Comparison of PBMC extraction methods to improve SARS-CoV-2 T cell research

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BACKGROUND

It has become apparent that T cells play a dominant role in immune protection against severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2). There is a requirement of developing reliable methods, which are easy to use and allow upscaling, to measure the specific T-cell response.
One purpose of this study was the optimization of an automatized method of magnetic-bead purification for the extraction of peripheral blood mononuclear cells (PBMC) with respect to the sensitivity in a SARS-CoV-2 Interferon-gamma release assay (IGRA). The second goal was to research the longevity of SARS-CoV-2 specific T cells in individuals with multiple antigen contacts.

METHODS

Employees of a medical laboratory with one, two or three booster vaccinations and/or infections agreed to blood collection 2 weeks to 6 months after vaccination or infection. The magnetic-bead extractions were done with the T-Cell Select™ kit by Oxford Immunotec and the AllSheng Auto-Pure20B (AP-20B). The T-SPOT®Discovery SARS-CoV-2 kit by Oxford Immunotec was used for the IGRA, forming visible spots where Interferon gamma had been released by sensitized T cells. Statistical analysis for the comparison of IGRA sensitivity after using different volumes of magnetic beads for the PBMC extraction was realized by using a Bland-Altman plot while the Mann-Whitney-U test was used to understand if there are significant differences in the T-cell response over time.

RESULTS

A comparison between magnetic-bead based PBMC extraction methods and classic density gradient centrifugation had revealed the pre-eminence of the magnetic-bead based methods. In extended tests we were also able to demonstrate that a smaller volume of bead reagent (40 µl compared to 60 µl) during PBMC extraction is increasing the number of spots in the SARS-CoV-2 IGRA panels further (n=66).
Additionally, our research shows that the number of spike-specific T cells remains consistent over a period of at least six months ($p > 0.05$). A very small cohort (n=3) with individuals tested more than six months after the last antigen contact hints to an even longer persistence. In contrast, the number of nucleocapsid- and membrane-specific T cells decreased significantly over the course of six months ($p << 0.05$).

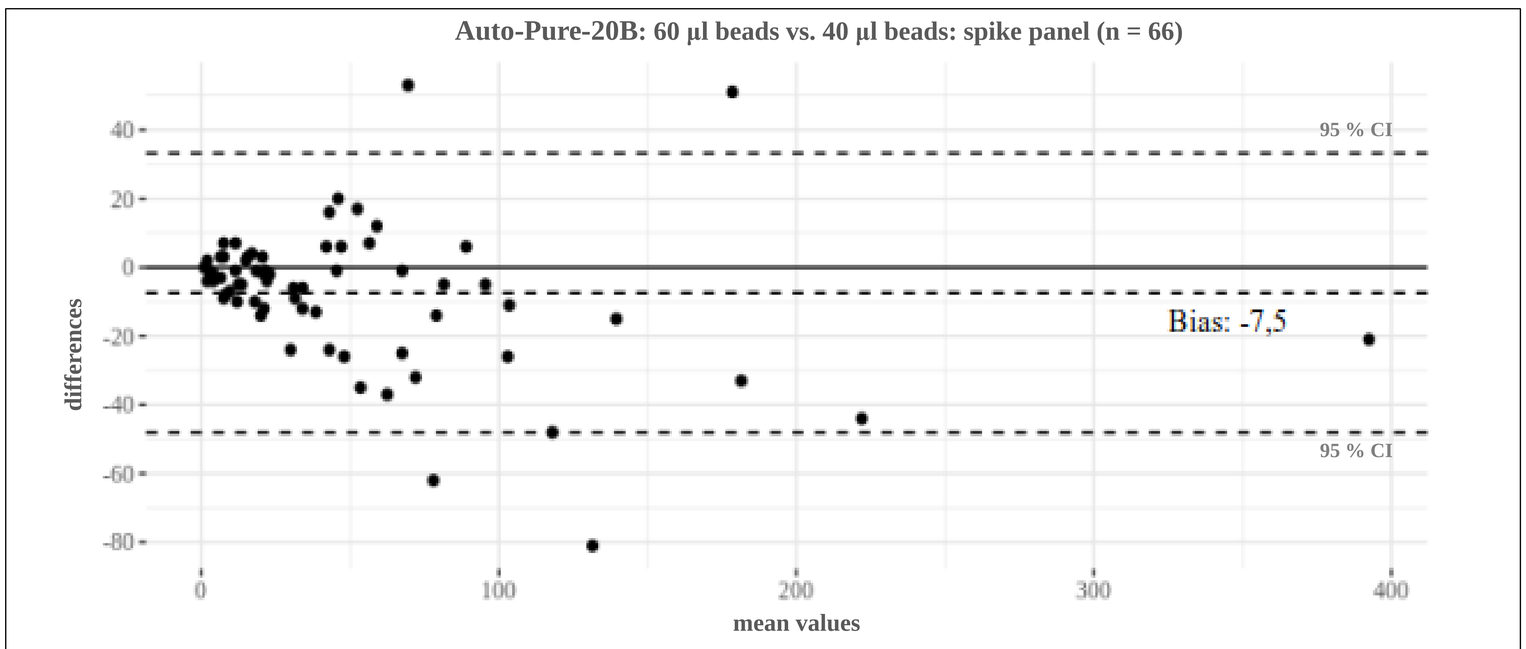

Fig. 1: Bland-Altman plot: number of spots in the spike panel after PBMC extraction with 60 µl magnetic beads (method 1) and 40 µl magnetic beads (method 2).

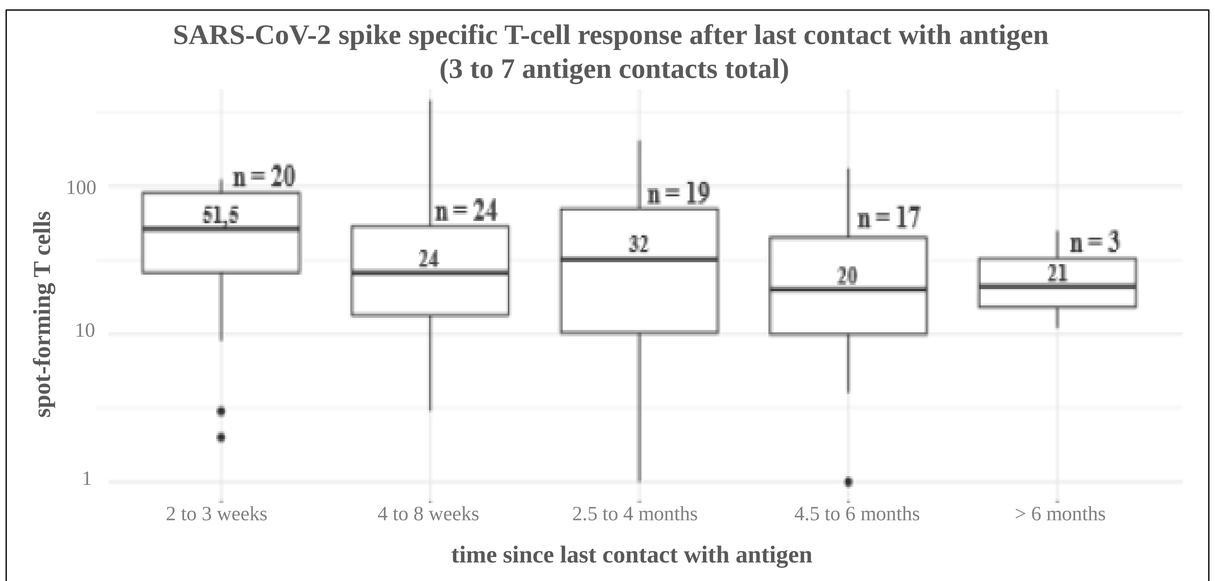

Fig. 2: Number of spot-forming T cells in the spike panel 2 weeks to 6 months after last antigen contact (through SARS-CoV-2 infection or vaccination). The value within a boxplot represents the respective median.

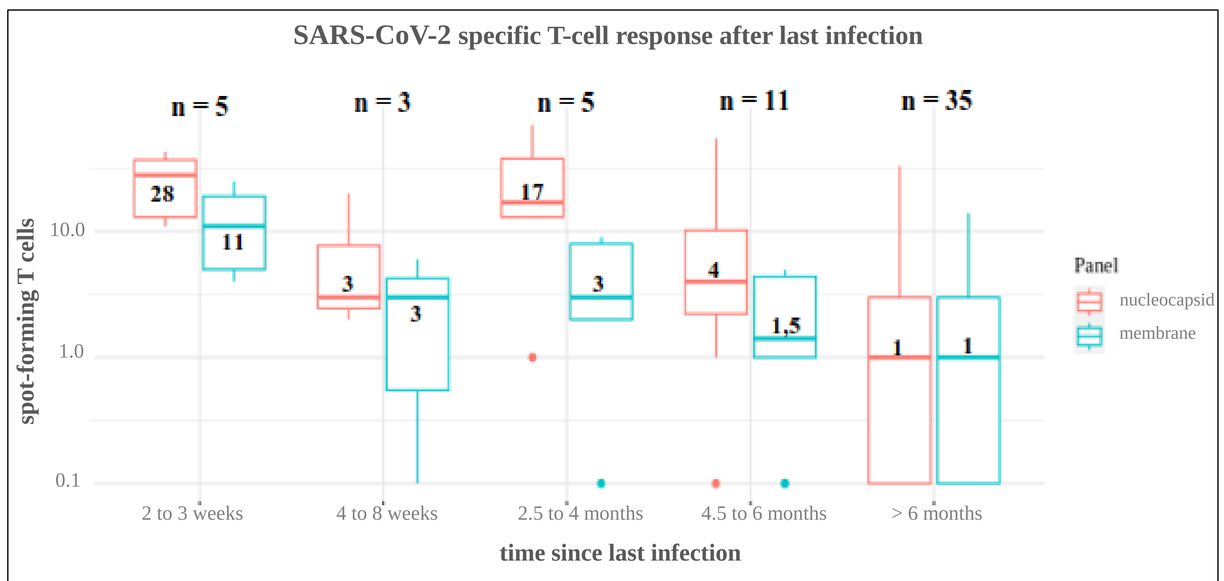

Fig. 3: Number of spot-forming T cells in the nucleocapsid and mebrane panels 2 weeks to over 6 months after last SARS-CoV-2 infection. The value within a boxplot represents the respective median.

CONCLUSIONS

For SARS-CoV-2-related T-cell research, automatized magnetic-bead mediated PBMC extraction with the T-Cell Select™ kit can be optimized by using a volume of 40 µl beads instead of the suggested 60 µl beads as a reduction of volume enhances the sensitivity in the T-SPOT®Discovery SARS-CoV-2 assay. The SARS-CoV-2 specific T-cell response is robust over a period of at least 6 months. However, only spike-specific T-cells show that kind of persistence while other SARS-CoV-2 antigens, like nucleocapsid- or membrane peptides, persisted substantially shorter in this investigation.