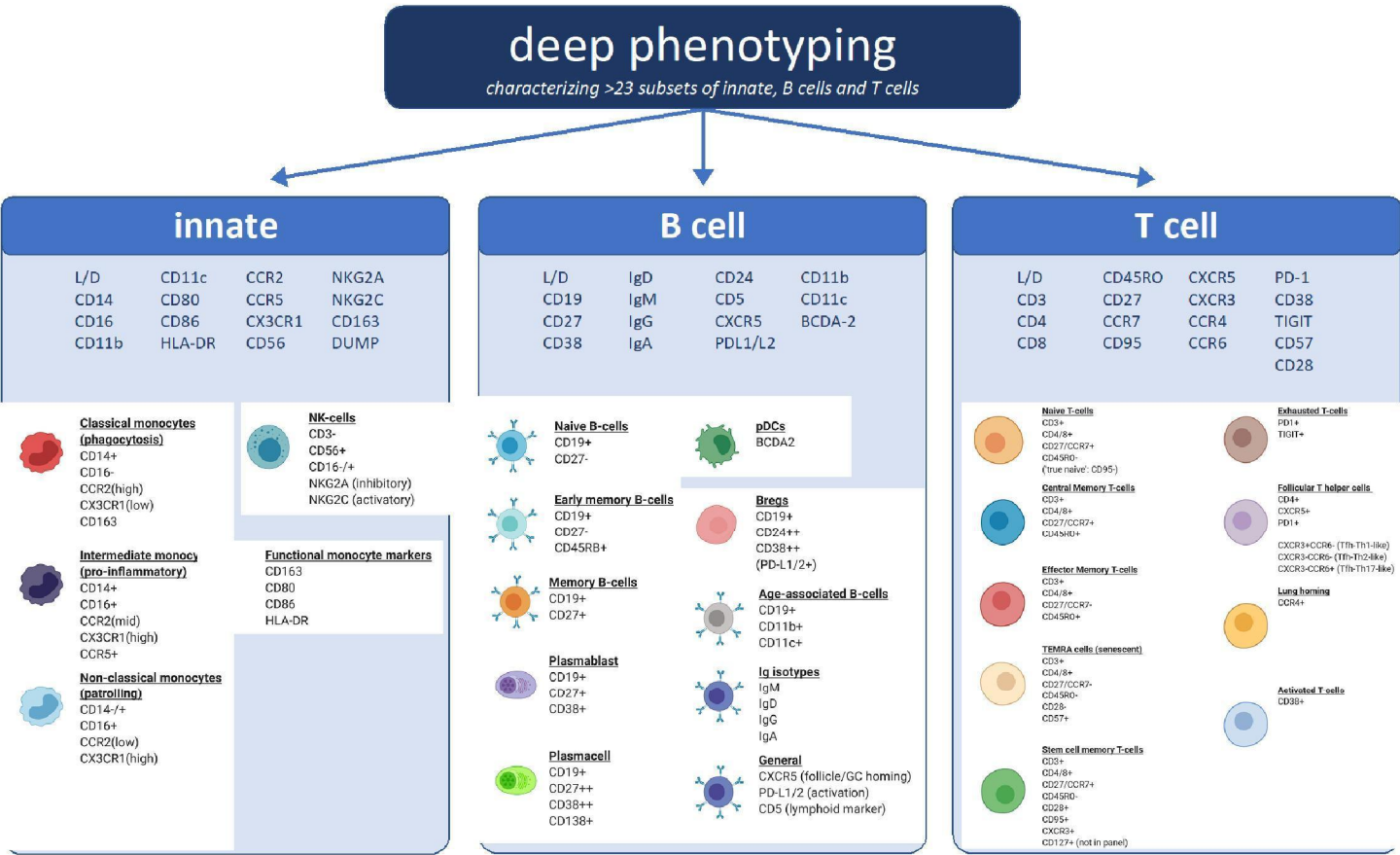




## innate response

test capacity and type of innate response to SARS-CoV-2

### Stimulation of total PBMCs with:

- Medium control
- Heat-inactive SARS-CoV-2
- TLR7/8 (R848)
- TLR2 (HKLM)
- TLR4 (LPS)
- TLR4 + h.i. SARS-CoV-2
- TLR7/8 + h.i. SARS-CoV-2
- TLR2 + h.i. SARS-CoV-2

### Analysis

- Cytokine/chemokine secretion in supernatant (IFN $\alpha$ 2, IFN $\gamma$ , IFN $\beta$ , IFN $\lambda$ 1, IFN $\lambda$ 2/3, IL6, IL8, IL10, TNF $\alpha$ , IL12p70, GM-CSF, CXCL10, IL1 $\beta$ )
- Cellular response by flow cytometry (CD14/CD16, CD80, CD86, CD11b, CD11c, CD163, CCR2, CX3CR1)
- Optional: RNA-seq of stimulated cells

## T cell response

test frequency (and type) of SARS-CoV-2 specific T cells

### Stimulation of total PBMCs with peptide pools:

- DMSO, negative control
- PHA, positive control
- SARS-CoV-2 spike (S1)
- Heat-inactivated SARS-CoV-2
- OC43 (spike)
- SARS-CoV-2 spike (S2)
- SARS-CoV-2 NCP
- Recombinant spike protein

### Analysis

- IFN- $\gamma$  ELISpot
- Supernatant for other cytokines produced (GM-CSF, IL2, IL4, IL5, IL13, IL17A, IL10, TNF $\alpha$ )
- Cellular analysis for T-cell subsets and activation markers (CD3, CD4, CD8, CD69, CD137, CD25, OX40, CD154, L/D)
- Optional: 6-day culture to expand specific T cells for CD4 T-helper cell responses and intracellular cytokine stainings

## B cell response

test frequency (and type) of SARS-CoV-2 specific B cells

### B-cell ELISpot:

- Expansion with R848 or CpG

### Analysis

- Spike protein IgG ELISpot
- Nucleo protein IgG ELISpot
- Cellular analysis for B-cell subsets and activation markers (CD19, CD27, IgM, IgG, IgA, CD38, CD138)

### B-cell clonality (CD40L system):

- Expansion with CD40L + cytokine mix

### Analysis

- SARS-CoV-2 specific Ab-production in supernatant