FCRN – ROMANIAN CONTRIBUTIONS

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Abstract

The neonatal Fc receptor (FcRn) was discovered in 1973 and characterized in 1989, but its existence was envisaged by Sir Frances Brambell in the 1950-1960’. One of the most important figures in this field, Victor Ghetie, managed to group around him several Romanians that brought important contributions to the characterization and understanding of the receptor, especially in defining its dual role in IgG transcytosis and protections against catabolism. Despite a long history, FcRn remains still of interest for research, many features awaiting to be unveiled.

The neonatal Fc receptor stands in the center of a biomedical scientific field that started in 1892, when Paul Ehrlich published the first data regarding the transmission of immunity from mother to newborn [Ehrlich, 1892]. He was the one to show that this phenomenon is different from one species to another and is also restricted to a well defined period.

However, the real father of this domain was Sir Frances W. Brambell, the first to envisage that this transmission can be performed due to the existence of a dedicated receptor. He showed that there is a certain selectivity, favoring certain „species” of IgG, that the transmission can be blocked with „interfering” proteins (hence demonstrating the competition for a substrate and a saturable transportation system), and that this blocking is not permanent (thus showing that the receptor can release its ligand). Remarcably, it was also Brambell to suggest that the same receptor involved in IgG transportation is probably involved in IgG protection against catabolism. He addressed all these issues in his last publication, a monography published in 1970 [Brambell, 1970].

After Brambells’s death, the domain was rather forgotten, and the progress was slow. In 1973, Waldmann and Jones succeeded in isolating the receptor from the neonatal rat gut, and this is how the molecule received its name: neonatal Fc receptor (FcRn). Furthermore, they were also able to demonstrate the pH dependency of the ligand binding/release (pH 6 for binding and neutral pH for release) [Waldmann and Jones, 1973]. Later on, various groups showed the presence of the receptor in adult tissues, as well in the human placenta [Simister et al., 1996], but the molecule is still named, even today, “neonatal”.

One major breakthrough came in 1989, when Simister and Mostov [Simister and Mostov, 1989] managed to characterize the structure and revealed that is a heterodimeric glycoprotein, consisting of beta 2 microglobulin and a heavy chain similar to the MHC I alpha chain. However, the gene encoding the FcRn alpha chain is not found within the MHC complex, but on CRS 19 [Mikulska et al., 2000], and under the control of a different set of regulating elements [Kandil et al., 1995; Mikulska and Simister, 2000].

But in the early 90’, the connection between immunoglobulin transport and catabolism was forgotten and it was only brought back to attention by a set of papers published by Victor Ghetie and E. Sally Ward. In fact, it all started with a 1983 paper coming from the group of Victor Ghetie [Dima et al., 1983] (working in the Victor Babes Institute in Bucuresti at that time), who stressed that the protein A from *Staphylococcus aureus* (SpA) is able to decrease IgG half life. Based on this observation, their hypothesis was that, most probably, SpA is binding to the same site as the neonatal receptor. The next set of experiments were performed in UT Southwestern (Dallas, Texas, USA) and focused on this particular site. Using site directed mutagenesis,

assessing IgG half life and maternofetal transfer, Ghetie and Ward were the first ones since Waldman and Jones to consider the dual functions of FcRn.
Taking into consideration the interface between the second (CH2) and the third (CH3) domains of the murine IgG1 as SpA target, and using Fc fragments produced in *E. coli*, they mutated several key residues that are in close vicinity, as shown by the three dimensional structure: Ile-253, His-310, Gln-311, His-433 and Asn-434. The results enabled them to show for the first time that this is the site controlling also the IgG1 catabolic rate. It was becoming evident that SpA and the molecule controlling the IgG half-life share the same binding site [Kim et al, 1994, a].
The next paper was published soon afterwards, in fact in the same volume of the European Journal of Immunology. Using the same approach, they have studied the effects of the above mentioned mutations on the Fc fragments intestinal transfer in neonatal mice. Direct transfer as well as competitions assay demonstrated elegantly that the mutations responsible for the IgG1 half-life decrease were also responsible for blocking the intestinal transfer. Since FcRn was already known to be the key molecule for IgG transfer across the neonatal gut, it became clear that the same receptor is responsible for both processes. Furthermore, the pH dependency of the binding could now be explained by the presence at the CH2/CH3 interface of two key Histidine residues (His-310 and His433) [Kim et al, 1994, b].
The paper addressed another issue, regarding the number of FcRn binding sites required for this interaction. For that purpose, Ghetie and Ward constructed a hybrid made of a wild type together with a mutated Fc fragment and compared the efficiency of the transfer with a wild type Fc homodimer. The results demonstrated that the heterodimer transfer is much affected, thus suggesting the requirement of two binding sites for one FcRn molecule.
Given the presence of key histidines at both the CH2 and CH3 interfaces, it was important to clarify if both domains are important for the IgG Fc-FcRn binding. Another paper, published also in 1994, showed the results of pharmacokinetic studies using Fc-hinge, CH2-hinge (both monomer and dimer), CH2 and CH3 fragments, demonstrating that indeed both CH2/CH3 domain interfaces are required for IgG1 persistence, thus FcRn binding [Kim et al., 1994, c]. Interestingly, these results preceded and were later confirmed by cristalography studies [Burmeister et al., 1994; Raghavan et al., 1994].
However, the CH2/CH3 interface creates a conformational binding site, the three dimensional shape being also influenced by a mini hinge region connecting the two domains. Several Fc fragments, with and without the hinge disulphide bonds, were produced and tested in the same manner, in pharmacokinetic studies, showing that this bond is very important for the longer survival of immunoglobulins G. This also lead to the understanding that differences in half-life can be explained by differences of the binding site conformation [Kim et al., 1995.
In 1995, Neil Simister's group takes advantage of the newly developed beta2 microglobulin KO mice (thus also FcRn KO) and publishes a paper showing that these mice lack FcRn expression in the gut, are not able to transfer IgG to the pups and their IgG levels are only 1/10 as compared to normal mice [Israel et al., 1995]. Their conclusion was that the explanation was, most probably, a decrease in IgG production, and the connection between transport and protection seemed to be lost again. The very next year, using the same mice, Ghetie and Ward showed that the low IgG levels are rather the expression of a defective protective mechanism, and that FcRn is involved in IgG homeostasis [Ghetie et al., 1996], and this was later confirmed by Junghans and Anderson [Junghans and Anderson, 1996].
The demonstration of the FcRn role in maternofetal transmission continued in 1996, by analyzing the transfer of radiolabeled wild-type and mutated Fc fragments from the circulation of pregnant

females to *in utero* fetuses. Ile-253, His-310, Gln-311, His-433 and Asn-434 proved again to be critical for this process, thus showing that FcRn in indeed involved in IgG transcytosis [Medesan et al., 1996].

The quest for pinpointing key residues for the IgG-FcRn interaction took now into account both transyctosis and catabolism and a new set of evidence were published in 1997, revealing that FcRn and SpA display a partial but not complete overlap of the binding site [Medesan et al., 1997].

The knowledge regarding the interaction between IgG1 and the receptor involved in its protection allowed Ghetie and Ward to push things further and the next step was to design mutations targeting Thr-252, Thr-254 and Thr-256 so that they were able to obtain an IgG fragment with a longer half-life than the normal counterpart. These results, opening a new path in antibody engineering, were published in Nature Biotechnology [Ghetie et al., 1997].

Somehow in contrast with the results obtained by Ghetie and Ward using mouse IgG, Pamela Bjorkman’s group, using rat immunoglobulins, came up with slightly different results regarding the key residues involved in the interaction with FcRn, especially His-435 [Raghavan et al., 1995]. To respond to this issue, new mutations were designed and this led to the identification of new aminoacids contributing to the formation of the FcRn binding site (257, 307, 309) and to the exclusion of a loop involving residues 386-387 [Medesan et al., 1998].

This line of investigation was then extended to the human IgG and this study revealed that there is a strong similarity between the mouse and human immunoglobulins, the interaction site being placed at the CH2/CH3 interface, but with His-433 playing no role in regulating the catabolic rate of human IgG1 [Kim et al., 1999].

The endothelial cells are still considered the main candidate for the immunoglobulins catabolism, and since FcRn plays a major role in this process, another field of research aimed at the identification of the receptor’s expression in these particular cells. A collaboration with the Institute of Cellular Biology in Bucuresti led to a paper in which it was demonstrated the FcRn presence in the endothelium of small arterioles and capillaries in adult mouse liver and muscle [Borvak et al., 1998]. Felicia Antohe continued this research in Bucuresti and using an endothelial cell line established from human placenta she managed to demonstrate not only the expression but also the neonatal receptor’s functionality as a bidirectional transporter [Antohe et al., 2001].

The FcRn expression in human placenta was well documented since 1996 [Simister et al., 1996], but the practical demonstration of the receptor’s role in human placental transfer had to wait until 2001, when a very elegant *ex-vivo* model could be used [Firan et al., 2001].

However, placenta is not the only organ involved in the passive transmission of immunity from mother to young, so the next tissue targeted for investigation was the mammary gland. There is an important difference between humans and mice regarding the IgG transport: while humans use only the placental transfer for this purpose, mice use also milk, so that suckling pups receive also maternal immunoglobulins by FcRn mediated intestinal transfer. The investigation of beta2 microglobulin KO mice revealed that milk IgG concentration was similar to the one of normal mice and this led Simister’s group to the conclusion that IgG transfer to milk is not FcRn mediated [Israel et al., 1995]. However, the same paper was indicating a 20 fold lower seric IgG concentration for the deficient mice, thus suggesting that IgG transportation is rather independent of the blood concentration. On the other hand, other literature data were indicating a corelation of this process with IgG concentration [Gitlin et al., 1976], and this was the reason we decided to investigate murine lactating mammary glands. The initial aim was to evidence the expression of

the neonatal receptor and this was done by immunhistochemistry and *in situ* hybridization. Furthermore, we have used not only an anti-FcRn antibody, but also mouse IgG1 at pH 6.0 and pH 7.2, taking advantage of the pH dependency of the binding. The FcRn's expression came as no surprise, the acinar epithelial cells showing positivity for this molecule. However, we could not detect the presence of the receptor in any of the investigated endothelial cells [Cianga et al., 1999]. The real surprise came when we have investigated the transfer efficiency, hence FcRn functionality. We used radiolabeld wild type Fc fragments, mutated Fc fragments, and 3 IgG isotypes in normal Swiss-Webster and SCID mice, and measured the suckling pups radioactivity. The results were showing that the molecules having the lowest afinity for FcRn were transfered better to milk. So it seems that FcRn could play in this particular tissue a distinctive role than in the intestine, that it is it could act rather to recycle IgG and return it to the mother's circulation, or it could play a dual role transport/recycling. Yet, if recycling would be the main FcRn function in the mammary gland, this means that the amount of immunglobulins passing to the secretion is simply the one surpassing the receptor's binding capacity.

Since in humans the transmission of IgG from mother to child is performed mainly *in utero* during a limited period of time and the amount of IgG drops from 1g/l in the colostrum to about 50 mg/l in the human milk secretion [Mehta et al., 1991], the investigation of human mammary gland became of obvious interest. One of the major obstacles that had to be surmounted in this respect was the availability of normal human tissue. We took advantage of oncology surgery, and we were able to analyze healthy tissue from the tumoral periphery. Immunohistochemistry allowed us to evidence the presence of the receptor in the acinar epithelial cells, but again, the capillary endothelial cells proved to be negative [Cianga et al., 2003] Furthermore, we were able to identify FcRn in various types of human mammary carcinomas, and also in the metastatic cells that migrated to the draining lymph nodes, thus showing that the receptor's expression is stable, unaffected by the malignant transformation.

Since pharmacokinetic studies using radiolabeled fragments cannot be performed in humans, it can only be speculated that, in a similar manner with the murine counterpart, FcRn functions in the mammary gland primarily as a recycling receptor. Since the amount of IgG decreases dramatically from colostrum to milk, it could be also speculated that FcRn'expression might be up-regulated by factors involved in setting up the lactation, but this was not yet demonstrated.

The presence of the neonatal receptor was further investigated in two widely used human mammary adenocarcinoma cell lines, MCF-7 and SKBR-3, and it came as no surprise to detect the presence of this molecule. However, the rather intriguing aspect was to evidence the heterogeneous expression, with some cells displaying a strong signal, while others being completely negative (unpublished results).

It was becoming increasingly clear that the so called „neonatal" receptor is in fact expressed in adult tissues as well and many different types of cells are FcRn positive, indicating that this molecule functions in a wide array of tissues. Besides the presence in the human mammary gland, we were able to show FcRn's expression in keratinocytes, epithelial cells of the hair follicle and sebaceous glands, but also melanocytes. This investigation showed again the negativity of the endothelial cells [Cianga et al., 2007]. If this is the case, it may be that the theory regarding the endothelial cells as a major site of catabolism and transcytosis could be revised.

To verify this, we are currently investigating the expression of FcRn in a variety of tissues, and the preliminary results were communicated at the 21st European Immunogenetics and Histocompatibility Conference in Barcelona [Cianga et al., 2007]. The data we have obtained so

far are again showing that most of the endothelial cells are negative leading us to an intriguing hypothesis: is there an alternative mechanism able to perform IgG transcytosis from bood to tissue?

The outstanding contribution of Victor Ghetie and E. Sally Ward in this field of research was acknowledged by the invitation to write several reviews in prestigious journals like Immunology Research and Annual Review of Immunology.

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