

CD137 for Isolation and Expansion of Ag-specific T cells using Dynabeads®

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Aim

Develop a protocol for isolation of expandable, viable and functionally intact antigen-specific CD8⁺ T cells

Background

CD137 (4-1-BB), a member of the TNFR-family, functions as a costimulatory molecule promoting proliferation and survival of activated T cells. CD137 identifies recently activated human CD8⁺ and CD4⁺ T cell and represent a promising approach for the isolation and sustained expansion of viable antigen-specific T cells.

Materials and Methods

Dynabeads® FlowComp™ technology isolates activated antigen-specific T cells by use of an agonistic anti-CD137 antibody conjugated to a modified biotin and nitrated streptavidin coated Dynabeads®. The modified biotin and nitrated streptavidin facilitates a gentle release mechanism and the procedure enables isolation of bead-free antigen-specific T cells. For further expansion of the isolated cells, Dynabeads® Human T-Activator which are magnetic beads conjugated with agonistic antibodies specific for CD3, CD28 and CD137 were used.

Results

In a virus model, PBMC from HLA-A2⁺ donors were stimulated with CMV-peptide for 24 h. Activated CD137 positive cells were isolated by use of FlowComp™ technology using antagonistic anti-CD137 antibodies to enrich the antigen-specific CD8⁺ T cells. The separated CD137⁺ T cells were further expanded *ex vivo* and after 8 days 50-85% of the cells expressed the antigen-specific TCR as detected with CMV-specific pentamer staining (Fig 1). These cells were tested in killing assays and demonstrated to express CD107a, secrete IFN- γ and to be cytotoxic when co-cultured with target cells (Fig 2). For expansion of the antigen-specific T cells, Dynabeads® Human T-Activator CD3/CD28/CD137 were used (Fig 3). The results demonstrate that CD137-ligation was critical to sustain expansion of viable antigen-specific central memory T cells and the phenotype indicate a central memory phenotype (Fig 4).

Discussion

Dynabeads® FlowComp™ allows for specific isolation of anti-viral CD8⁺ and CD4⁺ T cells directly from PBMC in an easy-to-use *in vitro* procedure. Isolated and expanded antigen-specific cells maintain their specificity and function and can be further expanded using Dynabeads conjugated with anti-CD3, anti-CD28 and anti-CD137 antibodies. The use of Dynabeads® represent an efficient way to isolate and expand highly pure and viable antigen specific T cells for further characterization or use in adoptive immunotherapy.

Ordering info

Dynabeads®	Cat.no
Human T-Activator CD3/CD28/CD137	111.62D
Mouse T-Activator CD3/CD28/CD137	114.54D
FlowComp™ Flexi	110.61D
DynaMag™-15	123.01D

Isolation of CMV-specific CD8⁺ T cells using Dynabeads®

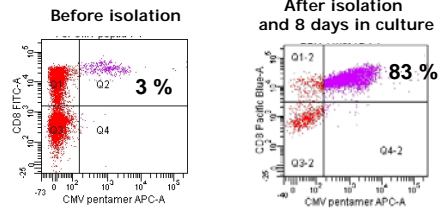


Fig 1: MNC from a HLA-A2⁺ donor was stimulated for 24 hours with CMV-peptide prior to isolation of activated CD137⁺ CMV-specific CD8⁺ T cells using Dynabeads® FlowComp™ Flexi. Before isolation 3 % of the CD8⁺ T cells were stained with CMV pentamer. After isolation and culture for 8 days, the percentage of CD8⁺ CMV⁺ cells was increased to 83 % as analyzed by FACS.

Functionally active CMV-specific CD8⁺ T cells after isolation of CD137⁺ cells using Dynabeads®

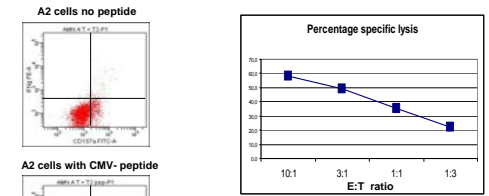


Fig 2: CMV-specific CD8⁺ T cell, isolated using Dynabeads® FlowComp™ Flexi and CD137 antibodies were grown in culture for 8 days prior to restimulation with peptide-pulsed A2 cells. After 4 hours cells were analyzed for CD107a expression and INF- γ secretion (left) and specific lysis (above).

CMV-specific T cell lines retain antigen-specificity after *in vitro* expansion with Dynabeads® T-Activator CD3/CD28/CD137

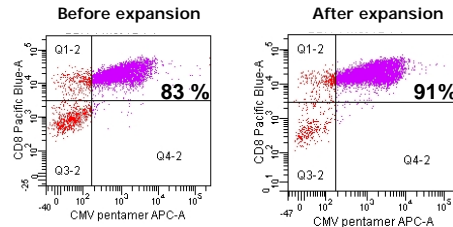


Fig 3: T cell lines (CMV-specific) were expanded *in vitro* using Dynabeads® Human T-Activator CD3/CD28/CD137 for 8 days at bead to cell ratio 1:10 in the presence of IL-2 and IL-7.

T cells expanded using Dynabeads® Human T-Activator CD3/CD28/CD137 express surface markers associated with T central memory cells

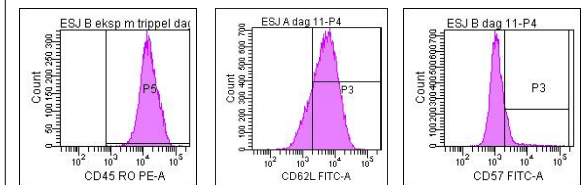


Fig 4: T cell lines expanded *in vitro* for 8 days with Human T-Activator CD3/CD28/CD137 express CD45RO, CD62L and were negative for CD57 indicating a central memory phenotype.

CONCLUSIONS

- Dynabeads® FlowComp™ technology allows for isolation of antigen-specific T cells
- Isolated and expanded T cells exhibit expected functions, such as killing of target cells, CD107a expression and INF- γ secretion
- Antigen-specific T cells expanded with Dynabeads® Human T-Activator CD3/CD28/CD137 retain the percentage of antigen-specific CD8⁺ T cells
- T cell lines expanded with Dynabeads® Human T-Activator CD3/CD28/CD137 express markers associated with central memory phenotype