Szent István University
Faculty of Veterinary Science, Postgraduate School

Tissue localization of the neonatal Fc receptor (FcRn) expression in ruminants of different physiological status

Summary of thesis

Balázs Mayer

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Szent István University
Faculty of Veterinary Science, Postgraduate School

Supervisor and advisors:

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Dr. László V. Frenyó, PhD
professor
Szent István University, Faculty of Veterinary Science,
Department of Physiology and Biochemistry

Dr. Imre Kacskovics, PhD
senior scientist
Szent István University, Faculty of Veterinary Science,
Department of Physiology and Biochemistry

Dr. Veronika Jancsik, PhD
professor
Szent István University, Faculty of Veterinary Science,
Department of Anatomy and Histology

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Balázs Mayer

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Introduction

In the first weeks of life, the immune system of the newborn is considerably undeveloped and therefore, it is not able to prevent infections. This temporary immunodeficiency is compensated by maternal immunoglobulins that provide specific protection against different pathogens. Maternal immunotransport is a process, in the course of which the mother gives significant amount of immunoglobulins to the new individual to ensure its survival in early life. This system can be considered as mediating immunological experience since maternal antibodies have been produced against pathogens existing in the environment of the mother. Inasmuch as the natural environment of the newborn and its mother are the same, this immunological experience is also efficient against pathogens threatening the newborn.

Multiple mechanisms have been evolved to enable the transfer of maternal immunity to the offspring. Maternal antibodies ensure passive immunity in the first period of life until the developing immune system is able to maintain protection against infections.

In ruminants, maternal immunity is exclusively mediated by colostral immunoglobulins. In the cow, serum concentration of immunoglobulin (Ig) G1 decreases rapidly several weeks

prepartum, and IgG1 is selectively transported across mammary gland acinar cells from maternal serum into the colostrum. The ingestion of colostral immunoglobulins by the newborn appears to be non-specific, but subsequently, a large proportion of the absorbed IgG1 is specifically recycled back by the crypt epithelial cells of the small intestine to the lumen, where it contributes to local immune protection. Besides mammary gland and small intestine, mucosal secretions of other organs (e. g. lung) and tissues contain considerable amount of IgG1.

The predominance of IgG1 in mucosal fluid supports the concept of a special role for IgG1 in mucosal immunity in ruminants, which can be explained by the fact that IgG1, similarly to IgA, is more resistant to proteolysis than IgG2.

The trans-epithelial transport of secretary IgA through epithelial cells has been characterized and the polymeric Ig receptor involved in this process has been identified. However, the precise mechanism by which IgG crosses epithelial barriers to provide defence on mucosal surfaces is still unknown.

The transport receptor for maternal IgG in the small intestine of neonatal rodents and in the human placenta is the neonatal Fc receptor (FcRn). FcRn consists of a heterodimer of an integral membrane glycoprotein, similar to MHC (major histocompatibility complex) class I α-chains and β2-

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in the proximal tubular epithelial cells of the bovine kidney may have the function, similarly to human FcRn, to bind the small amount of IgG in the primary filtrate and recycle it to the blood circulation.

To date, little is known about changes in FcRn expression level or regulation of gene expression. The analysis of the promoter segment of FcRn α-chain has been set out by several research groups since better understanding and influence on the regulation of gene expression could be of interest for basic science and has relevance also in the clinic.

Influencing ruminant FcRn expression and hereby the colostral IgG transport could enable to obtain large amount of bovine IgG or human IgG from transchromosomic cow for veterinary purposes or human therapy in the future.

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microglobulin. This receptor binds IgG in a pH-dependent manner (pH 6.0: binding, pH 7.5: dissociation). FcRn expression has been described not only in epithelial cells but also in endothelial cells. The receptor expressed in endothelial cells plays a role in IgG homeostasis, binds IgG molecules and protects them from lisosomal degradation.

Previously Kacskovics et al. (2000) cloned and characterized the bovine FcRn and confirmed its expression by Northern blot in multiple tissues like the mammary gland and the small intestine. My PhD thesis analyzes tissue localization of FcRn expression in ruminants. In our investigations we tried to answer the questions, whether FcRn is expressed in epithelial or endothelial cells in bovine mammary gland, small intestine, lung and kidney, whether its expression changes during colostral IgG transport in the mammary gland and what its role could be in IgG secretion.

Aims and Scopes

- Cloning and characterization of the ovine FcRn α-chain cDNA
- Development of an appropriate *in situ* hybridization method on transfected cells and tissue sections to detect FcRn α-chain mRNA

- Localization of FcRn α-chain mRNA on bovine and ovine mammary gland and bovine lung sections
- To raise an FcRn α-chain-specific antiserum
- Establishment of an immunohistochemical method to localize FcRn α-chain
- To detect FcRn α-chain on bovine and ovine mammary gland and small intestine sections and on bovine lung and kidney sections
- Localization of FcRn α-chain expression and detection of changes in expression pattern in the mammary gland around the time of parturition
- To confirm pH-dependent binding of IgG to mammary epithelial cells on tissue sections

Materials and methods

Based on the bovine FcRn α-chain cDNA sequence and the expected strong homology, the ovine FcRn α-chain cDNA was cloned using bovine FcRn α-chain-specific primers and cDNA was also subjected to 3'RACE (rapid amplification of cDNA ends) technique. For the detection of FcRn α-chain mRNA, single stranded digoxigenin-labeled cDNA probes were used that were prepared in a linear PCR. The probes, hybridized to mRNA in transfected cells or tissue sections *in situ,* were detected with an alkaline phosphatase conjugated

FcRn in the lamb and adult cow duodenal crypt epithelial cells suggest that FcRn plays an important role in epithelial IgG1 secretion.

Besides of these indirect evidences, we consider that analyzing the affinities of IgG1 and IgG2 for this receptor is critically important to settle our argument.

Concerning the second criterion, the cytoplasmic region of the ruminant FcRn molecules is shorter by ten amino acids compare to their rodent and human counterparts. Based on transport studies with truncated rat FcRn, one may speculate that the lack of this segment may lead to a significant shift to the basolateral to apical transport in ruminants. Additionally the triptofan based endocytosis signal of the cytoplasmic domain in ruminants are different from those in the rat. Further studies on the cytoplasmic region are required to evaluate these questions.

The expression of FcRn beside mammary gland and small intestine in epithelial cells of other tissues like the epithelium of lower airways indicates the presence of a hitherto neglected IgG1 secreting system in ruminants, which contributes to local mucosal immunoprotection against infections.

The presence of bovine FcRn in the capillary endothelial cells indicates that this receptor has a role in IgG homeostasis, similarly to its mouse and human counterparts. FcRn expressed

cells of the bovine kidney using immunohistochemistry.

Discussion

The transport receptor for ruminant IgG from serum to colostrum would have to fulfill at least two requirements: 1) it should prefer IgG1 in the binding and/or in the transport process, and 2) it should mediate basolateral to apical IgG transport in epithelial cells. To the first point we have indirect evidence that we have found the presence of FcRn within mammary acinar and duodenal crypt cells where previous studies detected IgG1. Therefore, we propose that ruminant FcRn most probably favours binding to IgG1.

Our data also suggest that the transcription of the FcRn α-chain is not down-regulated markedly, if at all, in association with the increased lactogenic activity and decreased secretion of IgG1. These data are in good agreement with a recent study, which has found constant level of FcRn mRNA in the bovine mammary gland throughout lactation, whereas in contrast, increased level of β2-microglobulin mRNA in the mammary gland correlated with the time of active IgG-transfer into milk. The presence of the FcRn in acinar and ductal epithelial cells in the mammary gland and the obvious change of its distribution before and after parturition moreover the apical localization of

anti-digoxigenin antibody. The alkaline phosphatase coupled to the anti-digoxigenin antibody was visualized using NBT/BCIP substrate.

To raise FcRn-specific antiserum, rabbits were immunized with an oligopeptide derived from a segment of the FcRn α-chain (the aminoacid sequence of this segment was the same in the cow and sheep). The antiserum was affinity purified using Sulfolink affinity column and tested with Western blots. By immunohistochemistry, this affinity purified serum, biotinylated anti-rabbit IgG and avidin-biotin peroxidase complex kit were used. 3,3'-diaminobenzidin was applied as a color substrate.

Tissue samples for *in situ* hybridization and immunohistochemistry were collected at a local slaughterhouse or harvested from the mammary gland with an automated biopsy instrument around the time of parturition. The samples were fixed in paraformaldehyde and then embedded in paraffin and sectioned.

The pH-dependent binding was investigated with cyanin 2-labeled bovine IgG on mammary gland sections after antigen retrieval in citrate buffer. The binding of the labeled IgG was evaluated using fluorescent microscopy.

Results

1. We detected FcRn α-chain mRNA in epithelial cells of acini and ducti in the mammary gland of non-lactating cow using *in situ* hybridization.
2. The ovine FcRn α-chain cDNA was cloned and characterized.
3. Mammary gland biopsies were collected from ewes around the time of parturition. *In situ* hybridization was carried out on the biopsy sections with ovine FcRn-specific cDNA probe. FcRn α-chain mRNA was detected exclusively in the acinar and ductal epithelial cells of the mammary gland, which are responsible for colostral IgG1 transport. The expression level of FcRn α-chain revealed no major changes before and after parturition.
4. Immunohistochemical analysis demonstrated that the cytoplasm of the epithelial cells of the acini and ducti in the mammary gland biopsies stained homogeneously before parturition, although a marked difference was observed in the pattern after lambing, when only the apical side of the epithelial cells were stained. At involution, the diffuse localization in the cytoplasm reappeared.

5. We found FcRn α-chain expression in the lamb and adult cow duodenal crypt epithelial cells, which were demonstrated to secrete IgG1 in newborn calves.
6. In another experiment, we demonstrated that in cow FcRn α-chain showed the same subcellular redistribution in the mammary acinar cells as observed in ewes.
7. We confirmed the pH-dependent binding of IgG to the mammary epithelial cells on sections from the prepartum period (pH 6.0: binding, pH 7.4: almost undetectable binding).
8. We analyzed the FcRn α-chain expression in the bovine lung, and demonstrated the receptor at mRNA and protein level in the bronchial and bronchiolar epithelial cells and in the alveoli. We could not detect FcRn α-chain expression in the epithelial layer of the trachea either by immunohistochemistry or by *in situ* hybridization.
9. FcRn α-chain expression was shown in the endothelial cells of the lamina propria in the bovine small intestine and in the proximal tubular epithelial