COLLEGE DOCTORAL BRETAGNE SCIENCES DE LA VIE ET DE LA SANTE

Year 2022-2023

THESIS TOPIC

Subject N° (to be completed by the ED):	**FUNDING:** ☐ **Requested (50%)** ☒ **Acquired. (50%)**	**Funding origin: ANR (Grant AID-G4-CSR)**
Thesis title: **How G quadruplexes control B-cell fate**		3 keywords: Genetics & immunity / immunopathology / immunotherapy
Research Unit / team: B Cell & Ig Remodeling Singularities (BIGReS) – UMR Inserm 1236		
Director's name: **Michel Cogné / Brice Laffleur**		Phone number: 02 23237004 / 0681262155 Email address: michel.cogne@inserm.fr / brice.laffleur@inserm.fr Year of HDR (s) : 1992 / 2022

Socio-economic and scientific context (approximately 10 lines):

During immune responses, activated mature B cells diversify immunoglobulin (*Ig*) genes through somatic hypermutation (SHM) and class switch recombination (CSR). In parallel, negative selection operates through multiple pathways to get rid of cells with low affinity, autoreactivity, or having lost their B cell receptor. Gene remodeling in activated B cells is promoted by the activation-induced cytidine deaminase (AID) *via* transcription-dependent cytidine deamination of single-stranded DNA targets which are rich into structured DNA such as G-quadruplexes (G4s). The precise role of G4s in transcription, AID targeting, CSR and associated illegitimate recombination remains poorly understood, although it could be of therapeutic interest. We additionally discovered G4 DNA at unexpected locations of Ig genes. Our expertise in molecular biology of normal and pathological B cell development, notably regarding the IgH super-enhancers which control the locus reshaping by AID, will help elucidating new roles of G4 DNA.

Working hypothesis and aims (approximately 8 lines):

Aim 1: Regulatory role of non-classical DNA repeats and G4s at the human IgH locus.
We have discovered new types of illegitimate recombination that lead to the deletion of critical sequences within the IgH locus. By using human cell lines and primary B cells, G4 ligands and tools for inhibition of G4-rich RNA processing, we will explore in-depth their regulatory roles on transcription, alternative splicing, and recombination of Ig genes.

Aim 2. G4-repeats and the "dark side" of AID as killer of IgH expression.
We demonstrated that besides CSR, AID-mediated locus suicide recombination (LSR) car also terminates IgH expression. Our working hypothesis is that LSR contributes to B cell homeostasis. We will focus on the furtive stage before negative selection and cell death and determine how the specific genetic events involving G4-rich repeats lead to major functional consequences regarding B cell homeostasis.

Main milestones of the thesis (approximately 12 lines):

- Pharmacological exploration of the role of G4s in switch transcription and CSR
- B cell stimulation, mapping of recombination breakpoints by LAM-HTGTS/NGS
- Generation of CRISPR variants deleted for G4 DNA of interest
- Evaluation of phenotype resulting from G4 experimental deletions
- RNA degradation machinery inactivation and physiological impact
- Repertoire of LSR human cells
- Single cell analyses of LSR-induced cells
- Analysis of data
- Writing of scientific reports / participation to scientific meetings (scientific communication)
- Preparation of post-doctoral projects
- PhD defense

Scientific and technical skills required by the candidate (2 lines):

Cellular and molecular biology / immunology / english speaking / bio-informatics (initial knowledge or at least agreeing for learning bio-informatics)

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3 publications from the team related to the topic (last 5 years):

RNA exosome drives early B cell development via noncoding RNA processing mechanisms.

B Laffleur[#], CR Batista, W Zhang, J Lim, B Yang, D Rossille, L Wu, J Estrella, G Rothschild, E Pefanis, Uttiya Basu.

Science Immunology, 2022, DOI: 10.1126/sciimmunol.abn2738 ([#] co-corresponding author)

Noncoding RNA processing by DIS3 regulates chromosomal architecture and somatic hypermutation in B cells.

B Laffleur, J Lim, W Zhang, Y Chen, E Pefanis, J Bizarro, CR Batista, L Wu, AN Economides, J Wang, U Basu.

Nature Genetics, 2021, DOI: 10.1038/s41588-020-00772-0

Locus Suicide Recombination actively occurs on the functional IgH allele in B-cells from inflamed human lymphoid tissues.

Dalloul I, Boyer F, Dalloul Z, Pignarre A, Lacombe G, Fest T, Chatonnet F, Delaloy C, Durandy A, Aldigier JC, Peron S, Cook-Moreau J, **M Cogné.**

Plos Genetics, 2019, DOI: 10.1371/journal.pgen.1007721

National and international collaborations:

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