Transplant Institute

Beth Israel Deaconess Medical Center

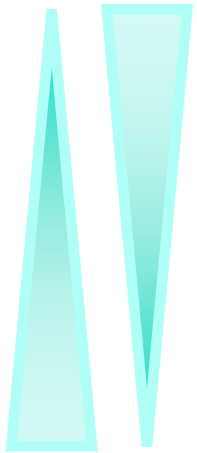
A teaching hospital of Harvard Medical School

Tolerance is best achieved if you don't hurry

Terry Strom

Mechanism of donor specific unresponsiveness in the absence of total and permanent deletion of donor reactive T cell clones *in vivo*

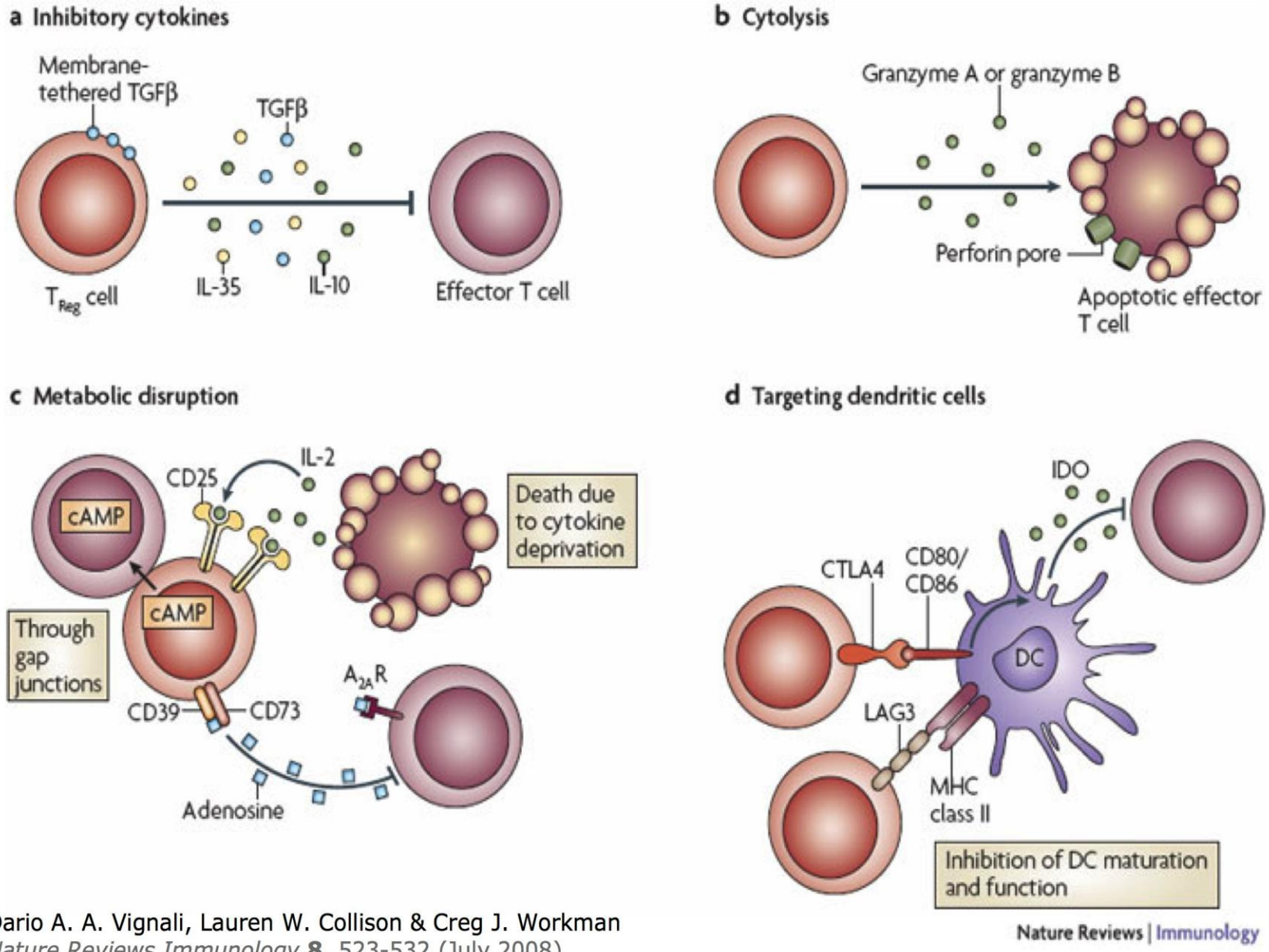
Depletion of donor reactive cells



Immunoregulation/Suppression

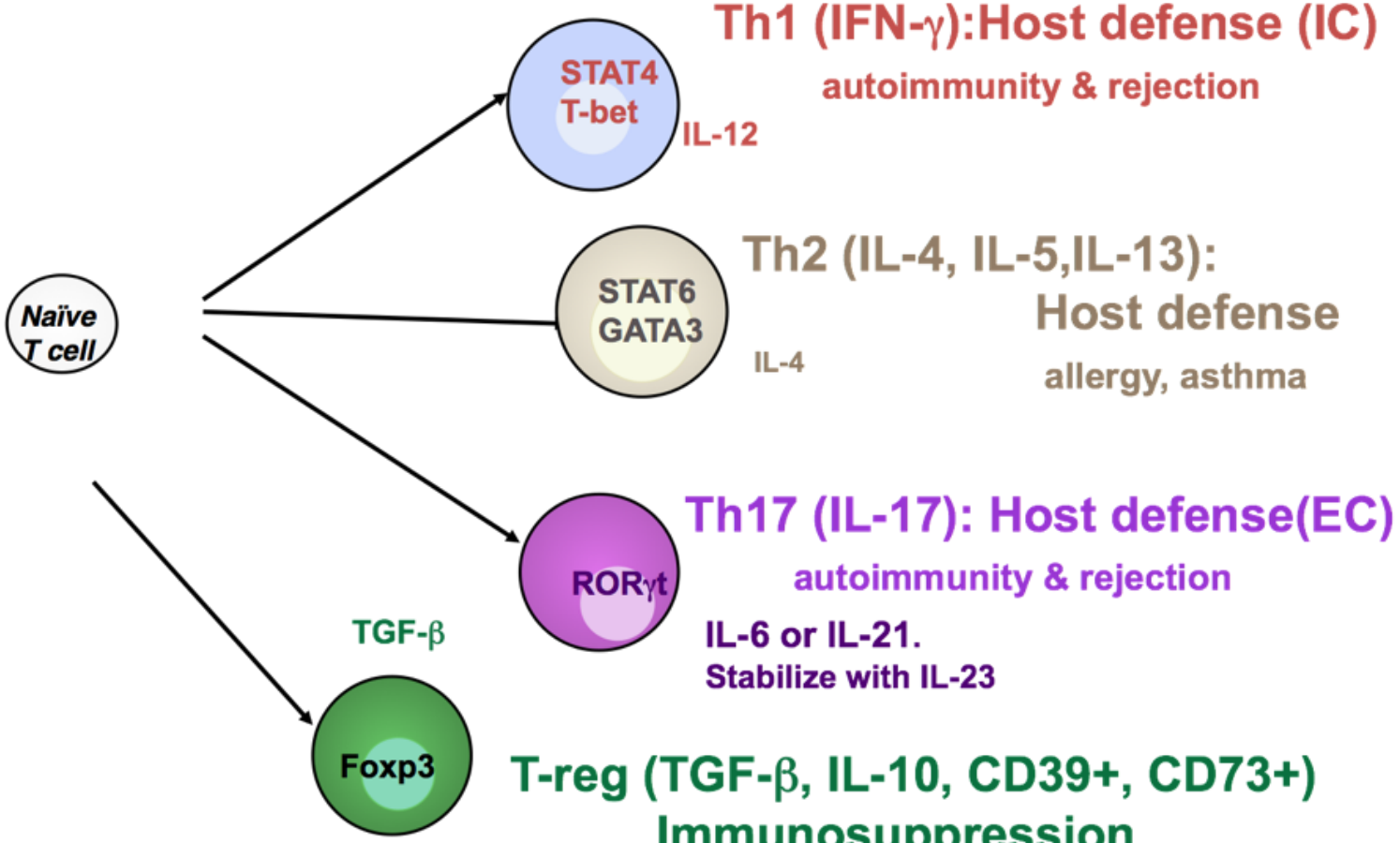
Li et al Nat Med 1999
Wells et al Nat Med 1999

How do Treg function?



Dario A. A. Vignali, Lauren W. Collison & Creg J. Workman
Nature Reviews Immunology **8**, 523-532 (July 2008)

Fates of CD4 T cells: Bettelli et al Nature 2006; Korn et al Nature 2007



Inflammation rules immunity

- "Adverse" inflammation is a tolerance blocker/ breaker.

- IL-21 and IL-6 block polarization of Th0 cells to Tregs while these cytokines as well as IL-1β and TNFα disrupt the Treg program @ the molecular and functional levels.

- Hence transplant tolerance will not be *readily* accomplished in the face of active inflammation produced as a consequence of ischemia reperfusion injury (IRI) or hypoxia.

Inflammation rules immunity

- "Adverse" inflammation, i.e., expression of pro-inflammatory with resultant activation of cytodestructive innate and adaptive immune cells.

- Expression of anti-inflammatory cytokines (TGFβ, IL-10, IL-1RA) and alpha1 antitrypsin, an inflammation induced acute phase protein, favor functional dominance of immunoregulatory, anti-inflammatory cytoprotective networks over rejection and adverse inflammation.

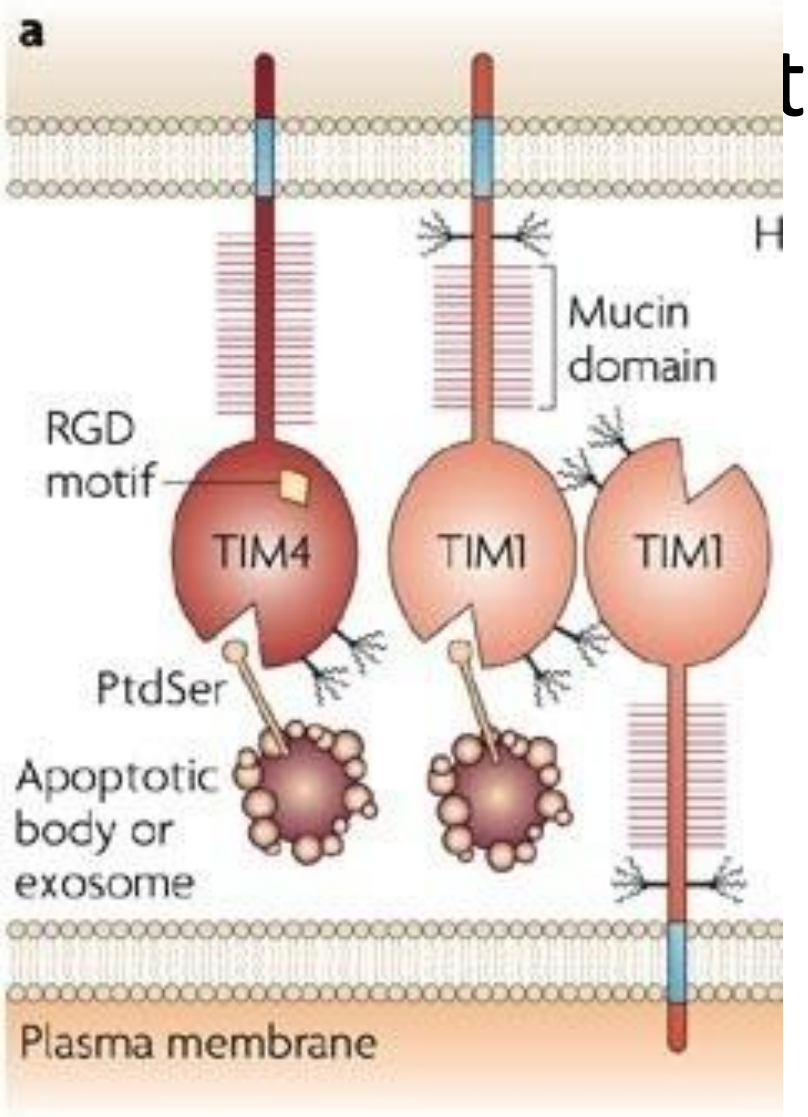
Conventional Anti-rejection Therapy: It's all about (recipient) T cells

- Conventional anti-rejection therapies impair recipient effector T cell responses

- "Next Generation" therapies bolster recipient regulatory T cells, thwart effector T cells

- ***PROBLEM:*** Both tactics ignore donor innate immune immunity which precedes T cell activation and is activated by Ischemia Reperfusion Injury (IRI) and the inflammatory death of cell transplants.

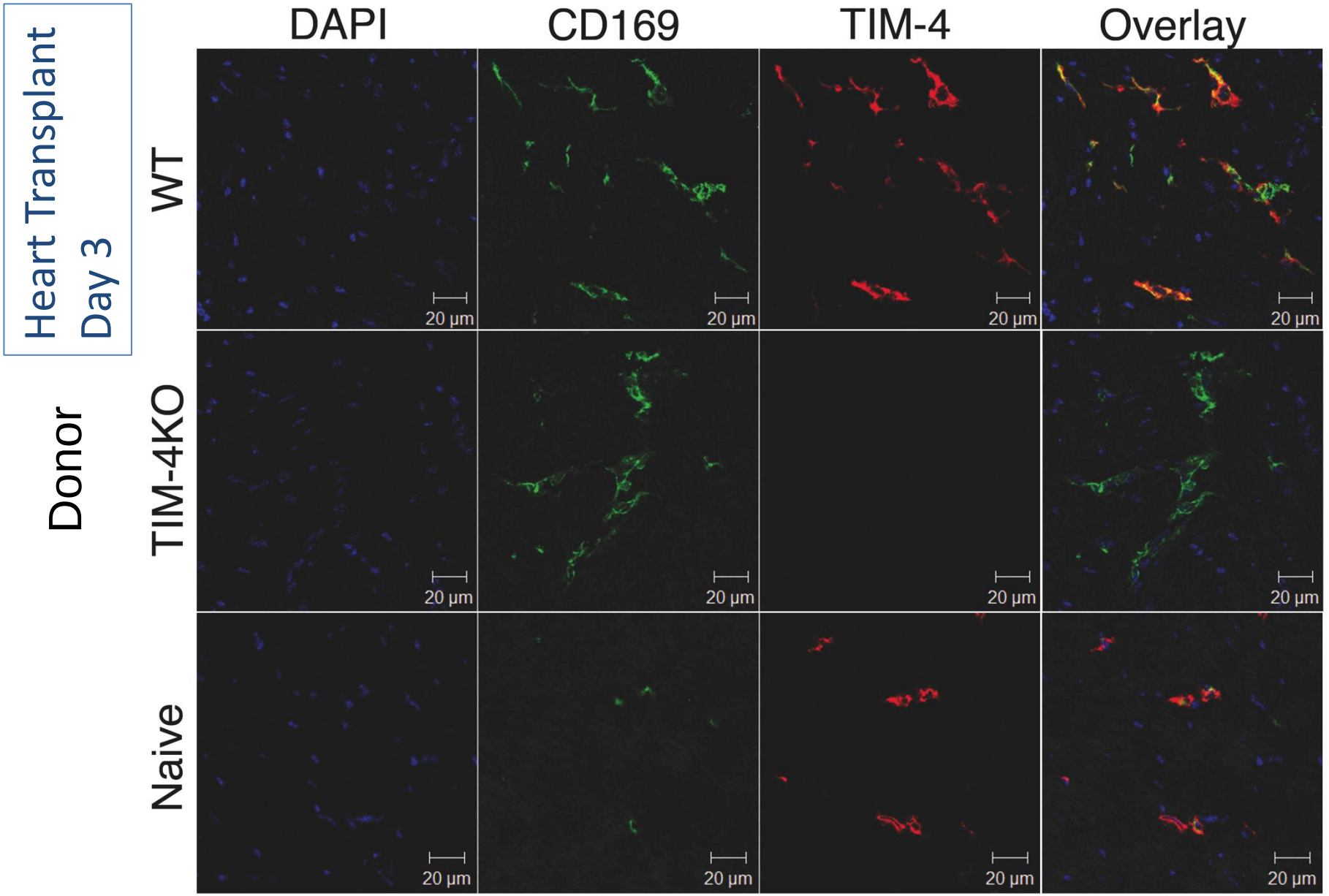
TIM-4: T cell Immunoglobulin & Mucin taining protein 4



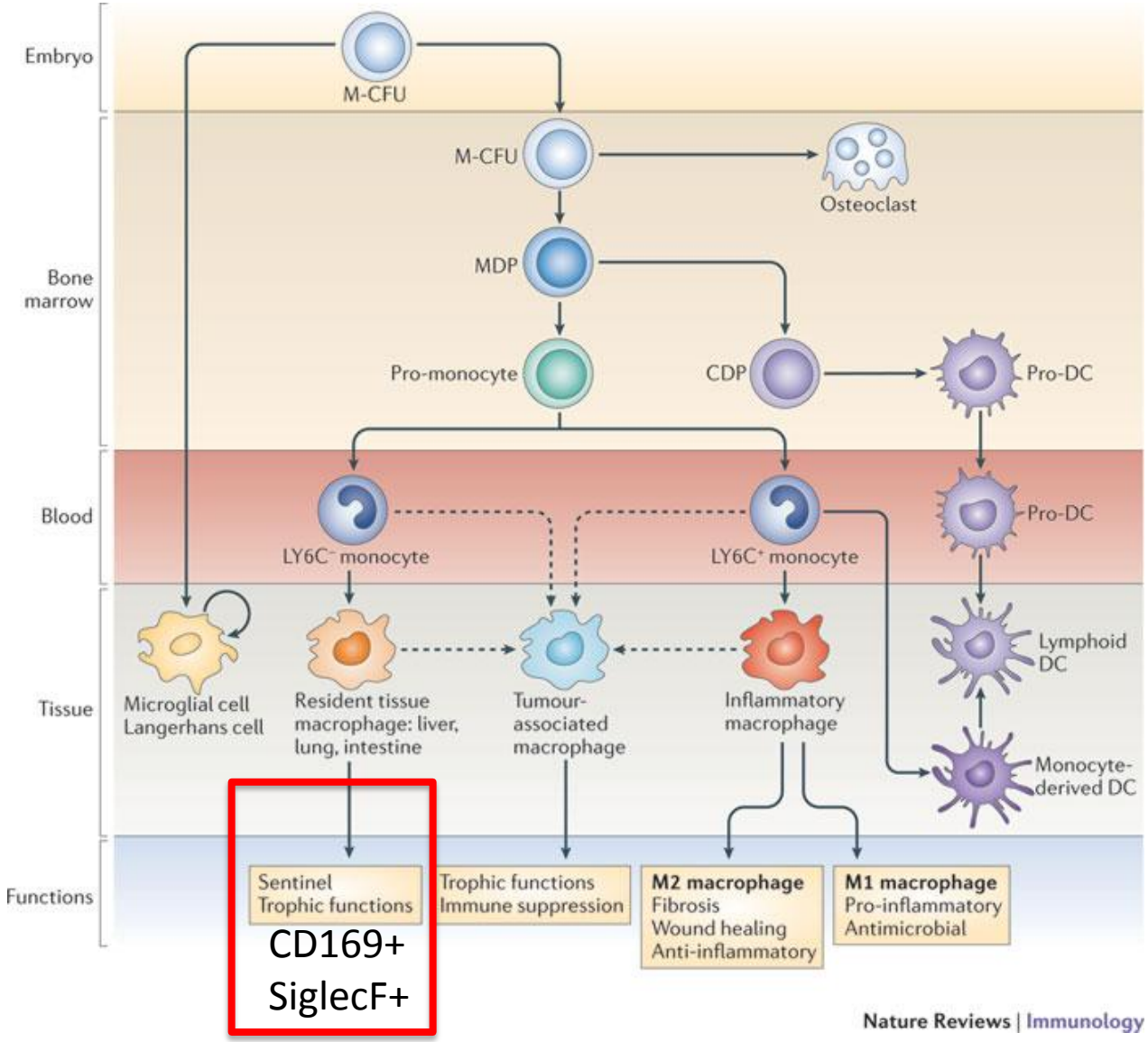
Kuchroo V et al. *Nat Rev. Immunol.* 2008.

- TIM-4 is expressed on subsets Mϕs and DCs.
- TIM-4 functions as a costimulatory ligand of TIM-1 on T cells but unlike other TIMs does not signal.
- Binds to phosphatidylserine (PtdSer) & thereby initiates phagocytosis of apoptotic cells.

CD169+ Donor Heart Mϕs are TIM-4+

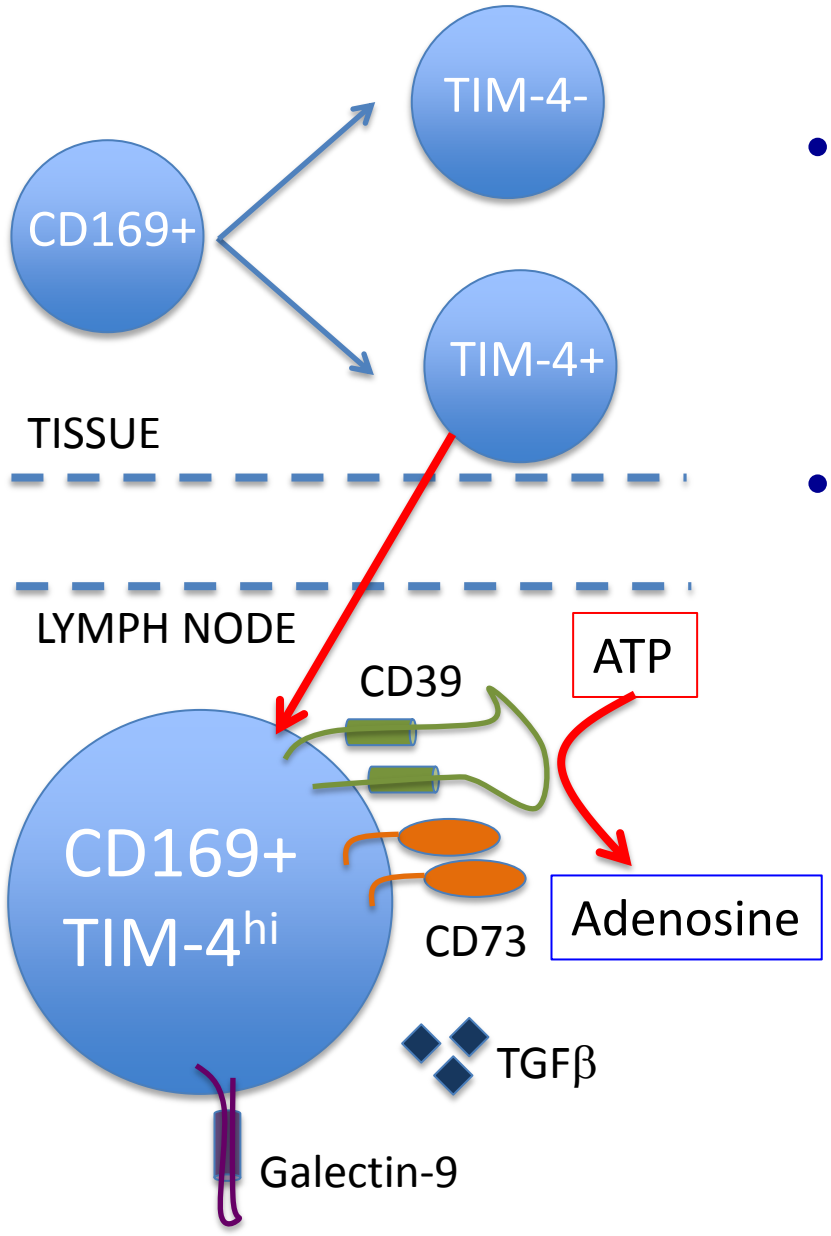


Many Tissue-Resident Mϕs are CD169+ / Siglec-F+



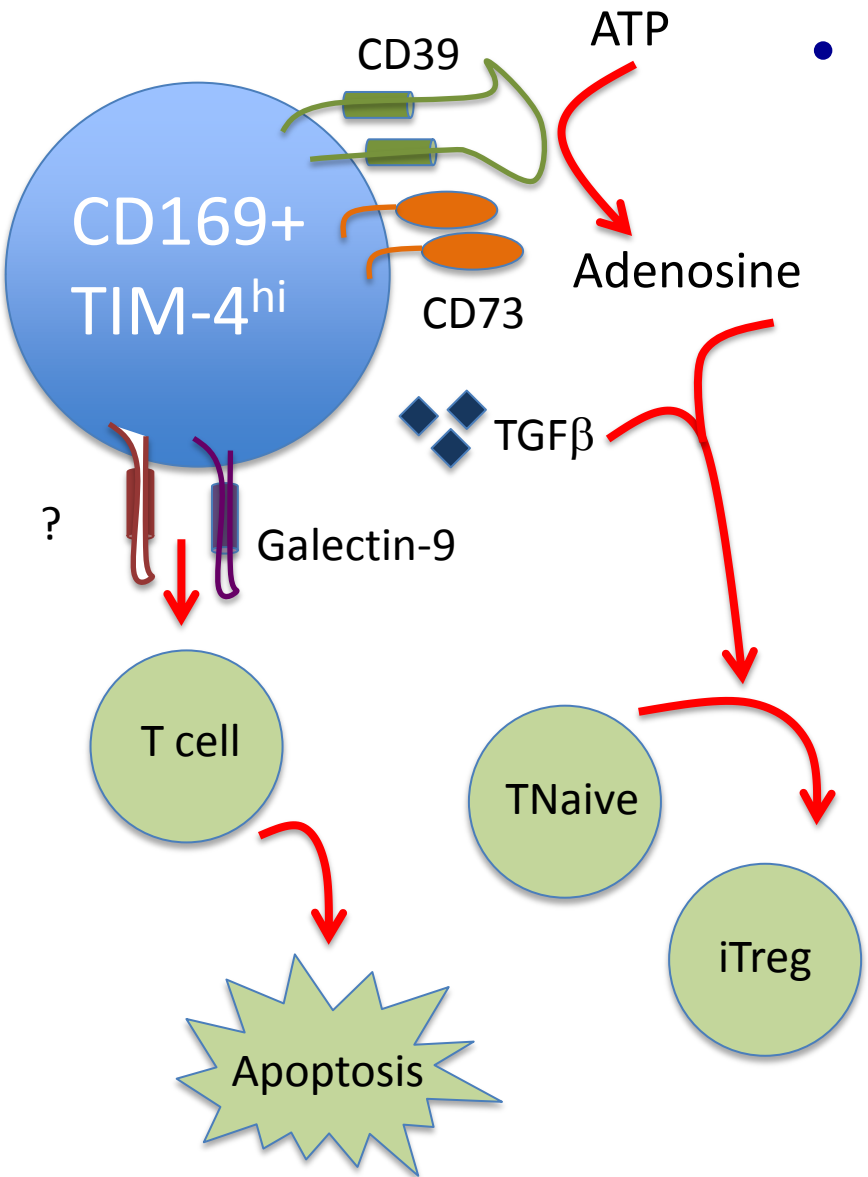
T Lawrence and G Natoli. 2011.

CD169^{+}TIM-4^{hi} Tissue-Resident Mϕs are migratory & have an immunoregulatory phenotype TB Thornley JCI 2014



- *Migratory*
  - Migrate to the draining lymphoid node in response to oxidative stress
- *Immunoregulatory Phenotype*
  - *CD39/CD73:* Cleave ATP and generate Adenosine collectively
  - TGFβ: induces iTregs
  - Galectin-9: induces apoptosis in TIM-3+ Th1/ Th17 effector T cells
  - Low levels of MHCII/ antigen presentation & costimulatory CD80, CD86, CD40 molecules

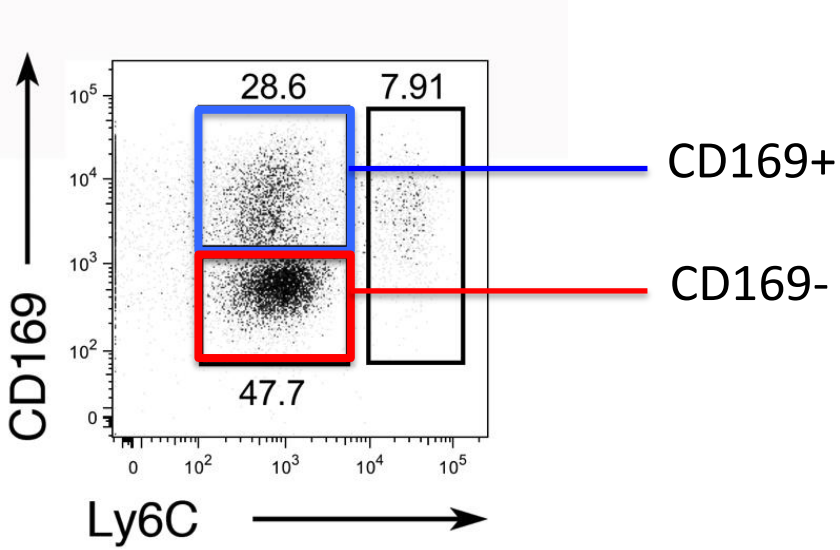
$CD169^{+}TIM\text{-}4^{hi}$ Tissue-Resident Mϕs are functionally immunoregulatory

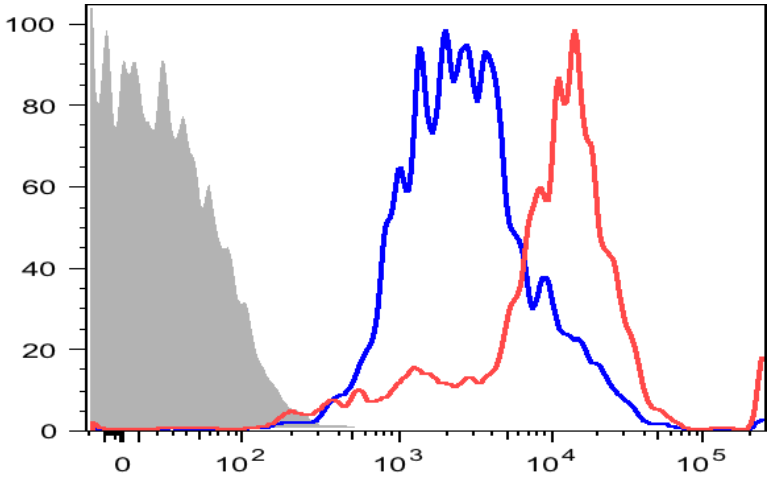


- *Influence on adaptive immunity*
  - Poorly Stimulate T cell proliferation
  - Induce more iTregs than $CD169^{-}TIM\text{-}4^{lo}$ stimulators
  - Fail to support robust T cell survival (data not shown)

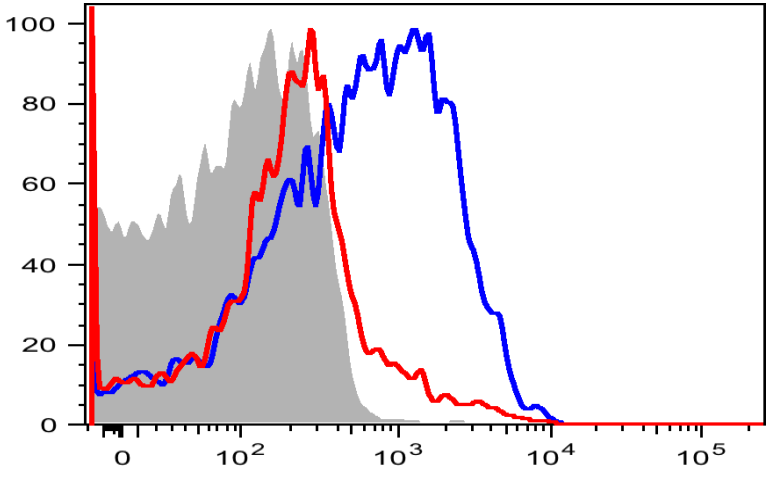
CD169^{+}TIM-4^{hi} Tissue-Resident Mϕs that migrate to the dLN are hypostimulatory & immunoregulatory

FITC Painting Model

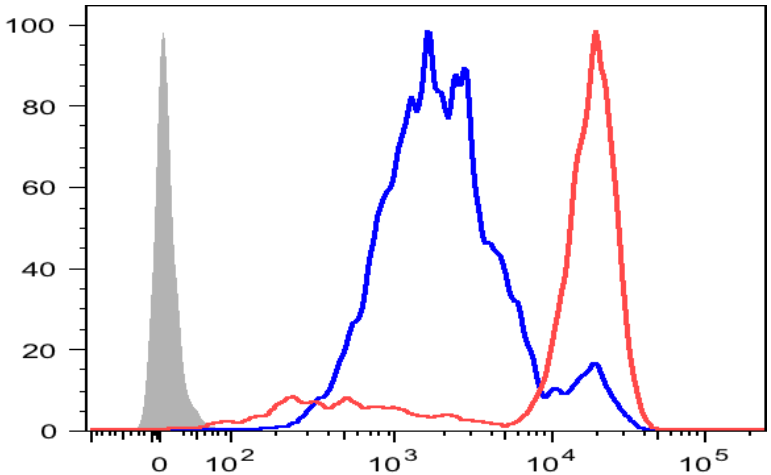




CD86



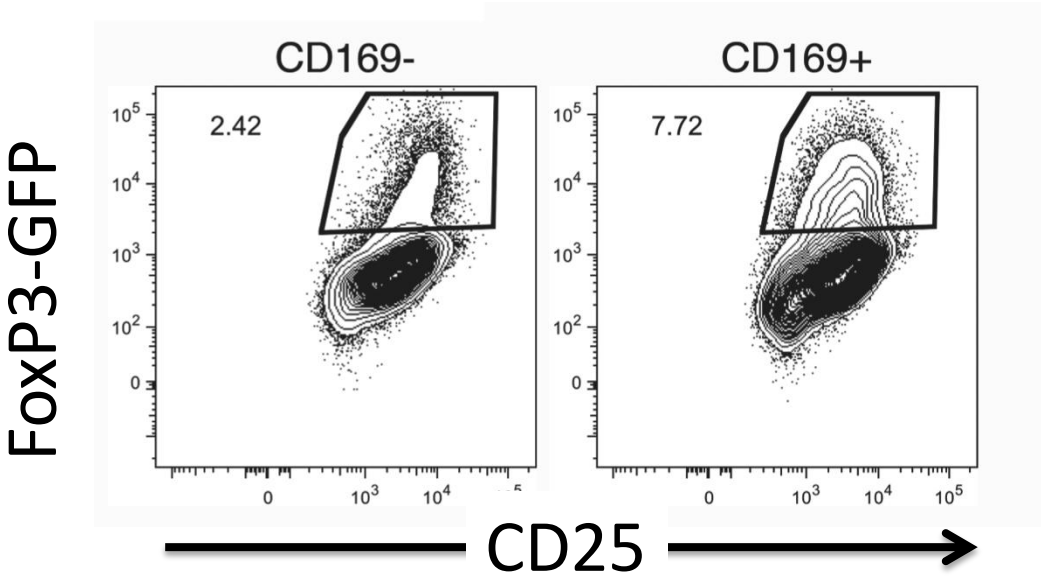
CD73

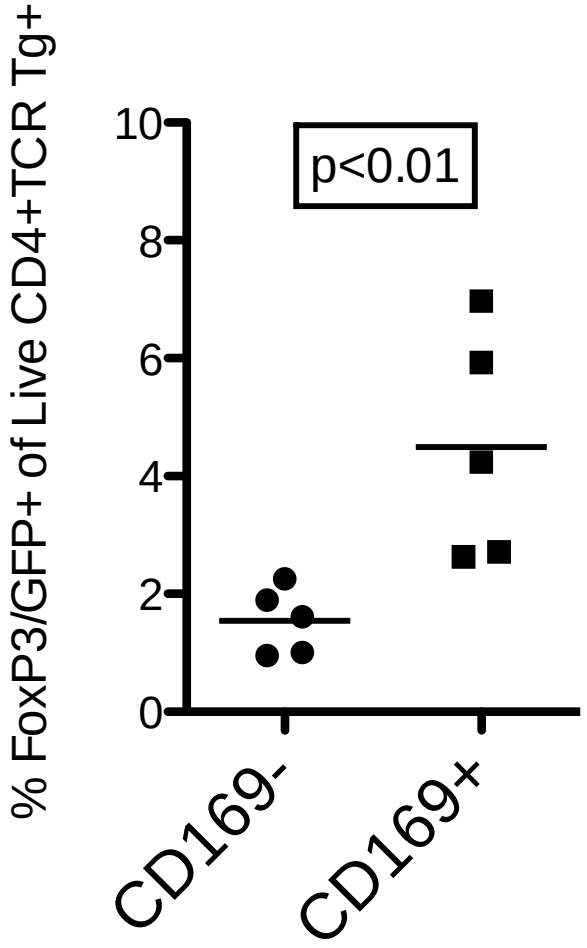


MHCII

CD169^{+}TIM-4^{hi} Tissue-Resident Mϕs induce more iTregs than CD169^{-} APCs

MLR





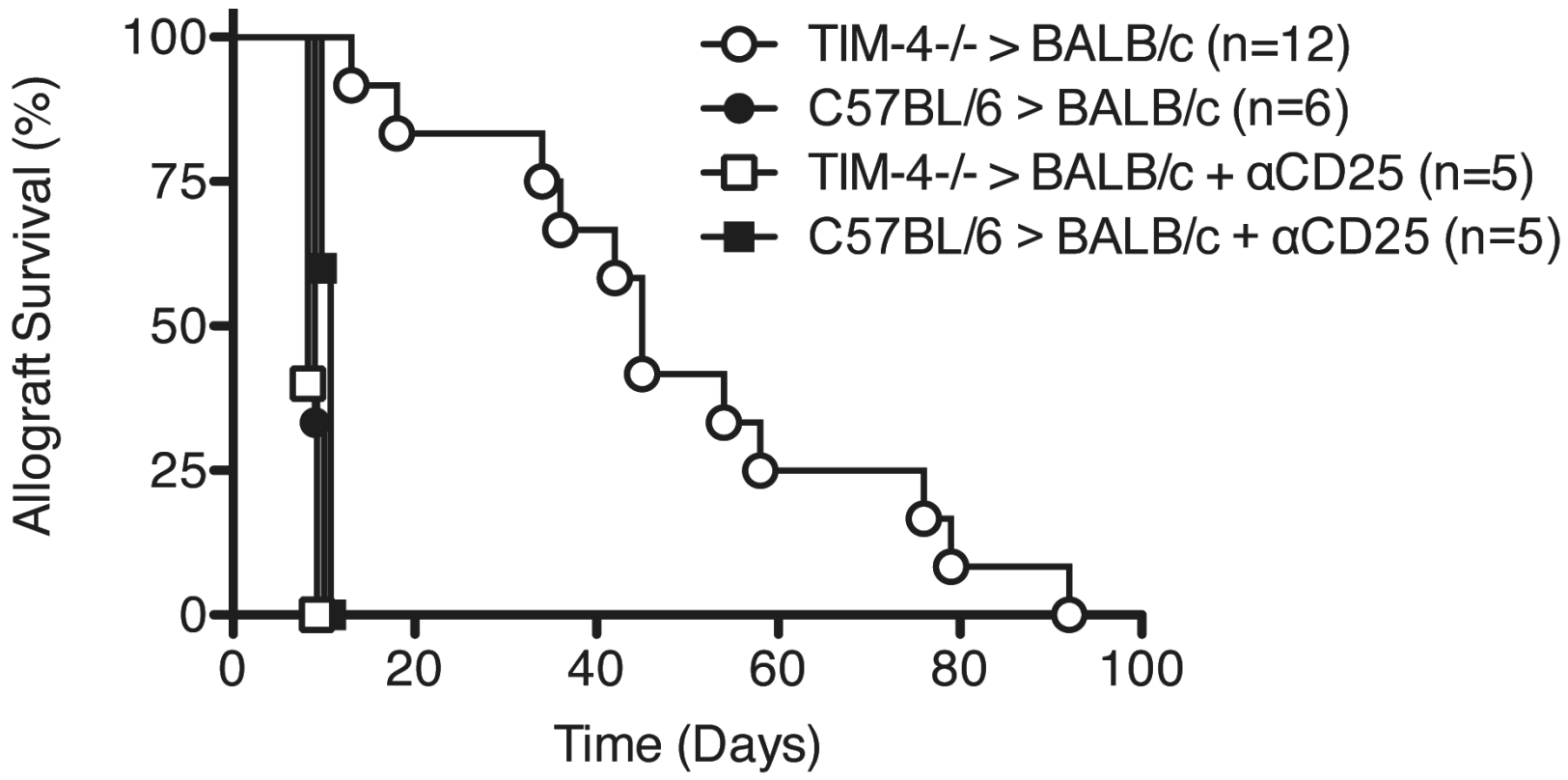
What is the functional role of TIM-4 on these fragile CD169^{+} Tissue-Resident Mϕs?

- TIM-4,CD169+ macrophages disappear just be fpre rejection.

- Hyypoyhesis: Genetic deletion of TIM-4 will increase the migration of CD169^{+} tissue-resident Mϕs that home from tissue to the dLN because they will not be retained by TIM-4 to PstSer interactions and killed, thereby prolonging engraftment.

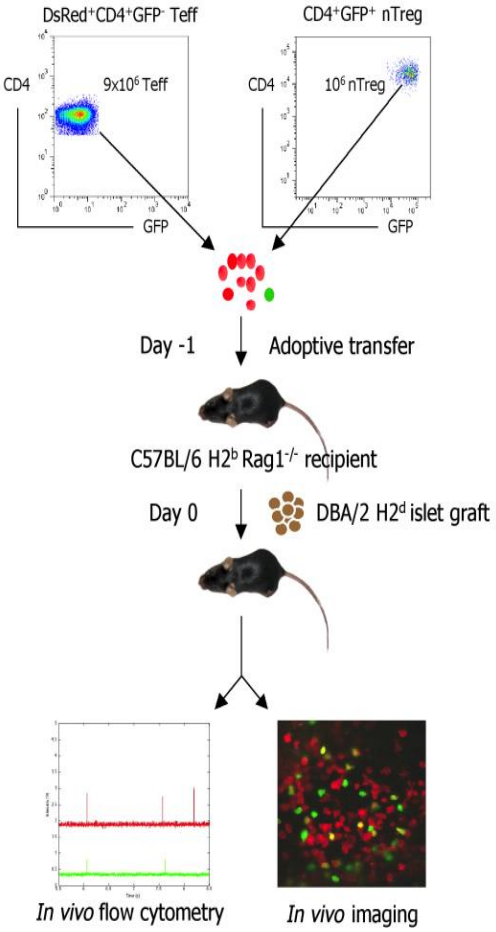
TIM-4$^{-/-}$ heart transplants exhibit prolonged Treg-dependent survival

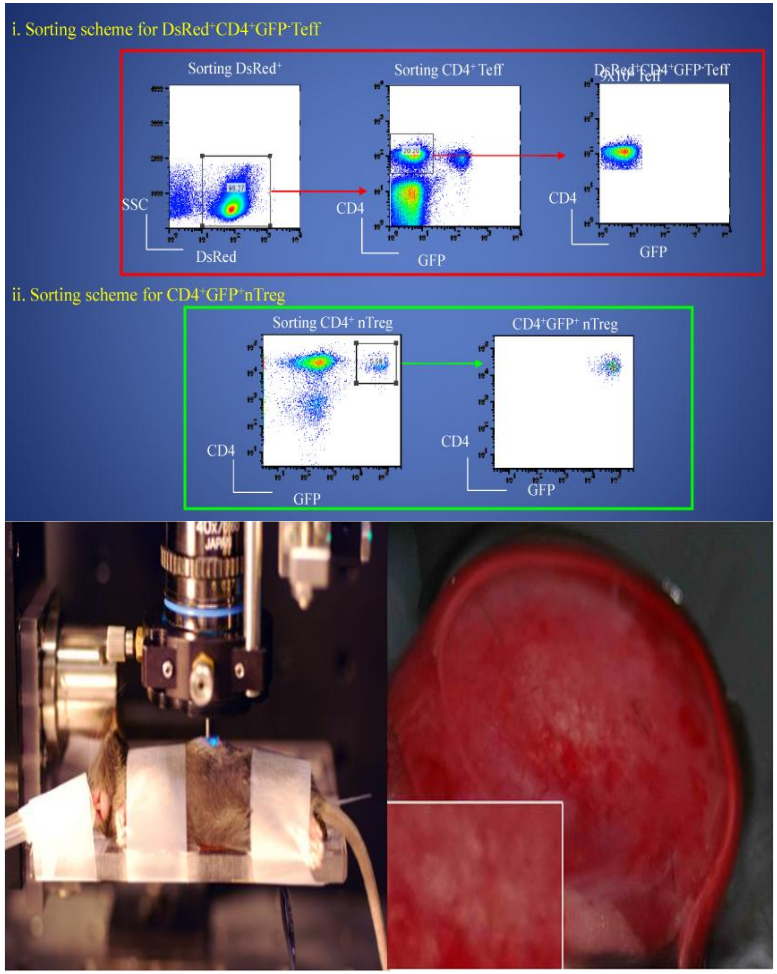
Heart Transplant



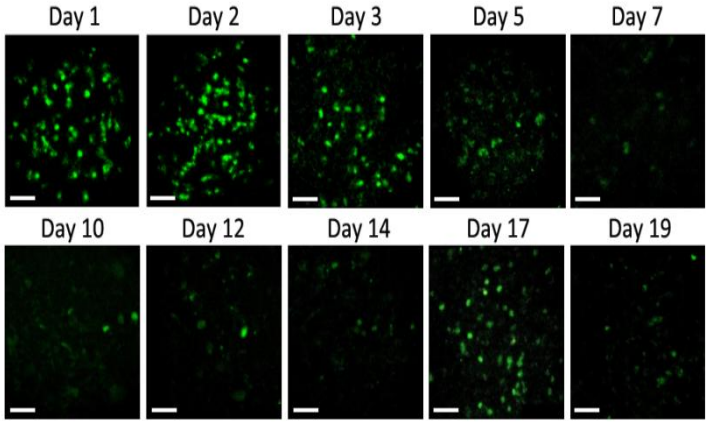
In vivo imaging by minimally-invasive endoscopic confocal microscopy:

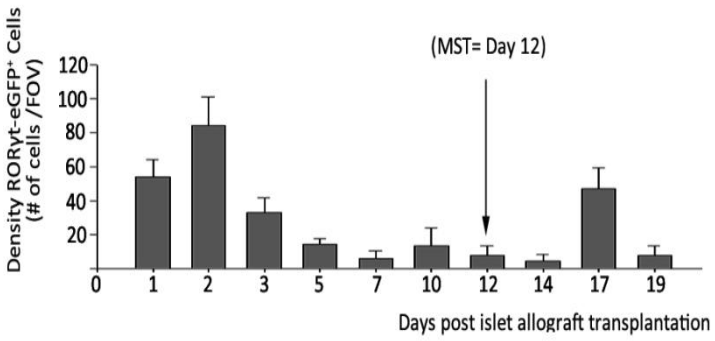
Z.Fan et al Nature Medicine 2010

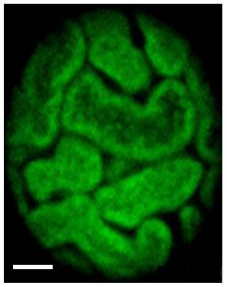




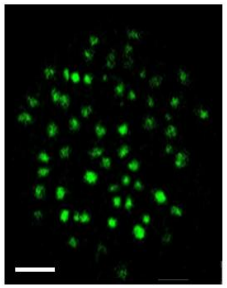
Peak of islet allograft infiltration by RORγt⁺ cells coincides with inflammation not rejection







Undisturbed contralateral kidney

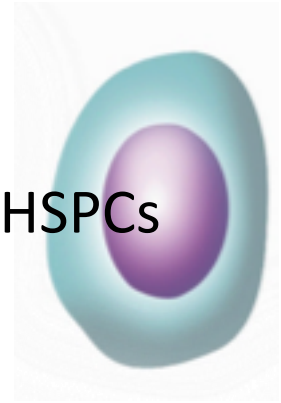


RORγt-eGFP⁺ cells infiltrate syngeneic islet grafts on Day 1

At least 5 animals were studied at each time points; 8-10 representative images were analyzed at each time points from each animal. The data was calculated and their mean value was used for comparison and analysis. Error bars represent standard error of the mean (s.e.m.)

Adaptive Hematopoiesis: The root of inflammation

HSCs/ HSPCs



•Fully functional hematopoietic stem cells HSCs and progenitors infiltartet inflammatory sites, i.e.,/transplants (Z Fan et al., Am J Transplant, 2014).

•Intramedullary HSCs are immunoregulatory (CD39/73+ et al) but loose immunoregulatory function S/P transplantation.

•HSCs rapidly differentiate into NOVEL primative myeloid and lymphoid cells including immunoregulatory and immunostimulatory phenotypes.

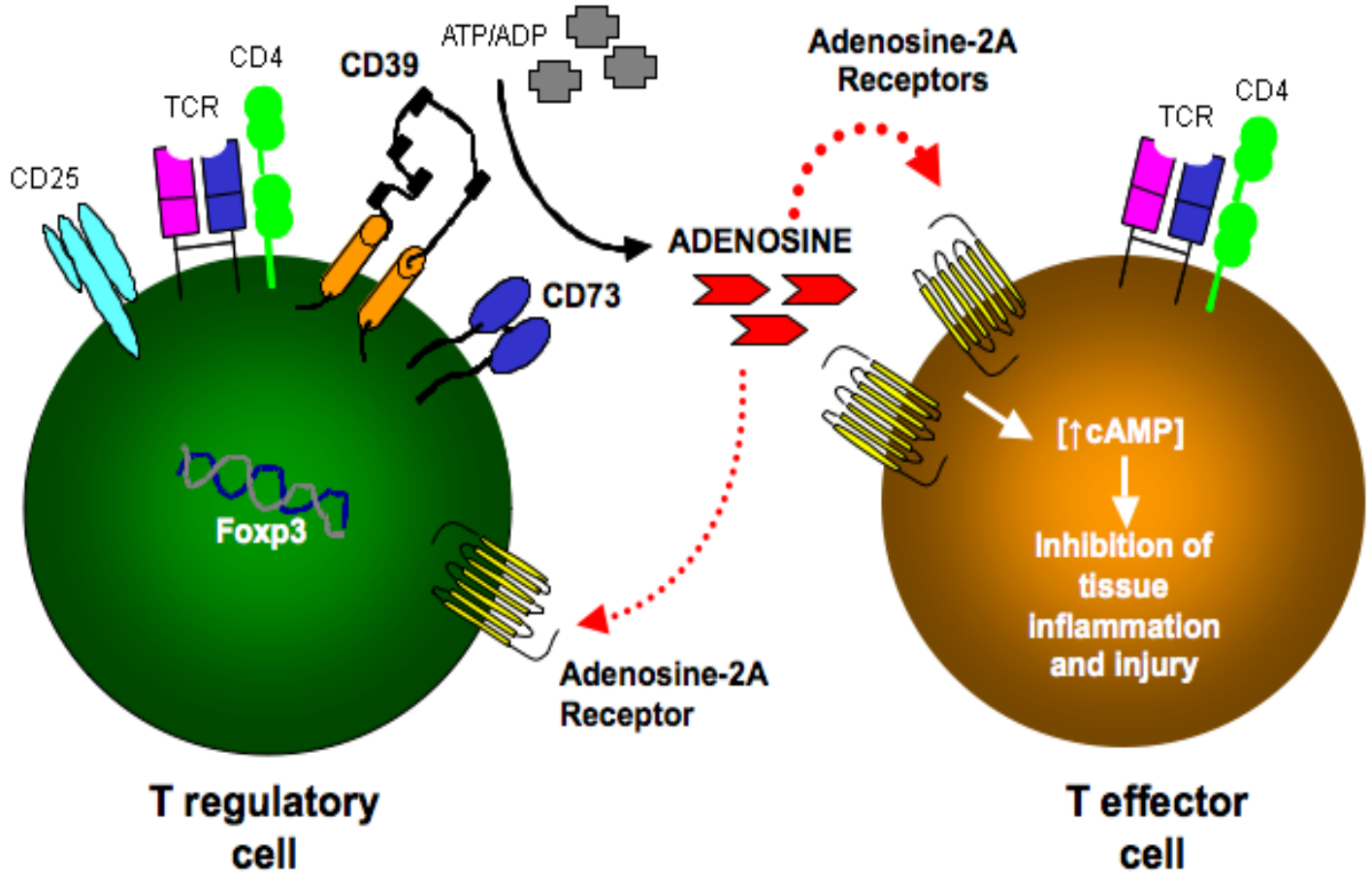
•Differentiation can be diverted to immunoregulation by drugs and genetic manipulation.

The Roots of Intragraft Inflammation

- Tissue resident macrophages.
- Graft infiltrating HSCs give rise to a diverse and esoteric population of myeloid and lymphoid cells….some of which have not been previously identified.
- The phenotype and function of these cells is governed by the inflammatory texture of the transplant (IRI, hypoxia) which can be altered by drug treatment and/or genetic manipulation.

Adenosine: a mediator of immunoregulation

S Deanglio et al JEM 2007

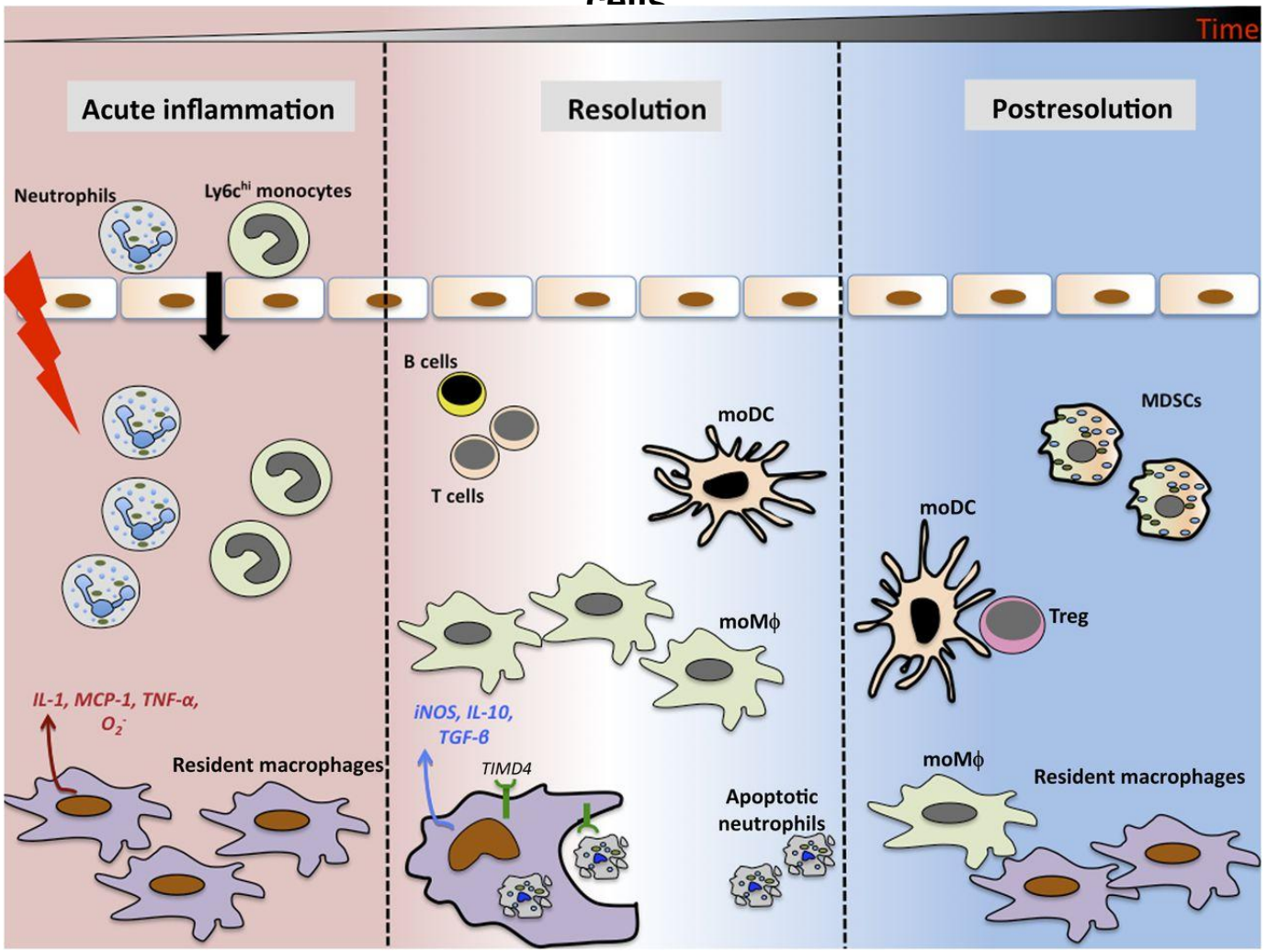


Immunoregulatory cells

- Tregs & Tr1 cells
- Mesenchymal stem cells
- M2 & a tissue resident macrophage subset
- “Virgin or Managed” HCSs
- Bregs: mature & pro-B cell

At least some of these cells loss immunoregulatory function and molecular phenotype in the inflammed microenvironment S/P transplantation.

The acute phase of inflammatory response is accompanied by rapid (within minutes) influx of neutrophils and monocytes in response to soluble mediators released by tissue resident cells



Bagaitkar J Blood 2014;124:1701-1703

Inflammation rules immunity

- Tolerance can be produced or favored through use of agents that profoundly and favorably change the texture of immunity from adverse- to healing-type immunity…the post resolution state.

- For example, alpha1 antitrypsin, now used in very promising T1D trials and about to be tried in islet transplantation, blunts expression of numerous pro-inflammatory cytokines, activation of the NF-kB pathway but does not *directly* act upon T cells.

Tipping the balance of immunity toward transplant tolerance.

- Eliminate adverse inflammation with Rx (AAT and other select agents) or wait for cessation of adverse inflammation from sites in which T cells recognize relevant histocompatibility antigens.

- Utilize other drugs such as IL-2 or IL-2.Ig (XX Zheng et al Immunity 2005) that bolster the abundance and strength of Tregs without strengthening adverse inflammation/ destructive type immunity.

Alpha 1 anti-trypsin

Biological Background

- Member of the Serpin family; a serine-protease inhibitor
- Abundant in blood (1.5-3.5mg/ml)
- Its concentration increases 4-4x during infection and inflammation in response to IFNα
- Neutralizes neutrophil elastase and other serine proteases -> protects the lung from proteolysis (emphysema)
- Single chain 394aa (expressed as pro-peptide of 418aa) (52KDa)

Involved in different pathologies

- Mutations cause aggregations that in the liver (Q242KZ-mutant)
- Aggregation -> Hepatitis, cirrhosis, emphysema
- Antitrypsin deficiency

Mechanism

- Oxidation M358 leads to emphysema
- Oxidized forms identified in smokers, persists after smoking for years

Alpha 1 anti-trypsin

- Anti-thrombotic, blocks complement activation & expression of IL-1β, IL-6, TNFα and activation of the NFκB pathway.

  M Koulmanda et al PNAS 2008; AJT 2015

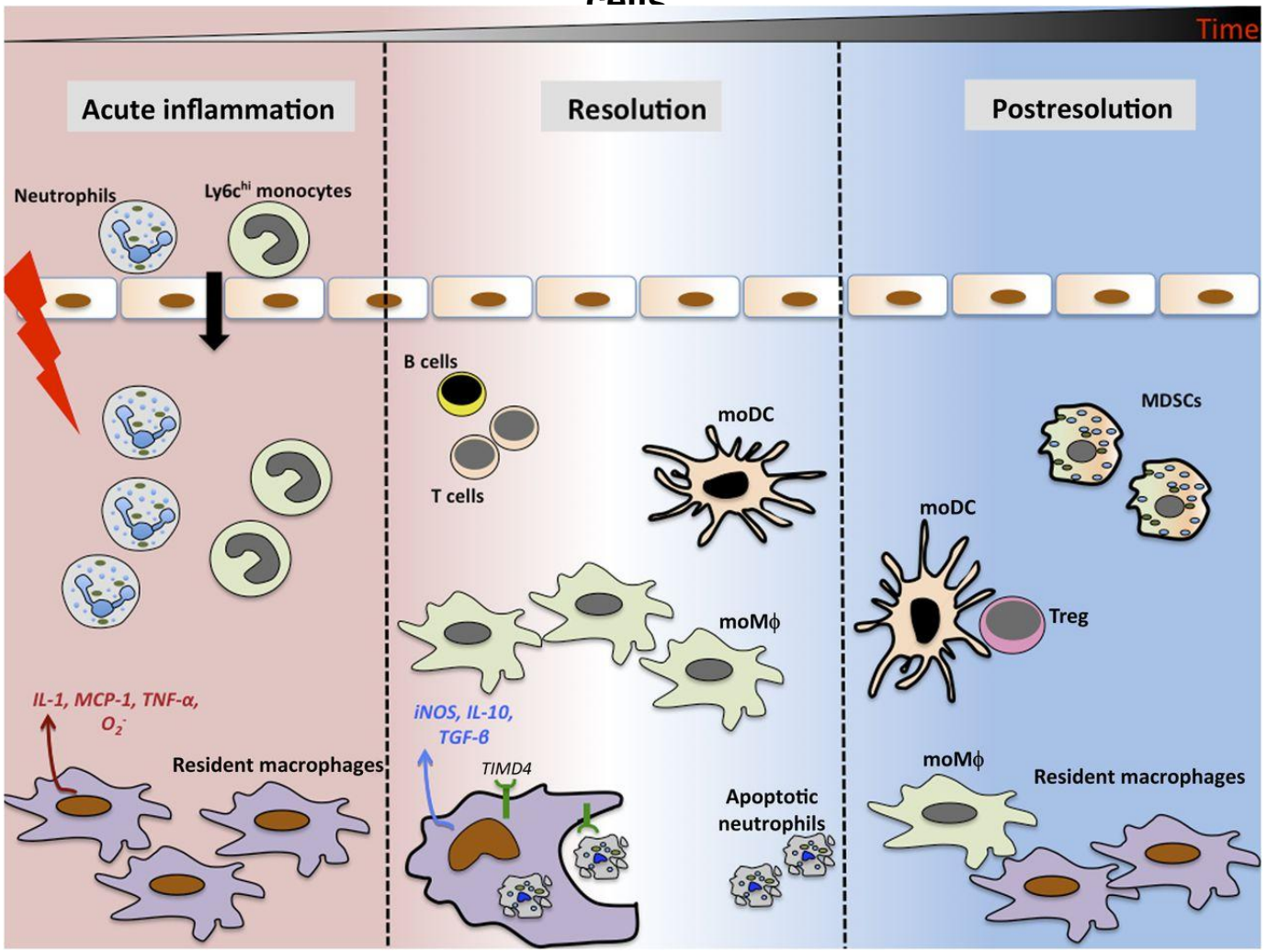
- 2 wk course restores euglycemia and tolerance in NOD model of new onset T1D. *PNAS 2014*

- 2 wk course blocks inflammatory loss of autologous islets in NHP model of minimal dose transplantation. *AJT 2015*

Alpha 1 anti-trypsin

- Short term AAT restores euglcemia and self tolerance in the NOD new onset T1D. Human trials in new onset T1D are very promising.
- 2 wk course of AAT prevents over expression of pro-inflammatory cytokines, chemokines invasion of macrophages, activation of NFkB and universal graft loss of autologous islets by 6 mos. in minimal dose islet monkey model. A clinical trial in hu autografts is enrolling patients
- Short term AAT + anti-CD40 + RPM produces drug free islet allograft survival in NHP model. AAT as an adjunctive Rx in human islet allografts is enrolling patients.

The acute phase of inflammatory response is accompanied by rapid (within minutes) influx of neutrophils and monocytes in response to soluble mediators released by tissue resident cells

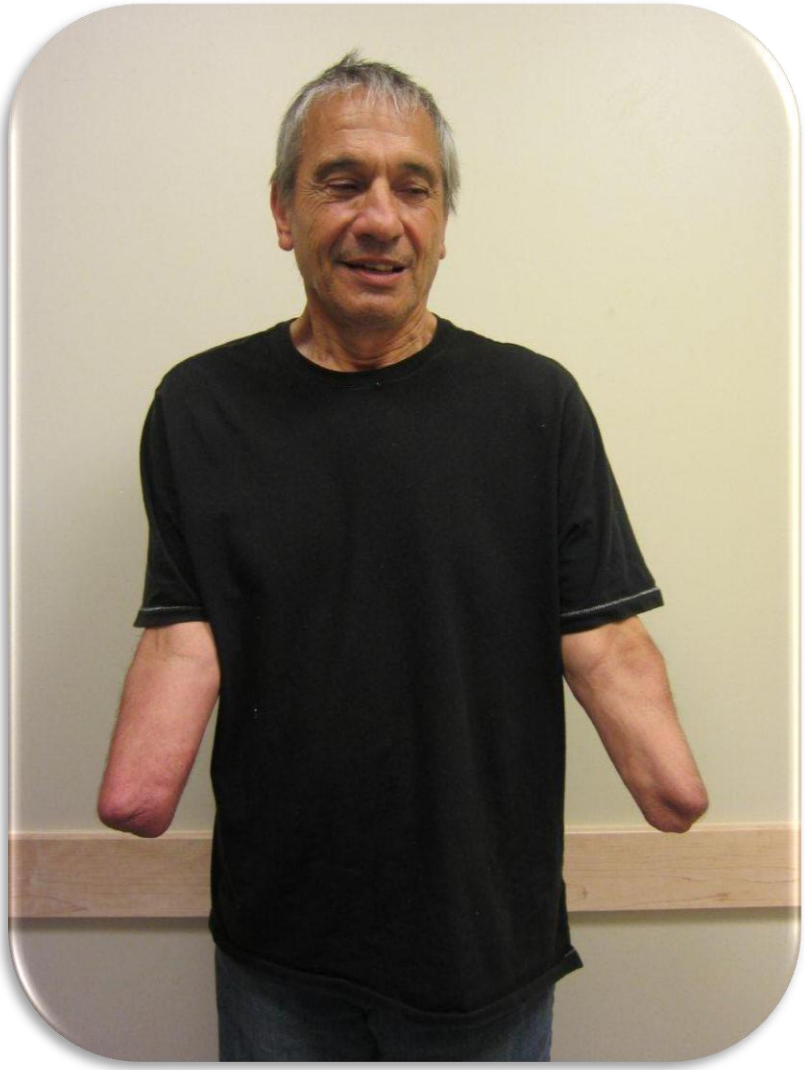


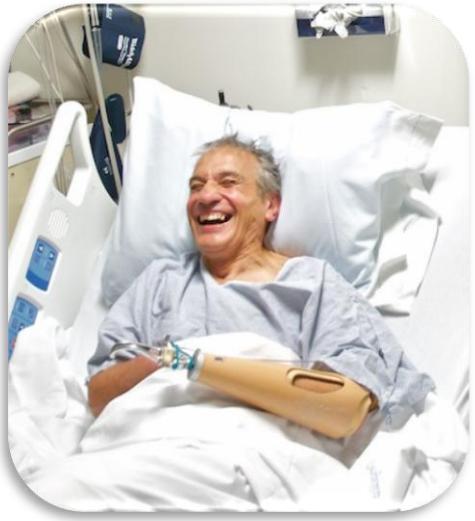
Bagaitkar J Blood 2014;124:1701-1703

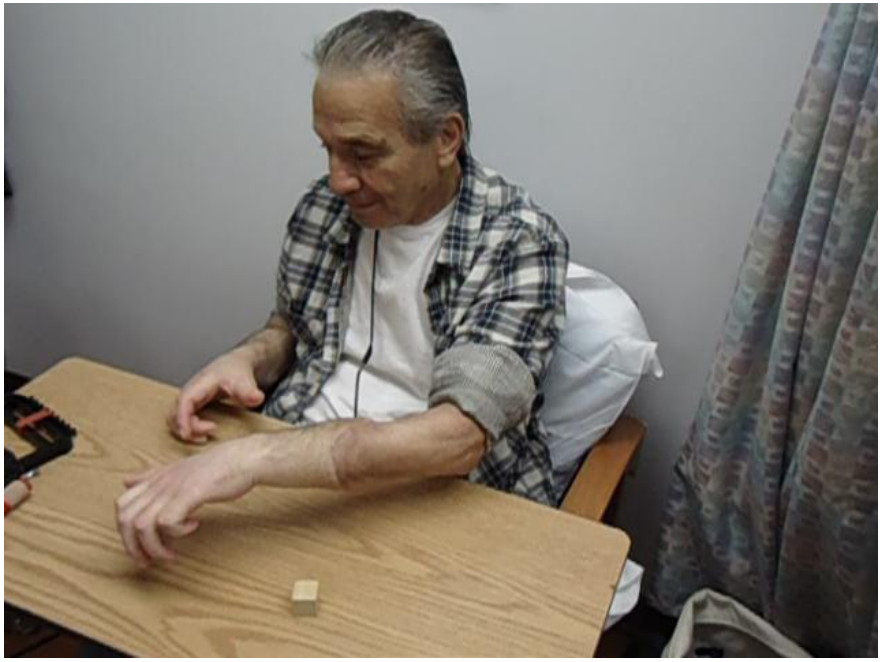
Tolerance induction strategy

1) Induce tolerance in patients without detrimental peri-and early post-transplant inflammation.

2) Alter the balance of immuno-regulatory to effector type immunity.







Why tolerize hand transplants?

- Life-long immunosuppression may not be warranted to preserve a transplant that is not life saving.

- Recipients often tolerate radical reduction in dosage and number of their maintenance immuno-suppression (tacrolimus, MMF, mTOR inhibitors, corticosteroids)

- Rejection is easily diagnosed early on enabling timely resumption of antirejection therapy, thereby minimizing accumulation of memory T cells.

Why tolerize liver transplants?

- Life-long immunosuppression is not required in all liver transplant recipients.

- Owing to the spare functional capacity of the liver, anti-rejection Rx select patients can be safely undertaken. Withdrawal succeeds in some and re-introduction of anti-rejection preserves transplant function in the “failures”.

- With Alberto Sanchez-Fueyo we will try to withdraw IS from select liver transplant recipients long folllowing transplantation.

Targeting Activated Immune and Inflammatory Cells

- LOW DOSE IL-2/ IL-2.Ig promotes expansion of regulatory type T cells and apoptotic death of recently activated effector T cells.
- HIGH DOSE IL-2/ IL-2.Ig activates inflammatory cells.
- Potent effects in tough mouse allograft models, NODs (90% permanent remissions), and subhuman primate transplant models.

X. Zheng et al Immunity 2003; M Koulmanda et al PNAS 2007; & AJT 2012.

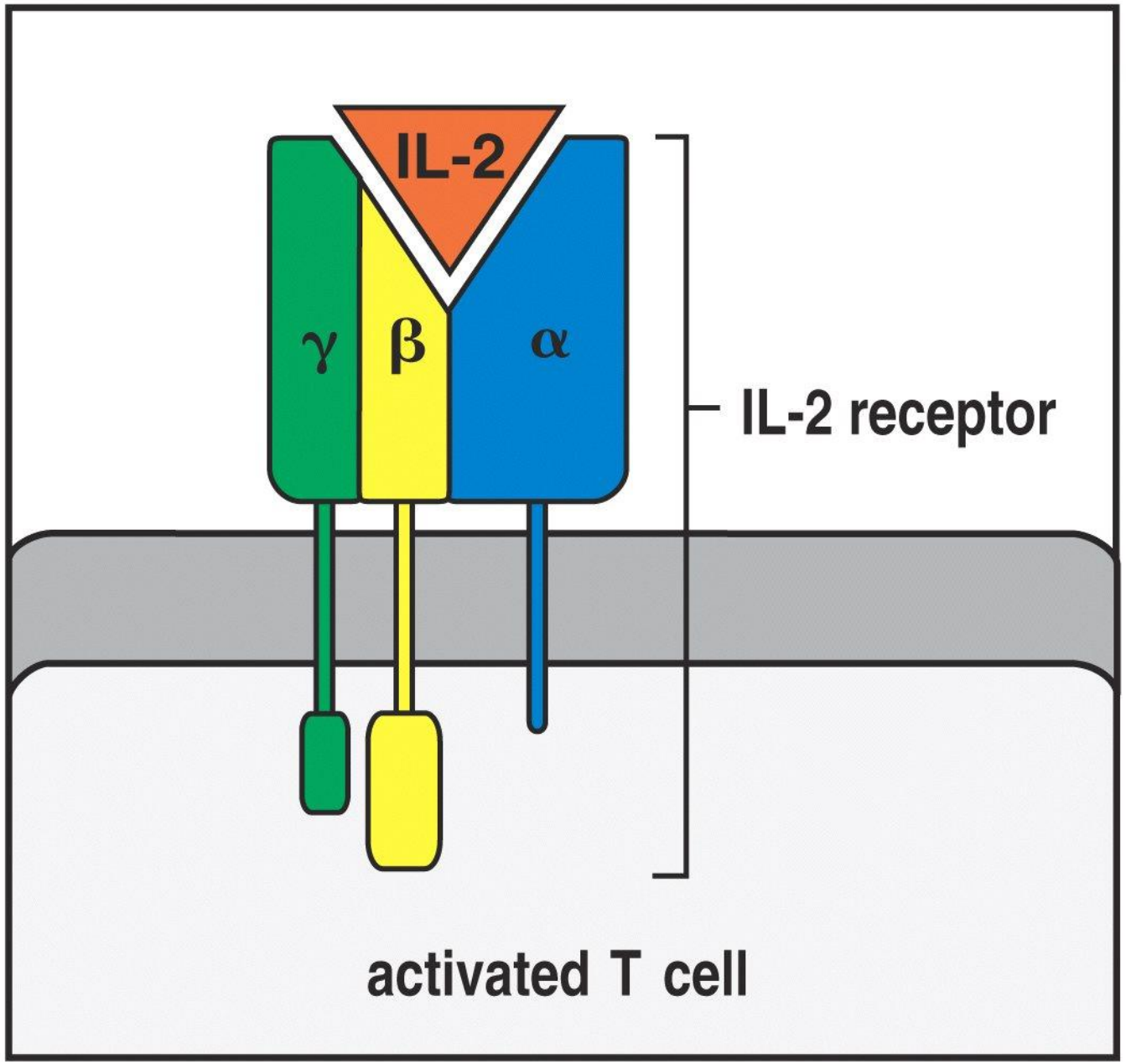


Figure 8-19 Immunobiology, 6/e. (© Garland Science 2005)

Selective Targeting Activated Immune and Inflammatory Cells

- Recently activated effector T cells and regulatory T cells express a trimolecular, high affinity receptor for IL-2 (Affinity = 10×10^{-11} M).

- Many innate immune cells (eosinophils, NK cells, macrophages, etc.) bear an intermediate affinity IL-2 receptor (Affinity = 10×10^{-9} M).

Tilt the balance of immunity!

- Unlike other immunosuppressives used in transplantation which block equally the activity of effector-type and and regulatory-type T cells, sirolimus (rapamycin) aids commitment of T cells to the regulatory tissue protective phenotype.

IL-2: A potent immunoregulatory molecule

- IL-2 or IL-2 related proteins (IL2.Ig) administered at **low dose** can promote peripheral tolerance to allografts in pre-clinical models.

- Low-dose IL-2 has been used safely for expansion of Tregs in patients hepatitis C induced vasculitis (Paris; NEJM 12/11) or corticosteroid resistant GVHD (HMS; NEJM 12/11) with therapeutic benefit

Shifting the balance of immunity toward tolerance

Collaborators

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