Stepping up in immunomonitoring – EliSpot five colour cytokine release Assay

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Since the end of the year 2019 the Severe acute respiratory syndrome coronavirus 2 is omnipresent in all health care related fields. Because of the pandemic prevalence and multifarious aspects concerning different disease stages, including vaccination pre or post infection, during active infection and after viral clearance with ongoing symptoms, various aspects must be considered to meet all requirements for adequate diagnostics.

In order to fulfil all these challenges a new five colour EliSpot assay system has been developed for the simultaneous detection of Interferon gamma, Interleukin-2, IL-6, IL-17A and Tumor necrosis factor alpha on single cell level. To refine the outcome of a standard EliSpot the PBMCs were additionally separated into $CD4^+$ and $CD8^+$ T cells via magnetic bead separation. This procedure led to five samples for each patient; untouched PBMCs, $CD4^+$ or $CD8^+$ cells exclusive and whole PBMCs without $CD4^+$ or $CD8^+$ cells.

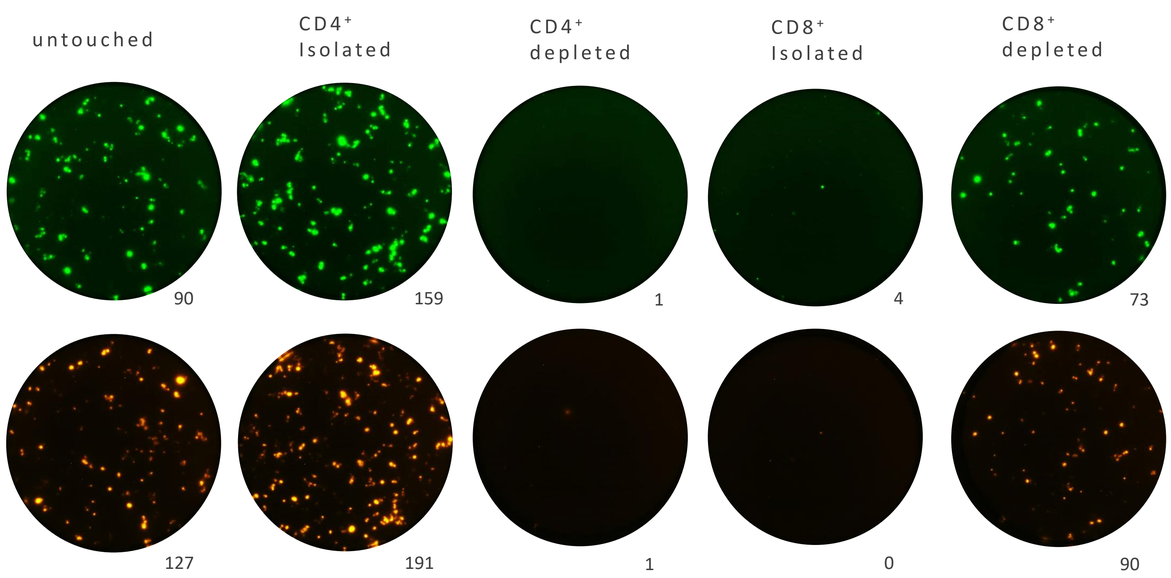

Fig.3: Representative results of the CE IVD 2-colour fluorescent EliSpot assay AID CoV-*i*Spot SARS-CoV-2 including $CD4^+$ and $CD8^+$ separation. For the purpose of a simplified explanation the following samples are based on a standard two colour IFN-γ / IL-2 *i*Spot assay. Shown is the SARS-CoV-2 specific secretion of IFN-γ (green) and IL-2 (orange) of untouched whole PBMCs, isolated $CD4^+$, PBMCs CD4 depleted, isolated $CD8^+$ and PBMCs CD8 depleted. Negative and positive controls (valid) are not shown. Fig. 3 A shows representative results of a naturally infected donor approximately 8 weeks post infection. Fig. 3 B shows representative results of a healthy donor approximately two weeks post vaccination with the second dose of mRNA based COVID-19 Vaccine. Both samples show a strong induction of the secretion of IFN-γ and IL-2 predominantly driven by $CD4^+$ t helper cells.

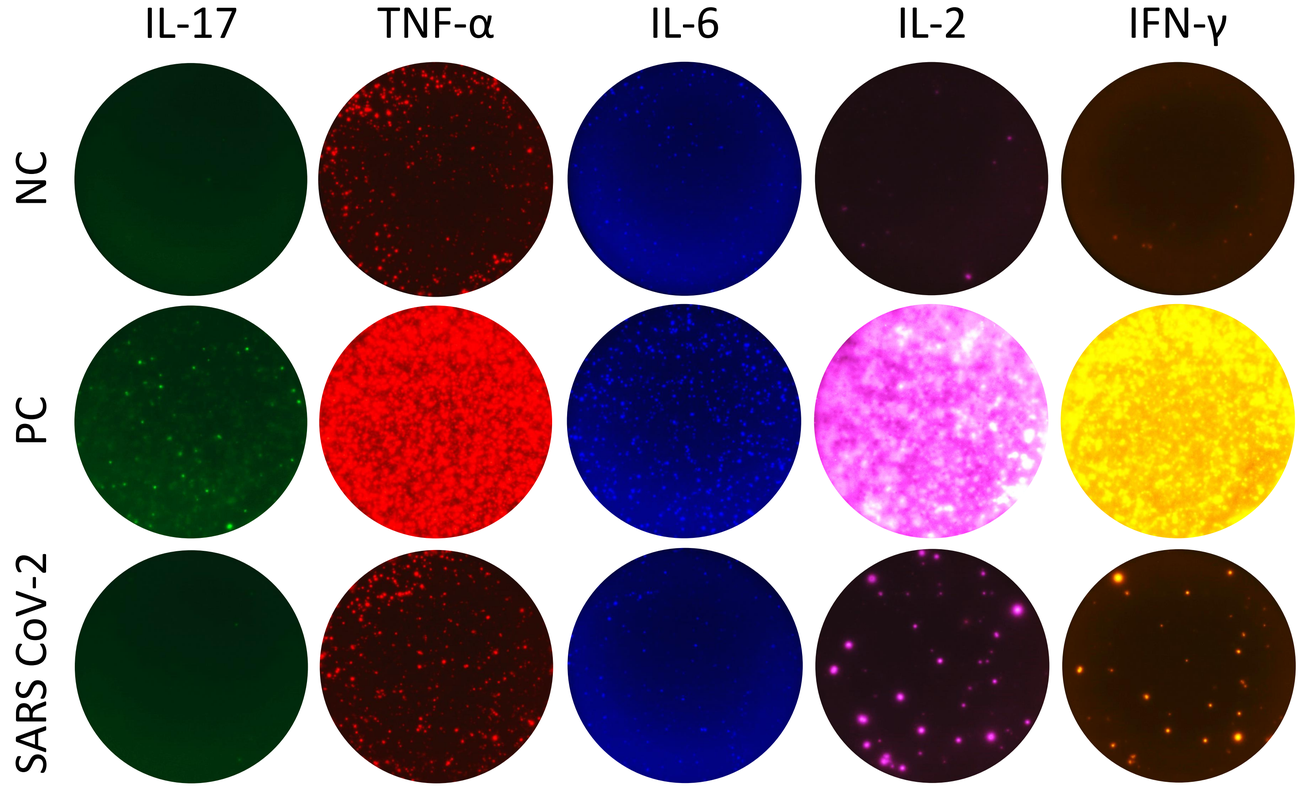

FIG. 1: Representative exemplary results of the new five colour fluorescent EliSpot assay of whole PBMCs post COVID-19 vaccination. The negative (NC) and positive control (PC) wells are shown for visualization of correct EliSpot assay performance and to ensure a sufficient number of stimulatable T cells. The NC is performed with PBMCs in cell culture media only, without any stimuli and the positive control with the lectin Pokeweed mitogen for a broad range stimulation of lymphocytes. The SARS-CoV-2 Peptid-Mix consists of peptides from the spike, nucleocapsid, matrix and envelope protein. In more detail this peptide mix is capable to induce T cell reactions after natural infection or vaccination. Pictures were taken with the upcoming new AID EliSpot Reader *i*Spot Spectrum model 9 equipped with selective filter sets for FITC (green), Cy5 (red), DAPI (blue), Cy7 (purple) and Cy3 (orange).

Interleukin 17A (IL-17, green), Tumour Necrosis Factor alpha (TNF-α, red), Interleukin 6 (IL-6, blue), Interleukin 2 (IL-2, purple) and Interferon gamma (IFN-γ, orange).

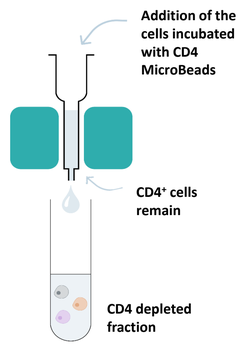

Fig.2: Schematic drawing of a T cell type specific isolation via positive selected T helper ($CD4^+$) and cytotoxic ($CD8^+$) cells by magnetic bead separation. Whole PBMCs were isolated and split into three portions for further purification. Example is showing the $CD4^+$ MicroBeads purification.
All fractions (PBMCs untouched, isolated $CD4^+$, run through of CD4 isolation, isolated $CD8^+$ and run through of CD8 isolation) were analysed by EliSpot in parallel.

The invented EliSpot assay platform can be used to identify the most effective vaccine available on the market, for further improvements of the vaccine itself or the used adjuvant. During infection, immunological measurements may be used to evaluate the disease outcome and help physicians to assess if a special treatment is indicated. Furthermore, such tools may be used for the stratification of future progression and therefore predict a mild ore severe disease progression. Last, this assay can be used to identify possible immunological dysregulation in long COVID patients to reach new treatment approaches and for the monitoring of the therapeutic progression.

For such a complex disease pattern along with very variable manifestations of different individuals or special patient groups extensive knowledge in combination with further analytical testing is needed. With this powerful tool deep insights into immunological details, in a variety of circumstances along SARS-CoV-2 infection, can be portraited.

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