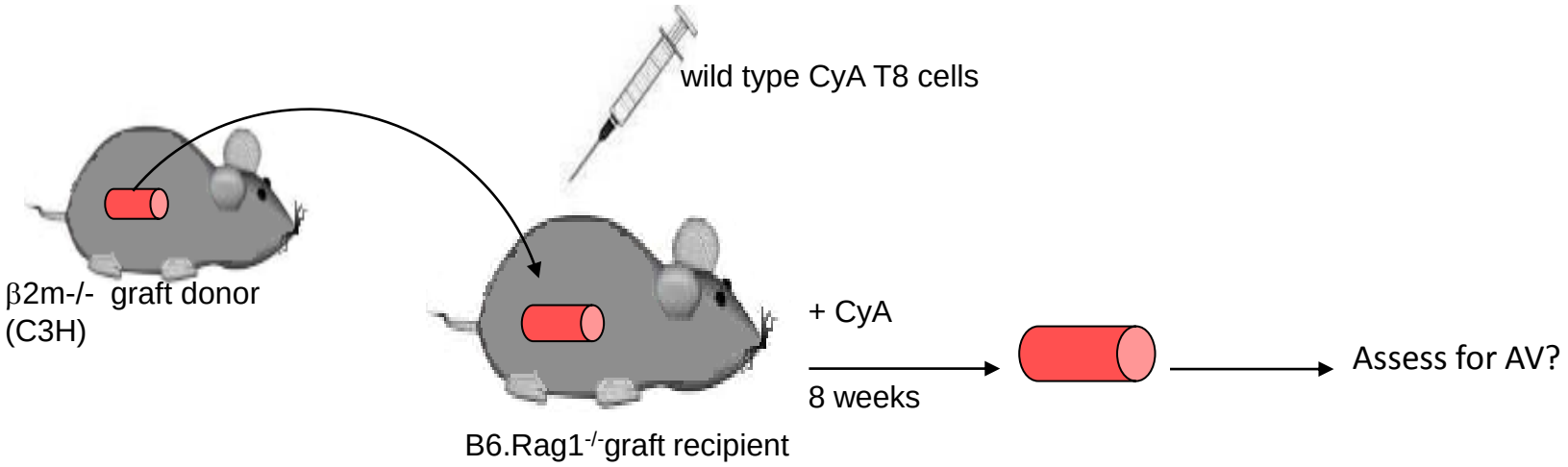


Only CD8+ T cells induce AV in RAG 1-/- mice in the presence of CyA.

Working Hypotheses

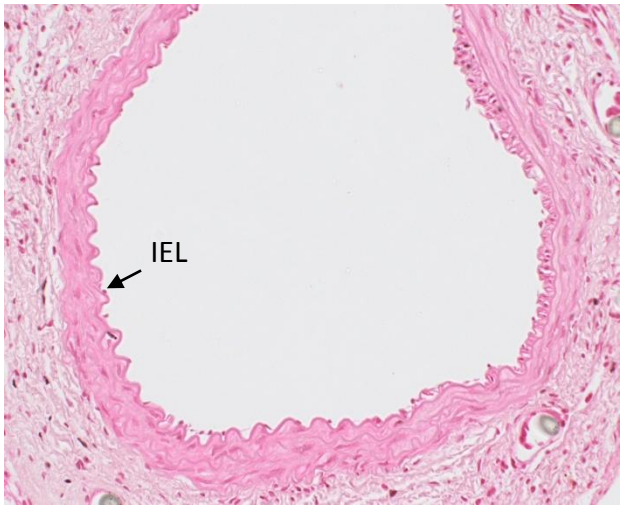
3. CD8+ T cells initiate AV by killing medial SMC.

Experiment 1:Role of Direct CTL



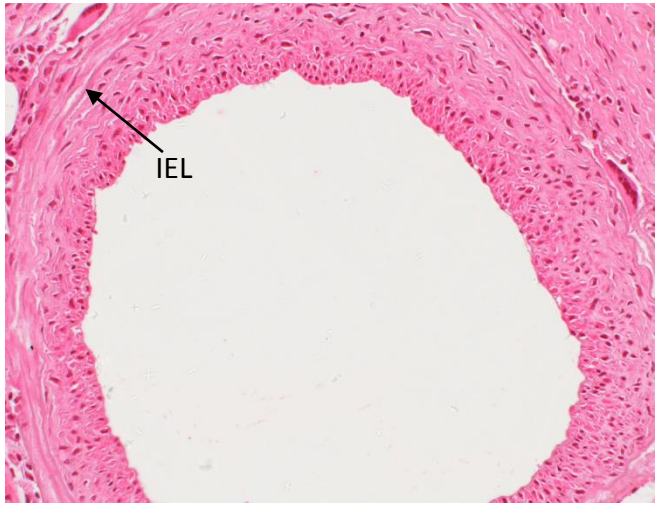
Lack of direct CTL activity ablates AV (under CyA)

a



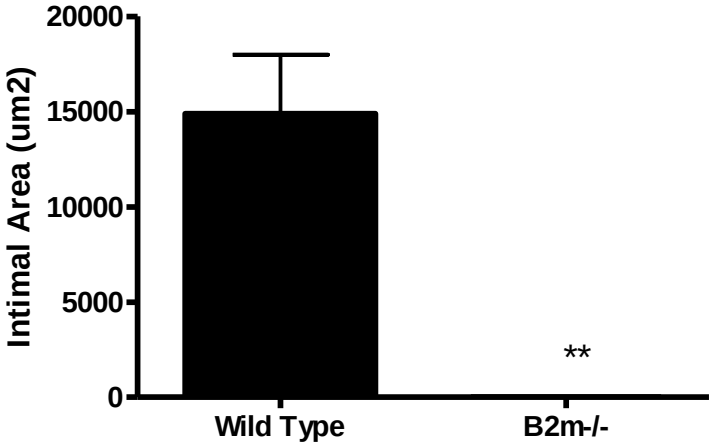
β2m graft

b

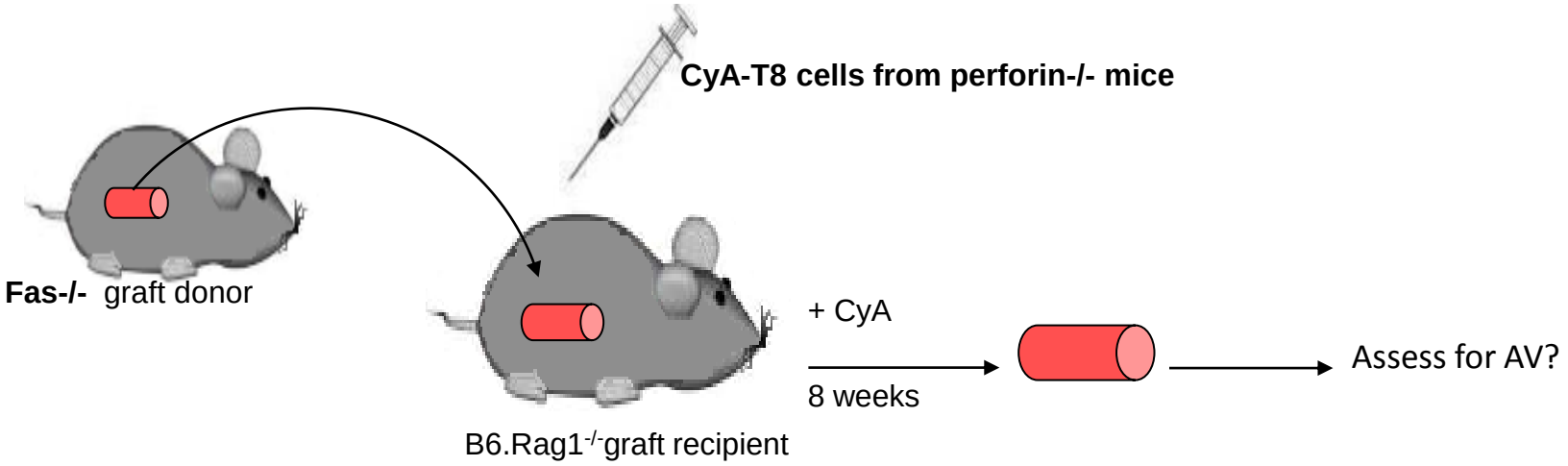


WT graft

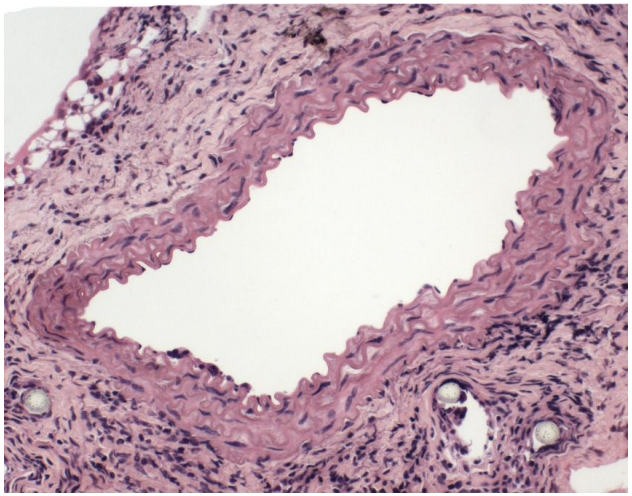
c



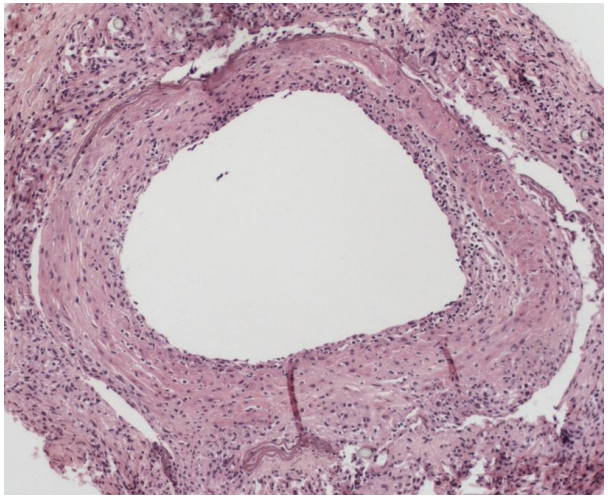
Experiment 2: Role of direct CTL mediators



CTL mediators are required for the development of AV under CyA

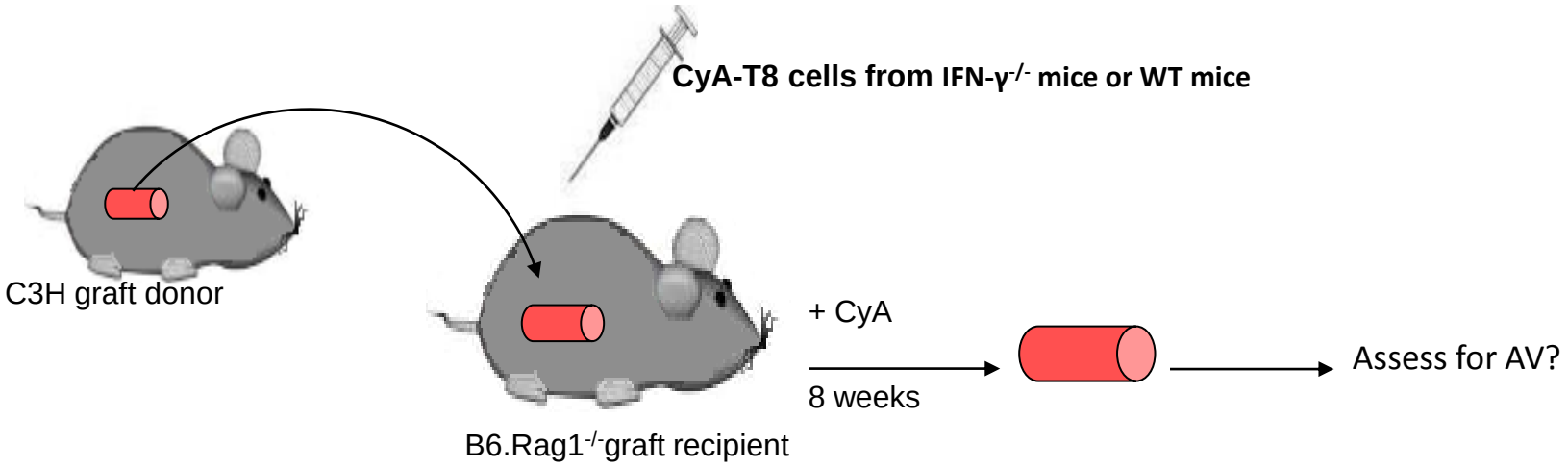


PFN^{-} FasL^{-}

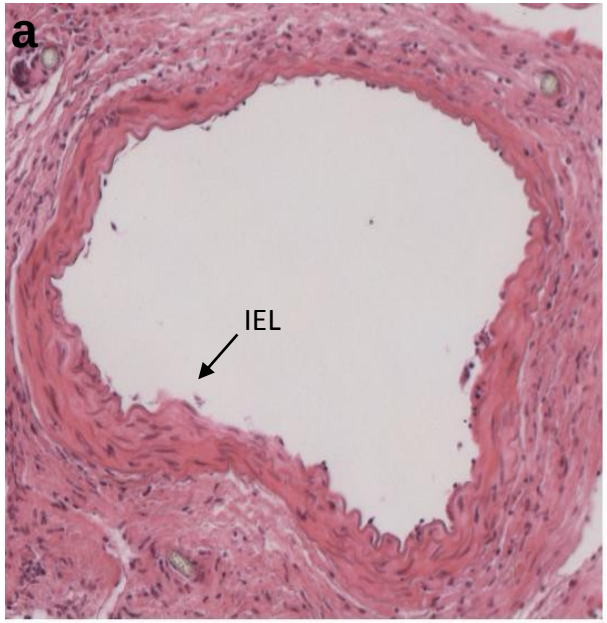


control

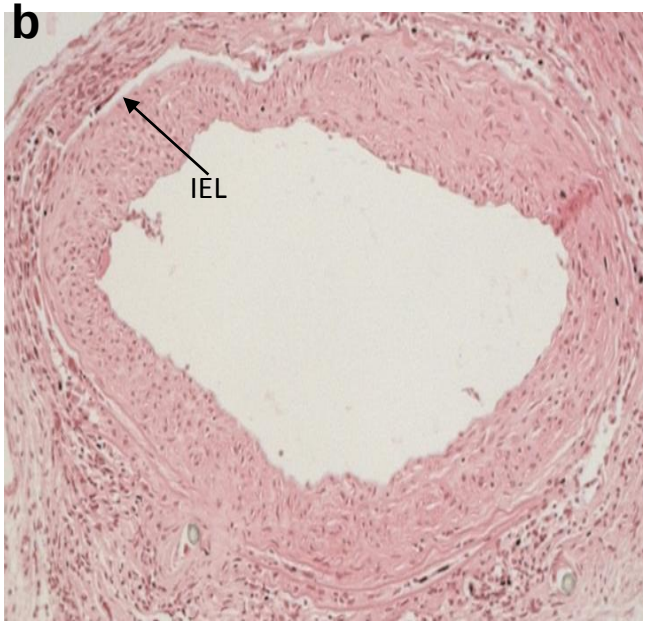
Experiment 3: Role of IFN-γ



IFN-γ producing T8 cells are required for the development of AV under CyA



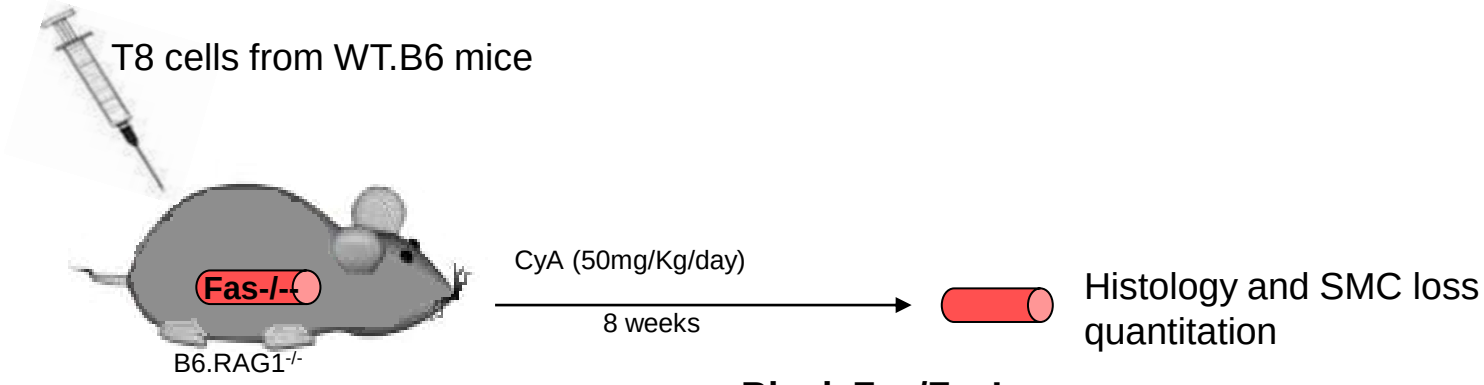
IFN-$\gamma^{-/-}$ CD8+ T cell



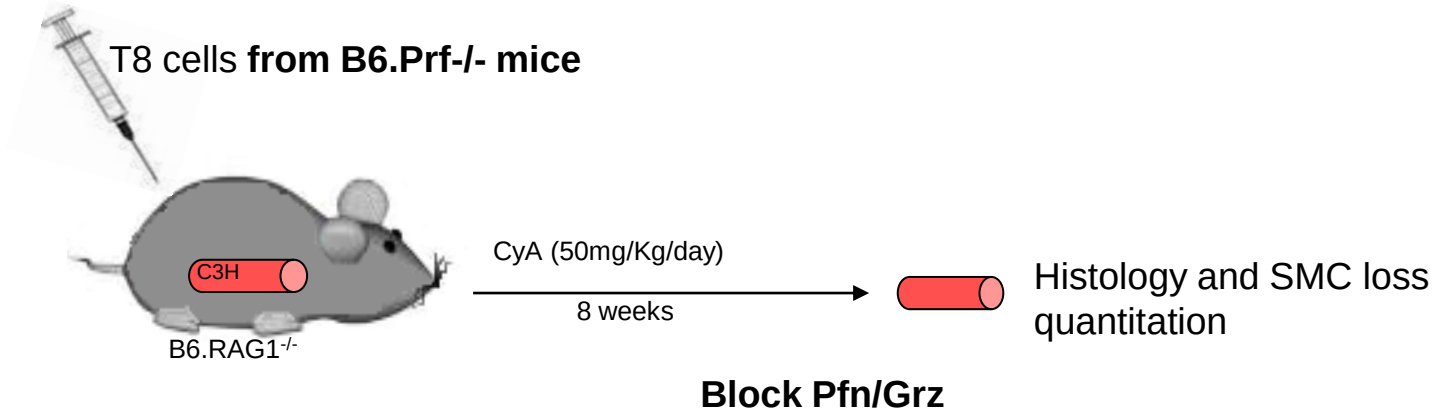
WT CD8+ T cell

Experiment 4:

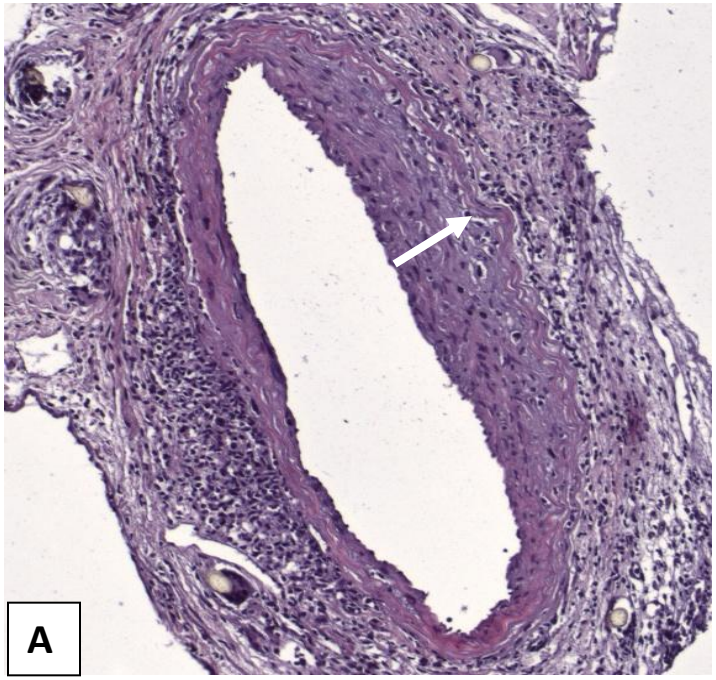
IFN-γ plus which CTL pathway required?



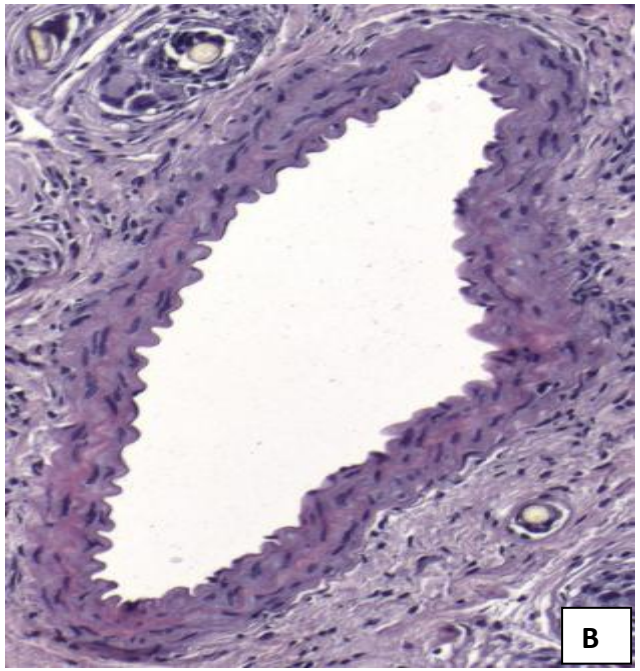
Note: Fas-/- graft



IFN plus Fas/FasL interaction needed to get lesion formation



Prf-/-



Fas-/-

Summary

- Lesion cells are recipient derived
- Lesion development in a CNI treated model requires CD8+ T Cells
- Direct CTL (Fas-Fas-L) and IFN- γ required

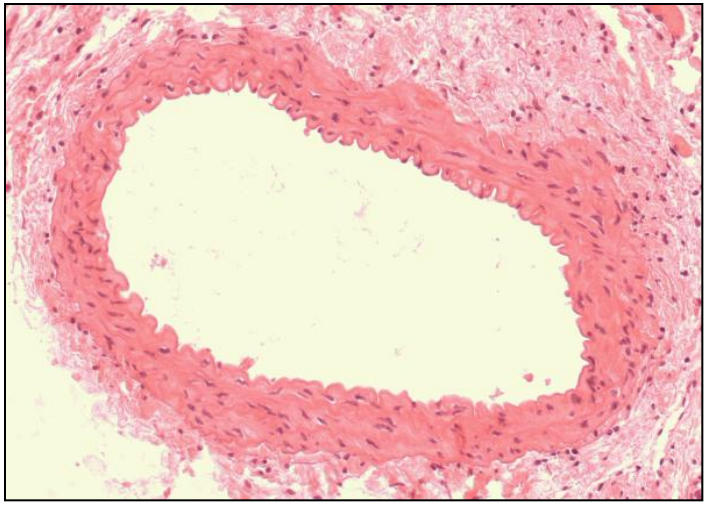
We argue we have a better model in that CNI immunosuppression in place

<u>but</u> human studies continue to demonstrate chimeric lesions, majority derived from donor

The Major Weakness of this Paradigm

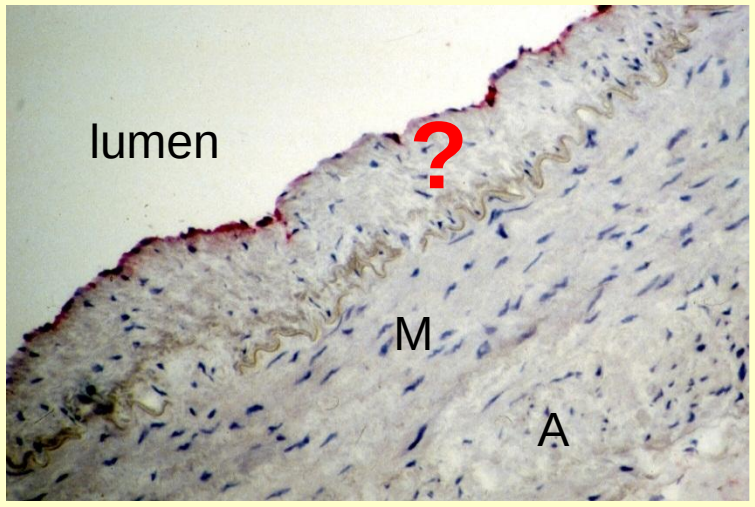
Model

Mouse



ACTR

Human



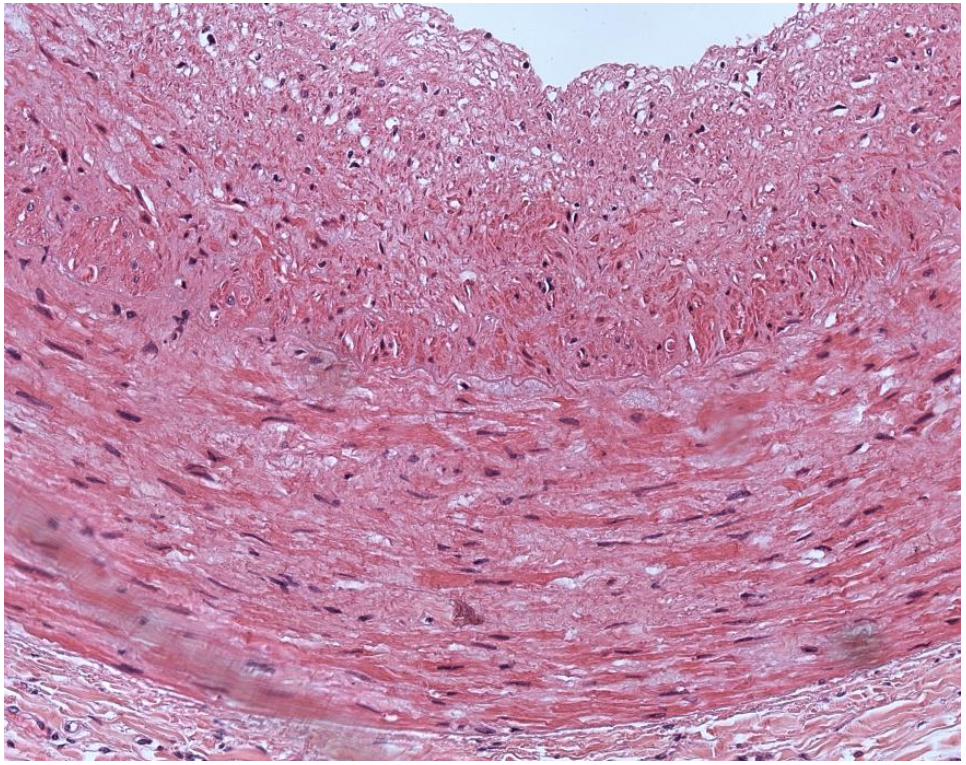
Imperial College

Initial Halifax Study (n=19)

What do donor coronary vessels really look like?

- “normal” hearts taken at autopsy
- All medical records available
- Hearts examined have potential “donor” status of hearts based on medical records
- Cardiac pathologist consulted on disease in the major coronary vessels

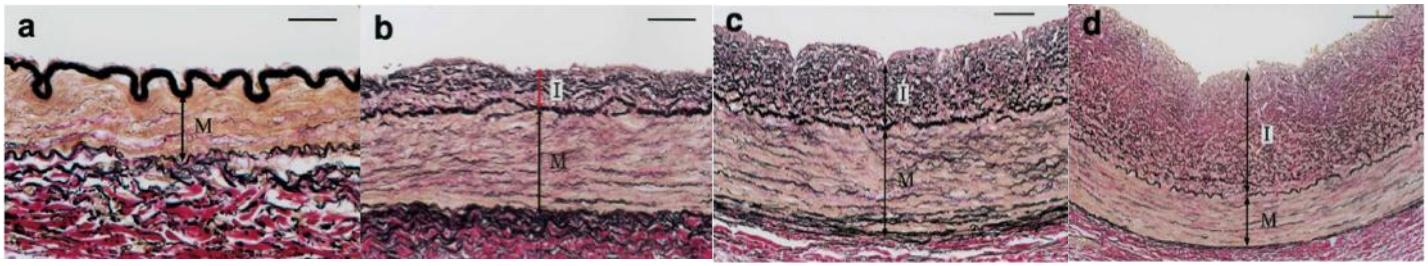
“Donor” coronary vessels



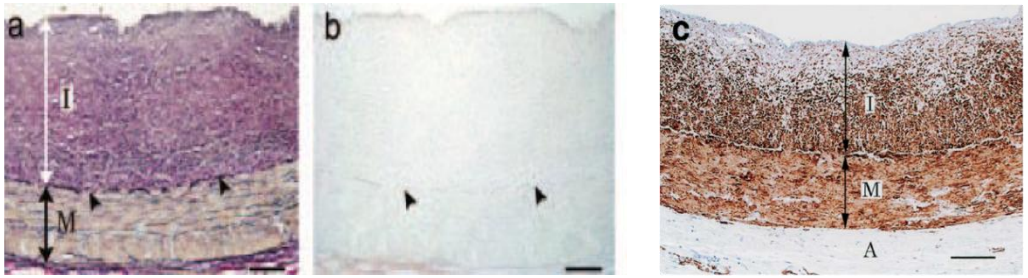
36 year old female who fit the heart donor criteria with no known cardiac risk factors

Benign Intimal Thickening in Human Coronary Arteries

7days 5 years 15 years 29 years



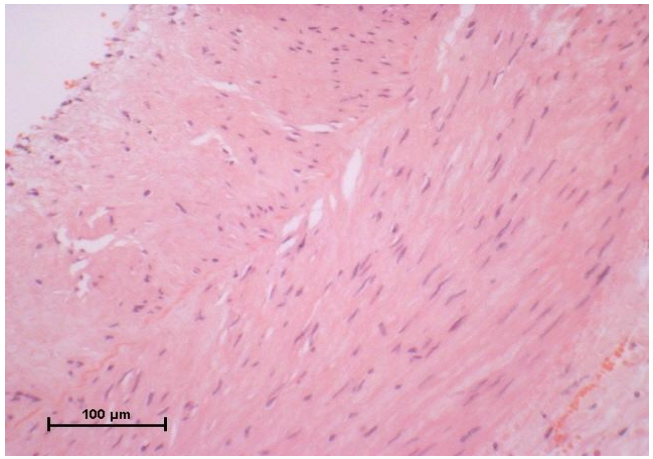
lipid smooth muscle cells



Nakashima et al. Virchows Arch 441:279-88

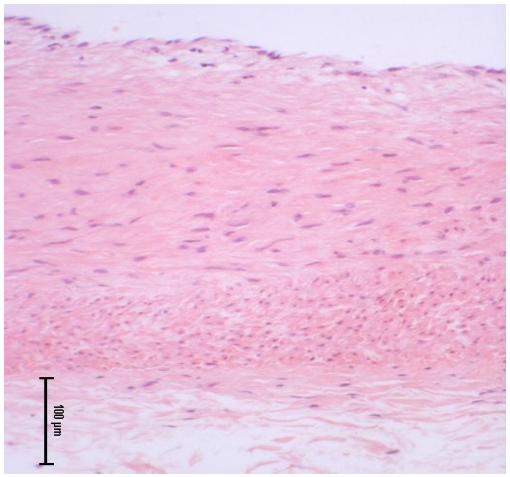
BIT is mostly longitudinally arranged smooth muscle cells

cross section



ACTR

longitudinal section

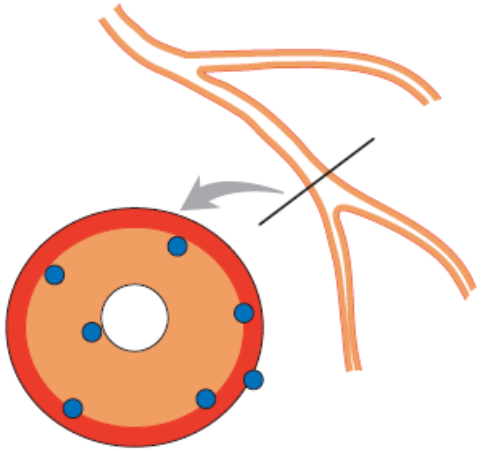


ACTR

Coronary arteries in donor hearts contain a pre-existing benign intimal thickening at the time of transplant

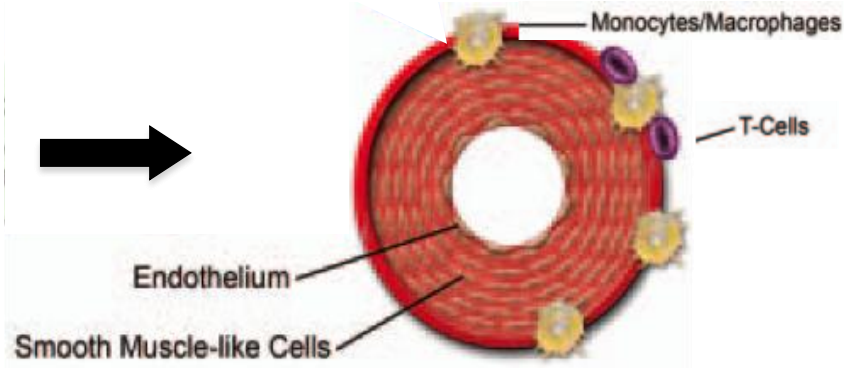
Brompton /Imperial
Clarify the role of BIT in CAV

- Retrospective study n=200
- Hearts taken at autopsy of transplant recipients
  - (1-15 yr post tranplant
- Examine RCA, LAD and Circumflex
- Examine at the proximal, mid and distal levels
- Digital image analysis of intimal lesion size

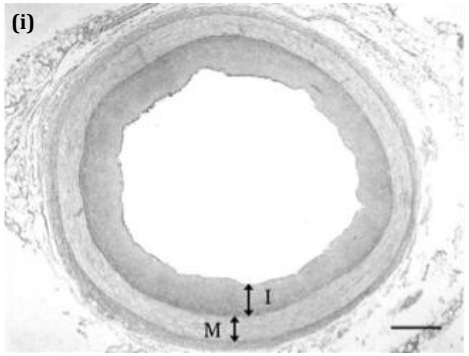


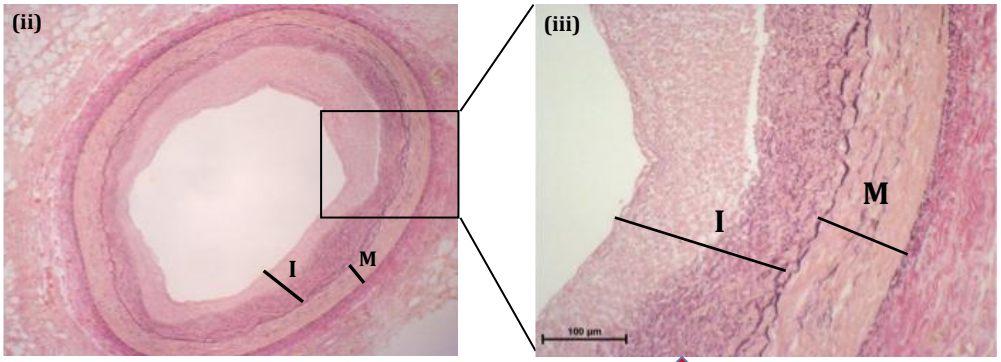
Initial results:

CAV Theory



CAV Reality



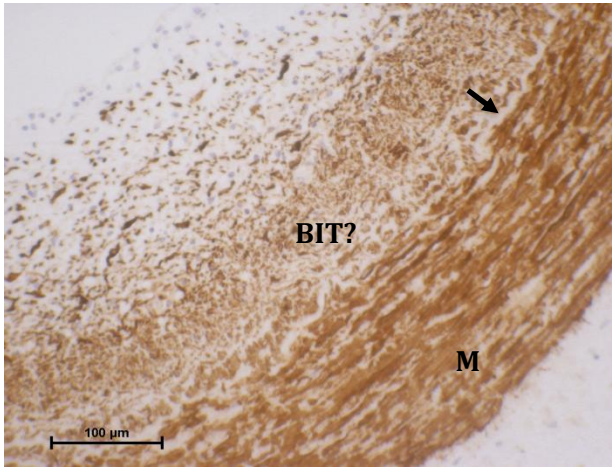


ACTR

BIT?

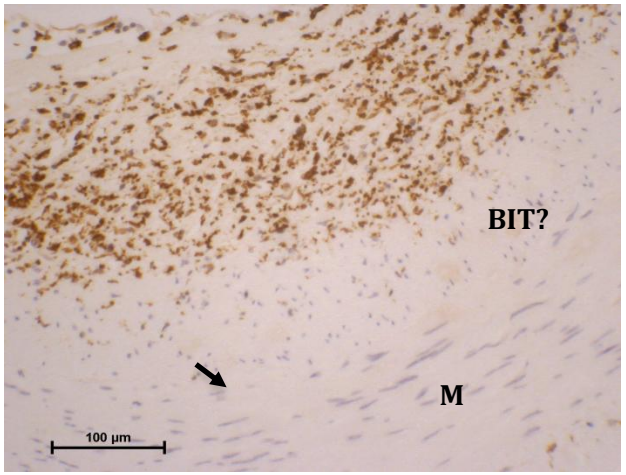
BIT-like layer underlays a less cellular macrophage rich, SMC poor intimal layer

smooth muscle



ACTR

macrophages



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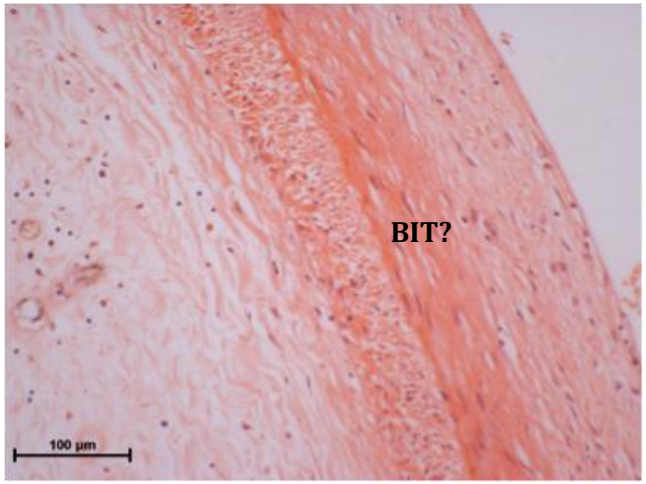
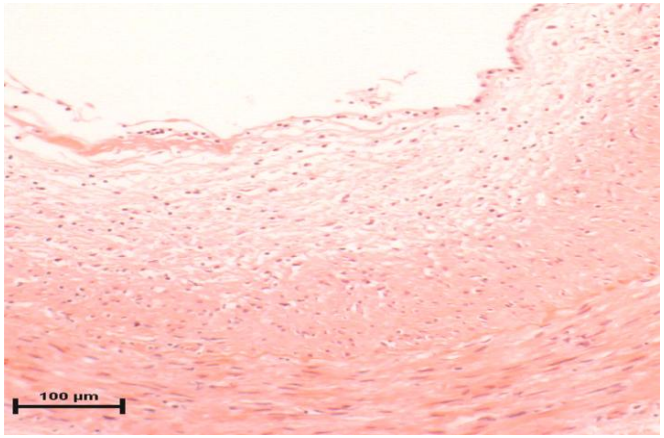


ACTR

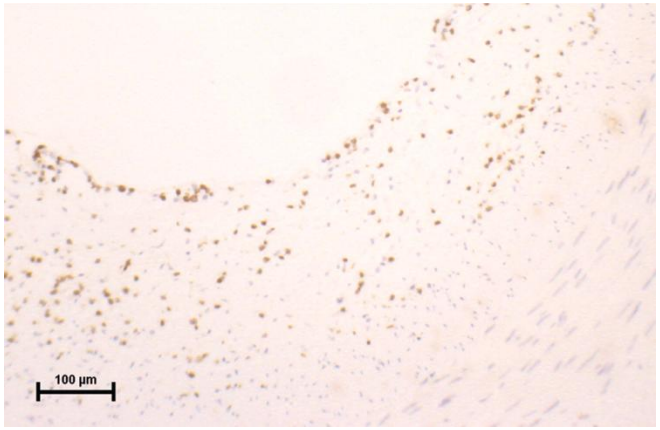
longitudinal section

Early CAV (< 1 year post transplant)



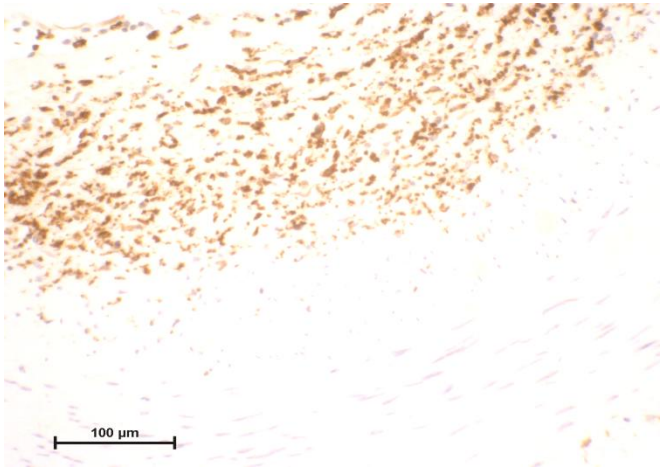
ACTR

H&E



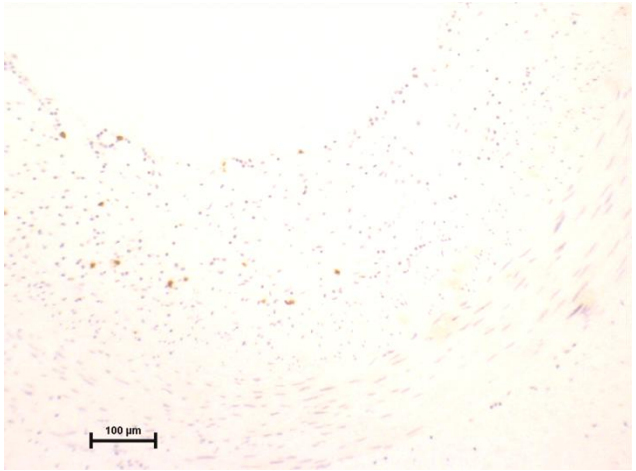
ACTR

T cells



ACTR

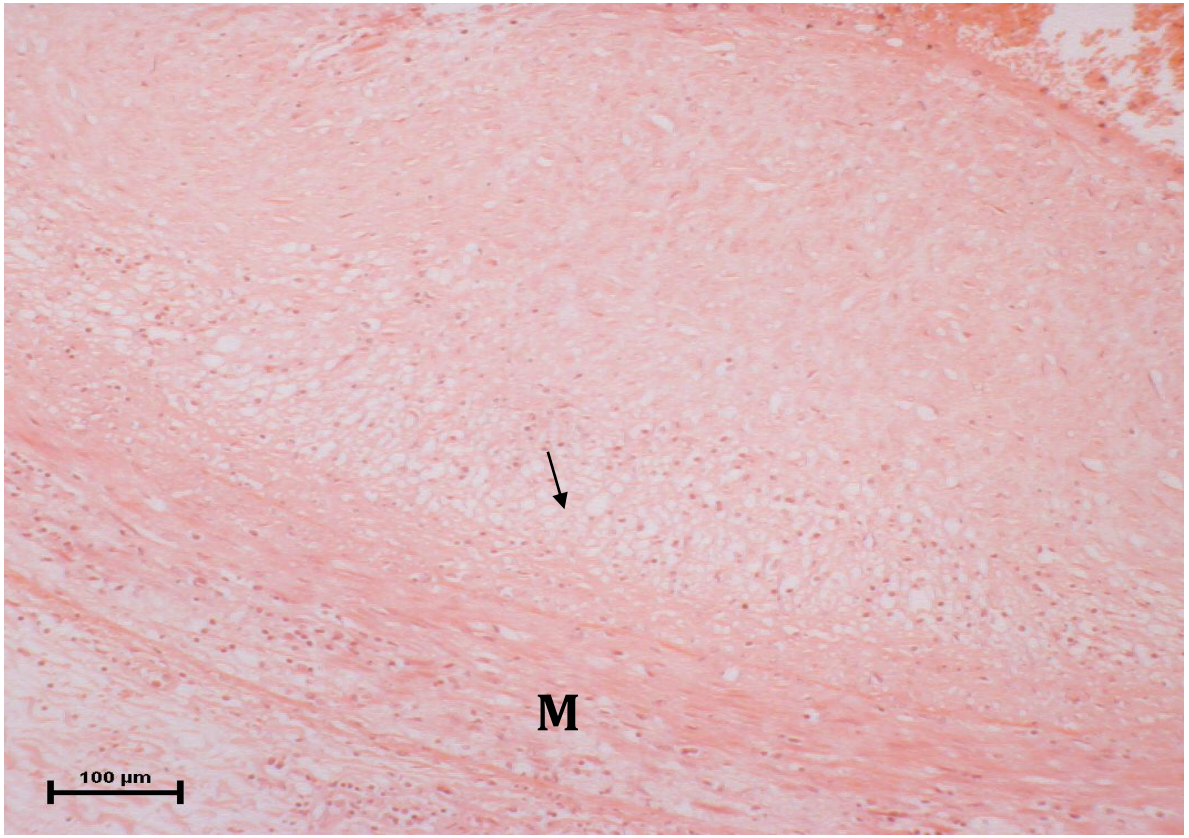
macrophages



ACTR

B cells

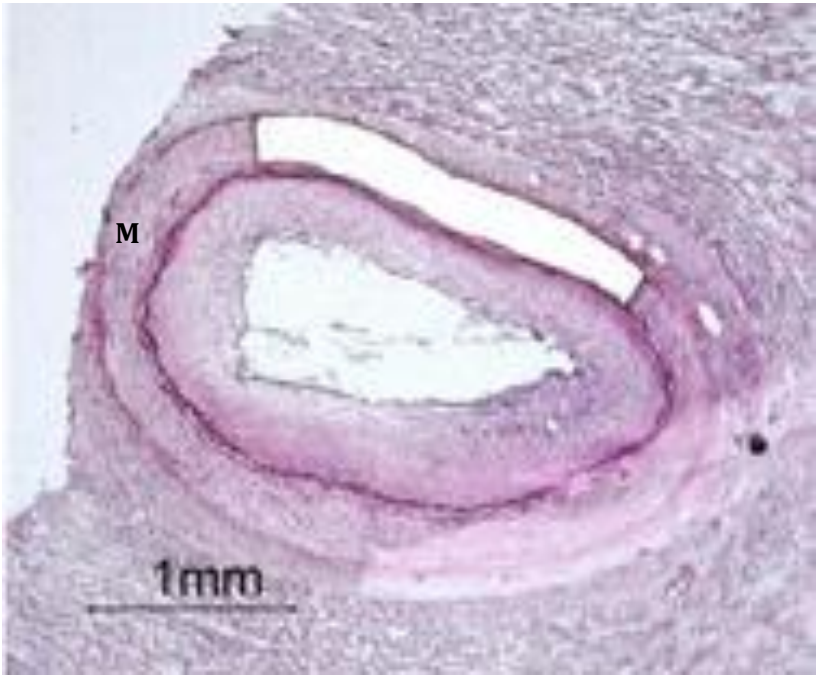
Late CAV looks like accelerated atherosclerosis

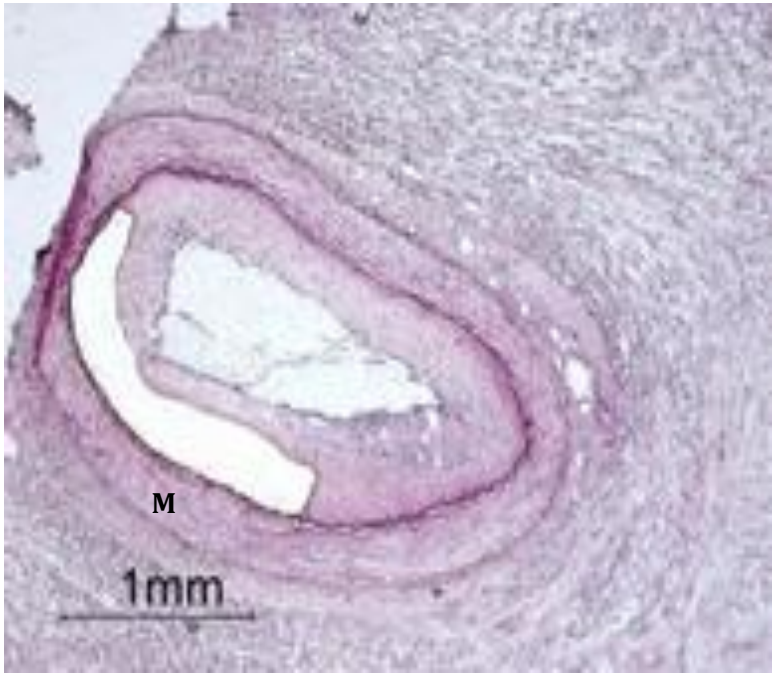


ACTR

Is the BIT-like layer in CAV actually carryover BIT?

Laser micro-dissection and polymerase chain reaction





De Weger et al Trans Immunol E-pub

Hypothesis: Etiology of human CAV

1. BIT layer is retained at transplant (SMC rich, macrophage poor, proteoglycan bound LDL)

2. I/R injury initiates inflammation in the BIT layer

3. Macrophages and T cell influx mediates chronic inflammation
4. Chronic vascular inflammation leads LDL uptake, foamy macrophages, atherogenesis and accelerated atherosclerosis

Lessons learned

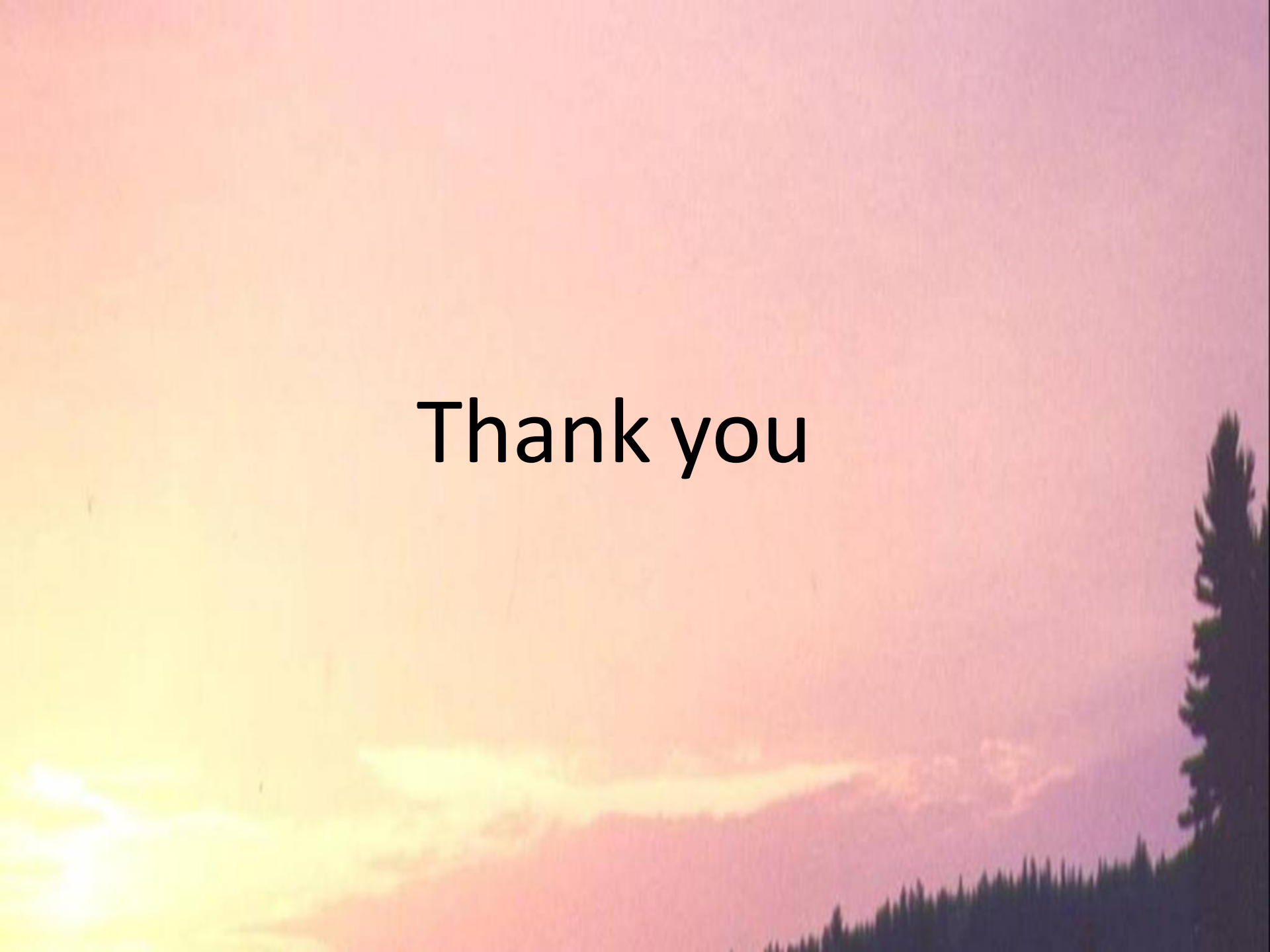
- *Necessary to check in with human data to assess validity of your in-vivo model (regardless of how superior you think it is)*

New Model

- Developing a rodent BIT model through intimal injury prior to transplantation (with CNI immunosuppression)

Acknowledgements

- Tim Lee
  - Anton Skaro
  - Ellen Vessie
  - Sara Nejat
  - Julie Jordan
  - Jen Devitt
  - Mike Matyas
  - Sean Murray


Thank you