Single-step isolation of highly purified, untouched human CD4$^+$ memory T cells by column-free immunomagnetic cell separation

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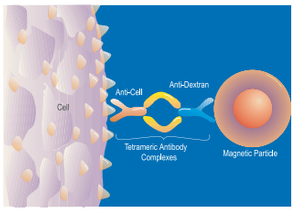
Summary

Memory T lymphocytes are a heterogeneous group of cells that enable the host to mount quick and effective immune responses against previously encountered antigens. Recent evidence suggests that they may also contribute to the onset of graft-versus-host disease during allogeneic bone marrow transplantation. Better understanding the various roles of memory T cells will require the ability to work with highly purified cell fractions. Most currently used memory T cell isolation protocols are cumbersome, requiring multiple steps often including FACS sorting. In this study, we developed a rapid and efficient method for the isolation of highly purified human memory CD4$^+$ T cells from peripheral blood mononuclear cells using column-free immunomagnetic negative cell selection. We also optimized the method for use with the RoboSep® fully automated cell separator. Both the manual and fully automated separation procedures took less than 30 minutes and provided average CD4$^+$ memory T cell purities above 90% combined with average recoveries above 60%. Naïve CD4$^+$ T cells accounted for less than 0.1% of total purified cells. Polyclonal antigenic stimulation of isolated CD4$^+$ memory T cell fractions using CD3 and CD28 coated beads lead to robust proliferation responses, as determined by CFSE dilution using flow cytometry. Furthermore, whereas antigen recall responses to tetanus toxoid were detectable in the purified memory CD4$^+$ T cell fractions from two donors, no response was detected in purified naïve CD4$^+$ T cell fractions from the same donors. This confirmed that the isolated cells retained memory function. We report here the successful development of a new one-step method for isolating highly purified and functional memory CD4$^+$ T cells.

Methods

Figure 1a. Schematic drawing of EasySep® magnetic labeling of human cells



TAC are comprised of two mouse IgG_1 monoclonal antibodies held in tetrameric array by two rat anti-mouse IgG_1 monoclonal antibody molecules. One mouse antibody recognizes the specific cell surface antigen while the other recognizes dextran on the EasySep® magnetic particle.

Figure 1b. EasySep® procedure for column-free enrichment of human CD4$^+$ memory T cells by negative selection

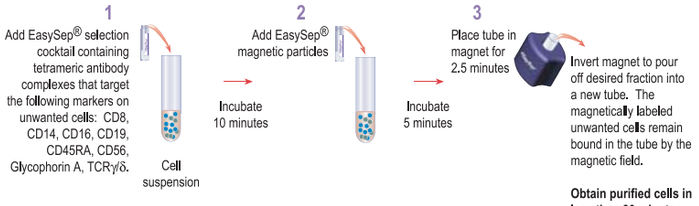
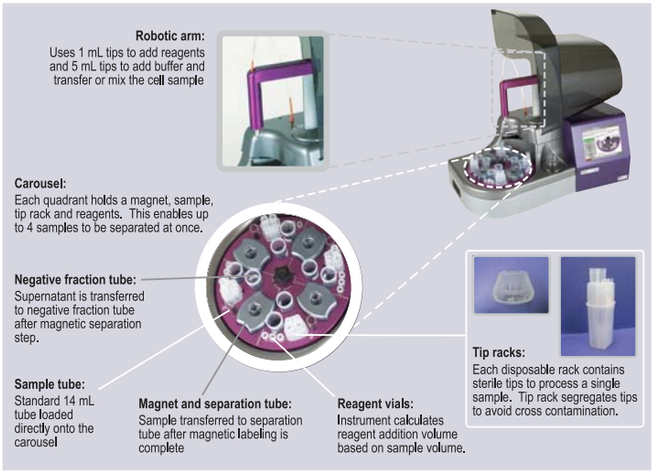


Figure 2. Fully automated enrichment of CD4+ memory T cells using RoboSep®



Results

Figure 3. EasySep® negative selection technology provides highly purified CD4$^+$ memory T cell fractions

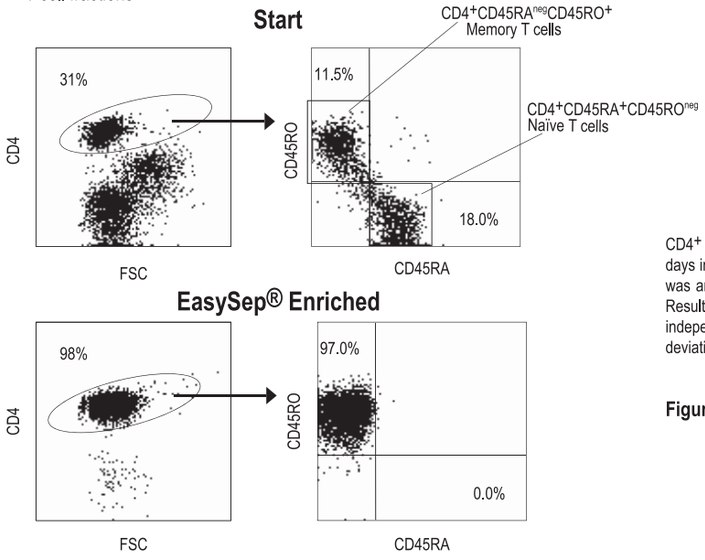


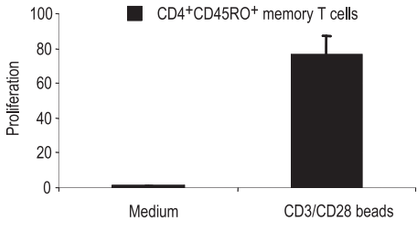
Table 1. Results obtained using EasySep® and RoboSep® technology for human CD4+ memory T cell enrichment from previously frozen mononuclear cells*

	%CD4$^+$CD45RA^{neg}CD45RO$^+$			Recovery from start	
	Start	EasySep®	RoboSep®	EasySep®	RoboSep®
AVERAGE (n=6)	11.8	92.7	93.3	68.2	69.3
SD	5.2	4.3	4.9	13.1	12.2

Contaminating naïve T cells (CD4$^+$CD45RA$^+$CD45RO^{neg}) accounted for fewer than 0.1% of total cells in the purified fractions (n=6)

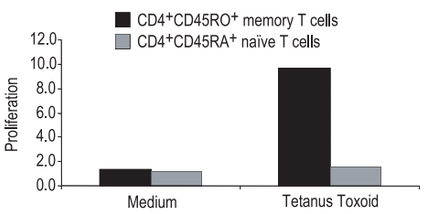
*Mononuclear cells obtained from peripheral blood Leuko Paks collected from normal donors using an apheresis machine were further processed using density centrifugation and cryopreserved in FBS containing 7.5% DMSO prior to use.

Figure 4. CD4$^+$ memory T cells isolated using EasySep® negative selection technology are highly responsive to polyclonal stimulation



CD4$^+$ memory T cells isolated using EasySep® technology were labeled with CFSE and cultured for 7 days in serum-containing medium in the presence or absence of CD3/CD28 coated beads. Cell division was analyzed by measuring CFSE dilution in gated viable CD4$^+$CD45RO$^+$ cells using flow cytometry. Results are expressed as % cells having undergone ≥ 1 cell division event. Bars are the means of 4 independent experiments, each performed with triplicate cultures, with Error Bars depicting standard deviations from the mean.

Figure 5. CD4$^+$ memory T cells isolated using EasySep® technology are capable of responding to antigen-specific stimulation



Antigen specific responses to tetanus toxoid by memory CD4$^+$ T cells isolated from two donors were measured by co-culturing cells with autologous monocytes in serum-containing medium for 7 days with or without the addition of tetanus toxoid at 1 µg per mL. Autologous monocytes were isolated using EasySep® CD14 positive selection technology, and used as antigen presenting cells at a 1:1 ratio. Naïve CD4$^+$ T cells purified from the same donors were used as a negative control in the assay. Cell division was analyzed by measuring CFSE dilution in gated viable CD4$^+$CD45RO$^+$ or CD4$^+$CD45RA$^+$ T cell populations using flow cytometry. Results are expressed as % cells having undergone ≥1 cell division event. Bars are the means of 2 independent experiments, each performed with triplicate cultures.

Conclusions

- Functional CD4+ memory T cells can be isolated in single step using column-free immunomagnetic EasySep® technology
- Highly purified cells can be obtained in less than 30 minutes
- The method can be fully automated using the RoboSep® cell separator

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