This report should be submitted within 2 weeks after you return to Japan. Please do not change the formatting

(Abroad) Domestic) Internship report form (Student) 2023.09. (Year/Month/Day)

Name	Erdenebat Temuulen
Laboratory	Veterinary Hygiene
Year (Grade)	D3
Internship institution	Laboratory of Persistent Viral Diseases TSE/Prion and Retroviral Pathogenesis Section, Rocky Mountain Laboratories, NIAID, NIH, USA
Internship period	Internship period: 07/24/2023 - 08/15/2023 Departure Date from Sapporo: 07/23/2023, Arrival Date in Sapporo: 08/25/2023) July 23: Dep. CTS 10:30 - HND12:10/ NRT16:35-DEN12:15/ DEN14:46- Arr. MSO 16:56 ＊ From Aug 16, I visited Colorado State University to have academic exchanges with Drs. Julie Moreno and Mark Zabel under the support of the Hokkaido University Dx fellowship program Aug. 16: MSO 10:41 – DEN 12:42 Aug. 24: DEN 11:50 – NRT 14:40 Aug. 25: HND 17:50 – CTS 19:20
Purpose	

- The reason why you chose this institute

TSE section at RML is one of the highest reputed research groups in this field and is interested in understanding how neuroinflammation and glial cell activation (astrocytes and microglia) influence pathogenesis and neurodegeneration in prion diseases TSE section at RML has a long history of receiving Ph.D. students from Hokkaido University to collaborate and to let them acquire knowledge and technical skills in studying prion diseases. Based on the long-lasting interaction, the TSE section and my laboratory have a good relationship with mutual trust and have done several collaborative studies.

I have been looking for an opportunity to learn RNA-seq data analysis to apply my research. Dr. James Carroll, whom I have been contacting, is now analyzing the roles of microglia and astrocytes in prion diseases using RNA-Seq analysis. Application of the RNA-seq to my research is invaluable for an understanding of the relationship between glial cells and neurons, which provides important information for the pathophysiology of prion diseases. Also, I can use RNA-seq data

analysis for any kind of molecular study in my future studies. Therefore, I decided to do my internship at the TSE section under the instruction of Dr. Carroll

- Result of the activity （about 800 words、provide photos, tables and figures that clearly show the activities during the period）

Lifestyle in Hamilton town

Hamilton is a small town in Southwest Montana's pretty Bitterroot Valley area. About 5000 people live there, and it's part of Ravalli County. People in Hamilton are friendly and hospitable, and the town is quite safe, especially when compared to big cities in the US.

When I visited RML for a short time, I needed a place to stay. It was a bit tricky because I had to find a place that cost less than 17400JPY per night. During the summer, many tourists come to Hamilton for activities like fishing, hiking, and camping. This makes hotels more expensive and harder to book. Luckily, I knew some researchers at RML who helped me find a rental room.

Staying in the rental room had a lot of benefits. I got to meet new people who lived nearby, and I could make my own meals, which was cheaper than eating out. Plus, the rental was close to RML, which was really convenient.

Activities at RML

My laboratory work at RML was aimed at changes in astrocyte function and their reactive phenotype by monitoring the expression of astrocyte-specific genes along with markers of astrocyte reactive states in a culture of mouse cortical astrocytes with prion 22L strain conjugated with AF-488 (22L+AF488). Cortical glial cells were isolated from three-day-old mouse brain pups and plated in a T75 flask. Astrocytes were isolated from glial cells using CD11b Microbeads (Miltenyi Biotec) and magnetic-activated cell separation (MACS). Then astrocyte cultures were inoculated with 22L+AF488 for analysis of astrocyte function and their reactive phenotype. Analysis of phagocytosis gene activation of astrocytes at 6 and 24 hours post-inoculation showed no significant changes in increase or decrease in gene activation. Immunofluorescent analysis showed that more PrP^{Sc} particles were observed near the nuclei of astrocytes at 24 and 48 h than 6 h post-inoculation of 22L+488.

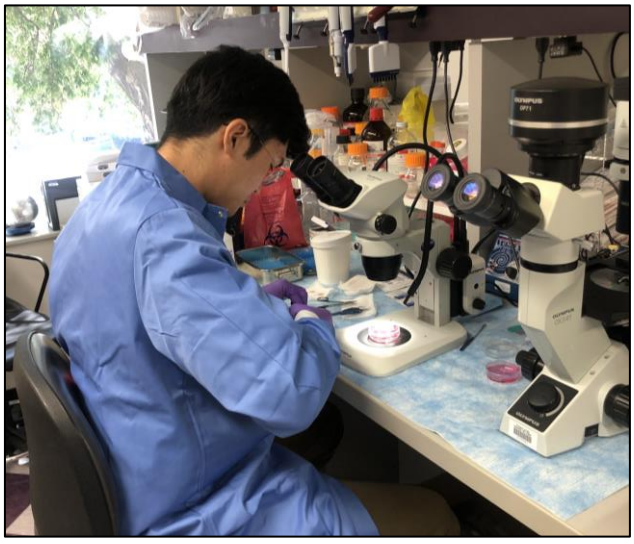
Besides the laboratory work, I participated in several seminars provided

by RML. The Laboratory of Neurological Infection and Immunity section holds Work In Progress (WIP) every Monday morning to share their laboratory's ongoing study. I joined 3 WIP seminars which gave me insight into the laboratory works of laboratories that study prion diseases. These seminars enlightened my knowledge of the background of prion diseases and I learned the efficient way to deliver the laboratory work and results of the research.

Activities outside of RML

Hamilton is surrounded by beautiful mountains Bitterroot, rivers, and lakes. It is a good place for hiking, fishing, camping, and hunting. My host Dr. Carroll took me to hiking every weekend during my stay in Hamilton. A researcher's job requires sitting on a chair all the time at the laboratory, but hiking helps the researchers stretch the body and refresh the mind for further work.

Daly Days is an annual traditional holiday in Hamilton. Marcus Daly was a businessman known as one of the three Copper Kings of Montana. He built his summer family mansion in Bitterroot Allay back in the nineteenth century and it was the beginning of the current Hamilton City. Now people are celebrating Daly Day between July 28th to 29th every day. I had a chance to join this holiday and it helped me to understand the local customs, traditions, and history of Hamilton city.



1. Training for isolation of cortical glial cells from three-day-old C57BL/10 mouse brain pups.

2. Overlook Blodget Trailhead. My 3rd hiking in Hamilton with my Hokkaido University T-shirt.

3. At Daly Day festival. From the left Dr. James Carroll (my host) and Dr. Cathryn Haigh.

- What do you think the positive impact of the activity will have on your further career path?

My research theme in Ph.D. study is “Brain region-specific mechanism of prion propagation and neuronal death” which is divided into two major parts. Firstly, now, I am using both mice and neuronal culture to analyze the neuronal cell-type tropism of prion propagation. Near soon, I start the second part of my study: the involvement of astrocytes in region-dependent neuronal loss in prion diseases. During my internship at RML, I learned RNA-seq-related skills that will be useful for the second part of my study. Moreover, I had a good opportunity to broaden my connections with experts in different disciplines who work at the TSE section which is composed of various researchers in the fields of cellular biology, structural biology, pathology, neuroscience, and immunology.

As Ph.D. students, we're always planning our careers after finishing our studies. Personally, I'm really interested in studying prion diseases further. I found out that the TSE section at RML would be a great place for me to work on prion

research. So, I reached out to ask if there's a postdoc position available and received a positive answer. I'm excited to join in, share what I know, learn from other researchers, and keep exploring the mysteries of prion diseases.

- Advice for your junior fellows

I'd like to share three tips with fellow junior researchers:

Preparation is Key: Before you start your internship in a lab, take time to read and understand their recent research papers. This will give you a strong foundation and context for your work.

Show Your Potential: If you're eyeing a postdoc position where you're interning, don't be shy to showcase your skills. Demonstrate your capabilities and contributions—it can leave a lasting impression.

Cultural Adaptation: Labs have unique cultures. While your current lab might differ from your internship lab, quickly adjusting to their way of doing things fosters friendly connections and collaboration. Embrace the culture!

Approval of supervisor	Institution・Official title・Name Laboratory of Veterinary Hygiene, School/Faculty of Veterinary Medicine Prof. Horiuchi Motohiro

※1 Send the electronic file to the WISE Program Office
※2 Attach a copy certificate of the content of internship activity that is prepared by the counterpart at the internship institution (any form with a signature of the counterpart).
※3 The Steering Committee for the WISE Program will first confirm the content of this report and report will be forwarded to the Educational Affairs Committee for credits evaluation.

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