Characterization of the immunogenome in domestic, wild, and ancient (domestic, wild) Old World camelids

We investigated the variation in the major histocompatibility complex (MHC) of Old World Camelids. In this group, so far very little is known about this important complex genetic region. Due to its crucial role in immune responses, MHC class II genes DRA, DRB, DQA, DQB and DYA were studied. Like other studies, we focused on their functionally most important domain, exon 2, encoding the antigen binding site (Janeway et al. 2001).

Thanks to the newly published whole genome sequences of camels (Wu et al. 2014), we were able to establish the general genomic structure of the camel MHC region. The order of regions "Centromere - Class II – Class III – Class I" and the presence of the DYA class II region revealed high genetic similarity to the MHC in cattle and related species. Therefore, the first planned objective of the project can be considered as accomplished.

By using PCR and cloning combined with Sanger and next generation sequencing, exon 2 genomic and haplotype sequences of all abovementioned loci in Camelus bactrianus, Camelus dromedarius and Camelus ferus were retrieved. For unknown reasons, the DYA gene could be amplified only in bactrian and dromedary camels. This result needs further investigations.

Very low level of polymorphism in all three species and high extent of allele sharing across them were found. For the DRA locus, 3 different exon 2 haplotypes shared across all three species sequences obtained from 109 samples. Surprisingly, only three different exon 2 DQA haplotypes were observed in n=76 camels. The DQB locus must be amplified with zoo-primers GH28/GH29 (Gyllensten et al. 1990) and therefore, just partial sequences were obtained. Similarly to DQA, only two exon 2 haplotypes were observed. The identification of DYA genomic sequences previously described only in cattle and sheep in the MHC of Camelids is one of important discoveries of this project. Camels are the only non-ruminant species where this genomic region was identified. All bactrian camels analyzed had a stop codon in the exon 2 reading frame, while in the dromedaries, a single nucleotide variation (SNP) with one potentially functional (full-length) allele and with one allele containing the stop codon were observed at this nucleotide position.

In conclusion, basic characteristics of the functionally important genetic variation in camel MHC class II genes were provided based on analyses of tens of animals of all three species

originating from different geographical regions. This means that the second goal of our project was completed. As planned in the original 2-year version of our project, it created an opportunity for a follow-up study of MHC diversity in various camel populations. Based on these results, we are planning to submit a follow-up application to Aktion.

The third goal of the project was to study the genetic variation in MHC class II genes in ancient camels specimens. Besides the complexity of MHC genes as a genetic marker, the recovery of the endogenous ancient DNA from degraded and chemically modified samples from desert regions still remains the main challenge. Here, we show our progress in obtaining endogenous camel DNA (nuclear and mitochondrial) from poorly preserved specimens of dry and arid regions. The DNA extraction from the total number of 23 samples (15 early domestic and 8 wild dromedary) was performed in a designated ancient DNA laboratory in Munich (LMU university) using two different extraction methods (Rohland et al. 2007 & Dabney et al. 2013). The historic dromedary specimens were collected from a sampling site in Jordan (Aqaba), Syria (Apamea, Palmyra), Turkey (Sagalassos). The age of the domestic samples approximately ranged from 1700-500 years ago based on archaeological records; however the wild specimens are dated to older than 3000 years ago. We attempted to sequence the exon 2 of the two MHC genes (DQA 249bp, and DRA 246bp) from both domestic and wild camel specimens. Each gene was sequenced in two overlapping regions. The fragment lengths of 138 bp and 237 bp were successfully amplified from DQA and DRA genes respectively. In 15 domestic samples, we had a success rate of 20% (3 samples) of obtaining endogenous camel sequence. In 70% of the PCR products the obtained sequences were non-camel MHC sequences. The MHC genes are highly conserved in mammals. Considering the ubiquity of the contaminant mammalian DNA, and the scarcity of the endogenous ancient DNA, a contamination of the samples analyzed is really likely. Regarding the wild samples, we successfully amplified 500 bp of the d-loop region of mtDNA; however obtaining the endogenous nuclear marker (MHC) was 100% unsuccessful in all wild samples. This is probably due to the age (>3000 years ago), to the level of the preservation of wild specimens, and to the scarcity of nuclear DNA in comparison to mtDNA. Nevertheless, our results are very valuable since they showed that even at extremely unfavorable conditions, ancient MHC can be studied.

All the results listed above were included into a manuscript, which is just finalized by the two groups and which is anticipated to be submitted in February 2015. The title and its abstract are attached to this report.

Conclusions:

1. The scientific contents of the project.

We successfully met out initial goals described in the research proposal (AKTION_Austria_Czech_FWF 68p7). We were able to determine the overall organization of the MHC region in camels, we determined the extent of polymorphism of their exon 2 genomic sequences in the most important class II genes and we have obtained unique information about the MHC in old Camelids by analyzing their ancient DNA.

2. The major goals of the AKTION programme

We are convinced that we also met successfully all objectives of AKTION. We have established a fruitful collaboration between two teams with complementary expertise. The Czech team took benefit of sharing precious samples and full genome data, as well of expertise in ancient DNA and bioinformatic analysis of nucleotide sequences. On the other hand, most of the molecular genetic analyses were performed in the Czech laboratory. Travel costs were used for coordinating meetings and especially for exchanging two PhD students (one from each side). The results lead to producing a manuscript with priority results.

References

1. Dabney, J., Knapp, M., Glocke, I., Gansauge, M. T., Weihmann, A., Nickel, B., ... & Meyer, M. (2013). Complete mitochondrial genome sequence of a Middle Pleistocene cave bear reconstructed from ultrashort DNA fragments. *Proceedings of the National Academy of Sciences*, *110*(39), 15758-15763
2. Gyllensten, U. B., Lashkari, D., & Erlich, H. A. (1990). Allelic diversification at the class II DQB locus of the mammalian major histocompatibility complex. *Proceedings of the National Academy of Sciences*, *87*(5), 1835–1839
3. Janeway, C. A. Jr, Travers, P., Walport M., et al. Immunobiology: The Immune System in Health and Disease. 5th edition. New York: Garland Science; 2001. The major histocompatibility complex and its functions. Available from: http://www.ncbi.nlm.nih.gov/books/NBK27156/.
4. Rohland, N., & Hofreiter, M. (2007). Comparison and optimization of ancient DNA extraction. *Biotechniques*, *42*(3), 343.
5. Wu, H., Guang, X., Al-Fageeh, M. B., Cao, J., Pan, S., Zhou, H., Wang, J. (2014). Camelid genomes reveal evolution and adaptation to desert environments. *Nat Commun*, *5*. Dostupné z http://dx.doi.org/10.1038/ncomms6188.

Brno, 27th January 2015

Prof. MVDr. RNDr. Petr Hořín, CSc., VFU Brno
Dr. med. vet. Pamela Burger, Vetmeduni Vienna