Purity and potency assay development

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Production Assistance for Cellular Therapies
National Heart Lung and Blood Program

Overview
Lessons from regulatory T cells

- Review of transplantation and regulatory T cells
- Mixed Lymphocyte Reaction (MLR) assay
- CFSE-based proliferation assay
- Lessons learned
  1. Choosing the right assay.
  2. Technical/Technician issues.

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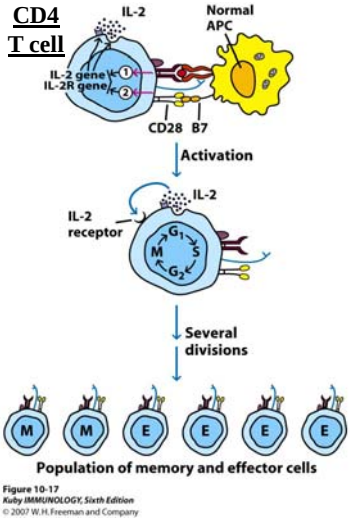
T cell activation



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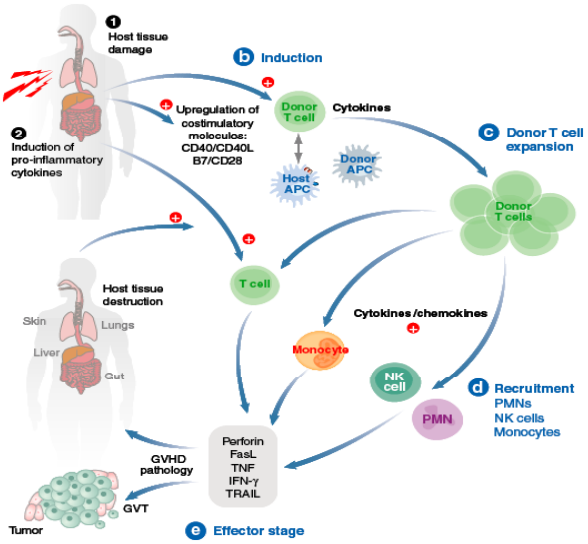
Graft-versus-host disease

- T cells in blood or marrow transplant cause GVHD
- T cells in blood or marrow also attack tumor (and suppress pathogens)
- T cell depleted blood or marrow has reduced engraftment and increased risk of infection.



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T cell activation in GVHD

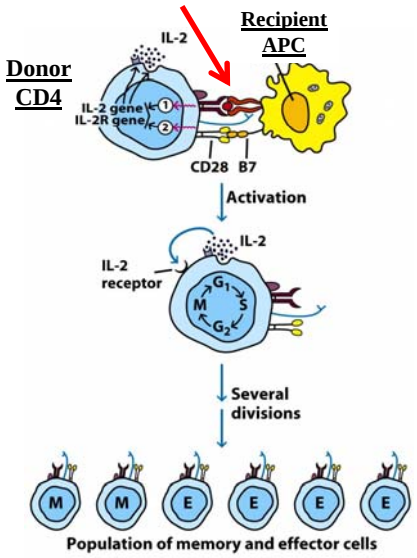

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Figure 10-17
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Treg suppress T cell activation

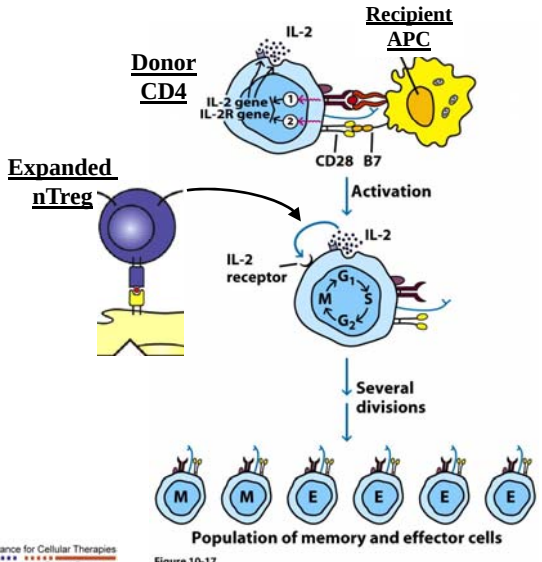

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Figure 10-17
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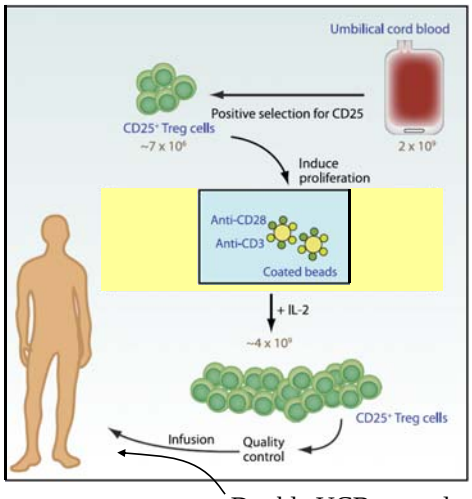
Schema for large scale production of nTreg from umbilical cord blood



Double UCB transplant

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MLR

i y e
x m a
e p c
d h t
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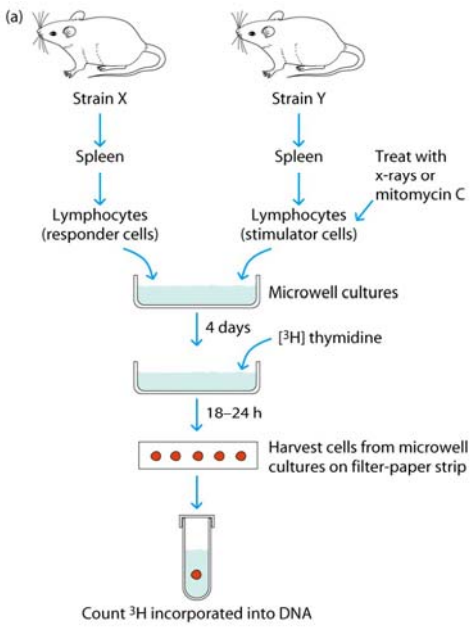
t
e

MLR = GVHD in a dish



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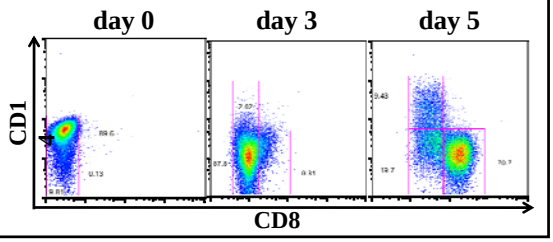
Large scale production of Stimulators for nTreg trial QC

- Stimulators (activated Macrophage DC, MoDC):
  1. Positively select immature cells using CD14 from apheresis unit (~10% or 300 million cells).
  2. Mature in vitro 5-7 days (requires IL-4, GM-CSF, and LPS; recovery ~30 million).
  3. Very hard to have fresh MoDC on hand, but cells freeze well.



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Large scale production of Responders for nTreg trial QC

- Responders (CD4+25- T cells):
  1. Negatively select CD25+ cells (deplete nTreg)
  2. Positively select CD4+ cells (~1x10^9 cells per apheresis unit).
  3. Freeze as per MoDC.
  4. Purified CD4 T cell recovery <25%

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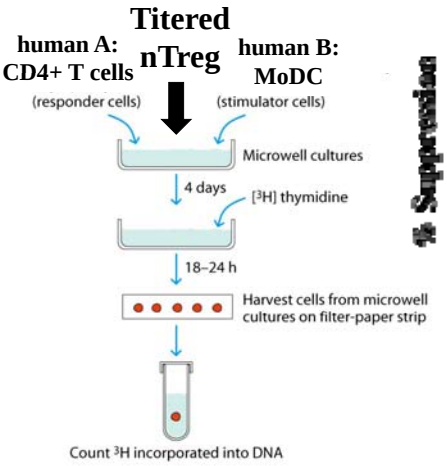
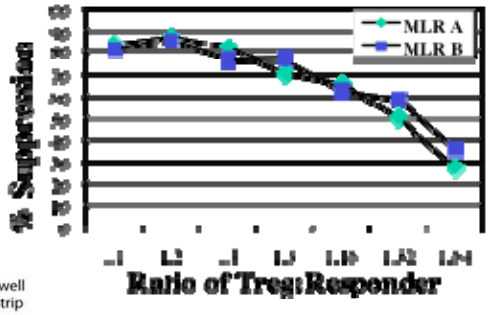
MLR assay for nTreg potency





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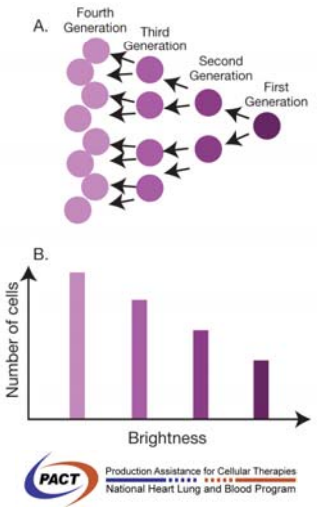
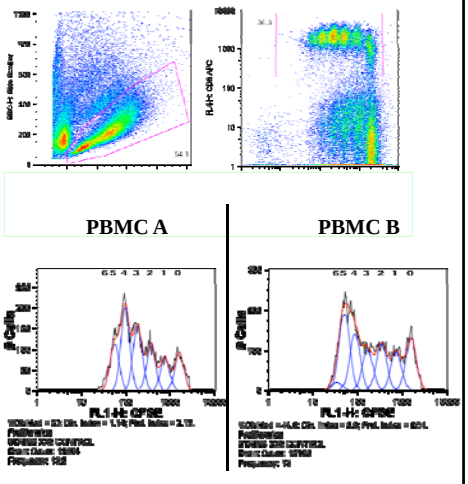
CFSE-based proliferation assay

Theoretical



Actual



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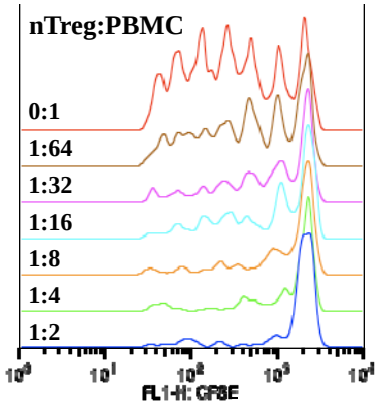
CFSE-based assay for nTreg potency



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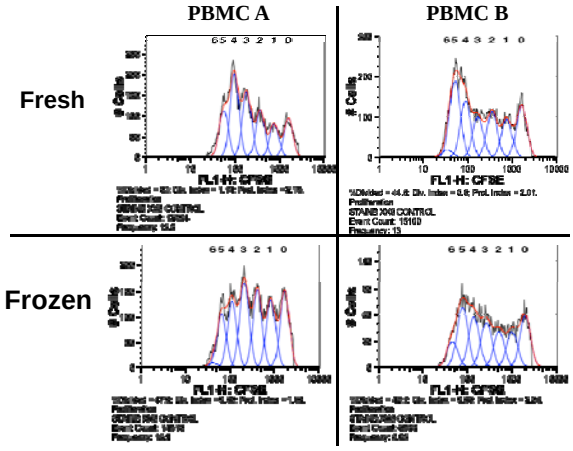
CFSE-labeled PBMC maintain proliferative capacity after freeze/thaw



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Lessons learned from Quality Control in production of regulatory T cells

1. How to choose the right assay:
   - Cost (per assay, who is paying!)
   - Fresh vs. Frozen
2. Technical/Technician issues:
   - Who does the assay
   - Timing (fresh, frozen, ASAP)

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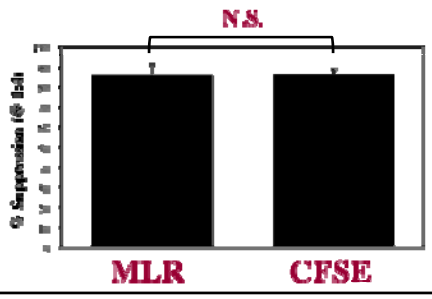
Choosing the right assay

MLR

- Benefits
  1. Mimics disease
  2. Amenable to high throughput.
- Drawbacks
  1. Cost.
  2. Radioactivity.
  3. Different Resp/Stim pairs were more or less reactive based on number of HLA mismatches).

CFSE

- Benefits
  1. Cost
- Drawbacks
  1. More labor intensive.
  2. Flow cytometry based.



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Who does the assay

1. Shipping
2. Timing
3. Backup

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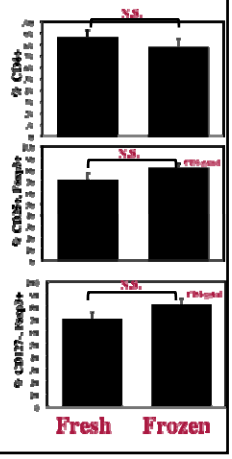
Timing (when to assay)

To overtime or not to overtime, that is the (HR) question

- Fresh: Functional assay (suppression)
- Frozen: Phenotype (FACS)
- ASAP: Critical product characteristics (Foxp3)



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