Magnetic enrichment of antigen-specific CD4+ T cells enables the in-depth characterization of vaccine-induced circulating follicular T helper cells

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Introduction

Importance of circulating follicular helper cells (cTfh)

Follicular T helper cells (Tfh) play a crucial role in supporting germinal center induction and responses by providing B cell help needed for affinity maturation and generation of B cell memory. Circulating (cTfh) have moved to the center stage of immunological research since it becomes increasingly apparent that these cells are providing insights into the immune responses occurring in the germinal centers of lymphoid organs. Typically, these lymphatic tissues are not accessible when studying human immune responses, thus making Tfh an attractive target for surrogate markers of immunity. The main identifying marker is CXCR5 which is a chemokine receptor required for lymphocytes to enter the B cell compartments of lymphoid tissues, while other markers such as CXCR3 and CCR6 have been used to characterize Tfh subsets. Here, we report on the feasibility of an in-depth characterization of Tfh subsets in humans immunized once with a promising Ebola (rVSV-ZEBOV) vaccine. The described method allowed us to demonstrate successfully that the frequency of Tfh and in particular, cTfh17, was associated with ZEBOV-specific antibody titers. Moreover, applying this analysis panel may reveal similar associations and therefore, advance our understanding of immune mechanisms associated with protective immunity.

Results

Enrichment of antigen-specific cTfh cells

The main hurdle for the characterization of antigen-specific cells, and in particular, subsets within these cells, is the low frequency of cells which requires very large numbers of cells to be acquired for flow cytometric analysis. To overcome this limitation, we adapted the enrichment of CD4+CD154+ antigen-specific T cells to a panel that identifies subsets of cTfh cells. Multiple antigen-specific T cell enrichments were performed via the use of the integrated pre-enrichment column of the MACSQuant® Analyzer 10. This workflow allowed us to stain, enrich, and analyze multiple samples in an automated fashion increasing the throughput and reproducibility of the experimental set-up.

The objective was to determine whether this T cell subset serves as immune correlates or surrogate marker of protection for vaccines that supposedly mediate protection through antibodies. We tested the analysis strategy first on samples from a recent Ebola vaccine, where study subjects received a single immunization with the vaccine. The results of the study attest to the high sensitivity of this experimental approach. Cryopreserved peripheral blood mononuclear cells (PBMCs) were cultured with a ZEBOV-GP peptide pool at 1.0 µg/mL or medium alone (control stimulation). Cells were cultured for 16 hours (37 °C, 5% CO₂) in RPMI-1640 containing 10% human serum at a concentration of 5×10⁶ cells/mL. CD196 (CCR6)-APC, human and CD40 pure – functional grade, human were added to the culture at a 1:10 and 1:100 dilution, respectively. Following stimulation, cells were washed and stained with CD154-Biotin for 15 minutes at 4 °C in solution (0.5% human serum and 0.1% sodium azide in phosphate-buffered saline (PBS)). Cells were further incubated with Anti-Biotin MicroBeads UltraPure for 15 minutes at 4 °C. A pre-titrated and optimized antibody cocktail with fluorochrome-conjugated antibodies against CD3-VioBlue®, CD4-PercP-Vio® 700, CD185 (CXCR5)-PE-Vio770, CD183 (CXCR3)-VioBright™ FITC, CD154-PE, and Zombie Aqua™ Fixable dye was added and incubated for 45 minutes at 4 °C.
Cells were enriched (program Enrich-S) and acquired on a MACSQuant® Analyzer 10. Refer to figure 1 for the gating strategy. Data analysis was performed using Flowlogic™ flow cytometry analysis software.

Enrichment increased the signal of antigen-specific cells more than thirty fold (representative enrichment results depicted in figure 2).

Identification of antigen-specific cTfh subsets

The enrichment of CD154+ cells enabled the subsequent in-depth characterization of the cTfh subsets as originally described² and was modified to enable the analysis using a MACSQuant Analyzer 10: CD3+CD4+CD154+CXCR5–CXCR3–CCR6– cells are considered to be cTfh2, CD3+CD4+CD154+CXCR5+CXCR3–CCR6– cells are cTfh1, and CD3+CD4+CD154+CXCR5+CXCR3–CCR6+ cells are cTfh17.

We applied this experimental strategy to the evaluation of cTfh induction via a viral vaccine (figure 3). To determine the impact of enrichment on the ability to detect significant changes after vaccination, we acquired and analyzed aliquots of the samples prior and after enrichment.
Figure 5: Enrichment of CD154+ CD4+ T cells does not skew the composition of the cTfh subsets. PBMCs stimulated with antigen and then either acquired ("unenriched") or enriched based on CD154 ("enriched") were analyzed for (A) the frequency of CXCR5+ T cells and (B) the proportion of cTfh subsets within CD4+ CXCR5+ T cells. Boxplots represent data from 18 different samples obtained from subjects immunized with a viral vaccine.
Conclusion

(1) The enrichment of antigen-specific CXCR5+ T cells based on CD154 via the integrated pre-enrichment column on the MACSQuant® Analyzer 10 enables for a detailed characterization of cTfh subsets (using MACS® Antibodies) and results in statistically significant frequencies compared to pre-immune time point for the rVSV-ZEBOV vaccine.

(2) The enrichment did not skew the composition of the cTfh subsets thus allowing an unbiased analysis with a much higher resolution.

References


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