ISOLATION AND EXPANSION OF MOUSE CD4⁺CD25⁺ REGULATORY T CELLS USING DYNABEADS® MAGNETIC SEPARATION TECHNOLOGY

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BACKGROUND: **Regulatory CD4⁺CD25⁺ T cells** are a specialized subpopulation of T cells that act to maintain homeostasis within the immune system. Recent advances in the characterization of this cell population have firmly established their existence and their critical role in regulating the immune response. Interest in regulatory T cells has been accelerated by evidence from experimental mouse and human models demonstrating that the immunosuppressive potential of these cells can be utilized in the treatment of various diseases such as autoimmunity, infectious diseases and cancer.

Materials and methods: Start material: Secondary lymphoid organs (spleen, lymph nodes) from mice. Isolation of effector and regulatory T cells: CD25⁺ regulatory T cells were isolated using the Dynabeads® FlowComp™ Mouse CD4⁺CD25⁺ Treg Cells. This kit allows the isolation of both T effector (CD4⁺, CD25⁻) and T regulatory (CD4⁺, CD25⁺) populations (Fig. 1). Expansion of the CD25⁺ regulatory T cell population: Dynabeads® Mouse CD3/CD28 T Cell Expander was added to 1x10⁶ cells/ml for 12 days (2 beads/cell). The cultures were supplemented with 1000U/ml of IL-2 (Fig. 2). Suppression Assays: CD4⁺CD25⁻ effector T cells were stained with CFSE and mixed with CD4⁺CD25⁺ regulatory T cells. Dynabeads® coated with anti-mouse CD3 (3 beads/cell) were added to activate the effector CD25⁻ T cells and the suppression was measured 4 days later using CFSE staining.

The Three Step Isolation Procedure

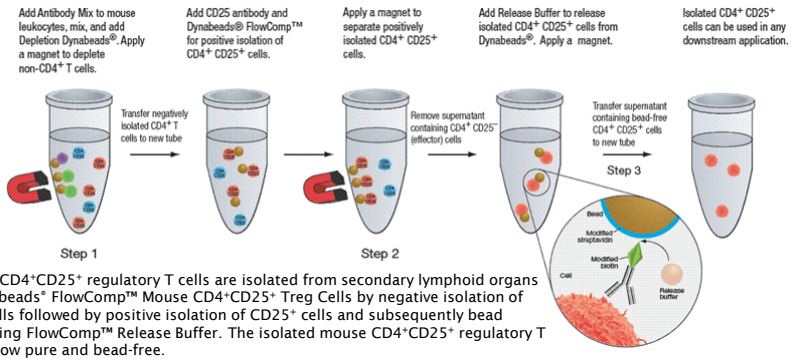


Figure 1. CD4⁺CD25⁺ regulatory T cells are isolated from secondary lymphoid organs with Dynabeads® FlowComp™ Mouse CD4⁺CD25⁺ Treg Cells by negative isolation of CD4⁺ T cells followed by positive isolation of CD25⁺ cells and subsequently bead release using FlowComp™ Release Buffer. The isolated mouse CD4⁺CD25⁺ regulatory T cells are now pure and bead-free.

Expansion of Treg Cells

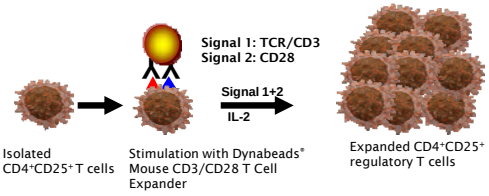


Figure 2. Expansion of mouse CD4⁺CD25⁺ Treg cells using Dynabeads® Mouse CD3/CD28 T Cell Expander.

AIM: Develop isolation and expansion protocols for mouse CD4⁺CD25⁺ regulatory T cells with characteristic phenotype and suppressive capacity.

Results: Highly pure (≥90%) regulatory CD4⁺CD25⁺ T cells were isolated from mouse secondary lymphoid organs using **Dynabeads® FlowComp™ Mouse CD4⁺CD25⁺ Treg Cells** (Fig. 3). A large majority of these cells expressed the transcription factor Foxp3 (avg. ≥ 88%, Fig. 4 and 6). Comparison of the Dynabeads® technology with a column-based isolation technology revealed that the tube-based isolation strategy from Invitrogen Dynal resulted in a significantly higher number of CD25⁺ T cells as well as Foxp3+ cells (Fig 7). Treg cells isolated with Dynabeads® FlowComp™ Mouse CD4⁺CD25⁺ Treg Cells suppressed the proliferation of CD4⁺CD25⁻ effector T cells in the presence of CD3 activation (Fig 5), showing that the isolated regulatory T cells retain their normal function. Low number of regulatory T cells can be a road block for scientists to perform functional and/or adoptive cell transfer experiments. As shown in Fig 8 Treg cells expanded using the Dynabeads® Mouse CD3/CD28 T Cell Expander retain Foxp3 expression.

Presence and percentage of CD4⁺ CD25⁺ cells during the three step isolation process

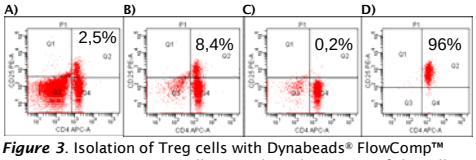


Figure 3. Isolation of Treg cells with Dynabeads® FlowComp™ Mouse CD4⁺CD25⁺ Treg Cells **A)** In the spleen, 2,5% of the cells are CD4⁺CD25⁺. **B)** After negative isolation of CD4⁺ cells, 8,4% of the CD4⁺ cells express CD25. **C)** After positive isolation of CD25⁺ cells, 0,2% of the low expressing CD25⁺ cells remain in the CD4⁺CD25⁻ fraction. **D)** The isolated CD4⁺CD25⁺ cells are 96% pure.

Purity of CD4⁺CD25⁺Foxp3⁺ cells

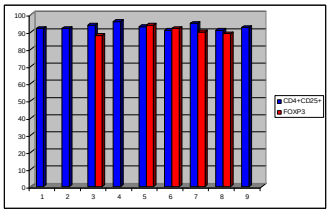
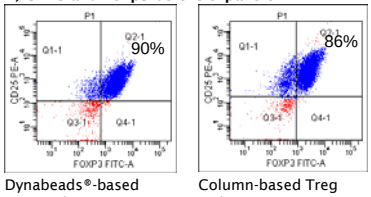


Figure 6. Treg cells were isolated from mice in 10 different experiments using Dynabeads® FlowComp™ Mouse CD4⁺CD25⁺ Treg Cells, and analyzed for purity as identified by expression of CD25 (n=10) and Foxp3 (n=5).

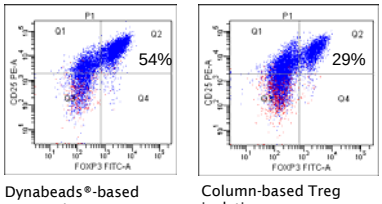
Expansion of isolated regulatory T cells

A) CD25 and Foxp3 before expansion



Dynabeads®-based Treg isolation | Column-based Treg isolation

B) CD25 and Foxp3 after expansion



Dynabeads®-based Treg isolation | Column-based Treg isolation

Figure 8. Purity of Treg cells isolated with Dynabeads® FlowComp™ Mouse CD4⁺CD25⁺ Treg Cells and a column-based method before **(A)** and after 10 days of expansion with Dynabeads® Mouse CD3/CD28 T Cell Expander **(B),** as measured by CD25 and Foxp3 expression.

Treg phenotype after isolation

A) CD25⁺FOXP3⁺ **B) CD25⁺FR4⁺** **C) Foxp3**

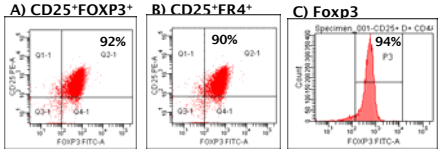


Figure 4. Isolation of Treg cells with Dynabeads® FlowComp™ Mouse CD4⁺CD25⁺ Treg Cells **A)** The isolated Treg cells are 92% pure. **B)** Within the CD4⁺ CD25⁺ Treg cell population, 90% express Foxp3 and FR4. **C)** In total, 94% of the CD4⁺CD25⁺ Treg cells express Foxp3.

Higher number of Foxp3⁺ T cells after isolation using Dynabeads® compared to a column-based method

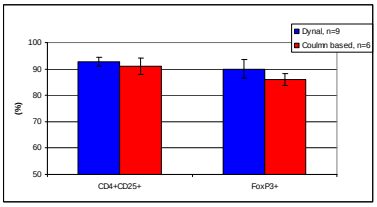


Figure 7. Treg cells were isolated from mouse secondary lymphoid organs using Dynabeads® FlowComp™ Mouse CD4⁺CD25⁺ Treg Cells or a column-based method. Purity was assessed by flow cytometric analysis with anti-CD25 and anti-Foxp3 antibodies.

Suppressive capacity of isolated Treg cells

A) CD4⁺CD25⁻ **B) CD4⁺CD25⁻ : CD4⁺CD25⁺**

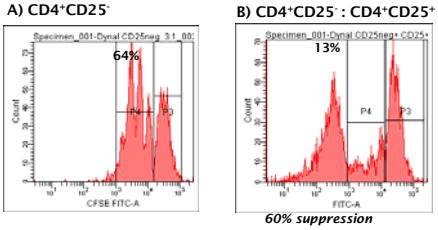


Figure 5. **A)** CD4⁺CD25⁻ cells were CFSE stained and stimulated with Dynabeads® coated with anti-mouse CD3 (3 beads:cell) for 4 days. On day 4, 64% of the cells were dividing as identified by flow cytometry **B)** CD4⁺CD25⁻ cells stained with CFSE were stimulated with Dynabeads® anti-mouse CD3 in the presence of CD4⁺CD25⁺ Treg cells in a 1:1 ratio. After 4 days, only 13 % of the CD4⁺CD25⁻ cells were dividing and 60% suppression of cell division was achieved in the presence of CD4⁺CD25⁺ Treg cells.

CONCLUSIONS

- **Dynabeads® FlowComp™ Mouse CD4⁺CD25⁺ Treg Cells** can be used to isolate ≥90% pure CD4⁺CD25⁺ T cells; more than 88% of the isolated CD25⁺ cells express the transcription factor Foxp3.
- Dynabeads® Mouse CD3/CD28 T Cell Expander expand mouse CD4⁺CD25⁺ regulatory T cells up to 10-fold during 10-12 days of culture while retaining their functional phenotype (Foxp3).
- Such expansion will facilitate further characterization of Treg cells as well as the evaluation of their potential in clinical applications by their use in *in vivo* transfer protocols.

Ordering information	Cat.no	Application
Dynabeads® FlowComp™ Mouse CD4⁺CD25⁺ Treg Cells	113.63D	Positive isolation of mouse Treg cells (bead-free)
Dynabeads® Mouse CD3/CD28 T Cell Expander	114.52D/53D	Expansion of mouse T and Treg cells
CD25, Rat Anti-Mouse (Alexa Fluor® 488)	RM6020	Flow cytometry staining antibody for CD25
CD4, Rat Anti-Mouse, (R-PE)	MCD0404	Flow cytometry staining antibody for CD4
CellTrace™ CFSE Cell Proliferation Kit	C34554	Cell proliferation kit for flow cytometry