Master 2 internship project
Year 2019-2020

Laboratory/Institute: IBS
Director: Winfried Weissenhorn
Team: MIT team
Head of the team: Franck Fieschi

Name and status of the scientist in charge of the project: Marie José Stasia **HDR: yes** X **no**☐
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Program of the Master's degree in Biology:

X Immunology, Microbiology, Infectious Diseases
☐ Integrative Structural Biology
☐ Physiology, Epigenetics, Differentiation, Cancer
☐ Neurosciences and Neurobiology
☐ Planta International

Title of the project:

Engineering stability of NADPH oxidase NOX2/p22phox toward its production for future protein therapy of X-linked chronic granulomatous disease

Objectives (up to 3 lines):

Recombinant expression of NOX2/p22phox (cytochrome b558) in HEK293T Lenti-X cells using lentiviral transduction (Elegheert Nature Protocols 2018).
Production of active proteoliposomes NOX2/p22phox (Brault et al. Int J Nanomedicine (2017)).

Abstract (up to 10 lines):

NADPH-oxidases are membrane enzymes producing reactive oxygen species and have several biological functions such as innate immunity, cellular signalling or cell differentiation. NOX2 is the main enzyme in phagocytes responsible for innate defense. X-linked Chronic Granulomatous Disease (CGD) is a rare innate immunodeficiency syndrome affecting child under 2 of age caused by NOX2/p22phox deficiency. We previously demonstrated the proof of concept of the efficacy of the proteoliposome (PL) therapy to restore NADPH oxidase activity of human XCGD iPSC- derived-macrophages (Brault et al. Int J Nanomedicine (2017)). However the cell free protein expression does not permit to obtain enough PLs. In addition post-translational modifications are not possible using E. Coli lysate. We propose to expressed NOX2 and p22^{phox} together by lentiviral transduction in the human mammalian cells HEK293T using an efficient protocol for expressing membrane proteins (Elegheert Nature Protocols 2018). Purified NOX2/ p22phox will be incorporated into liposomes and the NADPH oxidase activity will be tested in a cell-free-system assay (Beaumel et al. Biochem J (2014)).

Methods (up to 3 lines):

NOX2 and p22phox will be cloned in transfer plasmids and co-transfected in HEK293T Lenti-X cells with envelope and packaging plasmids to generate viral particules. Expression of NOX2/p22phox will be done by western-blot and flow cytometry. NADPH oxidase activity of NOX2/p22phox incorporated into liposomes, will be controlled in a cell free system assay with recombinant cytosolic partners p47phox, p67phox and rac routinely produced in the lab.

Up to 3 relevant publications of the team:

1. Brault J, Vaganay G, Le Roy A, Lenormand JL, Cortes S, **Stasia MJ.** Therapeutic effects of proteoliposomes on X-linked chronic granulomatous disease: Proof of concept using macrophages differentiated from patient-specific induced pluripotent stem cells Int J Nanomedicine (***2017***) 12:2161-2177
2. Hajjar C, Cherrier MV, Dias Mirandela G**,** Petit-Hartlein I, **Stasia,MJ**, Fontecilla-Camps JC, **Fieschi F**, Dupuy J **(*2017*)** The NOX Family of Proteins Is Also Present in Bacteria. mBio 8(6). pii: e01487-17. doi: 10.1128/mBio.01487-17
3. Beaumel S, Grunwald D, **Fieschi F, Stasia MJ**. Identification of NOX2 regions for normal biosynthesis of cytochrome b558 in phagocytes highlighting essential residues for p22phox binding. Biochem J. (***2014***) 464(3):425-37

Requested domains of expertise (up to 5 keywords): Protein expression; protein purification; Cell culture; flow cytometry; cloning