No.	K22-1059	
研究課題名	Mesenchymal Stromal Cell Therapy to Prevent Neurodevelopmental Disorders related to Low-Birth-Weight	
研究代表者	COQ Jacques-Olivier (Centre National de la Recherche Scientifique・教授)	
研究組織	受入教員	長村 登紀子 (東京大学医科学研究所・准教授)
	分担者	Coq, Jacques-Olivier (Centre National de la Recherche Scientifique・Professor/Senior Reasearcher)
	分担者	Nagamura-Inoue, Tokiko (Department of Cell Processing and Transfusion・Professor)
	分担者	Tsuji, Masahiro (Kyoto Women’s University/Department of Food and Nutrition・Associate Professor)
	分担者	Mukai, Takeo (Department of Cell Processing and Transfusion・Associate Professor)
	分担者	Sei, Kenshi (Department of Cell Processing and Transfusion・PhD student)
	分担者	Vianefe, Maele (Aix Marseille University・Master 2 student)
	分担者	Satoshi Uematsu (Division of Metagenome Medicine・Project Professor)
	分担者	Kosuke Fujimoto (Division of Metagenome Medicine・Project Assistant)
	分担者	Vanessa Da Silva Souza (Institut des Sciences du Mouvement (ISM, UMR7287 CNRS/Aix-Marseille Université)・PhD student)
	分担者	Lauren Jantzie (Dept of Pediatrics and Dept of Neurology, John Hopkins University・Associate Professor)

(Form 2)

Principal Investigator
Coq JO (France)

IMSUT International Joint Usage/Research Center Project <International>

Joint Research Report (Annual/Project Completion)

Project Completion Report

Report

Principal Investigator	Institution: Institut des Sciences du Mouvement (ISM, UMR7287 CNRS/Aix-Marseille Université), Marseille, France
	Name: Coq, Jacques-Olivier (PhD, Senior Researcher at CNRS)
IMSUT Host Researcher	Division: Department of Cell Processing and Transfusion, Professor
	Name: Tokiko Nagamura-Inoue
Project Title	Mesenchymal Stromal Cell Therapy to Prevent Neurodevelopmental Disorders related to Low-Birth-Weight
Duration	From 04/01/2022 to 03/31/2025

Project Members

Position, Institution	Name	Position, Institution	Name
PI CNRS, France	Coq, J-Olivier	PhD student, AMU, France.	De Souza Silva, Vanessa
Associate Prof., IMSUT	Nagamura-Inoue, Tokiko	Assistant Prof. UTokyo	Mukai, Takeo
Prof, Kyoto Women's University/ Dept. of Food and Nutrition	Tsuji, Masahiro	PhD Student, IMSUT	Zhang, Teitei

Report (Progress Report)

Children with low birthweight (LBW) and/or experiencing fetal growth restriction (FGR) have higher risk for neurodevelopmental disorders, such as attention-deficit/hyperactivity disorder (ADHD) and autism spectrum disorder (ASD), associated with sensorimotor disturbances, several deficits in memory, learning and cognition, as well as anxiety and psychiatric disorders. Prof Tsuji (Kyoto) and Dr Coq (Marseille, France) developed a rat model of FGR and LBW, based on a stenosis of intrauterine arteries at embryonic day 17 (MIUH: mild intrauterine hypoperfusion). Rats born with LBW exhibit brain damage, inflammation and neurodevelopmental disorders (Delcour et al., 2011, 2012ab; Ohshima et al., 2016; Coq et al., 2018, 2020; Tsuji et al., 2018). Mesenchymal stromal cells (MSCs) produce cytokines and neurotrophic factors that suppress inflammation and exhibit immunomodulatory, neuroprotective, and neurorestorative properties. Prof Nagamura-Inoue (IMSUT host researcher) has succeeded to produce clinical grade umbilical cord-derived MSCs (UC-MSCs) (Nagamura-Inoue & Nagamura, 2023). Thus, we injected UC-MSCs intravenously at postnatal day 1.

Below are presented the main results of this collaboration (Tsuji et al., 2023; Chebbani et al., in preparation):

1) Compared to sham-control rats, MIUH with vehicle treatment induced:

a) reduced body weight from birth to adulthood in LBW rats,

b) reduced neuronal density in the hippocampus,

c) hyperexcitability in the lumbar spinal cord at early and adult stages,

d) delayed performances in early sensorimotor reflexes,

e) disturbed sociability and hyperactivity in open-field in adolescent and young-adult LBW males,
f) reduced anxiety in young-adult LBW rats and,
g) no significant changes in pro-inflammatory cytokine expression in the serum and cerebrospinal fluid at postnatal day 2, but increased expression of pro-inflammatory cytokines (9/27 tested cytokines) at postnatal day 14.

2) Compared to MIUH with vehicule, LBW rats treated early with MSCs showed:
a) relative improvement of body weight in adulthood,
b) restoration of the neuronal density in the hippocampus of LBW-MSC treated rats,
c) early and late restoration of excitability in the lumbar spinal cord attested by electrophysiology and western blotting of KCC2, which regulates chloride homeostasis and cell excitability,
d) the emergence of relatively typical sensorimotor reflexes in LBW-MSC treated pups,
e) partial improvement of sociability and slight but significantly positive impact on hyperactivity in open-field in young-adult LBW-MSC males,
f) partial restoration of anxiety in young-adult LBW-MSC rats in open-field and elevated plus maze testings that became comparable to that of control animals,
g) no changes in the expression of pro-inflammatory cytokines at postnatal day 2, but a slight decrease in the expression of pro-inflammatory cytokines (3/27 tested cytokines) at postnatal day 14.

These interesting results related to the early MSC therapy suggest restorations of normal organization of the sensorimotor circuitry and the early emergence of sensorimotor reflexes, along with partial normalization of some executive functions (sociability, inhibition and hyperactivity), indicating possible improvements in the hippocampal, cortical and cognitive networks. Thus, early administration of UC-MSCs show a great potential for improvements and restoration of neuroanatomical networks underlying sensorimotor, cognitive and executive functions. In addition, early UC-MSC administration reduced the expression levels of the few pro-inflammatory that were activated in MIUH-induced LBW pups. We postulate that MIUH only induced a mild perinatal inflammation that might be not enough for MSCs to fully exert their anti-inflammatory and neuroprotective capacities.

During the last visit of Prof Tsuji (Kyoto) and Dr Coq (France) at the IMSUT host researcher's lab of Prof Nagamura-Inoue in September-October 2024, we developed with a PhD student (Zhang T) two novel models of intrauterine inflammation, based on the prenatal injection of lipopolysaccharides (LPS, cell membrane components of *E. Coli* and *Salmonella*) or of polyI:C (polyinosinic:polycytidylic acid, an immunostimulant that simulates viral infection, leading to inflammation). After several trials, we successfully induced FGR since all pups from dams infected with either LPS or PolyI:C were born LBW. Offspring pups of the two models exhibited early and adult behavioral deficits, memory and social deficits, and anxiety that need to be consolidated, as well as the beneficial effects of early administration of UC-MSCs. Preliminary statistics indicate that these new results are very promising and encouraging. The inflammatory expression levels in these rat models of intrauterine inflammation are under progress.

In addition,to increase intrauterine levels of inflammation, we extended our model of MIUH by adding the intraamniotic injections of LPS at E17. The preliminary results show high levels of spontaneous abortion and difficulties of pups to survive. The survivors of MIUH+LPS treatment display higher levels of early sensorimotor delays and deficits that are not compensated over time. These survivors appear to have significantly greater deficits than MIUH-submitted pups in several tasks, such as hyperactivity in open-field, anxiety in elevated plus maze and greater memory deficits. These promising results need to be confirmed and the impact or early therapy with UC-MSC are ongoing, as well as levels of inflammation.

<Publications>

1) Chebbani R, Vianefe M, DaSilva Sauza V, Omar Toiouil N, Zhang T, Cayetanot F, Bodineau L, Kitase Y, **Nagamura-Inoue T, Tsuji M, Coq JO**: Promising restoration of neurodevelopmental disorders related to low birthweight after early therapy with umbilical cord-derived mesenchymal stromal cells, to submit in Stem Cells
2) **Tsuji M**, **Mukai T**, Sato Y, Azuma Y, Yamamoto S, Cayetanot F, Bodineau L, OnodaA, **Nagamura-Inoue T, Coq JO**. Umbilical cord-derived mesenchymal stromal cell therapy to prevent the development of neurodevelopmental disorders related to low birth weight. Sci Rep. 2023, 7;13(1):3841. doi: 10.1038/s41598-023-30817-3.
3) **Mukai T**, Galindo R, **Coq JO**. Neonatal and pediatric brain injury: novel therapeutics and perspective, Front Pediatr, 2023, 16:11:1210749, doi 10.3389/fped.2023.1210749.

<Patent Applications>

Frequency of visits to IMSUT (Details are on Exhibit)

In 2020, the researchers could not visit IMSUT.

*Please include even visits without travel allowances.

*For this fiscal year only, if the project members could not visit IMSUT due to the pandemic of COVID-19, please report the number of times of online meetings, discussions via Email or communication tools such as Slack etc.. *For the "Age," please select from the followings: 35 or younger / 36 to 39 / 40 or older. Information about sex, age shall be used only for the purpose of writing statistical reports submitted to the Ministry of Education, Culture, Sports, Science and Technology, and for no other purpose what so ever. *Please write the details in the "Exhibit" section.

Name	Position, Institution	Sex	Age	Total Days of Visits
Coq, J-Olivier	Senior researcher	**Male**	**40 or older**	September 15th to October 5th, 2024

Name	Position, Institution	Sex	Age	Number of Times of Online Meetings
Coq, J-Olivier	PI	**Male**	**40 or older**	ZOOM or on-site meeting x 3
Tsuji, Masahiro	Professor	**Male**	**40 or older**	ZOOM meeting x 3
Nagamura-Inoue, Tokiko	Associate Prof.	**Female**	**40 or older**	ZOOM meeting/Month x 1
Zhang, Teitei	PhD	**Male**	**35 or younger**	ZOOM meeting/Month x 1

Name	Position, Institution	Sex	Age	Number of Times of discussions via Email or communication tools such as Slack *Please write about the discussions via Email or communication tools such as Slack with project members since April 1st, 2022.
Coq, J-Olivier	PI	**Male**	**40 or older**	Email x 37
Tsuji, Masahiro	Professor	**Male**	**40 or older**	Email x 37
Nagamura-Inoue, Tokiko	Associate Prof.	**Female**	**40 or older**	Email x 15
Kitase, Yuma	Lecturer	**Male**	**40 or older**	Email x 26
Zhang, Teitei	PhD student	**Male**	**35 or younger**	Email x 8

Usage of Facilities

Name of Facility	Machines	Number of Times	Number of hours
FACS Core Laboratory	FACS Aria (BD) FACS Calobur(BD)	N/A	
Medical Proteomics Laboratory	Orbitrap QSTAR Elite	N/A	
Imaging Core Laboratory	Zeiss Multiphoton Microscopy (LSM710NLO)	N/A	
Gene Manipulated Mouse Section	Creation and cryopreservation embryo of Knockout mouse	N/A	
Human Genome Center	Supercomputer	N/A	
Amami Laboratory of Injurious Animals	Experimental lab	N/A	120
Other Nagamura-Inoue's laboratory	• FACS CantoII • IMSUT CORD (injections of UC-MSCs)	• 1 • 2	8

Usage of Scientific Resources *Please enter'0' or 'N/A' if you have not used any facilities.

Name of Scientific Resource	Number of Times
Serum (BioBank Japan)	N/A
DNA (BioBank Japan)	N/A
Knockout mouse	N/A
Pathogenic bacteria	N/A
Other: human umbilical cord-derived mesenchymal stromal cells from IMSUT CORD	1

Usage of Database *Please enter'0' or 'N/A' if you have not used any databases.

Name of Database	Number of Times
N/A	

Exhibit

Details of the Visits to IMSUT etc.

*Please include even visits made without travel allowances.
*Please write about online meetings and discussions via Email or communication tools such as Slack as well.
*Please list by person.
*Please add new rows as needed.

Name	Position
Nagamura-Inoue, Tokiko Tsuji, Masahiro Coq, J-Olivier Kitase, Yuma Takeo Mukai Zhang, Teitei	Professor Professor PI Lecturer, Narita Univ Assistant Prof. PhD student

Schedule	Purpose/Description
03/12/2025 18:00-19:00 (Japanese time) (ZOOM)	Coq, Tsuji and Kitase to plan MIUH+LPS experiments
04/18/2025 18:00-20:00 (Japanese time) (ZOOM)	