Szent István University
Postgraduate School of Veterinary Science

The transcriptional regulation of the bovine neonatal Fc receptor

Summary of thesis

written by

Márton Doleschall

2007

Szent István University
Postgraduate School of Veterinary Science

Supervisor and advisors:

…….
Dr. Imre Kacskovics, Ph.D.
Department of Physiology and Biochemistry, Faculty of Veterinary Science, Szent István University (until September 2006)
Department of Immunology, Institute of Biology, Faculty of Science, Eötvös Loránd University (from September 2006)

Prof. Dr. Gabriella Sármay, Ph.D., D.Sc.
Department of Immunology, Institute of Biology, Faculty of Science, Eötvös Loránd University

Prof. Dr. László V. Frenyó, Ph.D.
Department of Physiology and Biochemistry, Faculty of Veterinary Science, Szent István University

…….
Márton Doleschall

The neonatal Fc receptor (FcRn) is composed of an α-chain and a β_2-microglobulin (β2m). This receptor has been detected in the bovine mammary gland, small intestine, lower respiratory system, and endothelial cells. While epithelial FcRn is involved in IgG transport through these barriers, this receptor is responsible for regulating the IgG catabolism in the capillary endothelial cells. Due to these crucial immunological functions, the gene regulation of the bovine FcRn (bFcRn) may contribute to the immune homeostasis. Although gene expression is controlled at multiple levels, one of the most important is the regulation of transcription, whereby an mRNA is produced from a gene to encode a particular protein. The transcriptional regulation is achieved by the coordinated cooperation of transcription factors and their binding sites located on the transcription regulatory DNA region of a particular gene. In order to fulfil their role, transcription factors can bind transcription factor binding sites on DNA in sequence-specific manner and can influence the transcription of genes. Accordingly, the sequences of the human and mouse FcRn α-chain transcription regulatory DNA region have been published and their preliminary examination has been performed, although their transcriptional regulation has not been adequately unravelled in relation to immunologically relevant transcription factors.

The primary aim of our study was to investigate the transcriptional regulation of the bFcRn α-chain. As FcRn is a heterodimer, the

transcriptional co-regulation of the bovine β2m (bβ2m) and FcRn α-chain was also analyzed. To perform these aims, the methods for the investigation of the transcription regulatory DNA region, such as luciferase reporter gene assay, gel retardation assay and site-directed mutagenesis, had to be set up in our laboratory. During the experimental work of the present study, the demand arose to generate a fully bovine-specific cell model of the transcription factor NFκB gene regulation, therefore the cloning and functional characterization of the bovine p65 (bp65) subunit of NFκB was added to the original aims.

The present study was mainly based on two methods, known as luciferase reporter gene assay and gel retardation assay. The reporter gene assay accompanied by the site-directed mutagenesis represents the single technique, which can reveal the contribution of an individual transcription factor binding site to the transcription of a gene. The definition of gel retardation assay collects highly similar techniques that are suitable for the *in vitro* investigation of the interaction between the transcription factor and its binding site.

In order to reveal the transcriptional regulation of the bFcRn transcription, the transcription regulatory DNA region of the bFcRn α-chain gene was sequenced and isolated. In the luciferase reporter gene assay of human and bovine cell models, the bFcRn α-chain transcription regulatory DNA region was induced by the transcription factor NFκB, which plays an indispensable role in

controlling both innate and adaptive immunity among other crucial regulatory functions. Three functional κB binding sites were identified in the transcription regulatory DNA region using site-directed mutagenesis accompanied by luciferase reporter gene assay. It was verified that these κB sites were responsible for the complete NFκB responsiveness of the bFcRn transcription regulatory region. The κB binding sites were also tested in gel retardation assay verifying their binding ability to NFκB with p65 content. These *in vitro* findings indicated, in accordance with the present *in vivo* data, that the bFcRn was under the control of this important transcriptional pathway, which is activated during infection and inflammation.

The β2m is a chaperone of FcRn and other MHC class I (-like) proteins ensuring the appropriate function of these molecules. To fulfil this function, it is expressed ubiquitously under constitutive and immune-related transcriptional control. The transcription factor binding sites of the β2m transcription regulatory DNA region have been experimentally well characterized in human, and it has been found that a κB site and an ISRE site are responsible for the immune-related transcriptional regulation of this gene. (The ISRE site is bound by the members of the immunologically relevant IRF transcription factor family.) The 5'-flanking sequence of the bβ2m was isolated and cloned in order to assess its immune-related gene expression in relation to FcRn. Although ISRE was conserved in the cattle, there was a deletion in the bβ2m κB site compared to the

human orthologue, and there was no NFκB responsiveness of the bβ2m transcriptional regulatory DNA region in luciferase reporter gene assay of human and bovine cell models. Nevertheless, the deleted bβ2m κB site did bind the NFκB complex with p65 content in gel retardation assay rendering these *in vitro* results controversial. *In vivo* data from analyzing the mRNA level of the bβ2m upon LPS induction are also contradictory, therefore the NFκB inducibility of the bβ2m transcription regulatory region cannot be deduced from the present data. The functionality of the conserved ISRE site was confirmed *in vitro* by gel retardation and luciferase reporter gene assays, thus the bβ2m ISRE site mediated the IFN-γ induction through the IRF family members similarly to its human orthologue, and there were no differences in the ISRE-mediated transcriptional regulation of this gene in cattle and human.

In order to establish a species-specific system that can be used to analyze gene regulation in bovine, the full length coding sequence of the bp65 subunit of NFκB was isolated and cloned. The cloned bp65 was expressed in mammalian cells, and it induced the NFκB-specific luciferase reporter gene expression. Using gel retardation assay, it was demonstrated that the cloned bp65 did bind to the consensus κB sequence. The comparison of bp65 with its human and mouse orthologues at amino acid level showed high homology in both the DNA-binding domain, known as Rel homology domain (RHD) and the transactivation domain (TAD). The phylogenetic analysis at

DNA level provided a new insight into the evolution of the NFκB family, and it was able to resolve the topology of the mammalian p65 molecules. Although the RHD was conserved in vertebrates, the TAD sequences deviated from each other, and showed faster molecular evolution than RHD sequences, which might indirectly result in the modification of NFκB immune functions.

New scientific results

It has been proven using luciferase reporter gene assay that the transcription of the bovine FcRn α-chain is under the control of p65 in mammalian- and bovine-specific cell models. Three κB binding sites have been identified in the *cis*-regulator region of the bFcRn α-chain using site-directed mutagenesis accompanied by luciferase reporter gene assay and gel retardation assay, which are completely responsible for the p65 responsiveness of the bFcRn α-chain, and can bind the NFκB complex with p65 content.

It has been confirmed by luciferase reporter gene assay that the bovine β2m ISRE binding site mediates the IRF1 induction in mammalian cell models similarly to its human orthologue. In addition, the bβ2m ISRE site can bind the IFN-γ specific complex in EMSA.

The bovine p65 cDNA sequence has been cloned and its basic transcription factor specific features have been characterized by luciferase reporter gene and gel retardation assays.

The phylogenetic analysis has provided a new insight into the evolution of the NFκB family, and it has resolved the topology of the mammalian p65 molecules. Genetic analysis has proven that the mammalian TAD sequences have undergone faster molecular evolution than RHD sequences.

References

Kis Zs., Mayer B., Juhász V., Doleschall M., Frenyo L. V. and Kacskovics I. (2004) A szarvasmarha neonatalis Fc receptor (bFcRn) tőgybeli expressziója és IgG kötő képessége. [Expression in the udder and IgG binding capacity of bovine neonatal Fc receptor (bFcRn).] *Magyar Állatorvosok Lapja* 10, 598-605

Doleschall M., Zhao Y., Mayer B., Hammarstrom L. and Kacskovics I. (2005) Isolation of the gene encoding the bovine neonatal Fc receptor. *Vet Immunol Immunopathol* 108, 145-150

Mayer B., Doleschall M., Bender B., Bartyik J., Bosze Z., Frenyo L. V. and Kacskovics I. (2005) Expression of the neonatal Fc receptor (FcRn) in the bovine mammary gland. *J Dairy Res* 72, 107-112

Kacskovics I., Mayer B., Kis Zs., Doleschall M., Bősze Zs. and Bender B. (2005) Az IgG transzportját és katabolizmusát szabályozó FcRn génregulációs elemzések szarvasmarhában. [Investigation on the contribution of the FcRn gene regulation for the IgG transport and catabolism in cattle.] *Magyar Tudomány* 6, 714-23

Doleschall M., Mayer B., Cervenak J., Cervenak L. and Kacskovics I. (2007) Cloning, expression and characterization of the bovine p65 subunit of NFκB. *Dev Comp Immunol* 31, 945-961

Doleschall M. and Kacskovics I. (2007) Az NFκB és transzkripciós faktor tulajdonságainak vizsgálata [The NFκB and the methods for the investigation of its transcription factor features.] *Magyar Immunológia* (accepted)

Acknowledgement

I would like to express my sincere gratitude to my supervisor, Dr. Imre Kacskovics, for the important support throughout my doctoral work. His integral view on research and his logical way of thinking have been of great value for me. I wish to thank my advisors, Prof. Dr. Gabriella Sármay (Eötvös Loránd University, Budapest, Hungary) and Prof. Dr. László V. Frenyó, who kept an eye on the progress of my work. I warmly thank my close colleagues, Judit Cervenak, Zsuzsanna Kis, Dr. Anikó Zeöld (Institute of Experimental Medicine, Budapest, Hungary) and Dr. Balázs Mayer, for their valuable advice and friendly help. I wish to thank Dr. Yaofeng Zhao and Prof. Dr. Lennart Hammarström (Karolinska University Hospital, Stockholm, Sweden) for cloning the 5'-flanking region of the bovine β2m and FcRn and generating the initial luciferase reporter gene constructs to establish the work with luciferase reporter gene assay in our laboratory. I am grateful to our technicians, Ilona Horn, Ágnes Méhes and Zsolt Bíró, for supporting my laboratorial activities with their own work. I would also like to

thank all the members of Department of Physiology and Biochemistry, Faculty of Veterinary Science, Szent István University, I spent a good time with them during the doctoral work.

My warm thanks are due to Klára Hajdu, who revised the English of the manuscript. I owe my deepest gratitude to Zoltán Doleschall (National Institute of Oncology, Budapest, Hungary), who has not refused any requests and made the effort to perform high quality qPCR for bovine FcRn and β2m. I would like to acknowledge the help of Ágnes Major (Hungarian Natural History Museum, Budapest, Hungary) in the phylogenetic analysis. I also wish to thank Dr. Balázs Gereben (Institute of Experimental Medicine, Budapest, Hungary) for his help during our collaboration and his patience at the final stage of my doctoral work.

Especially, I would like to give my special thanks to my family for supporting me in all the time of research and writing of this thesis.