Project information

Project title

Exposure to pollutants and quantification by qPCR of the IFN-gamma gene expression in arctic wildlife – glaucous gull, hooded seal and polar bear

Year

2012/2013

Project leader

Jacques Godfroid, NVH

Participants

- Jacques Godfroid (project leader)
- Ingebjørg Nymo, Norwegian School of Veterinary Science (NVH)
- Geir Gabrielsen, Kjetil Sagerup, Norwegian Polar Institute (PI)
- Eldjørg Sofie Heimstad, Norwegian Institute for Air Research (NILU)

Flagship

Hazardous substances, Theme: Animal health and ecosystem

Funding Source

Fram Centre

Summary of Results

In order to achieve the goals of the project, three Work Packages (WP) had to be undertaken:

WP 1. Field work (PI):

Samples taken to measure the expression of cytokines (among which Interferon gamma) by RT-PCR, have to be taken and put in special buffer in order to be processed further in the lab. Two types of sampling were done: blood sampling and also, in the case of glaucous gull, lethal sampling (spleen). In parallel blood samples have been taken in order to measure total antibody titers as well as antibodies against specific pathogens.

Fire perioder med feltarbeid har vært gjort for innhenting av prøver til INF-gamma.

Juni 2011: 17 prøver polarmåke (blod + plasma), 14 dager feltarbeid Anja Haugerud, Kjetil Sagerup

August 2011: 10 prøver polarmåke (milt), 3 dagers feltarbeid Silje Mæhre, Kjetil Sagerup.

August 2011: 13 prøver ærfugl (blod), feltarbeid Ireen Vieweg, Mikko Vihtakari.

April 2012: 10 prøver isbjørn (blod), 1 mnd feltarbeid, Heli Routti, Jon Aarst,

I tillegg har vi vært på felt mai/juni 2012 i Ny-Ålesund uten å hente inn nye prøver til INF-gamma. Prøver til miljøgifter, spesielt PFAS (fluor), og hormonpåvirkning ble hentet inn.

WP 2. Setting-up of the RT-PCR technique in the laboratory:

This has been a very demanding process, necessitating a lot of control and validation work in the laboratory. A lot of emphasis has been put on the setting up and the validation of the technique in the mouse model. The technique is implemented in the laboratory and results have been presented at the 4th Norwegian Environmental Toxicology Symposium - October 2012, Tromsø, Norway. (Poster in annex). Of importance, the gene expression of other cytokines has also been validated and can also be performed Matandis Mutadis on cDNA extracted from immunological cells from different animal species.

Several steps are needed to perform the techniques on field samples. The first step, i.e., the extraction of good quality RNA has been done for the different samples. Excellent quality RNA has been extracted from both blood and spleen and has been reverse transcribed into cDNA for the glaucous gull samples and for the hooded seal samples.

The next step, i.e., the analysis per se of the expression of Interferon gamma will be performed for the glaucous gull samples) in November-December 2012. The analysis of polar bear and hooded seal samples will be performed during the first trimester of 2013.

WP3. Analysis for pollutants in field samples

Field samples, collected by PI have been transferred to NILU and analysis will be performed in November-December 2012.

Published Results/Planned Publications

Work performed at NVH has been presented to the following conferences:

- Ingebjørg H. Nymo, Carlos G. das Neves, Berit Djønne, Birte Graeber, Eva Breines, Ellinor Hareide, Elisabeth Lie, Vidar Berg, Morten Tryland, Jacques Godfroid. DOES EXPOSURE TO PCB 134 ALTER THE IMMUNOLOGICAL RESPONSE OF BALB/C MICE TO A PATHOGEN? [poster presentation]. The 4th Norwegian Environmental Toxicology Symposium – Emerging challenges and threats in the Arctic. October 2012, Tromsø, Norway.

- Ingebjørg H. Nymo, Berit Djønne, Birte Graeber, Elisabeth Lie, Vidar Berg, Morten Tryland, Jacques Godfroid. EXPERIMENTAL INFECTION OF BALB/C MICE WITH B. PINNIPEDIALIS FROM HOODED SEAL (CYSTOPHORA CRISTATA) AND CONCURRENT EXPOSURE TO PCB 153 [poster presentation]. The 61st international conference of the WDA and the 10th biannual conference of the EWDA. July 2012, Lyon, France.

Work performed at NVH will also been presented to the following conferences

- Ingebjørg H. Nymo, Carlos das Neves, Vidar Berg, Elisabeth Lie, Birte Graeber, Eva Breines, Ellinor Hareide, Berit Djønne, Morten Tryland and Jacques Godfroid. BRUCELLA PINNIPEDIALIS HOODED SEAL (CYSTOPHORA CRISTATA) STRAIN IN THE MOUSE MODEL FOLLOWING EXPOSURE TO PCB 153 [oral presentation]. The 65th Brucellosis Research Conference, Chicago, IL. December 2012, Chicago, USA.

Ingebjørg Nymo will hand in her PhD Thesis in Mai 2013. One publication related to the mouse model, realized thanks to the support of the FRAM Center, will be part of her dissertation. Two other publications are planned, based on the results generated from the field samples.

Communicated Results

Results obtained on the combine effect of exposure to POPs and infection with *Brucella*-bacteria in the mouse model, were presented during the FRAMdagen in November 2012.

Interdisciplinary Cooperation

The project is a true collaboration between infection biologists and toxicologists. Both disciplines were actively involved in the conception of the project.

The rationale of this project can be summarized as follow: the analysis of immune mediators is of value, if linked, either correlationally or mechanistically, to functional immune endpoints in order to get insight into the physiological or pathological consequences of the altered expression of the mediators.

Therefore, the combined effect of exposure to PCB153 and infection with *Brucella*- bacteria in the mouse model is an important validation study. Indeed, only combined analysis of POP exposure, immunological parameters and resistance to an infection *Brucella*-bacteria will allow us to draw conclusions related the effects of pollutants to the emergence of diseases.

Budget in accordance to results

- **In which way has the funding from the Fram Centre helped the project?**

The financial support provided by the FRAM Center allowed us to start this "Proof of Concept" project**.**

- **Did the Fram Centre funding act as a sufficient boost for completing the project through other sources of funding?**

The Fram center’s contribution was really necessary to allow the purchase of expensive reagents. Some travel arrangements Tromsø/Oslo were also made possible by the Fram Center funding. To summarize, the Fram Center funding was indispensable.

Could results from the project be subject for any commercial utilization

No

Conclusions

a) **Indicate future research and/or perspectives which the project results have led to:**

In vitro model of infection in macrophages (from mouse, human and seal origins) is now implemented at NVH (publication submitted).

We are planning further experiments with combined *in vitro* exposure of macrophage to pollutants and infection with *Brucella*-bacteria in order to analyze the immunotoxicity on metabolic pathways important for resistance to bacterial infection.

b) List and describe new methods or techniques that have been developed during the project or that the project has revealed a need for:

The RT-PCR technique is now implemented for the analysis of the transcription of genes of interest. The technique can be used for the expression of other cytokines'genes and for other genes of interest, for which close collaboration between NP, NILU and NVH will be developed in the next coming years.