

Key questions cellular research FFX

1. Why are some COVID-19 patients symptomatic and other asymptomatic, and can we predict severity of disease / successful outcome?
2. Why are children mostly asymptomatic?
3. Why do men get more severe symptoms?
 - **In depth characterization of the immune response of COVID-19 patients vs. controls**
assays: extensive ex vivo phenotyping for innate, B-cell and T-cell responses; plasma factors
timepoints: t=1, t=2, t=3
 - **Test capacity and type of innate responses of (pre-) COVID-19 patients**
assay: innate stimulation assay
timepoints: t=1
4. Is cellular immune acquired after infection?
 - **In depth characterization of the immune response of COVID-19**
assays: extensive ex vivo phenotyping for innate, B-cell and T-cell responses
timepoints: t=1, t=2, t=3
 - **Test SARS-CoV-2 specific T-cell and B-cell responses of COVID-19**
assays: T cell stimulation assay; B cell ELISpot;
timepoints: t=2, t=3

Other FFX-related questions for in depth research

5. Cross reactivity with other corona viruses?
 - **Antibody cross reactivity**
assays: protein microarray / MIA; clonal B cell analysis
timepoints: t=3
 - **T-cell cross reactivity**
assay: peptide stimulation assays
timepoints: t=3
6. Translation of ferret model to human immune response?
 - **Phenotyping of immune cells**
assays: protein microarray / MIA; clonal B cell analysis
timepoints: t=1, t=2, t=3
 - **Test T cell responses**
assay: peptide stimulation assays
timepoints: t=2, t=3
 - **Epithelial models**
assay: infection of human epithelial models
timepoints: NA

Assays in more detail:

In depth phenotyping (2x10 ⁶ PBMCs)
<i>Characterizing xx subsets of innate, T cells and B cells</i>
Flow cytometry with 3 main panels: - Innate (CD14, CD16, CD80, CD86, HLA-DR, CCR2, CCR5, CX3CR1, CD11c, CD163, CD93, CD120b, L/D, CD56, NKG2A, NKG2C) - T cell (CD3, CD4, CD8, CD45RO, CD27, CCR7, CD95, CXCR5, PD1, CCR4, CCR6, CXCR3, NKG2A, TIGIT, CD57, CD28) - B cell (CD19, CD27, CD38, CD24, CD5, CXCR5, CD1d, IgA, IgM, IgG, IgD, BDCA-4, CD45RB, L/D)
Innate stimulation assay (5x10 ⁶ PBMCs)
<i>Test capacity and type of innate response to SARS-CoV-2</i>
Stimulation of total PBMCs with: - o Medium control - o Heat-inactive SARS-CoV-2 - o TLR2 (HKLM) - o TLR2 + h.i. SARS-CoV-2 - o TLR7/8 (R848) - o TLR7/8 + h.i. SARS-CoV-2 - o TLR4 (LPS) - o TLR4 + h.i. SARS-CoV-2 - Triplicate → duplicates → single
Analysis: - Cytokine/chemokine secretion in supernatant (IFNα2, IFNγ, IFNβ, IFNλ1, IFNλ2/3, IL6, IL8, IL10, TNFα, IL12p70, GM-CSF, CXCL10, IL1β) - Cellular response by flow cytometry (CD14/CD16, CD80, CD86, CD11b, CD11c, CD163, CCR2, CX3CR1) - Optional: RNA-seq of stimulated cells
SARS-CoV-2 specific T-cell responses (4x10 ⁶ PBMCs)
<i>Test frequency (and type) of SARS-CoV-2 specific T cell responses</i>
Stimulation of total PBMCs with peptide pools: - o DMSO control - o SARS-CoV-2 spike pepmix (S1+S2) - o Heat-inactivated SARS-CoV-2 - o PHA, positive control - o SARS-CoV-2 NCAP pepmix - o OC43 spike (S1+S2) pepmix - o Recombinant spike protein triplicates
Analysis: - IFN-γ ELISpot - Supernatant for other cytokines produced (GM-CSF, IL2, IL4, IL5, IL13, IL17A, IL10, TNFα) - 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

SARS-CoV-2 specific B-cell responses (5x10 ⁶ PBMCs)
Test frequency of SARS-CoV-2 specific (memory) B cell responses
Analysis: - B cell ELISpot for SARS-CoV-2 (total, spike protein, nucleo protein)

First sample selection based on

- Index case, SARS-CoV-2 PCR+
- Amount of PBMC vials available (2 or more)
- Time inclusion after first symptoms
 - o Priority innate assay: 3-9 days
 - o Priority T cell assays 10-14 days

Sample selection innate assay:

T=1, T=2 & T=3

- 1) Deep phenotyping (2x10⁶ PBMCs)
- 2) Innate stimulation assay (5x10⁶ PBMCs)
 - a. Medium control
 - b. Heat-inactive SARS-CoV-2
 - c. TLR2 (HKLM)
 - d. TLR2 + h.i. SARS-CoV-2
 - e. TLR7/8 (R848)
 - f. TLR7/8 + h.i. SARS-CoV-2
 - g. TLR4 (LPS)
 - h. TLR4 + h.i. SARS-CoV-2
- 3) T cell response (4.5x10⁶ PBMCs)
 - a. DMSO control
 - b. SARS-CoV-2 spike pepmix (S1+S2)
 - c. Heat-inactivated SARS-CoV-2
 - d. PHA, positive control
 - e. SARS-CoV-2 NCAP pepmix
 - f. OC43 spike (S1+S2) pepmix
 - g. Recombinant spike protein
- 4) Any left-over cells:
 - a. Freeze cell pellet in qiazol

Sample selection T cell assay:

T=1, T=2 & T=3

- 1) Deep phenotyping (2x10⁶ PBMCs)
- 2) T cell response (4.5x10⁶ PBMCs)
 - a. DMSO control
 - b. SARS-CoV-2 spike pepmix (S1+S2)
 - c. Heat-inactivated SARS-CoV-2
 - d. PHA, positive control
 - e. SARS-CoV-2 NCAP pepmix
 - f. OC43 spike (S1+S2) pepmix
 - g. Recombinant spike protein
- 3) Innate stimulation assay (5x10⁶ PBMCs)
 - a. Medium control
 - b. Heat-inactive SARS-CoV-2
 - c. TLR2 (HKLM)

- d. TLR2 + h.i. SARS-CoV-2
- e. TLR7/8 (R848)
- f. TLR7/8 + h.i. SARS-CoV-2
- g. TLR4 (LPS)
- h. TLR4 + h.i. SARS-CoV-2

4) Any left-over cells:

- a. Freeze cell pellet in qiazol

Sample selection children samples:

T=1, T=2 & T=3

- 1) Deep phenotyping (**1.5x10⁶** PBMCs)
- 2) Innate stimulation assay (**1.2x10⁶** PBMCs)
 - a. Medium control
 - b. Heat-inactive SARS-CoV-2
 - c. TLR7/8 (R848)
 - d. TLR7/8 + h.i. SARS-CoV-2
- 3) T cell response (**0.8x10⁶** PBMCs)
 - a. DMSO control
 - b. SARS-CoV-2 spike pepmix (S1+S2)
 - c. Heat-inactivated SARS-CoV-
 - d. PHA, positive control
- 4) Innate stimulation assay (**0.6x10⁶** PBMCs)
 - a. TLR2 (HKLM)
 - b. TLR2 + h.i. SARS-CoV-2
- 5) T cell response (**0.4x10⁶** PBMCs)
 - a. SARS-CoV-2 NCAP pepmix
 - b. OC43 spike (S1+S2) pepmix
- 6) Innate stimulation assay (**0.6x10⁶** PBMCs)
 - a. TLR4 (LPS)
 - b. TLR4 + h.i. SARS-CoV-2
- 7) Any left-over cells:
 - a. Make duplicates → triplicates
 - b. Freeze cell pellet in qiazol