Two Strategies to Efficiently Purify Functional CD4+CD25+ Regulatory T Cells from Normal and Transgenic Mouse Strains

Cheryl D. Helgason[2], Lixin Xu[2], Zoe M. Raffard[2], Sharon A. Louis[1], Ana M. Lopez[1], Steven M. Woodside[1], and Terry E. Thomas[1]
[1]StemCell Technologies Inc, Vancouver, BC, Canada; [2]Cancer Endocrinology, British Columbia Cancer Agency, Vancouver, BC, Canada

Introduction

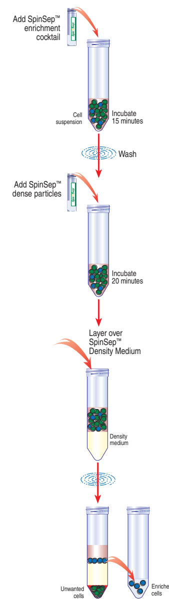
Disease progression in the LPB-Tag transgenic (Tg) mouse model of prostate cancer (Kasper et al, 1998) is accompanied by evidence of impaired T cell responses (Xu et al, manuscript submitted). Surprisingly, there is a correlative increase in the number of CD4+CD25+ T cells in the spleen and lymph nodes (LN) of the Tg mice. To address the possibility that regulatory T (Tr) cells within this population were responsible for the immunosuppression, we designed two strategies which combine negative (SpinSep™) and positive (EasySep™) separations to purify these cells. In the first strategy, CD4+ T cells were isolated using SpinSep™ negative selection, followed by CD25 immunomagnetic selection with EasySep™. In the second strategy, CD25+ T cells were positively selected with EasySep™ and then depleted of CD4- cells using SpinSep™. CD4+CD25+ T cells were enriched to similar purities using both strategies (80-89%). Both CD25+ and CD25- CD4+ T cell populations were recoverable using both strategies. The CD4+CD25- T cell population retained the capacity to respond to antigen stimulation, while the CD4+CD25+ T cell population was shown to contain functional Tr cells that inhibited the proliferation of the CD25- T cell population. These methods to purify CD4+CD25+ T cell populations containing functional Tr cells will facilitate further functional, phenotypic, and genomic characterization of this class of regulatory T cells and will ultimately be useful for studies of immunosuppression in mouse models.

Methods

Spleen and Lymph Node (LN) Cells

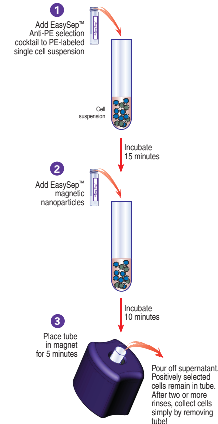
Spleen or lumbar LN cells were obtained from C57BL/61, as well as wild type (WT) or Tg CD1 mice and were suspended in PBS + 2% FBS at an appropriate cell density. Two strategies were used to purify CD4+CD25+ T cells. In the first strategy (**Strategy #1**), CD4+ T cells were isolated using standard SpinSep™ procedures (Figure 1) followed by CD25 immuno-magnetic selection with the standard EasySep™ procedure (Figure 2). In the second strategy (**Strategy #2**), CD25+ T cells were first purified with EasySep™ and then depleted of CD4- cells using SpinSep™. The purified cells from both strategies were analyzed by FACS and in cell proliferation assays. Cells were cultured at 1×10^5 cells/well in the presence or absence of anti-CD3 mAb (145-C11) at indicated concentrations. Proliferation was measured by incorporation of [^{3}H] TdR determined by liquid scintillation counting. Results are expressed as the mean ± SEM of triplicate determinations in each experiment.

Figure 1. SpinSep™ Depletion of CD4- Cells



Unwanted cells (including red blood cells) are linked to antibody coated dense particles via primary monoclonal antibodies that recognize cell surface antigens on these cells. CD4+ T cells were enriched using a cocktail of monoclonal antibodies specific for CD8a, CD45R, CD11b, GR-1 and TER119. Unwanted cells with the dense particles are pelleted in a density gradient centrifugation.

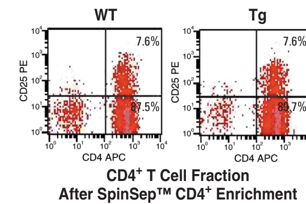
Figure 2. EasySep™ Positive Selection of CD25+ Cells



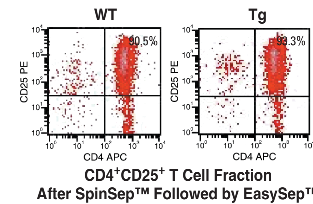
Cells are magnetically labeled with PE-conjugated anti-CD25 antibody. Antibody labeled cells are cross linked to the EasySep™ magnetic dextran coated particles using tetrameric antibody complexes which recognize PE and dextran. The separation occurs in a tube, no columns are necessary and the magnetic particles do not interfere with FACS analyses.

Results

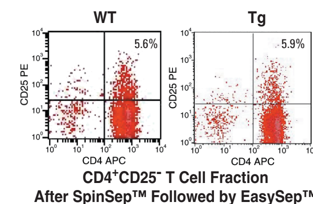
Figure 3. FACS Profiles Showing Purities of CD4+CD25+ and CD4+CD25- Populations After Enrichment with Strategy #1 (SpinSep™ → EasySep™)



CD4+ T Cell Fraction
After SpinSep™ CD4+ Enrichment

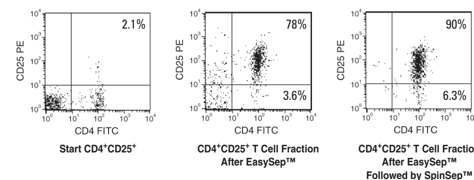


CD4+CD25+ T Cell Fraction
After SpinSep™ Followed by EasySep™



CD4+CD25- T Cell Fraction
After SpinSep™ Followed by EasySep™

Figure 4. FACS Profiles Showing Purities of CD4+CD25+ and CD4+CD25- Populations After Enrichment with Strategy #2 (EasySep™ → SpinSep™)



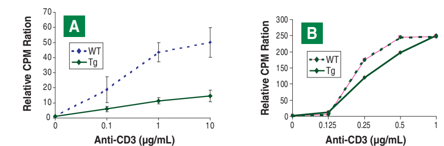
Start CD4+CD25+ | CD4+CD25+ T Cell Fraction After EasySep™ | CD4+CD25+ T Cell Fraction After EasySep™ Followed by SpinSep™

Table 1. Purity of CD4+CD25+ T Cells from C57BL/61 or WT CD1 and Tg CD1 Mouse Strains Using the Combined EasySep™ and SpinSep™ Strategies

	Strategy 1 SpinSep™ → EasySep™		Strategy 2 EasySep™ → SpinSep™	
Strain	n	% Purity[1]	n	% Purity[1]
C57BL/61	5	80 ± 6	5	87 ± 2
CD1 WT	4	89 ± 2	ND	ND
CD1 Tg	5	84 ± 8	ND	ND

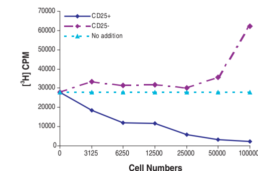
[1]Purities determined by flow cytometry; ND=not determined

Figure 5. Removal of the CD4+CD25+ T Cell Population Restores Tg T Cell Proliferation to WT Levels



A. Proliferation of WT (dotted line) and Tg (solid line) T cells, isolated from spleen and LN of mice >20 weeks of age, was determined by incorporation of [^{3}H] TdR.
B. CD4+CD25+ T cells were depleted using Strategy #1 above and the proliferation of the CD4+CD25- population was determined by incorporation of [^{3}H] TdR.

Figure 6. Inhibition of T Cell Proliferation by CD4+CD25+ Cells



Single cell suspensions of a spleen (10^5 cells per well) were stimulated with 10 μg/mL anti-CD3 antibody with the addition of the indicated numbers of CD4+CD25+ (solid line) or CD4+CD25- (dotted - dashed line) T cells. Proliferation was determined by incorporation of [^{3}H] TdR. The dotted line represents the baseline proliferation in the absence of any additional cells.

Conclusions

- CD4+CD25+ T cells can be enriched from spleen and/or LN of WT and Tg mice using SpinSep™ depletion of CD4- cells and EasySep™ CD25 positive selection in either order.
- **High Purity.** Purities close to 90% can be routinely achieved.
- Enriched CD4+CD25+ T cells inhibit T cell proliferation suggesting that the functional capacity of Tr cells is retained.
- Isolated CD4+CD25+ T cells retain their capacity to respond to antigenic stimulation.

StemCell Technologies
777 West Broadway, Suite 808
Vancouver • BC • Canada • V5Z 4J7
Tel: 604.877.0713 • Fax: 604.877.0704
Email: info@stemcell.com
www.stemcell.com