Regulatory T Cells Reduce Infarct Size, Improve LV Remodeling and Function after Experimental Myocardial Infarction

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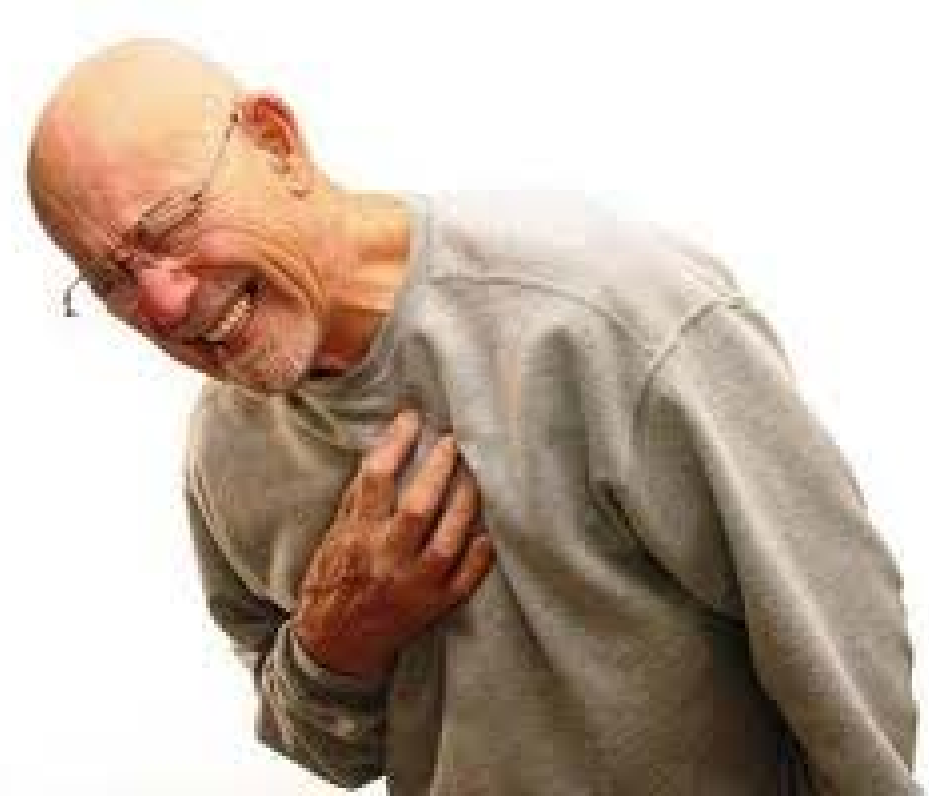
Heart Attack-Killer Number 1



Regulatory T cells (Tregs)

- Early 1970s: T cells that were able to suppress immune responses were first described.
- Mid-1990s: S. Sakaguchi showed that a minor population of CD4+ T cells is crucial for the maintenance of immunological self tolerance.
- These cells were subsequently named: Regulatory T cells (Tregs)
  - ”Induced Tregs”
  - ”$CD4^+CD25^+$ Naturally Occurring Tregs”-
    - Co-expressing the interleukin-2 receptor (IL-2R) α-chain(CD25)

Naturally occurring CD4+CD25+ Treg cells Are there specific molecular markers?

- **$CD25^{high}$**

  Activated effector T cells express low level of CD25 in contrast to Tregs, which express high level of CD25 ($CD4^{+}CD25^{high}$ Treg cells)

- **Foxp3 - Forkhead transcription factor**
  - Constitutively expressed by $CD4^{+}CD25^{+}$ Treg cells as well as by $CD4^{+}CD25^{-}$ T cells with regulatory activity.
  - The forced expression of Foxp3 in $CD4^{+}$CD25- cells or $CD8^{+}$ cells confer suppressor functions to these cells
  - Foxp3 is thought to program the development and function of Tregs

Foxp3 is the most unambiguous marker available to identify naturally occurring Treg cells

Naturally occurring CD4+CD25+ Treg cells phenotypic characteristics

- Constitute 5-10% of peripheral $CD4^+$ T cells and less than 1% of peripheral $CD8^+$ T cells in normal naïve mice and human.
- Partially 'anergic' *in vitro*:
  - Proliferate poorly upon TCR ligation.
  - Don't produce IL-2.
- Treg cells may modulate the function of effector T cells and reduce the damage induced by the infarct.
  - IL-10 and TGF-β may have beneficial effects in ischemia.

(Marc Gavin et al. 2003)

General Aim

To test the hypothesis that regulatory T cells have potential beneficial effect in ischemic environment.

General scheme of study plan

Evaluate the nature (level and activity) of Treg cells present in the local ischemic environment

In-Vivo mouse model of myocardial infarction and hindlimb ischemia

Ischemia in mice

Sham operated

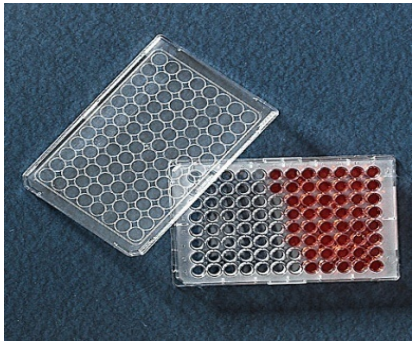
Spleen extract (splenocytes)

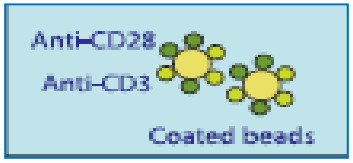
FACS

DIVA (quantitative analysis)

In-Vitro mouse model of myocardial infarction and hindlimb ischemia

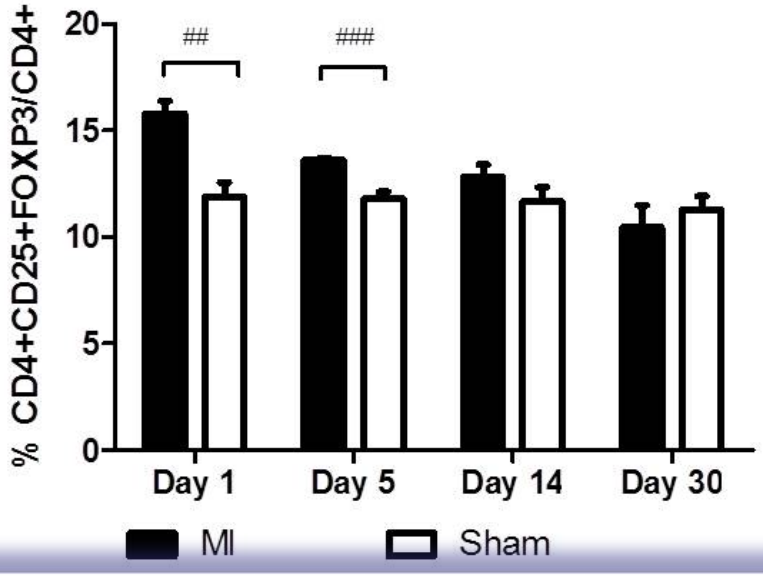
Functional suppression assay



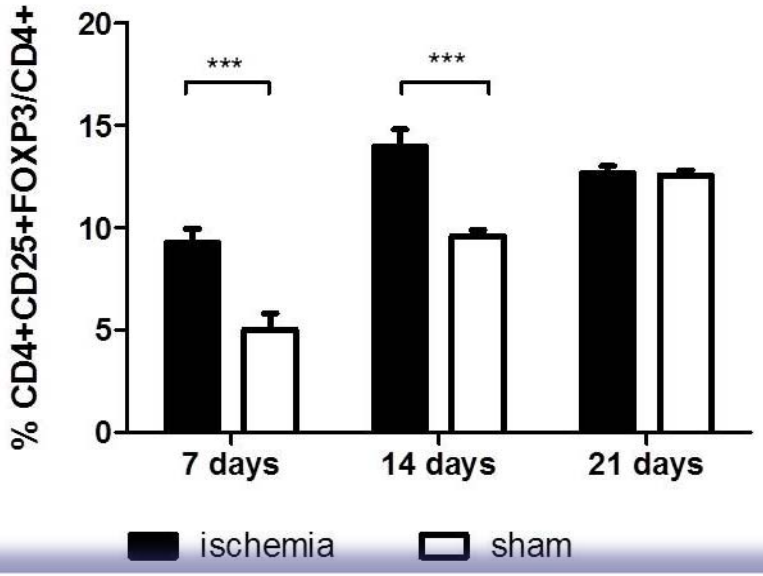


Kinetics of Treg Splenocytes

MI model



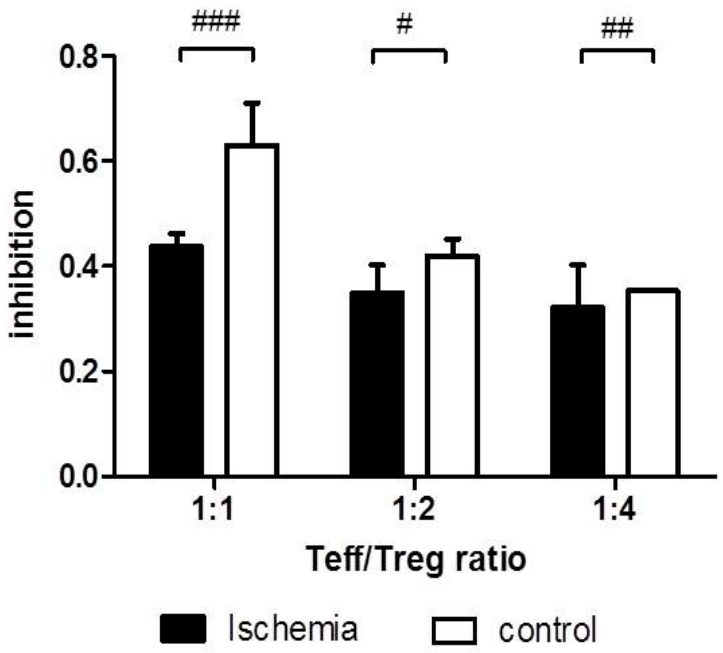
Hindlimb ischemia model



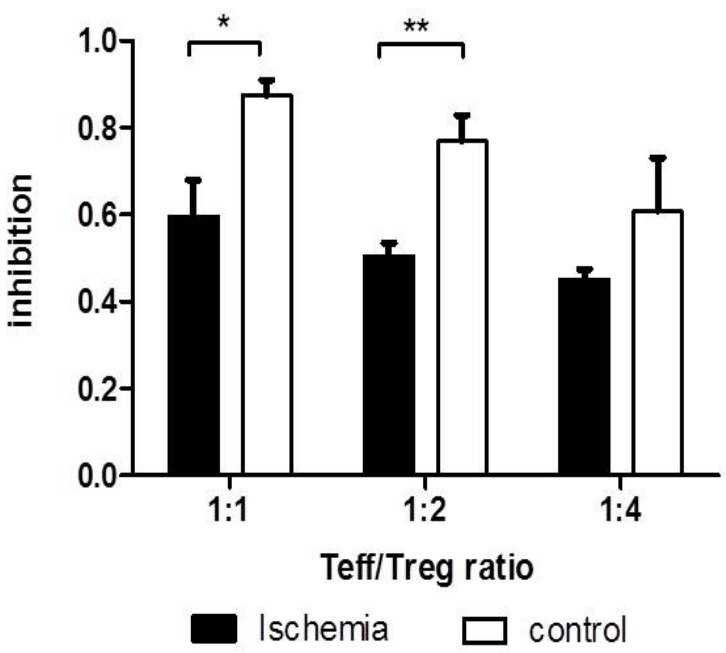
Following MI induction and hindlimb ischemia, levels of regulatory T cells are higher than in the control group

Suppressive properties of Treg Splenocytes

MI model



Hindlimb ischemia model



The suppressive properties of regulatory T cells upon ischemia are significantly reduced

General scheme of study plan

Assess the impact of adoptive transfer of regulatory T cells on the functional recovery of the post infarcted heart

MI or hindlimb ischemia induced mouse

Treg cells

PBS

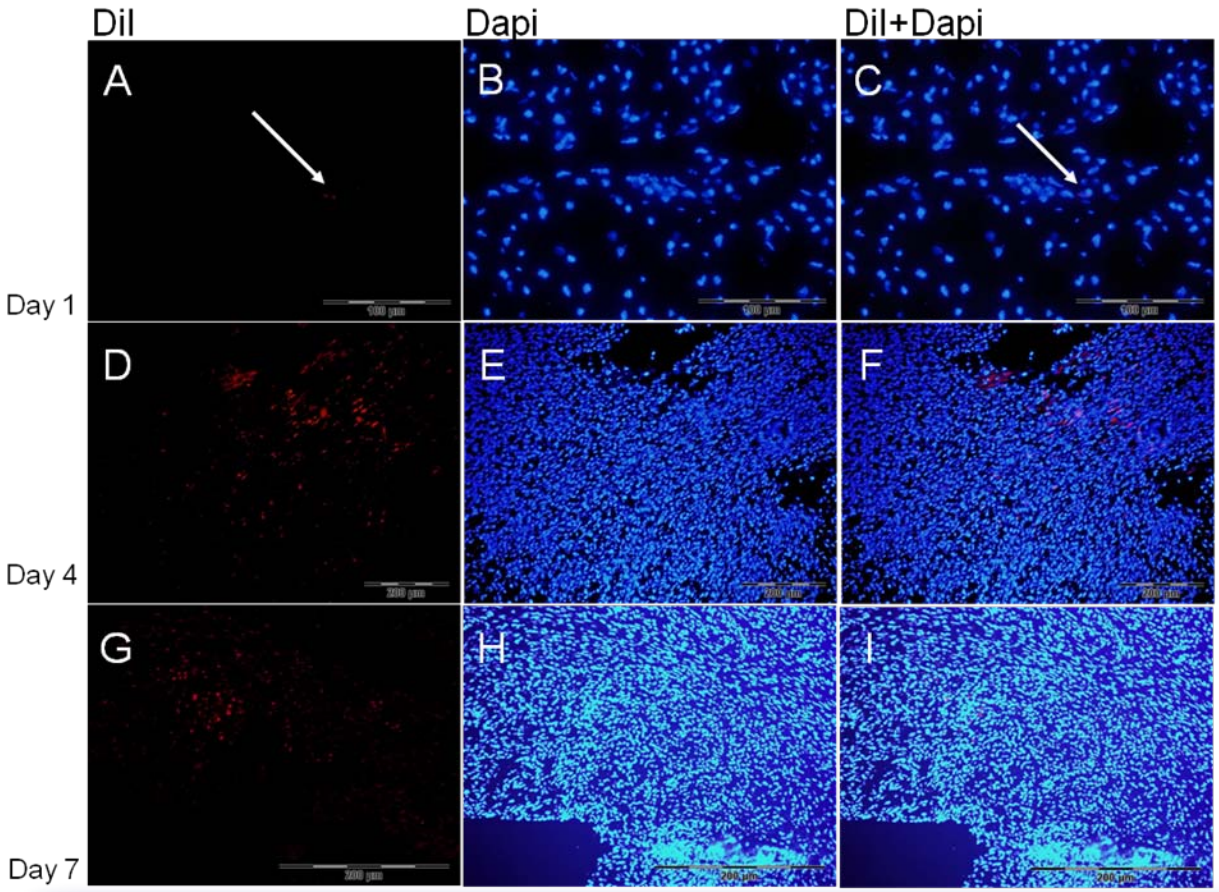
Cardiac performance and remodeling :

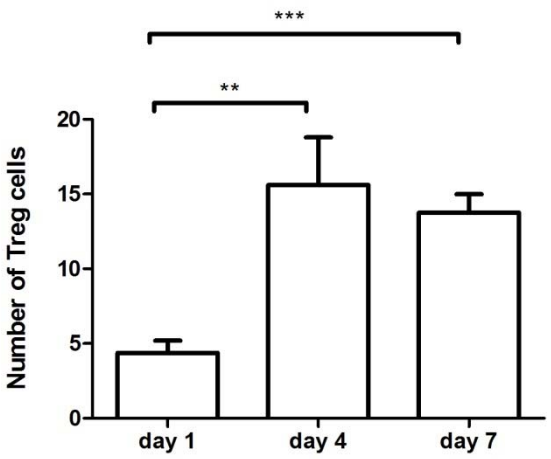
- Echocardiogram to assess cardiac function in day 30th
- Morphometric evaluation: H&E, Masson's Trichrome stain(infarct size)

Blood flow performance:

- Laser-doppler perfusion imaging (LDPI) demonstrated to assess blood flow in day 0, 7 and 14.
- Photomicrographs evaluation- CD31 staining

Homing of regulatory T cells to ischemic hearts after their intravenous injection

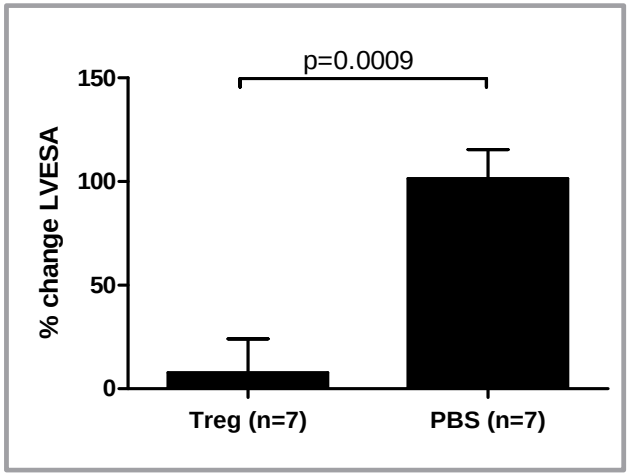




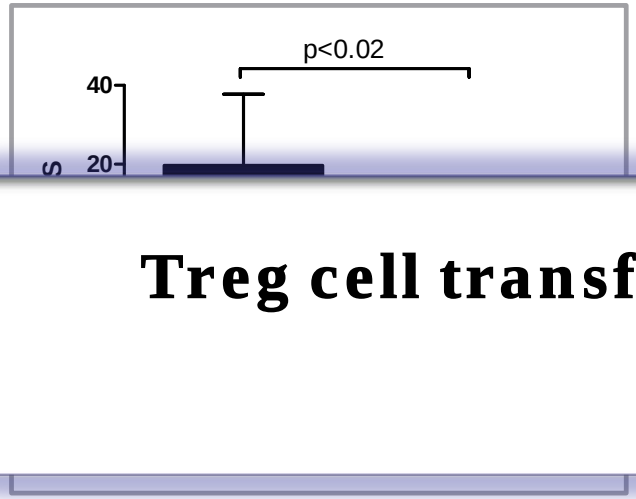
Tregs migrate to the peri-infarct region

Adoptive Transfer of Regulatory T Cells attenuate remodeling post MI

Change in LV end systolic area



Change in LV fractional shortening

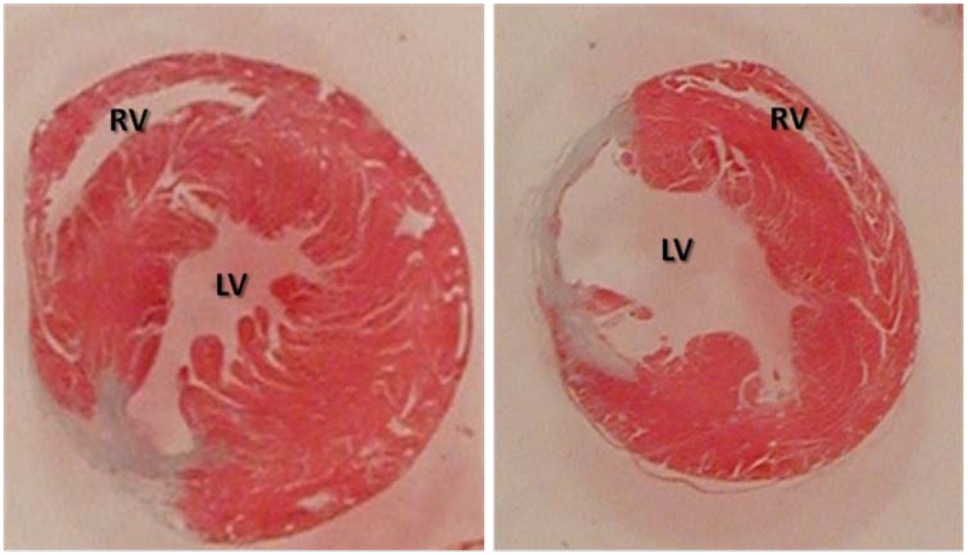


	Treg (n=7)	PBS (n=7)	P
LVDD, mm	5.6±4.5	21.06±4.9	0.04
LVSD, mm	4.89±7.5	41.48±6.75	0.003
LVDA, mm^2	10.8±10.2	31.25±7.9	0.1
LVSA, mm^2	7.8±16.3	101.4±13.9	0.0009
FS, %	19.6±18.1	-31.7±5.8	0.02
FAC, %	14.1±12.4	-26.9±7.5	0.015

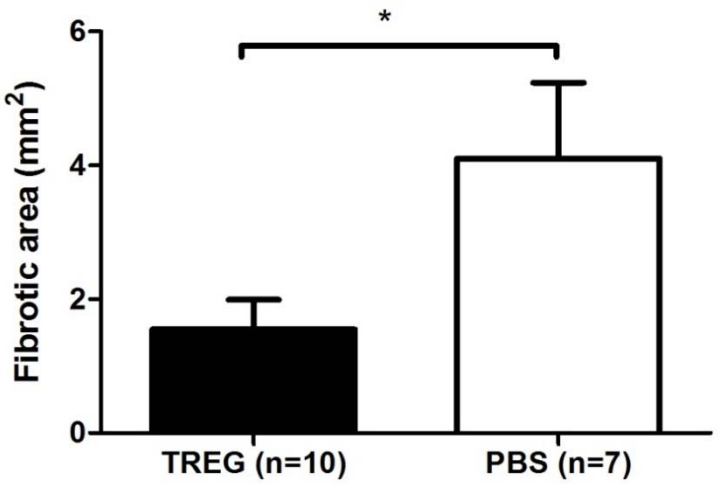
Treg cell transfer resulted in improved cardiac function

Adoptive transfer of regulatory T cells reduces infarct size

Masson's Trichome staining

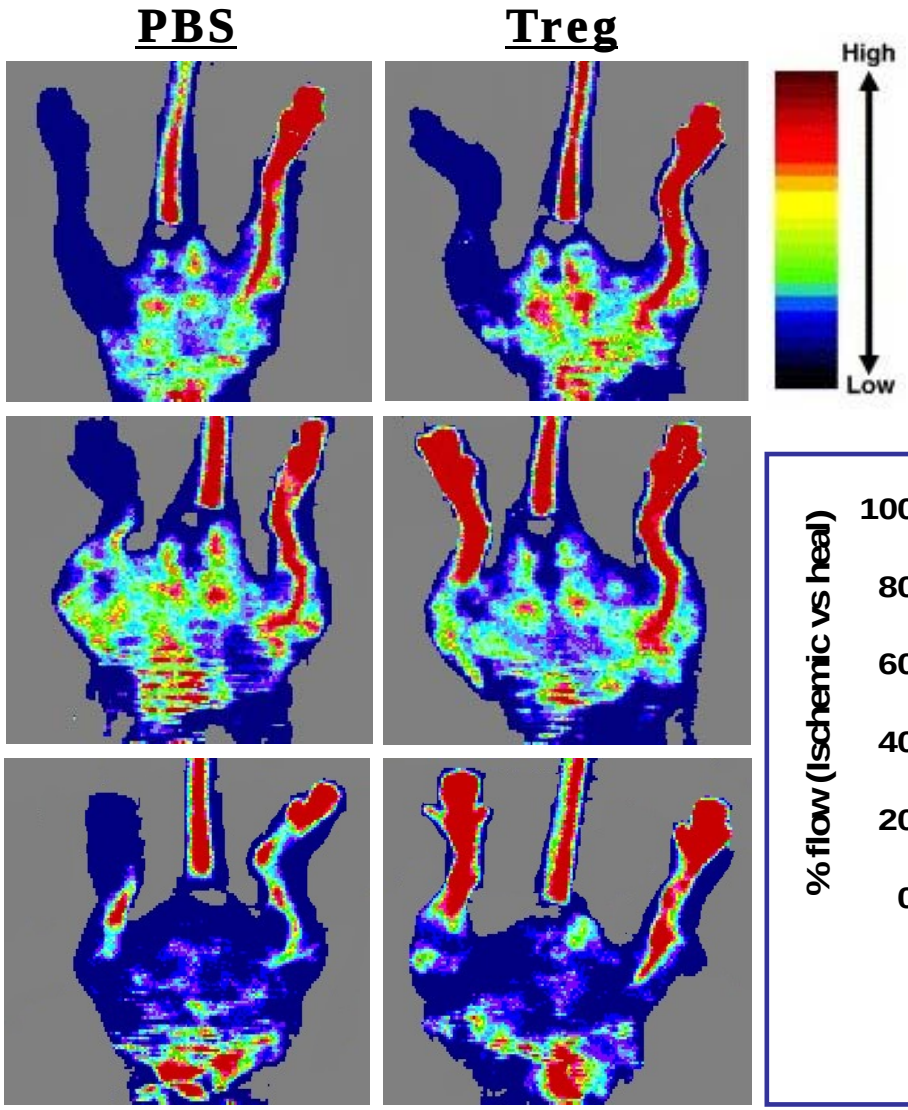


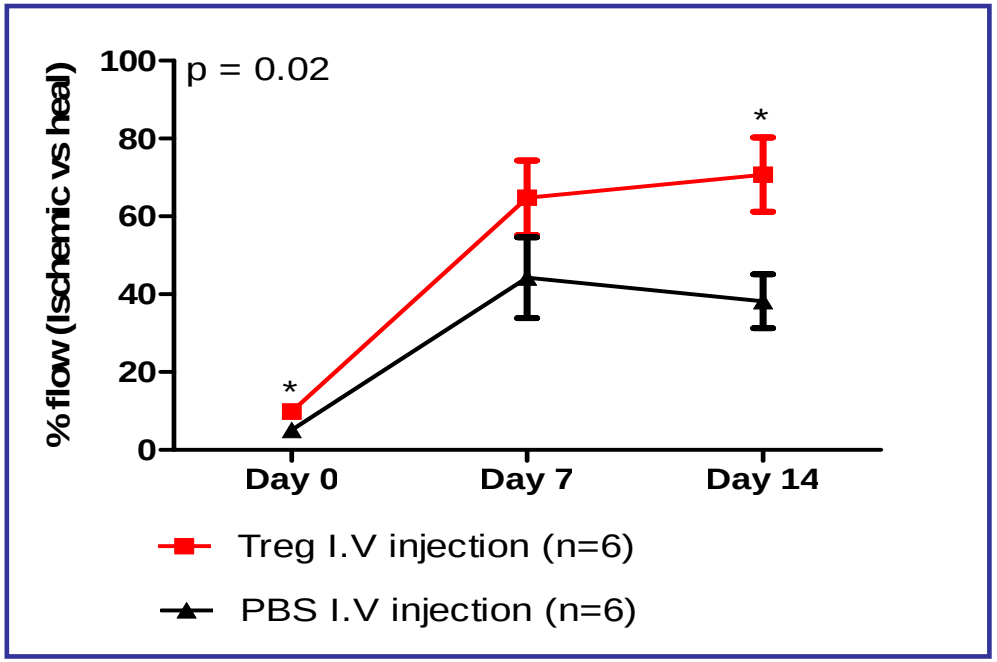
Treg PBS



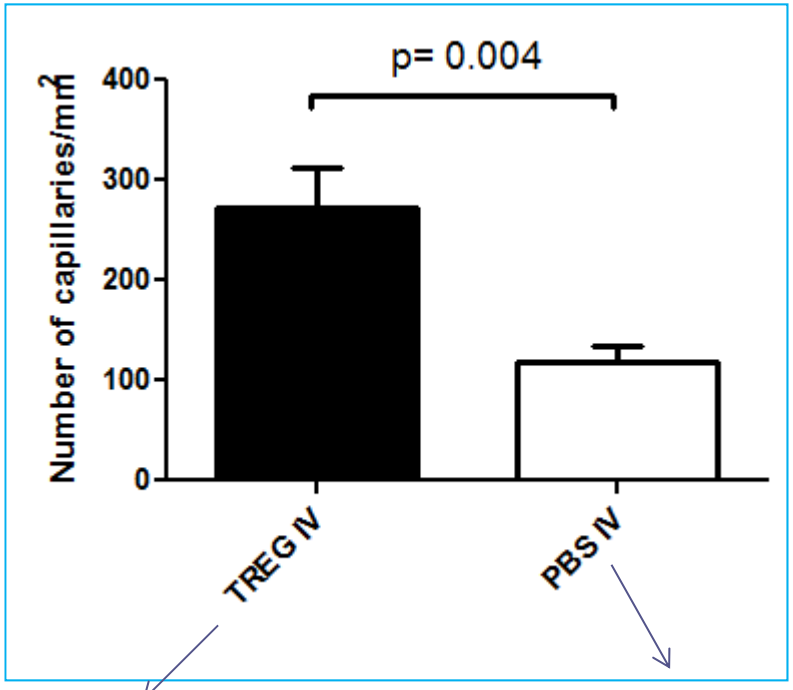
Treg cell transfer resulted in decreased infarct area

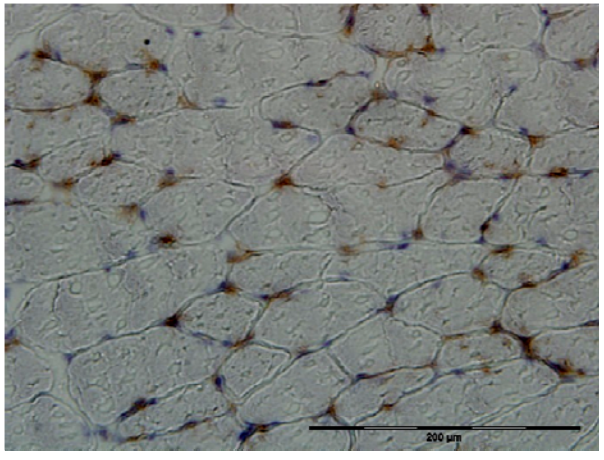
Measurement of blood flow after hindlimb ischemia after transfer of Treg or PBS

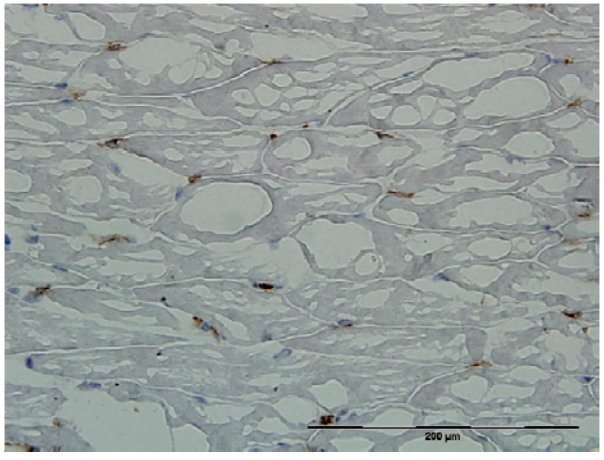




Capillary density in ischemic tissue at Treg transfer mice vs. PBS injected mice







Conclusions

- Following MI induction and hindlimb ischemia, levels of regulatory T cells are higher than in the control group.
- The suppressive properties of regulatory T cells of MI mice and hindlimb mice were significantly reduced 14 days post procedure compare to control.
- Tregs migrate to the site of ischemia and were found in the peri-infarct region.
- Treg cell transfer resulted in decreased infarct area and improved cardiac function / angiogenesis to the hindlimb.

Acknowledgments

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תודה על ההקשבה!

Evolution of acute MI

Reversible, irreversible injury:

0.5-4 hr: waviness

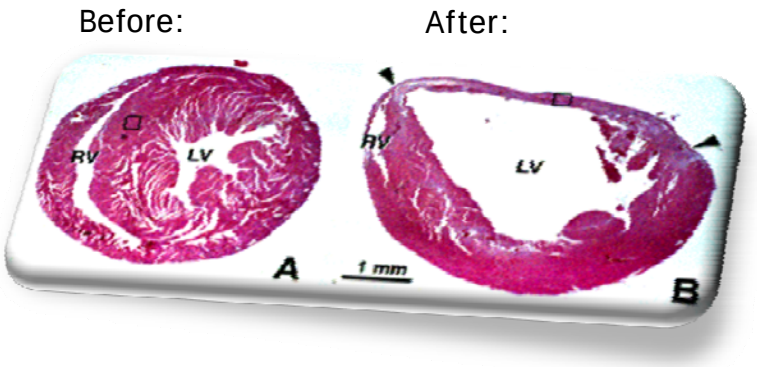
4-12 hr: beginning coagulation necrosis

12-24 hr: beginning inflammatory cells [polymorphonuclear leukocytes(PMN)

1-3 d: intense PMN

3-7 d: beginning mononuclear phagocytosis

10-14 d: granulation tissue



<u>1-2 months: scar !</u>