In vivo deuterium labeling of circulating immune cells: a feasibility study

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Introduction

Quantification of the life span of immune cells *in vivo* by deuterium labeling is an attractive methodology when studying disease or drug effects. It requires the isolation of immune cell subsets, which can be operationally challenging in clinical studies. Moreover, limited data is available on the variability of cellular lifespan between subjects. For this reason, a feasibility study was performed on the use of deuterium labeling for evaluation of lymphocyte dynamics.

AIM

To study the feasibility of *in vivo* deuterium labeling for quantification of the life span of lymphocyte subpopulations, in a clinical study settting.

Methods

In total 16 volunteers (8 healthy subjects, and 8 MS patients) participated in the study. These subjects received 70% deuterated water for 9 weeks and were followed for one year. Immune cell subsets were analyzed by:

- Sequential magnetic sorting (RoboSep)
- Flow cytometric phenotyping (MACSQuant 10)
- Deuterium incorporation (GCMS)

Results

Average purities exceeded 85% for CD19+ B-cells and CD4+ T-cells (Figure 1). Counts were generally above 30x10^4 for these cells subsets (Figure 2). However, for memory and naïve CD8+ T-cells average purities were much lower, coinciding with lower cell counts. Average life span was derived from deuterium incorporation (Figure 3), and amounted 271±232 days for CD19+ cells, and 209±283 days for CD4+ cells (Figure 4), with no difference between healthy subjects and MS patients. For CD8+ cell subsets, the purity was too low to reliably estimate life span.

Conclusions

- Customized automated sequential magnetic isolation protocols may result in impure cell populations when the original population size is relatively small;
- The life span of CD19+ B cells and CD4+ T cells was variable between subjects, and did not correlate with circulating cell numbers;
- With these limitations in mind, cellular life span may serve as a valuable readout measure in future clinical studies (investigating pathophysiology or drug effects).

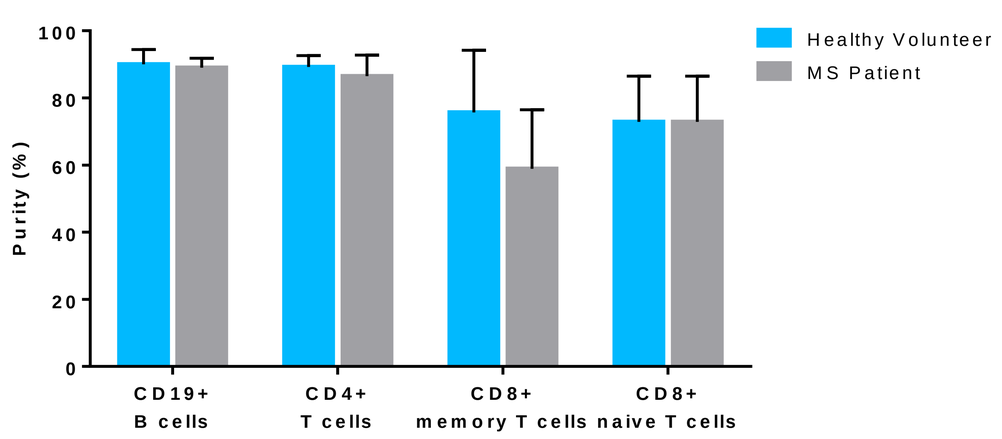

Figure 1: Purity of isolated cell populations

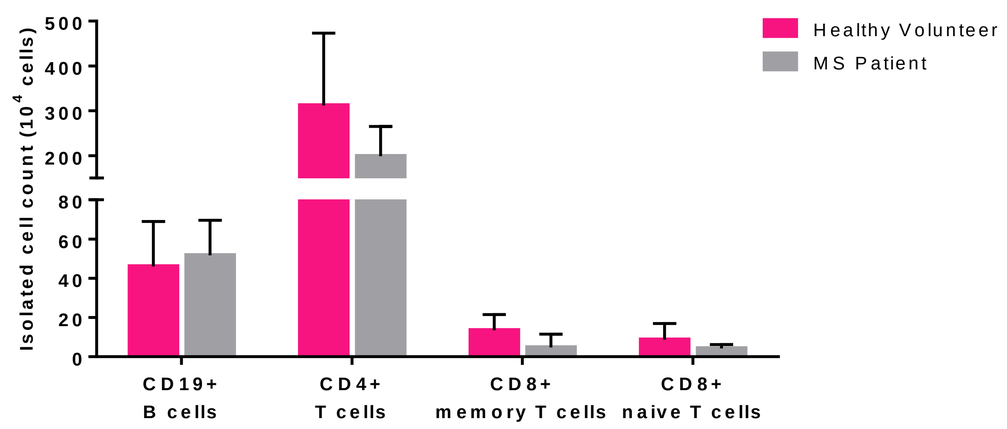

Figure 2: Isolated cell counts

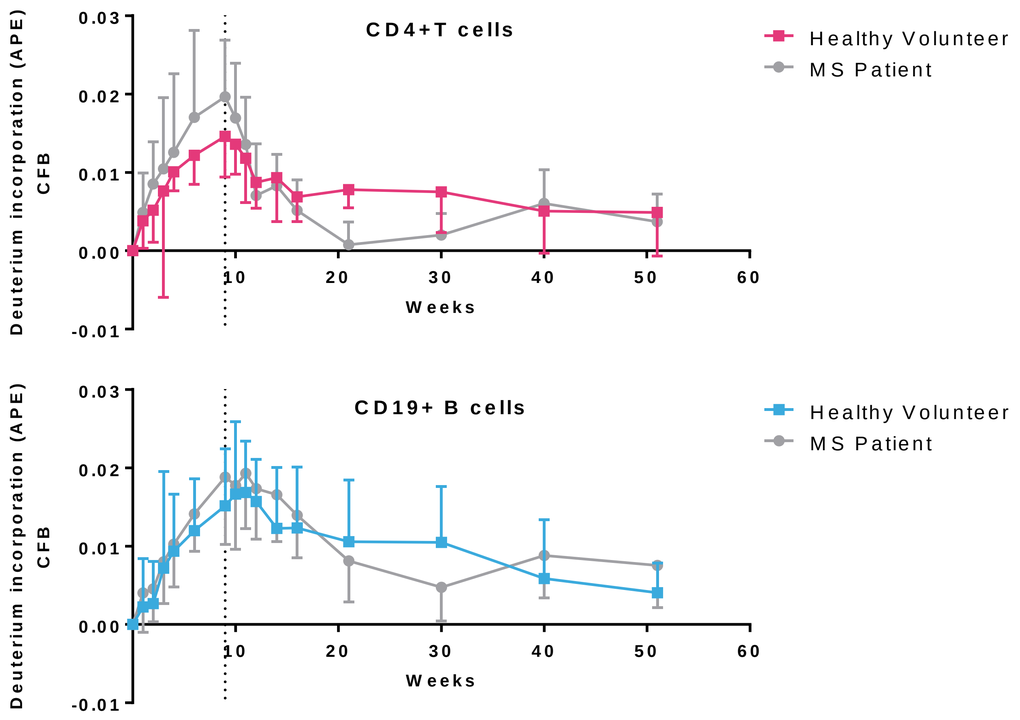

Figure 3: Deuterium incorporation

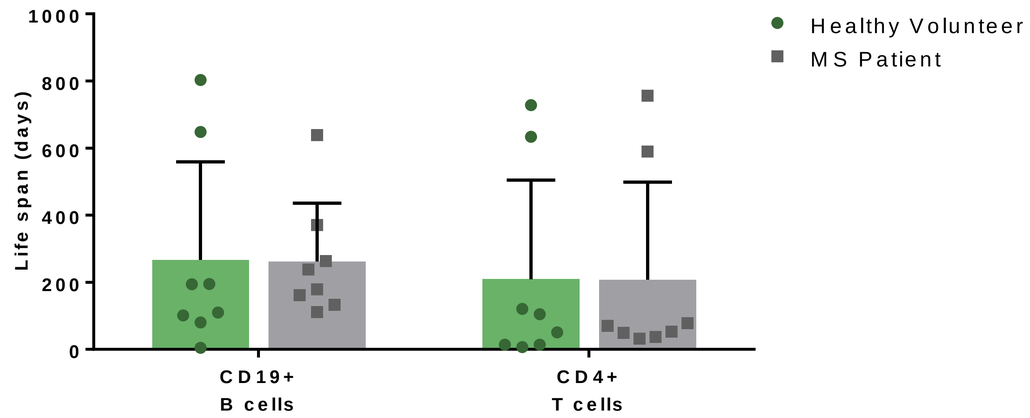

Figure 4: Average life span

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