

GENERAL INFORMATION

1. NAME OF THE CENTER AND LOCATION

Institute of Biology and Immunology of Reproduction, Bulgarian Academy of Sciences, part of Research Infrastructure Alliance for Cell Technologies (INFRA ACT)

2. TYPE OF THE RESEARCH INFRASTRUCTURE AND/OR SCIENTIFIC EXPERTISE

Identify the type of the RI, equipment/facilities/specific research, and in particular linked to COVID-19:

COVID-19 RNA epi-transcriptome analysis: Scientific equipment allowing for long read DNA/RNA sequencing, including RNA direct sequencing – Nanopore sequencing facility with 2 Oxford Nanopore Technologies (ONT) MinION sequencers, 1 MinIT for real time base calling, fluorescent based QC. Pending until the 3rd quarter of 2020 we are obtaining Qsep RNA/DNA fragment analyzer, automated library prep – ONT Voltrax, ONT GridION (middle range sequencer – up to 150 GB per project), coupled with ONT flongle adaptors for small projects (up to 1.5 GB).

Analysis of cell response to viral invasion: We have microfluidics equipment for single cell microchip-based encapsulation for single cell RNA-seq and similar studies (based on InDrop protocol and chip design), and flowcytometry analysis of cellular immune response. Fluorescence and luminescence reporters-based systems for analysis are also possible. CellINK Bio X bioprinter expected by the 3rd quarter 2020 for cell-cell interaction models. Since cytokine storm and septic shock are main lethality related pathogenic factors, and suppression of inflammasome Nlrp3 has been shown to prevent or ameliorate this process, we aim to investigate the role of its activation and incurred cell death in non-immune and immune cells.

KEY WORDS:

Expertise in inflammasome research in non-immune cells, cell death analysis, direct RNA sequencing, molecular methods – qPCR, molecular cloning, *in silico* docking of small molecules and peptides, differential scanning fluorometry ligand-receptor interaction studies

3. TYPE OF THE RESEARCH

Provide information on the research carried on or planned in regard

Planned research:

1. Direct RNA sequencing of novel SARS-CoV-2 from patient specimens or from passaged virus seeking for mutations (ex. in-dels) in regions encoding

with COVID-19 and other viruses

S-protein or E-protein. Two studies to date (2.4.2020) have sequenced directly the viral RNA, showing very complex viral transcriptome, 40+ new unknown ORFs, possibility for spike protein in-dels resulting in changes in the viral entry/exit rate. The data should be combined with disease outcome and sequencing data characterizing innate immunity and autophagy genes.

2. Analysis of the effect of wt/mutated spike proteins, and other proteins in *in vitro* systems of non-immune and macrophage cell lines using combination of microfluidics-based human lung model, or bioprinted models, and subsequent tissue disruption into single cells suspension and immunophenotyping of non-immune/immune cells, or single cell RNA-seq. Since cytokine storm and septic shock are main lethality related pathogenic factors, and suppression of inflammasome Nlrp3 has been shown to prevent or ameliorate this process, we aim to investigate the role of its activation and incurred cell death in non-immune and immune cells. We will follow the activation of the inflammasome in epithelial and endothelial cells and if this process could be ameliorated therapeutically.

4. WEBSITE

Provide the internet address:

<http://ibir.bas.bg/en/>

5. BACKGROUND, PUBLICATIONS AND OPEN DATA REPOSITORY

leading research team AND Scientific publications of the research group on the topics of related to coronavirus research results;

link to open data repository

Publications in the area of Nlrc3 inflammasome:

1: Hayrabedian S, Todorova K, Jabeen A, Metodieva G, Toshkov S, Metodiev MV, Mincheff M, Fernández N. Sertoli cells have a functional NALP3 inflammasome that can modulate autophagy and cytokine production. *Sci Rep.* 2016 Jan 8;6:18896. doi: 10.1038/srep18896. PMID: 26744177; PMCID: PMC4705529.

2: Hayrabedian S, Todorova K, Pashova S, Mollova M, Fernández N. Sertoli cell quiescence - new insights. *Am J Reprod Immunol.* 2012 Dec;68(6):451-5. doi: 10.1111/j.1600-0897.2012.01137.x. Epub 2012 Apr 24. PMID: 22531009.

3: Hayrabedian SB, Zasheva DY, Todorova KO. NLRs Challenge Impacts Tight Junction Claudins In Sertoli Cells. *Folia Med (Plovdiv).* 2015 Jan-Mar;57(1):43-8. doi: 10.1515/folmed-2015-0018. PMID: 26431094.

6. COORDINATOR

Full name of the coordinator organization;

**Research Infrastructure Alliance for Cell Technologies (INFRA ACT) -
Institute of Biology and Immunology of Reproduction, Bulgarian Academy of
Sciences**

Contact person;

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7. POSSIBLE PARTNERS

Indicate the partner
organizations

Potential long term partners:

Catholic University of Sacred Heart, Institute of general pathology, Rome, Italy –

Assoc prof Francesco Ria

Essex University, School of Biological Sciences, UK – Prof Nelson Fernandez,

Prof Metodi Metidiev

BioIncept LLC, New Jersey – Dr Eytan Barnea, CSO

Potential long term partners:

8. IMPLEMENTED AND RUNNING PROJECTS

Projects related to
virology, vaccines,
infection diseases ...

*We are currently running RI upgrade project aiming on next gen equipment in the
area of nucleic acid sequencing, tissue modelling, microfluidics-based cell models
and cell analysis.*

*Current projects related to the proposed topics – analysis of tumour plasticity using
single cell RNA-seq, and development of multiplexed protocol for simultaneous cell
surface antigen profiling and transcriptome analysis.*