deep phenotyping

characterizing >23 subsets of innate, B cells and T cells

innate

L/D CD14 CD16 CD11b
CD11c CD80 CD86 HLA-DR
CCR2 CCR5 CX3CR1 CD56
NKG2A NKG2C CD163 DUMP

Classical monocytes (phagocytosis)
CD14+
CD16-
CCR2(high)
CX3CR1(low)
CD163

Intermediate monocy (pro-inflammatory)
CD14+
CD16+
CCR2(mid)
CX3CR1(high)
CCR5+

Non-classical monocytes (patrolling)
CD14-/+
CD16+
CCR2(low)
CX3CR1(high)

NK-cells
CD3-
CD56+
CD16-/+
NKG2A (inhibitory)
NKG2C (activatory)

Functional monocyte markers
CD163
CD80
CD86
HLA-DR

B cell

L/D CD19 CD27 CD38
IgD IgM IgG IgA
CD24 CD5 CXCR5 PDL1/L2
CD11b CD11c BCDA-2

Naive B-cells
CD19+
CD27-

Early memory B-cells
CD19+
CD27-
CD45RB+

Memory B-cells
CD19+
CD27+

Plasmablast
CD19+
CD27+
CD38+

Plasmacell
CD19+
CD27++
CD38++
CD138+

pDCs
BCDA2

Bregs
CD19+
CD24++
CD38++
(PD-L1/2+)

Age-associated B-cells
CD19+
CD11b+
CD11c+

Ig isotypes
IgM
IgD
IgG
IgA

General
CXCR5 (follicle/GC homing)
PD-L1/2 (activation)
CD5 (lymphoid marker)

T cell

L/D CD3 CD4 CD8
CD45RO CD27 CD95
CXCR5 CXCR3 CCR4 CCR6
PD-1 CD38 TIGIT CD57 CD28

Naive T-cells
CD3+
CD4/8+
CD27/CCR7+
CD45RO-
('true naive': CD95-)

Central Memory T-cells
CD3+
CD4/8+
CD27/CCR7+
CD45RO+

Effector Memory T-cells
CD3+
CD4/8+
CD27/CCR7-
CD45RO+

TEMRA cells (senescent)
CD3+
CD4/8+
CD27/CCR7-
CD45RO-
CD28-
CD57+

Stem cell memory T-cells
CD3+
CD4/8+
CD27/CCR7+
CD45RO-
CD28+
CD95+
CXCR3+
CD127+ (not in panel)

Exhausted T-cells
PD1+
TIGIT+

Follicular T helper cells
CD4+
CXCR5+
PD1+

CXCR3+CCR6- (Tfh-Th1-like)
CXCR3-CCR6- (Tfh-Th2-like)
CXCR3-CCR6+ (Tfh-Th17-like)

Lung homing
CCR4+

Activated T cells
CD38+

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 (IFNα2, IFNγ, IFNβ, IFNλ1, IFNλ2/3, IL6, IL8, IL10, TNFα, IL12p70, GMCSF, CXCL10, IL1β)
- 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 NCAP
- Recombinant spike protein

Analysis

- IFN-γ ELIspot
- Supernatant for other cytokines produced (GM-CSF,IL2,IL4,IL5,IL13,IL17A,IL10,TNFa)
- 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