

# Amnion-Derived Multipotent Progenitor Cells Support Allograft Tolerance Induction

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# Introduction

## AMPs

- Amnion-derived multipotent progenitor cells (AMPs) are a subtype of hAECs.
- Human amnion epithelial cells (hAEC) are allogenic tolerated cells that suppress T-cell allogenic proliferation responses in MLR (mixed lymphocyte reaction) cultures.
- Hyporesponsiveness mediated via:
  - cell-to-cell contact
  - Soluble factors (PGE-2 and TGF- $\beta$ 1)
  - Inhibition of monocyte  $\rightarrow$  DC maturation

## Tregs

- Subpopulation of T-cells
- Modulating the immune system
- Treatment of autoimmune diseases, cancer and organ transplantation in mouse models
- CD4+ CD25+ Foxp3+

## Allo-MLR (mixed lymphocyte reaction) assay

- $5 \cdot 10^5$  **C57BL/6 splenocytes** with  $5 \cdot 10^5$  **BALB/c splenocytes**
- Complete culture medium for 5 days
- Pulsed with  $1,0 \mu\text{Ci}$  for 18 hours ( $[^3\text{H}]$ -thymidine)

## C57BL/6



- Common names: C57 Black, B6, B6J, Black 6
- C57BL/6J is the most widely used inbred
- Age related hearing loss
- Susceptible to diet-induced obesity, type 2 diabetes, and atherosclerosis.

## BALB/c

- Common names: c
- BALB/cJ is a commonly used inbred.
- Resistance to experimental autoimmune encephalomyelitis (EAE), and a susceptibility to developing the demyelinating disease upon infection with Theiler's murine encephalomyelitis virus.
- Susceptible to *Listeria*, all species of *Leishmania*, and several species of *Trypanosoma*.



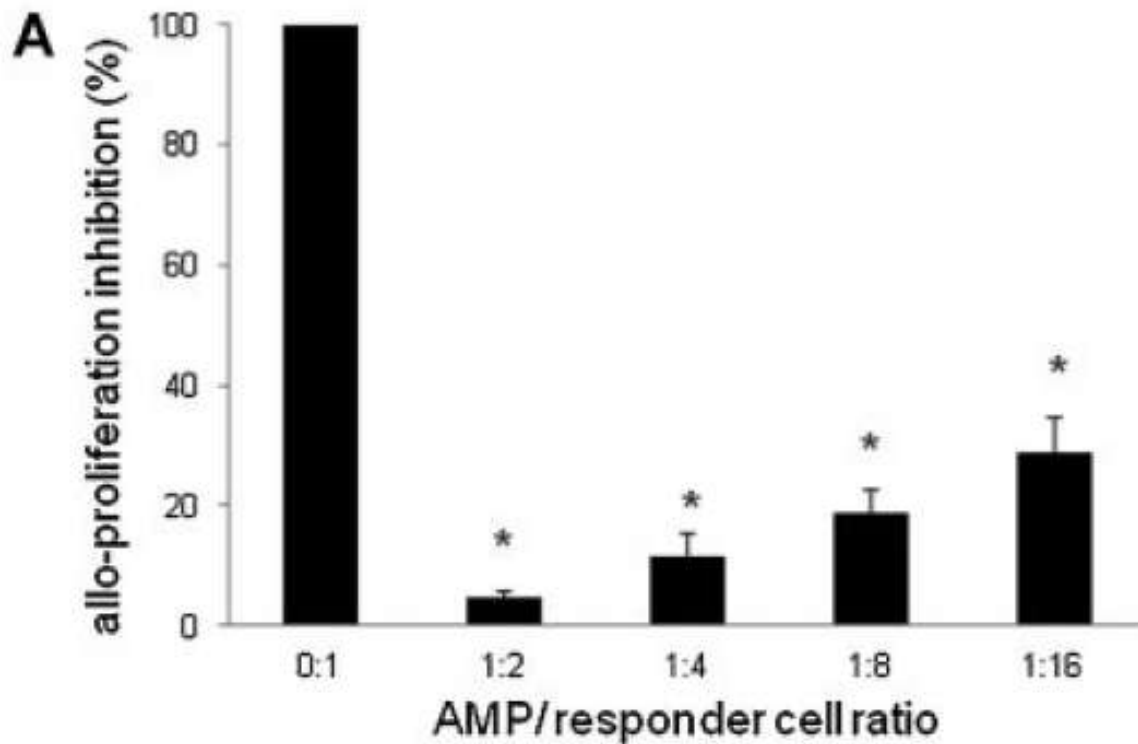
## C3H/HeJ



- Common names: C3, C3H Heston
- This strain is homozygous for retinal degeneration allele *Pde6b<sup>rd1</sup>*
- defective lipopolysaccharide response allele *Tlr4<sup>Lps-d</sup>*
- chromosomal inversion on Chromosome 6.

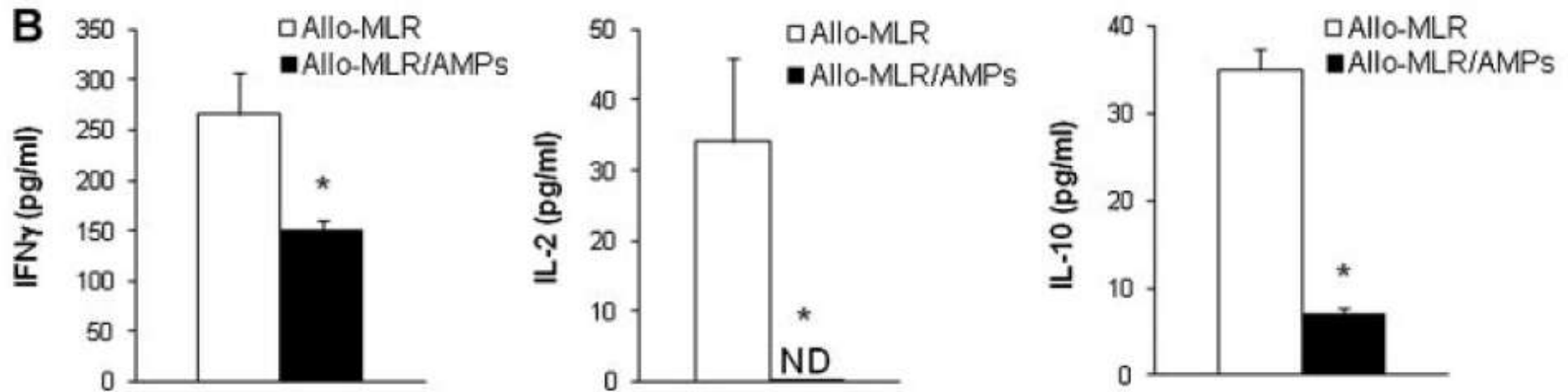


# Experiments

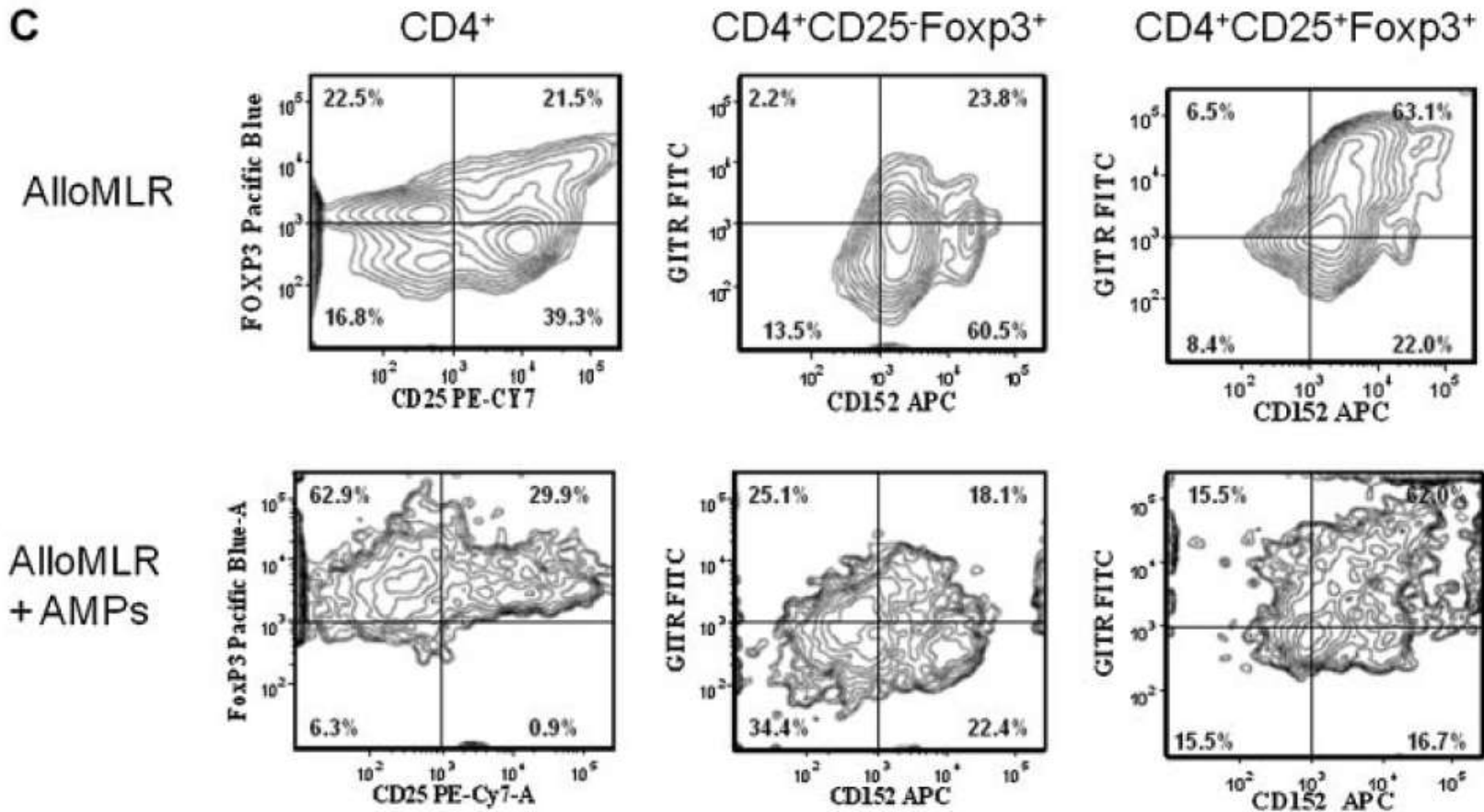


- Naive C57BL/6 splenocytes (responder) cultured 1:1 with irradiated naive BALB/c stimulatory cells.
- AMPS added at the onset of culture.
- Proliferation analyzed by  $^3\text{H}$ -thymidine incorporation during last 18h of 5day incorporation.

## Cytokine analysis of MLR culture supernatants after 48h.



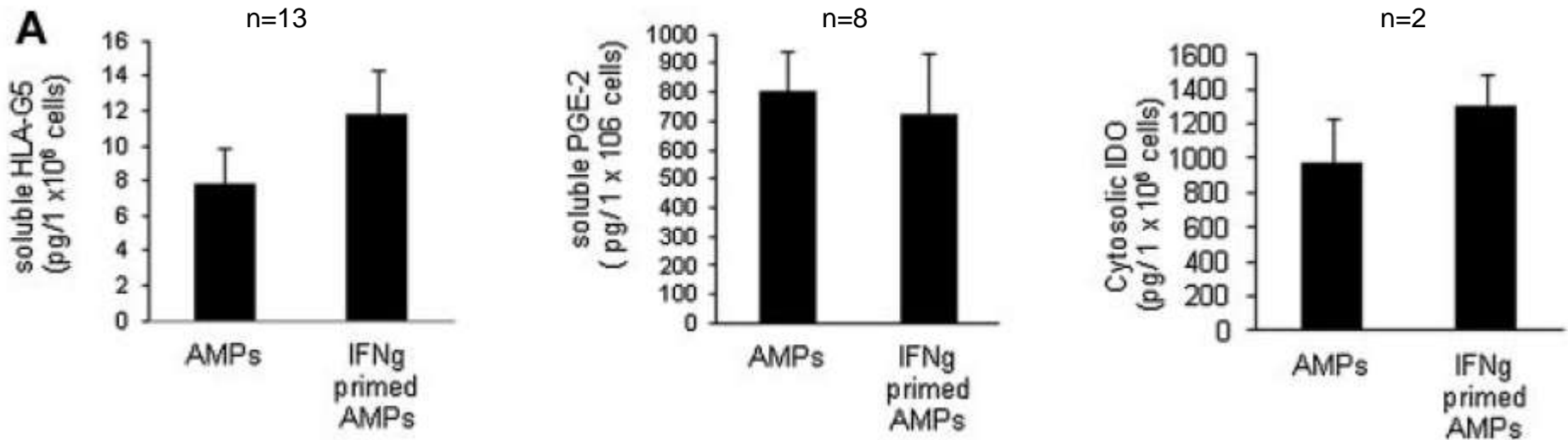
## Flow cytometric analysis of splenic Tregs after 48h.



GITR.. Glucocorticoid induced TNFR family related gene. Costimulant of t-cell activation

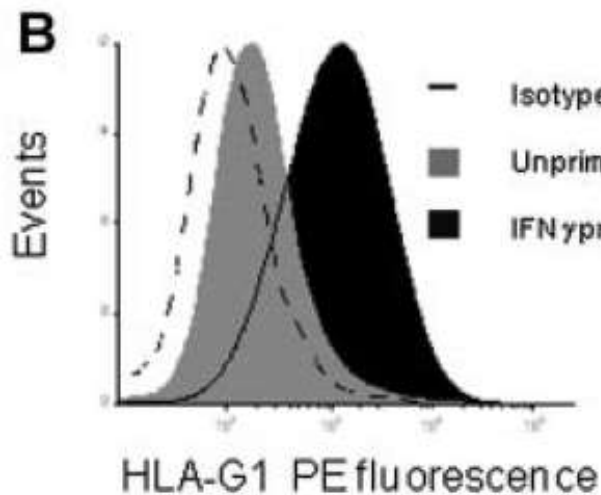
**AMPs inhibit alloantigen-dependent proliferation of T cells**

## Untreated and IFN $\gamma$ treated AMPS for 48h, measured by ELISA

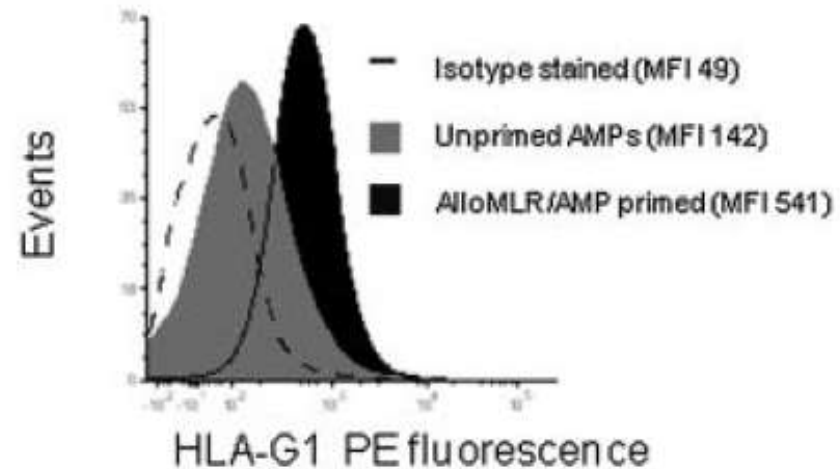


- sHLA-G5...soluble HLA-Gen 5
- sPGE-2... inhibits IFN $\gamma$  & IL-2 building
- IDO... Indolamin-2,3-Dioxygenase: decomposites tryptophan (needed for T-cell activation) and stimulates Treg recruitment

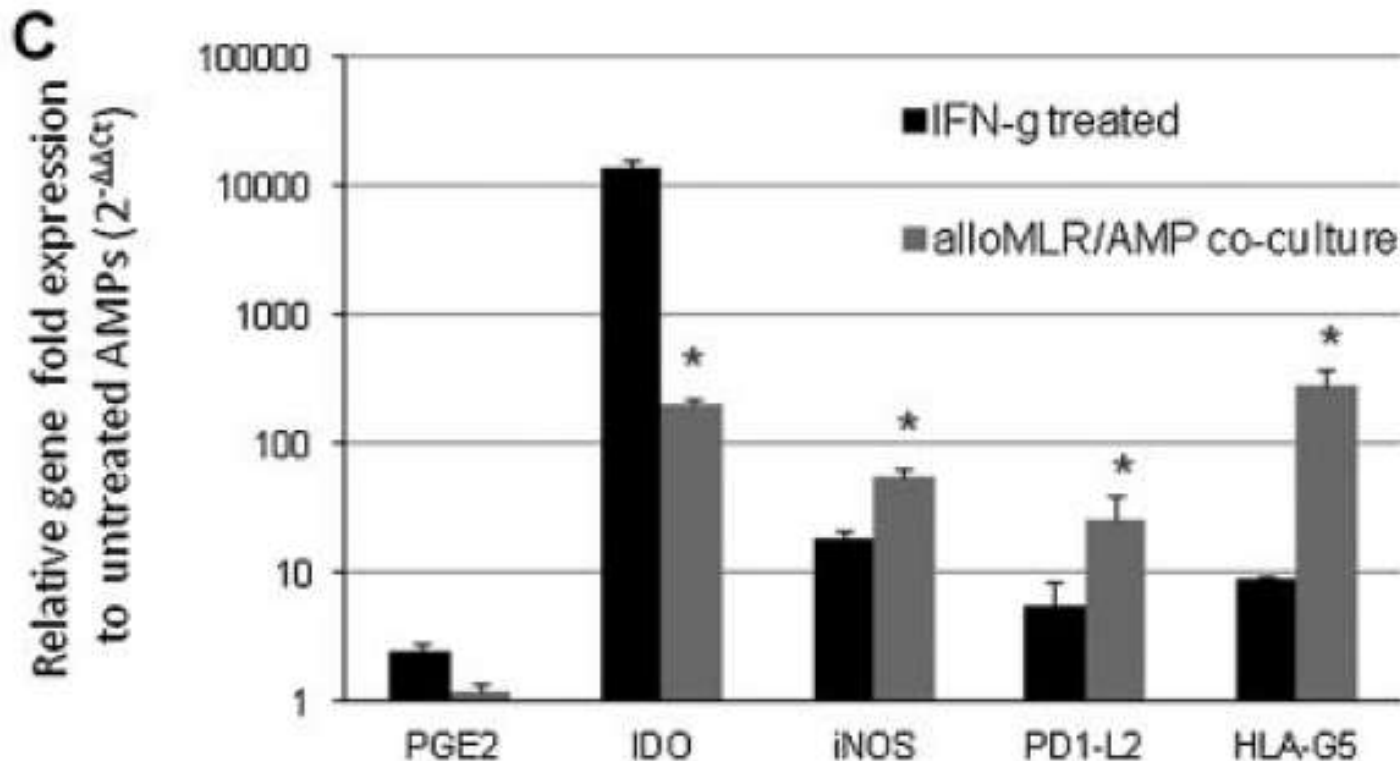
## Representative flow cytometry histograms after 48h of culture



n=5



## mRNA of immune modulating factors

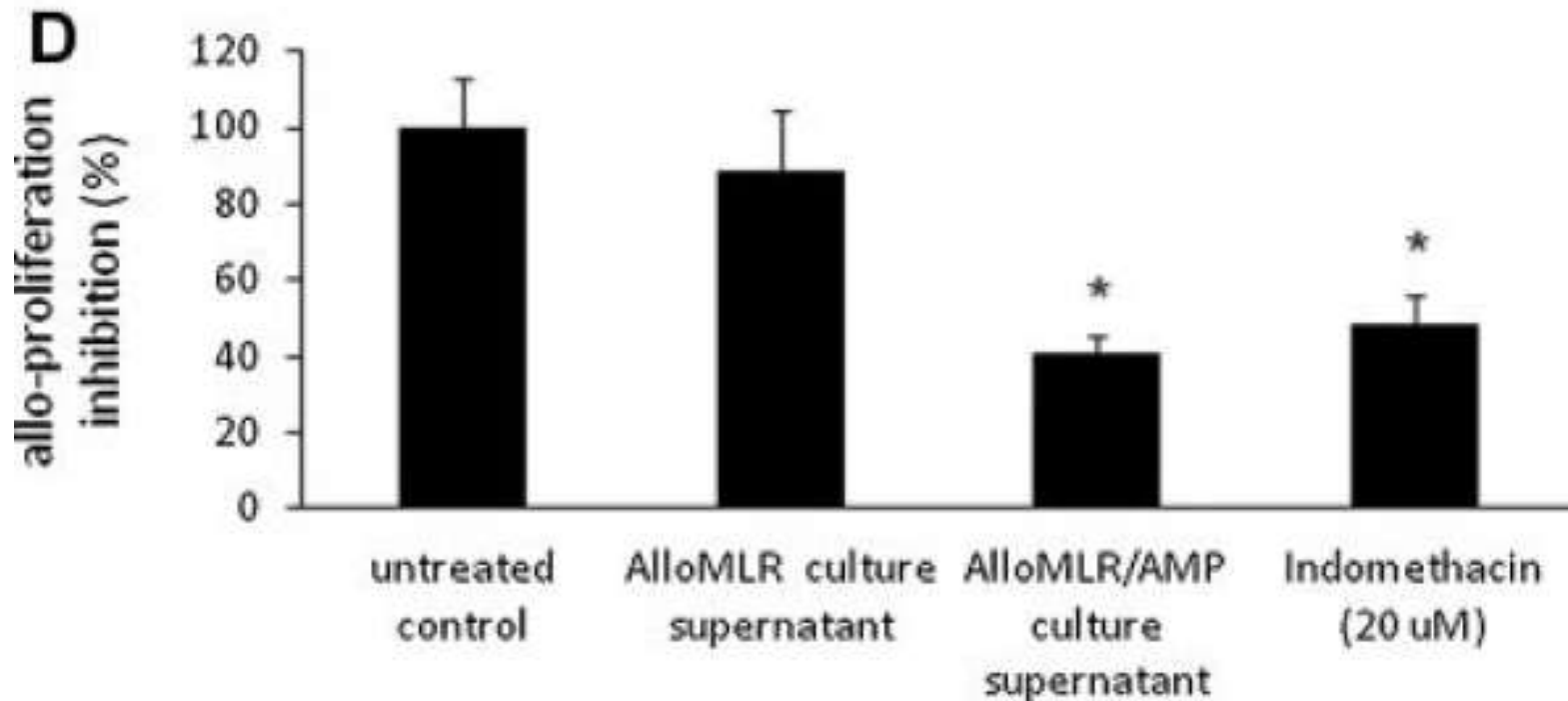


iNOS... produces cytotoxic doses of NO in macrophages

PD1-L2... Programmed cell death 1 – ligand 2, modulates T-cell activity



## Suppression of T-cell proliferation

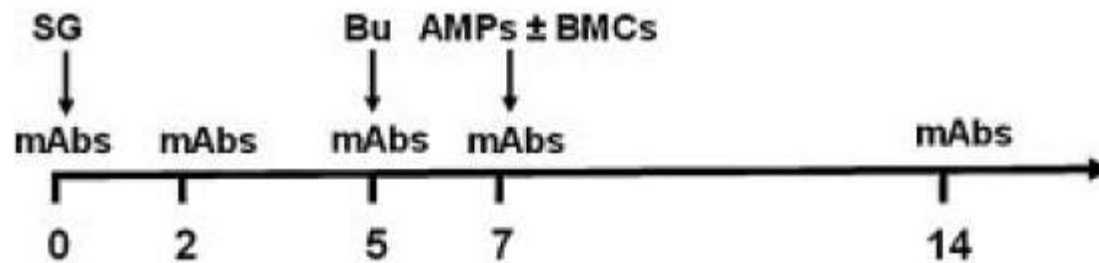


**Soluble factors from Allo-MLR/AMP culture supernatant  
suppress T-cell proliferation**

## Skin grafting

- Full thickness skin grafts 4cm<sup>2</sup>
- From BALB/c and C3H/HeJ mice onto C57BL/6 mice
- Adhesive bandage for 7 days

## Experimental protocol



mAbs = $\alpha$ CD4 + $\alpha$ CD8	Bu = 5 mg/kg
AMPs ( $5 \times 10^6$ )	BMCs ( $5 \times 10^5$ )

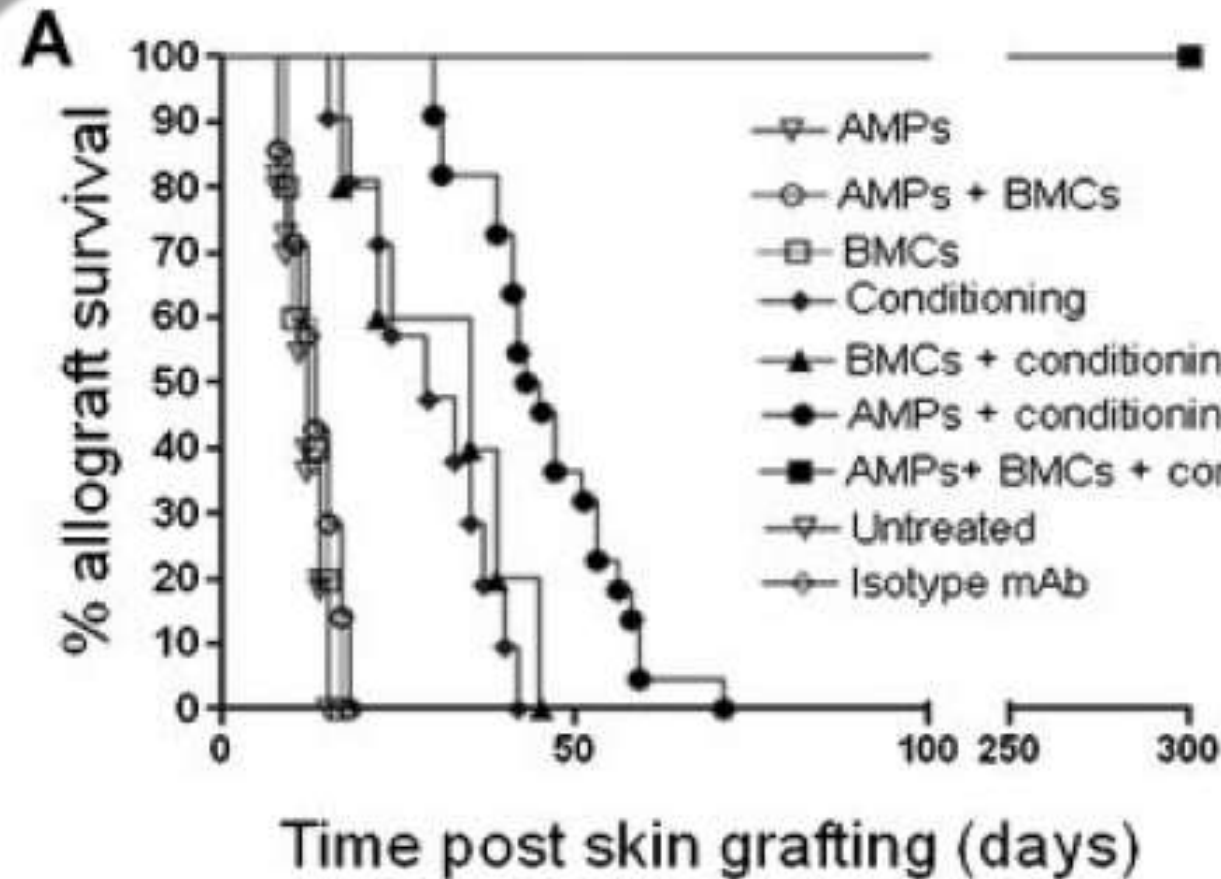
SG... skin grafting

mAbs...  $\alpha$ CD4 +  $\alpha$ CD8

Bu... Busulfan (5 mg/kg)

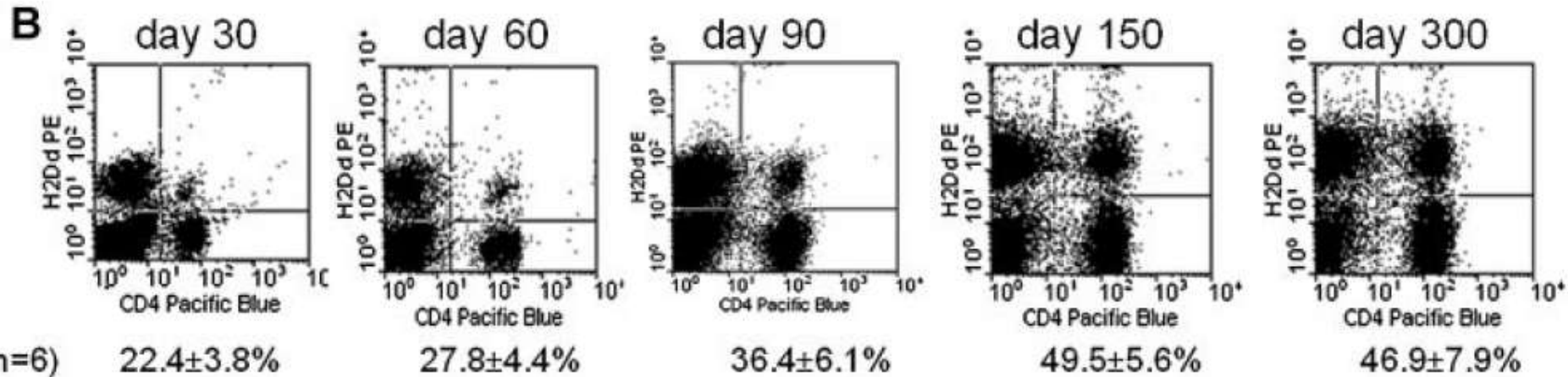
BMCs ...  $5 \times 10^5$  donor cells

AMPs...  $5 \times 10^6$  cells + heparin + DNase



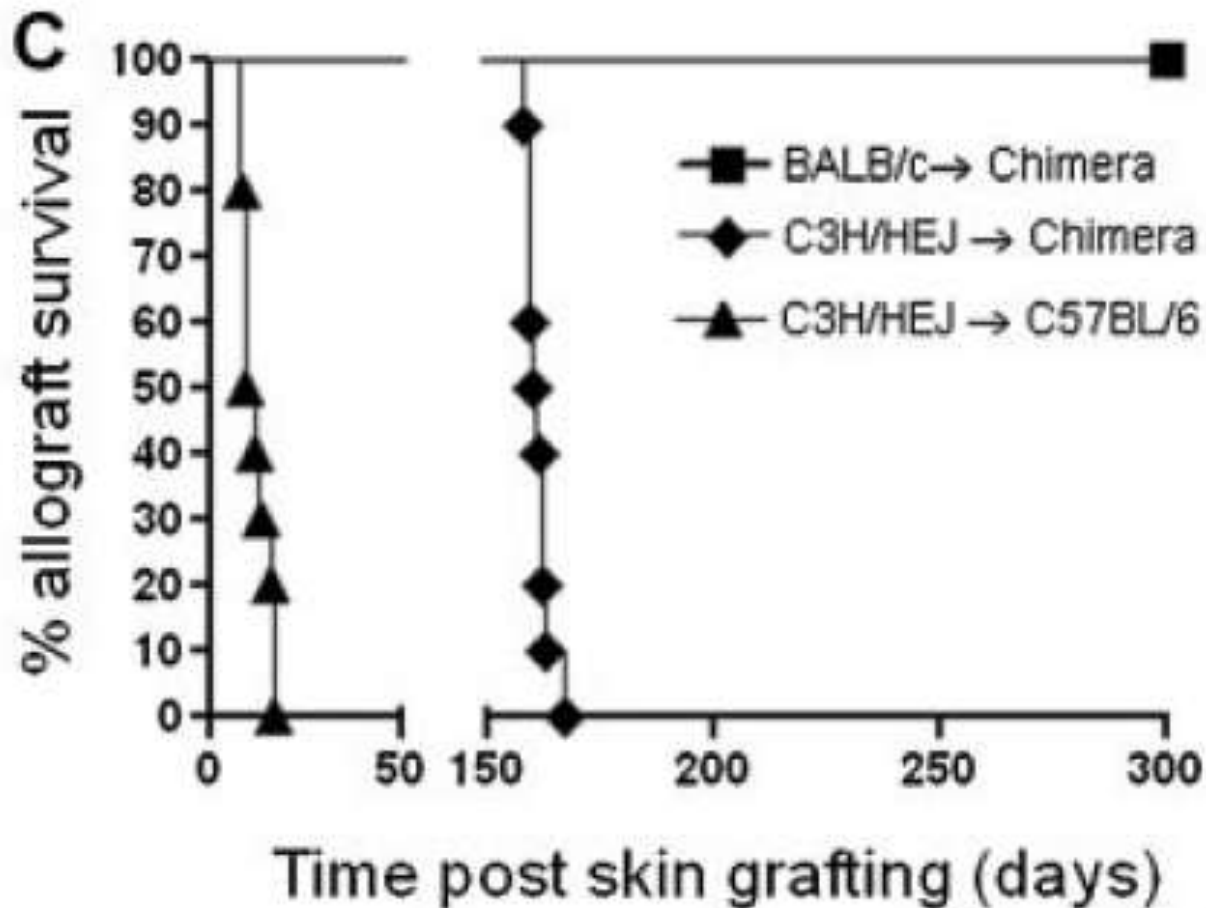
- (1) AMPs  
n=10
- (2) AMPs+BMCs  
n=14
- (3) BMCs  
n=10
- (4) cyto reduction conditioning  
n=22  
anti-CD4/CD8 mAb therapy  
plus 5 mg/kg busulfan
- (5) BMC conditioning  
n=10
- (6) AMPs + conditioning  
n=21
- (7) AMPs+BMC+conditioning  
n=13
- (8) Untreated  
n=16
- (9) isotype contro  
l n=12.

## Percentage of donor derived cells in chimeras



H-2d... donor cells (BALB/c)

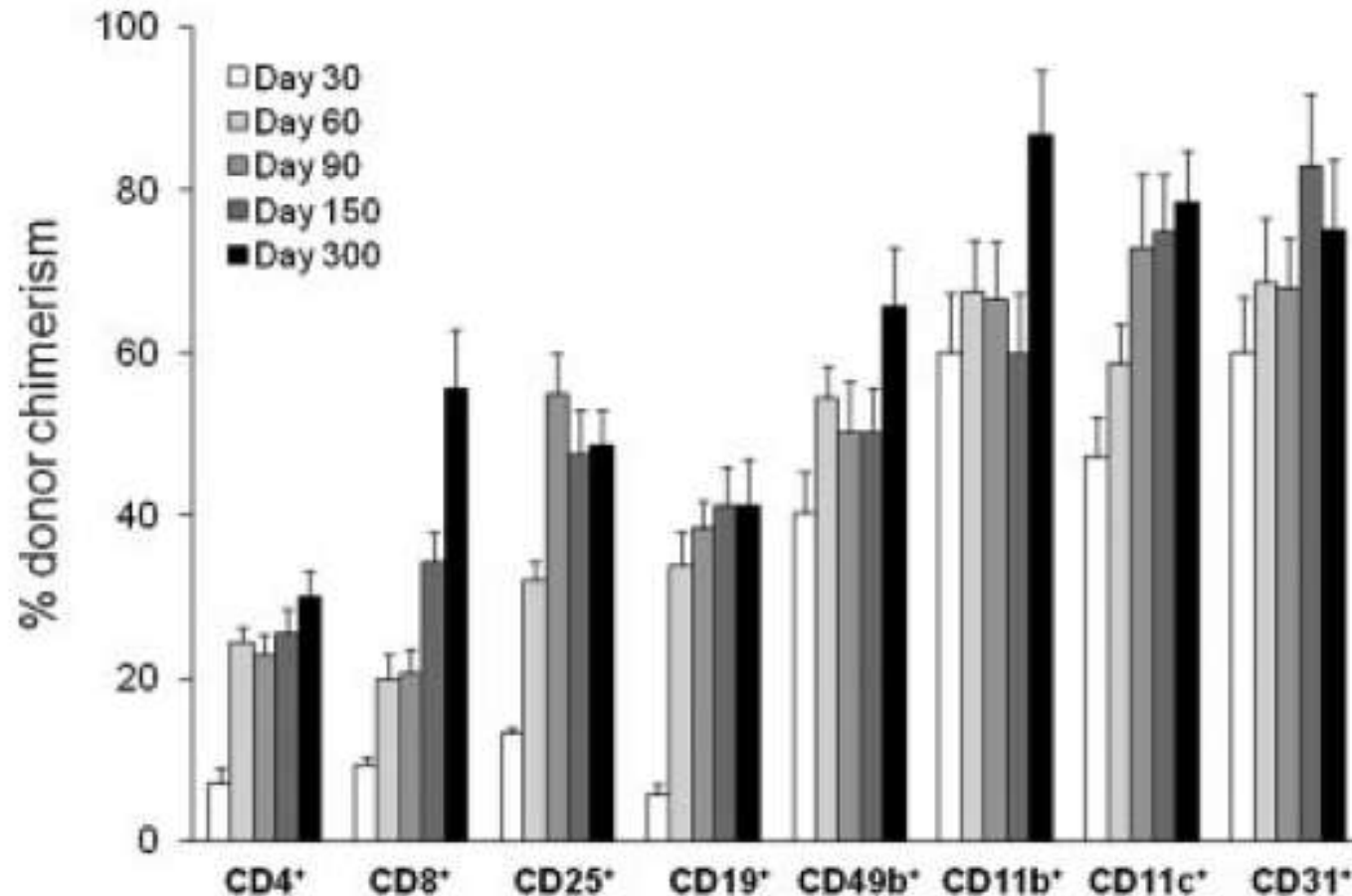
## Chimeras accept secondary donor-specific skin grafts but reject third-party grafts



**BMCs+AMPs induces indefinite skin allograft survival and mixed donor-recipient macrochimerism**



## Stable multilineage macrochimerism

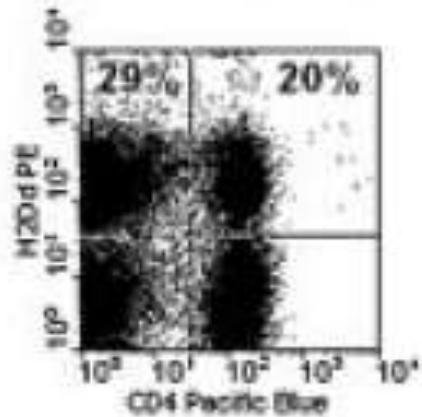


- **CD4:** Tcells, Monocytes, Macrophages
- **CD8:** CTC
- **CD25:** Tregs
- **CD19:** Bcells
- **CD49b:** NKTcells
- **CD11b:** monocytes, granulocytes, macrophages, NKC
- **CD11c:** DC
- **CD31:** platelets, monocytes, neutrophils

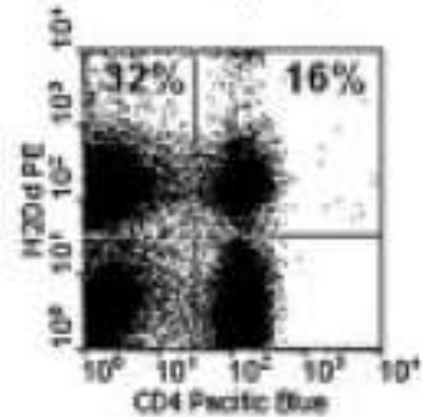
**Mice develop stable multilineage hematopoietic cell macrochimerism  
in the peripheral blood**

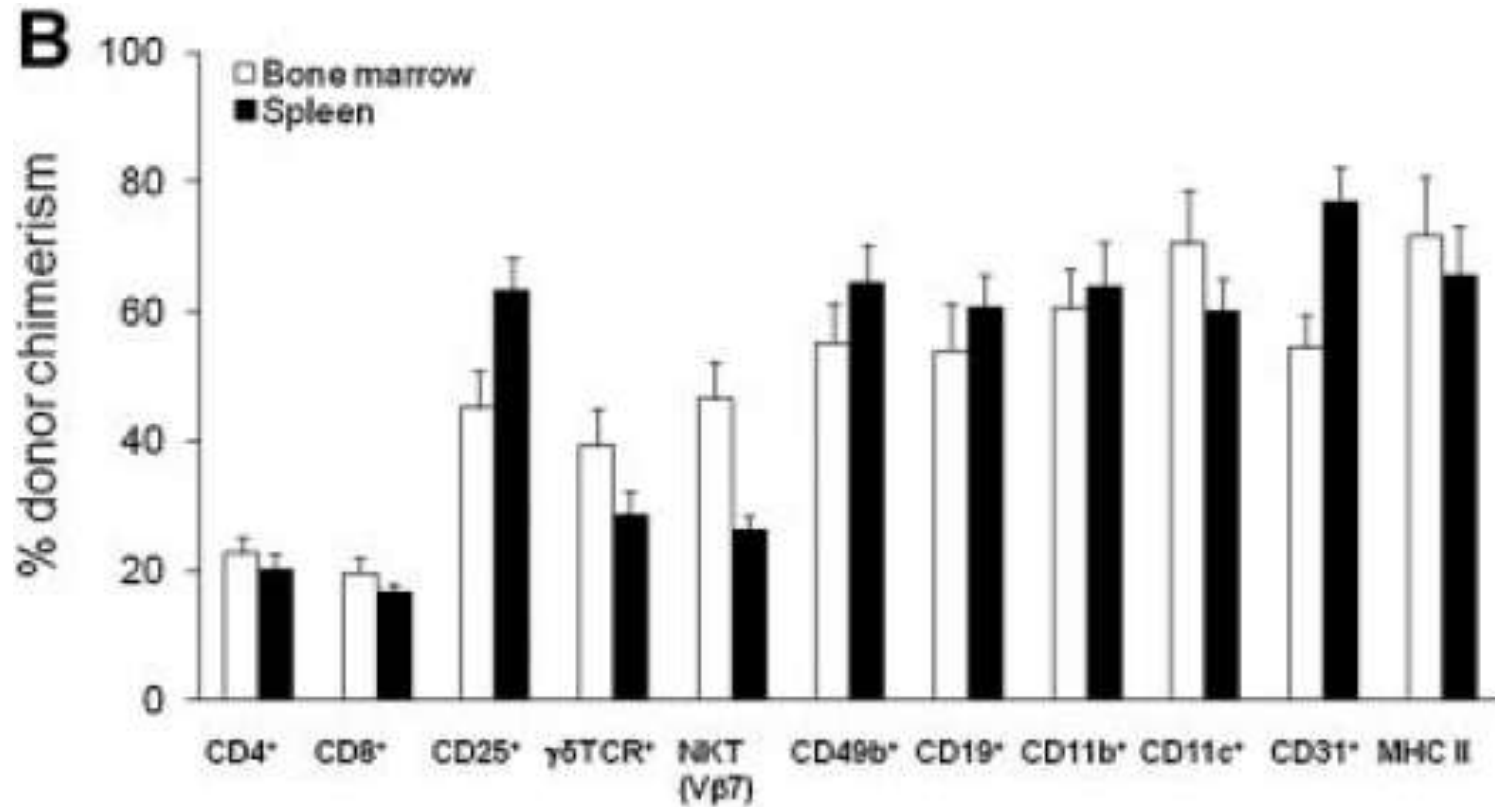
## Chimerism in the bone marrow and the spleen

**A** Bone Marrow  
45% ± 6 (n=6)



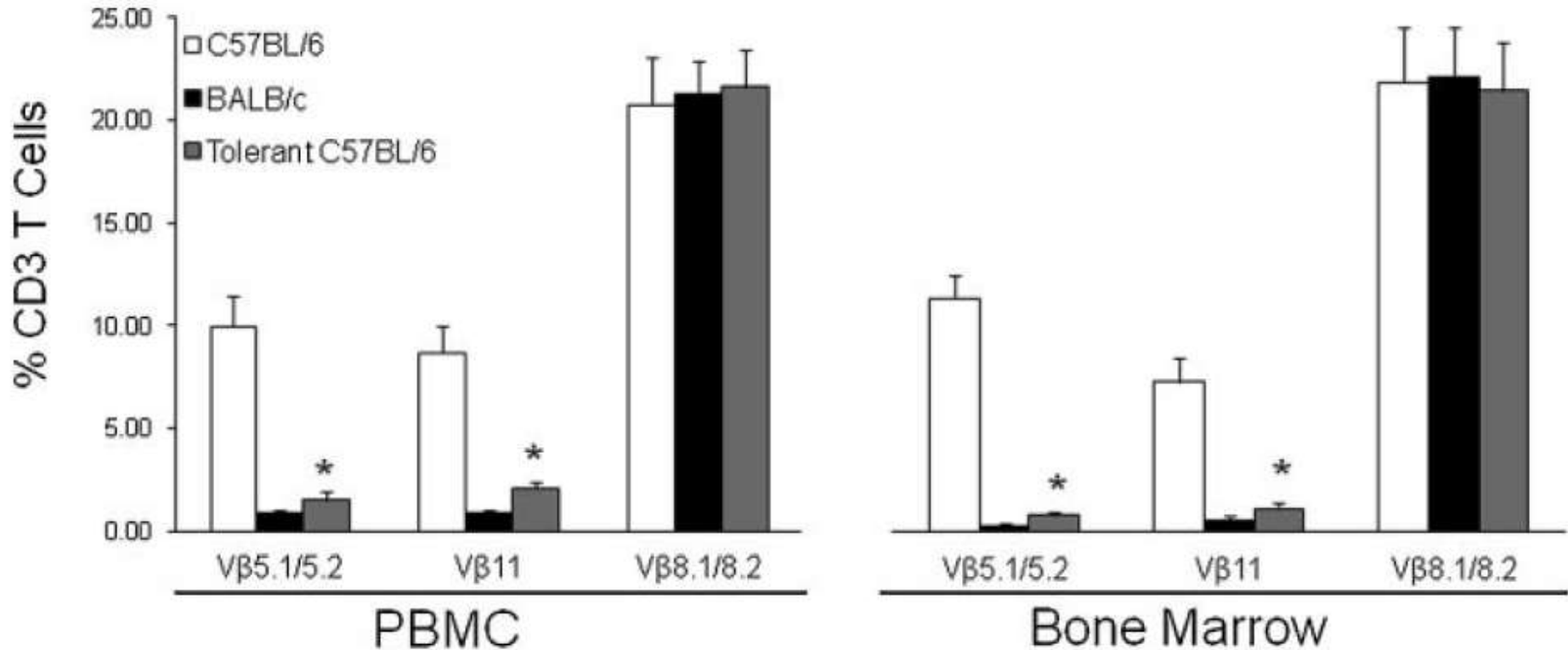
Spleen  
51% ± 6 (n=6)





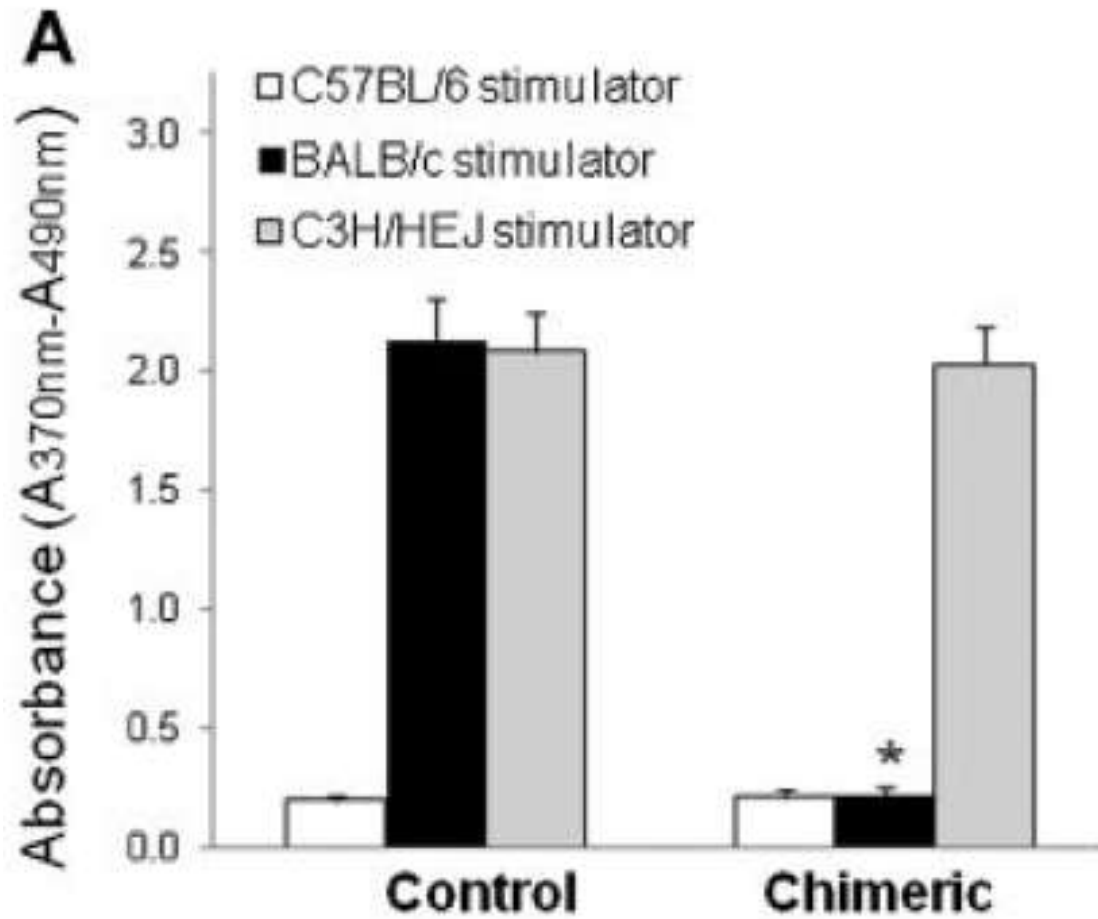
**Similar levels of chimerism among lymphoid and myeloid cell lineages**

## Differences of V $\beta$ expressions of CD3 T-cells

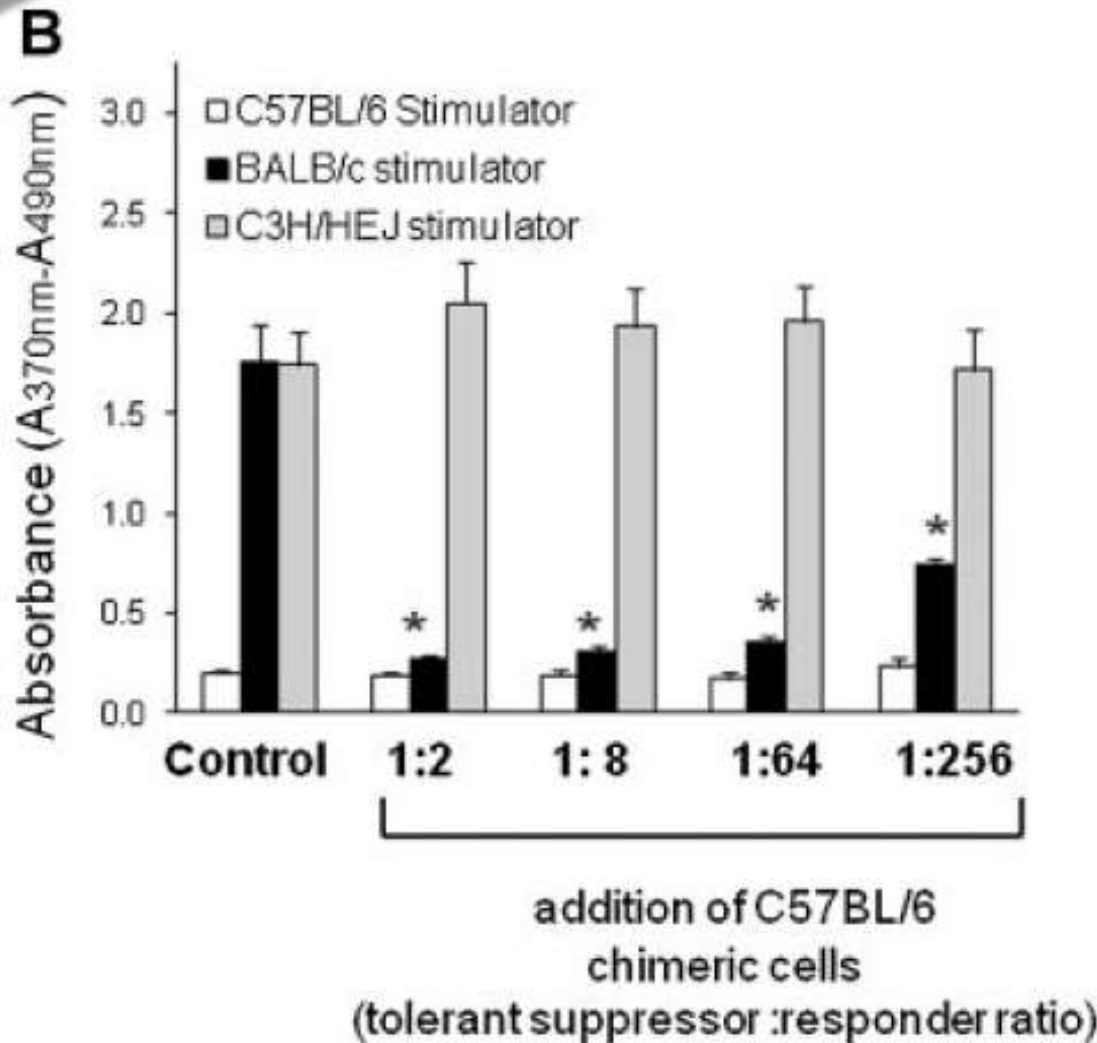


V $\beta$ ... TCR subtypes

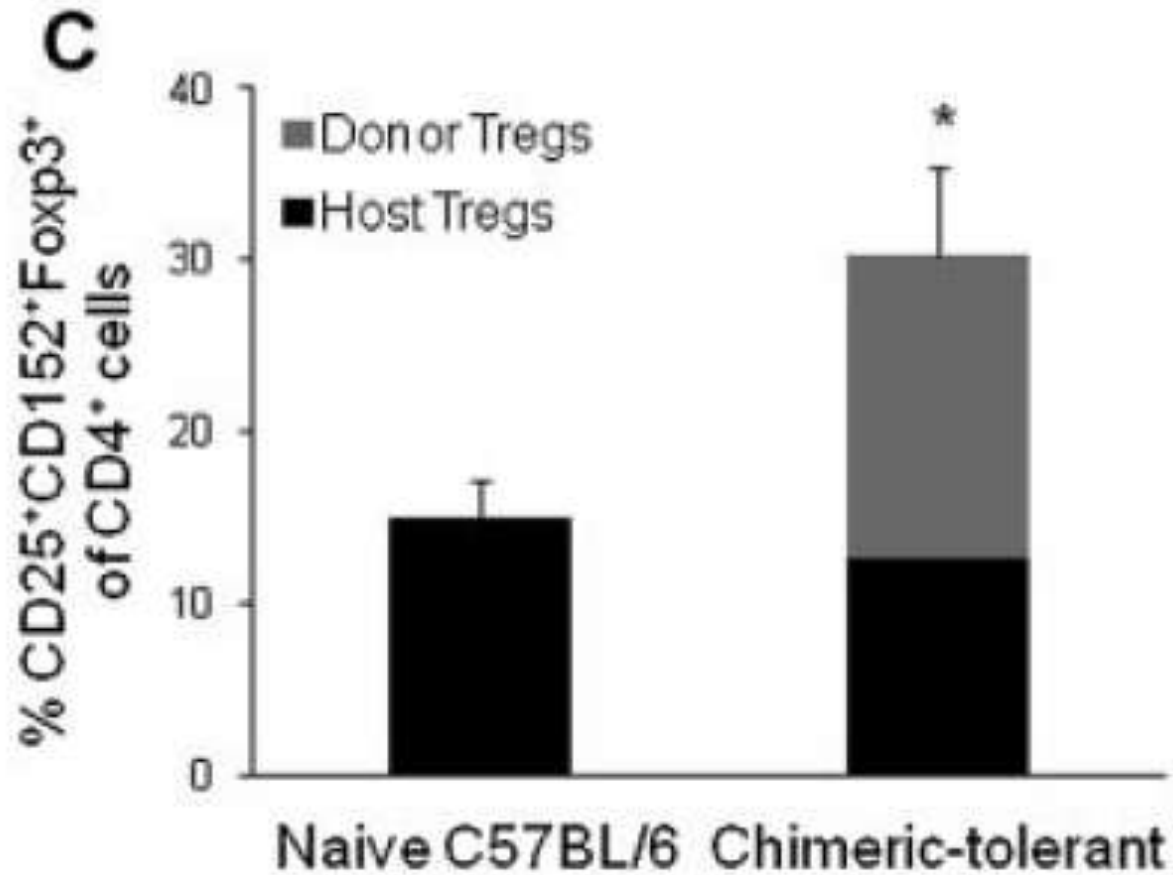
**AMP cell-mediated chimerism results in deletion of alloreactive T-cells, without any change of non-alloreactive Tcells**







- Naive C57BL/6 splenocytes, cocultured with irradiated naive C57BL/6 or BALB/c or C3H/HEJ splenocytes
- Tolerant C57BL/6 irradiated splenocytes as third-party regulatory T-cells



**Spleens of chimeric mice contain potent immunoregulatory  
suppressor cells**

## Conclusio

- AMPs in concert with immunological conditioning (anti-CD4/CD8 mAb therapy plus low-dose non-myeloblastive busulfan treatment), promotes the engraftment of donor BMCs across MHC barriers
- In the absence of BMCs, iv. administration of AMPs+conditioning led to a significant allograft survival
- BMCs alone failed to prolong allograft survival
- **AMPs may have pro-tolerogenic clinical efficacy for solid organ and stem cell transplant applications**

## Sources

- Immunologie 5.Auflage , Janeway, Spektrum Akademischer Verlag; 2002
- <http://www.ncbi.nlm.nih.gov/pubmed>
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**Thank you, for your attention!**