MASTER 2 BMC
PARCOURS GENOPATH
ANNÉE 2021-2022

Titre du sujet de stage :
Regulation of inflammation: *in vivo* (mouse) and cell Biology

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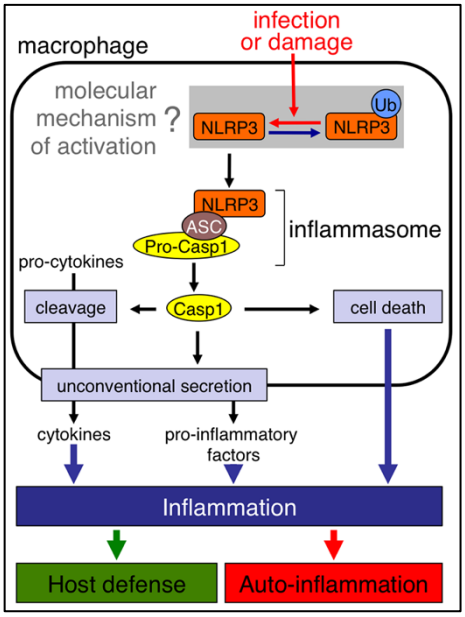
Sujet de stage :
Appropriate inflammatory response efficiently participates in protection against infections and mediates tissue repair. Adversely chronic or excessive inflammation fuels pathogenesis of a large set of conditions including gout and Alzheimer's diseases, type 2 diabetes, atherosclerosis and cancer, and causes deleterious genetic autoinflammatory syndromes that can be lethal. Therefore, we decipher the molecular mechanisms regulating inflammation in order to identify new therapeutic targets and genetic susceptibility factors for these multifactorial conditions.

At the molecular level, inflammation is triggered by the detection of pathogen- and damage-associated molecular patterns (PAMP and DAMP) through a repertoire of pattern recognition receptors (PRR). PAMPs are typically molecular structures essential and unique to microorganisms but absent from the host. DAMPs are inflammatory stimuli resulting from cellular damage or metabolic stress. PRRs are highly diverse in their specificities, subcellular localizations and downstream signaling pathways. We are currently focusing on NLRP3, a cytosolic PRR involved in numerous highly prevalent human pathologies aforementioned. In addition, mutations in *NLRP3* are associated with genetic autoinflammatory syndromes named CAPS. NLRP3 activation leads to the assembly of an oligomeric complex named inflammasome, serving as an activation platform for caspase-1. Caspase-1 protease then controls maturation and secretion of key proinflammatory cytokines and can trigger a proinflammatory form of cell death.



NLRP3 does not directly bind its diverse activators and we still know very little about the molecular mechanism of NLRP3 activation. **We discovered that inflammasome assembly is regulated through NLRP3 ubiquitination and identified a key ubiquitination site. We generated knock-in mice mutant for this ubiquitination site. Mutant primary macrophages from these mice show defect in negatively controlling the inflammasome and mutant mice are more susceptible to inflammatory models. These results confirm the key functional role of this modification in NLRP3 biology.** The recruited student will participate in characterizing the role and regulation of this ubiquitination in NLRP3 activation. The student will more deeply characterize the inflammatory phenotype of the mutant mice by *in vivo* study (with the help of an assistant engineer), as well as deciphering the signaling pathway in primary macrophages using a variety of approaches including cell biology (macrophages extraction from mice and purification of human monocytes, in vitro cell culture and treatment, siRNA transfection, immunofluorescence) and biochemistry (co-immunoprecipitation, western-blot, ELISA test).

Technologies utilisées :

- In vivo experiment on transgenic mouse model to study inflammation
- Cell biology: macrophages extraction from mice, in vitro cell culture and treatment, siRNA transfection, immunofluorescence and live automated microscopy
- Biochemistry: western-blot, ELISA test, co-immunoprecipitation

Mots clés :

Inflammation, biologie cellulaire, *in vivo*

Publications d'intérêt :

- Dufies O, Doye A, Courjon J, Torre C, Michel G, Loubatier C, Jacquel A, Chaintreuil P, Majoor A, Guinamard RR, Gallerand A, Saavedra PHV, Verhoeyen E, Rey A, Marchetti S, Ruimy R, Czerucka D, Lamkanfi M, **Py BF**, Munro P, Visvikis O, Boyer L : Escherichia coli Rho GTPase-activating toxin CNF1 mediates NLRP3 inflammasome activation via p21-activated kinases-1/2 during bacteraemia in mice. **Nat Microbiol.** 2021 doi : 10.1038/s41564-020-00832-5 PMID: 33432150

-Yuan J, Najafov A, **Py BF** : Roles of caspases in necrotic cell death. ***Cell***. 2016. 167(7):1693-1704.

-**Py BF**, Jin M, Desai BN, Penumaka A, Zhu H, Kober M, Dietrich A, Lipinski MM, Henry T, Clapham DE, Yuan J : Caspase-11 controls interleukin-1b release through degradation of TRPC1. ***Cell Reports***. 2014. 97(2):292-6.

- **Py BF**, Kim MS, Vakifahmetoglu-Norberg H, Yuan J : Deubiquitination of NLRP3 by BRCC3 critically regulates inflammasome activity. ***Molecular Cell***. 2013 49(2): 331-8.

- **Py BF**, Gonzalez SF, Long K, Kim MS, Kim YA, Zhu H, Yao J, Degauque N, Villet R, Ymele-leki P, Gadjeva M, Pier GB, Carroll MC, Yuan J : Cochlin produced by follicular dendritic cells promotes anti-bacterial innate immunity. ***Immunity***. 2013 38(5): 1063-72.