Functional specialization and cooperation between the neonatal- and classical IgG receptors, FcRn and FcR

Einarsdóttir, H.K.

Citation for published version (APA):
Einarsdóttir, H. K. (2014). Functional specialization and cooperation between the neonatal- and classical IgG receptors, FcRn and FcR

General rights
It is not permitted to download or to forward/distribute the text or part of it without the consent of the author(s) and/or copyright holder(s), other than for strictly personal, individual use, unless the work is under an open content license (like Creative Commons).

Disclaimer/Complaints regulations
If you believe that digital publication of certain material infringes any of your rights or (privacy) interests, please let the Library know, stating your reasons. In case of a legitimate complaint, the Library will make the material inaccessible and/or remove it from the website. Please Ask the Library: http://uba.uva.nl/en/contact, or a letter to: Library of the University of Amsterdam, Secretariat, Singel 425, 1012 WP Amsterdam, The Netherlands. You will be contacted as soon as possible.
GENERAL DISCUSSION
AND CONCLUSIONS
The objective of this thesis was to investigate the extent of FcRn involvement in several IgG mediated pathways, including maternal transport, phagocytosis, half-life extension and antigen presentation, and to see if we could discover novel factors that influence those processes. A special emphasis was placed on the investigation of feto-maternal antibody transport, trying to shed light on several unexplained aspect of this important mechanism, such as the possible involvement of other receptors. Furthermore we wanted to see whether the light chain isotype of IgG could influence interaction with FcRn or other Fc\gamma receptors. In addition, we studied the extent of FcRn involvement in the immune reaction against RSV following vaccination with IgG opsonized virus in mice. The main results of our research are discussed in detail below.

RECEPTORS INVOLVED IN FETO-MATERNAL TRANSPORT: EVIDENCE FROM STUDYING ALLOTYPES AND IgG-GLYCOSYLATION

FcRn is generally considered to be the most important, presumably even the sole, receptor mediating transport of IgG across the placenta by transcytosis; first through the multiple layers of placental syncytiotrophoblasts, then either through or in-between the underlying celllayers, passing through the endothelial cells of the umbilical cord before finally reaching fetal circulation. This transport is highly analogous to what we know of half-life extension in the adult’s circulation, where it has been shown that FcRn-expression and its biology is sufficient to explain the long half-life of IgG in several species. 1-4 In humans this leads to the long half-life of 21 days for IgG1, IgG2, and IgG4, but not for most allotypes of IgG3, which have only a half-life of 7 days. 5,6 In agreement with this, IgG3 transport across the placenta is also lower than for the other IgG subclasses, albeit still considerable, and surprisingly the transport of IgG2 is comparable to that of IgG3. 7,8 In order to address these puzzling inconsistencies a persistent alternative theory suggests the involvement of other Fc\gamma receptors; Fc\gammaRIIb being a historical suspect due to its expression in the placental endothelium. 9,10 The fact that albumin, FcRn’s other ligand, has not been shown to be efficiently transported from mother to fetus, lends some support to that theory. 11,12 However, none of our results implicate other Fc\gammaRs in the process and indicate FcRn to be, if not the only, then at least one of the rate limiting IgG receptors involved in and responsible for feto-maternal transport of antibodies. This is in agreement with other published works on this subject, including work excluding a role for Fc\gammaRIIb in transport across the mouse placenta. 13-15 However, it must be kept in mind during all interpretation of data regarding FcRn mediated IgG transport that while maternal FcRn facilitates antibody transport over the placenta, the child itself already expresses FcRn within its own tissues and is thus able to recycle IgG in serum. Therefore, when examining
serum concentrations of IgG subclasses in the fetal circulation, it is impossible to 
completely discriminate between effects on FcRn mediated placental transport 
and on serum half-life extension within the child.

Firstly, in chapter 2, our mass spectrometry analysis of IgG subclasses 1-4 failed 
to show any significant differences in Fc glycosylation patterns between mothers 
and their new-born children. Average levels of galactosylation, sialylation, bisecting 
GlcNAc and fucosylation were found to be very similar for matched fetal and maternal 
IgGs. Since affinity to classical Fcγ receptors (but not FcRn) is known to be affected 
by Asn297 sugar moieties on the Fc, involvement of those receptors in the transport 
would have been expected to result in a skewing of the glycosylation profiles of a 
new-born’s serum IgG compared to its mother’s. 16-20 In chapter 3, we furthermore 
found that a simple change from R435 to H435 in the IgG3 Fc tail, a key Histidine 
residue for pH dependent FcRn binding, (the lack of which explains the short half-life 
of IgG3) was sufficient to increase its placental transport to levels comparable to 
those for (H435-containing) IgG1. 6 By quantifying the placental transport of IgG3 
normalized against IgG1 transport in two groups of women, one expressing IgG3 
allotypes containing an R435 and the other expressing the H435 G3m16 allotype, 
we found that the mean child/mother ratio of IgG3 was 67.5 ±7.3% of IgG1 transport 
in the first group but 102.9±33.8% in the other. The difference in IgG3 transport 
between the two groups was significant, with a p<0.0001. This is in concurrence 
with a previous report by Stapleton et al., where it was shown that H435 containing 
IgG3 has a comparable half-life to IgG1; serum recycling being another mechanism 
mediated by FcRn. 6 Taken together these results substantiate and support the 
generally accepted premise that FcRn is either the sole or at least the rate limiting 
receptor responsible for feto-maternal IgG transport.

These results have important implications for populations where H435 containing 
IgG3 allotypes are prevalent. While these allotypes, collectively designated as IgG3 
G3m(s) or G3m16, are rare in Europe (around or under 1%), the frequency can rise 
up to almost 50% in some Asian populations. 21-24 The combination of an increased 
feto-maternal transport and extended half-life of H435 containing G3m16, as well as 
the strong effector functions of IgG3 in general, could certainly influence the severity 
and frequency of auto- and alloimmune diseases with frequent IgG3 responses 
(e.g. HDN) in ethnic populations where G3m16 is common. The next logical step 
would thus be to investigate whether populations with a higher frequency of G3m16 
indeed have more instances of IgG3 mediated auto- and alloimmune diseases, such 
as ITP, ABO/RhD-HDN and FNAIT, than western populations where R435 IgG3 is the 
norm. 25-27 Furthermore, it would be of interest to see whether the IgG3 phenotype 
presented by a patient influences the severity of these diseases. Clearly it would 
be preferable to perform this particular study in an Asian population where G3m16 
is prevalent. 24
Albumin transport – the missing link?

The puzzle remains unsolved why FcRn cannot recycle (i.e. rescue from lysosomal degradation) R435-IgG3 while the antibody is evidently transported across the placenta, although to a lesser extent than IgG1. Meanwhile, albumin is recycled (albeit to a lower degree than IgG) and inefficiently transported over the placenta (or not at all). Together these facts suggest either recycling to differ fundamentally from placental transport or we still have an unknown factor that is involved in IgG transport, which would then be responsible for the transport of R435-IgG3. A key to answer this question might be to study human albumin trafficking in the placenta in more detail.

Since our data, presented in chapters 2 and 3, does not suggest the involvement of other IgG receptors, the apparent absence of albumin transport continues to be a mystery. If some other Fc-glycosylation-independent IgG receptor expressed in syncytiotrophoblasts, or other cell layers within the placenta is selectively shuttling IgG across the placenta, but not albumin, we have no likely or known candidate; particularly since the involvement of FcγRIIb has been shown to be unlikely. Another possible explanation of albumin’s perplexing lack of maternal transport might be some sort of selective blocking by another protein or lipid. The structure of albumin binding to FcRn was recently solved offering a potential explanation. It suggest that the binding of albumin to FcRn, and consequently its transport and recycling properties, may be hindered when albumin is associated with long fatty acids (FAs) such as C16 and C18 lipids (Figure 1 and Figure 2). The binding of FAs to albumin changes the three dimensional structure of the protein by inducing a relative rearrangement of its three domain interfaces. This is apparently one of albumin’s biological functions; binding with high affinity to and transporting fatty acids and/or lipophilic small molecules such as steroid hormones, calcium, bilirubin and many drugs. Inhibition of the binding of albumin to FcRn by the lipid-cargo of the former protein would make sense, since if albumin were transported over the placenta then the many different molecules carried by it might be transported unchecked as well, with possibly disastrous results for the developing fetus. In addition, human children are perfectly capable of endogenous serum albumin production from early pregnancy on, so overall it would seem logical that there were a mechanism present that inhibits maternal albumin transport over the placenta. An inhibition of this sort might explain why recycling efficiency and therefore half-life extension of albumin is only 87% of that for IgG, since only albumin not carrying long fatty acids could efficiently be recycled (Figure 2).

But based on the same premise, one would then expect the defatted fraction of albumin to be transported over to the fetus as well- unless the proportion of defatted and lipid-carrying albumin at the site of placental transport were different from that at the site of recycling, although how this could be achieved is unclear. Figures 1-2
depict a model of FcRn mediated placental transport and plasma recycling based on this premise; i.e. assuming a mixture of FA-loaded and defatted albumin in plasma and mostly FA-loaded albumin at the site of placental transport.

Conversely, older literature suggests that when loaded with fatty acids, albumin’s uptake and transcytosis might actually be more efficient than for defatted albumin. However, a direct comparison between studies is problematic since in those papers, bovine albumin and either mice or bovine cells were used, while in the recent study of Schmidt et al. the analyses were performed with human protein and receptor. In addition, due to possible inter-species differences, there is the added complexity of albumin’s seven binding sites, each one presumably displaying a different affinity to the over 40 different fatty acids available in serum. Some of these lipid-binding sites (only some of which are predicted to directly interfere with FcRn binding when occupied) may have selective preference for certain lipids, suggesting that whereas some of the sites may block FcRn binding, others may hypothetically enhance it. This makes comparison difficult since composition and number of fatty acids bound to albumin could also be very different in the various studies.

Although both albumin and IgG bind FcRn in a similarly pH dependent manner, the stoichiometry of their respective interactions is not the same; IgG can interact with two receptors at a time while albumin binds only one. Thus an FcRn-IgG-albumin possibly consists of a complex between one IgG bridging two FcRn, each of which binding one albumin molecule (Figure 3). Indeed, the valency of the Fc-FcRn binding can indeed influence the efficiency of transport, as a mutated IgG possessing only one binding site was transported to a lesser extent than WT IgG across a canine epithelial layer. Furthermore, when IgG interacts with FcRn, the complex has been predicted to display an unusual “lying down” orientation, which might enable two FcRn receptors on opposing membranes in tubular vesicles to bind one IgG, given that the gap is small enough (Figure 3).

While IgG does not inhibit albumin binding to FcRn as measured on a chip, the “lying down” orientation of FcRn-IgG might not be compatible with albumin binding in vivo due to steric hindrance. As a result, albumin transcytosis, and maybe even recycling, could be impeded. However intriguing, this mechanism does not explain how maternal transport can be selectively blocked in the placenta, while other FcRn expressing tissues such as vascular endothelium and lung epithelium, have been described to able to transcytose both albumin and IgG effectively.

In these aforementioned tissues, albumin transcytosis seems to be regulated by a yet uncharacterized receptor known as GP60, an albumin binding surface glycoprotein located within lipid rafts containing caveolins, which are the main scaffolding proteins of caveolae vesicles. These caveolae are bottle-shaped invaginations on the cell membrane and unlike clathrin-coated pits, which are constitutively endocytosed, their release from the membrane requires a specific activation signal. Following GP60 ligand activation, the receptor is colocalized and physically associates with
Figure 1. Proposed model of FcRn mediated placental transport of IgG1, IgG2, IgG3 and albumin (predominantly FA-loaded) in a syncitiotrophoblast. Following non-specific uptake\(^1\), all IgG subclasses are transported\(^2\) across the placenta, but IgG2 and IgG3 transport is less efficient\(^2a,2b\) than for IgG1\(^2b\). FA, or equivalent cargo may interfere with albumin association with FcRn\(^a\) and inhibit placental transport of albumin. Whether or not albumin is transcytosed at all has not been conclusively demonstrated ("?"). However, this pathway is only valid for IgG3 allotypes containing R435, while H435-containing IgG3 allotypes (common in Asia) are transported normally.

Figure 2. Proposed model of FcRn mediated recycling of IgG1, IgG2, IgG3 and albumin (defatted or FA-loaded). Following endocytosis\(^1\), IgG3 and FA-loaded albumin are directed into a lysosomal pathway\(^a\) while IgG1, IgG2 and de-fatted albumin interact with FcRn and are sorted into a recycling pathway\(^b\), to be released at the surface of the cell\(^1\). However, as for transport, this pathway is only valid for IgG3 allotypes containing R435, as H435-containing IgG3 allotypes (common in Asia) are recycled normally.
caveolin-1 in vesicles that migrate in the basolateral direction. Activation also results in phosphorylation of caveolin-1, and blocking GP60 or disrupting the caveolin pathway inhibits albumin transport. FcRn, on the other hand, is predominantly expressed intracellularly in endothelial cells and alveolar epithelium, and its function seems to be neither clathrin-, nor caveolin-dependent. Intriguingly, syncytiotrophoblasts, the FcRn expressing cells credited with the feto-maternal transport of IgG, don’t express caveolin. One possibility would be that transcytosis and recycling of albumin are differentially regulated, and that entry by GP60 ligation is necessary for albumin to be properly coupled to the FcRn transcytosis pathway via acidified endosomes. There are indications that a lowered pH might encourage fatty acid dissociation from albumin, which would leave the defatted albumin available for binding to FcRn under the now optimal conditions (Figure 4).

It would be tempting to hypothesize that this lack of caveolin in syncytiotrophoblasts would mean that the only pathway remaining to the albumin would be a recycling one, especially since albumin has been reported to be recycled in human placental syncytiotrophoblasts, but in a clathrin-rather than caveolin-mediated way. However, albumin has been shown to be transcytosed together with IgA via a clathrin-, not caveolin, mediated endocytic pathway across epithelium of the lactating mouse mammary gland, meaning that at least in some cases, albumin can be transported in the absence of caveolin.

Certainly, further research is needed to determine whether, and then to what extent, serum albumin is actually transported over the placenta, and which fundamental difference between IgG and albumin causes such a divergence. The most straightforward way to investigate this would be using an in vitro perfused human placenta, which can be acquired relatively easily and is used by several groups...
studying the feto-maternal transport of drugs and carcinogens. \cite{14,64,65} Comparison of human serum albumin (HSA) and bovine serum albumin (BSA) transport would be interesting, as would comparing affinities of the respective albumins to human FcRn, for instance by surface plasmon resonance. Under these controlled conditions, it could be elucidated which factors (including fatty acid chains) influence the interaction between FcRn and albumin and hinder its transport over the placenta.

**THE INFLUENCE OF IgG2 LIGHT CHAINS ON Fcγ RECEPTOR INTERACTIONS**

Serum recycling and feto-maternal transport

Our goal in chapter 4 was to seek an explanation for the discrepancy between the reported normal serum half-life of IgG2, which is equal to IgG1 and IgG4, and its low transport across the placenta, which is comparable to the most common R435-bearing IgG3 allotypes. Based on findings reported in mice, showing differential half-life in mice of human IgG2 with different light chains, and that of a study showing that the affinity for FcRn of IgG2λ is higher than for IgG2κ, we tested if the low placental transport of

---

**Figure 4. Proposed model of albumin and IgG transcytosis in endothelium or epithelium.** Following GP60 ligation by albumin at the apical side, caveolin-mediated uptake brings the albumin to an acidified FcRn- and IgG-containing endosome. Due to some unexplained mechanism (perhaps pH influence on albumin affinity), albumin disassociates from GP60. The acidic environment encourages the release of FcRn-blocking fatty acids from their binding sites on albumin, leaving the protein free to interact with FcRn and be transported across along with IgG. The acidic environment encourages the release of FcRn-blocking fatty acids from their binding sites on albumin, leaving the protein free to interact with FcRn and be transported across along with IgG. The acidic environment encourages the release of FcRn-blocking fatty acids from their binding sites on albumin, leaving the protein free to interact with FcRn and be transported across along with IgG. The acidic environment encourages the release of FcRn-blocking fatty acids from their binding sites on albumin, leaving the protein free to interact with FcRn and be transported across along with IgG.
IgG2 could be attributed to this difference.\textsuperscript{66,67} However, we found neither an effect of the light chain isotype on maternal transport of IgG2, nor on half-life in humans (chapter 4). In addition, we were also unable to reproduce the results of Montano and Morrison showing the longer half-life of chimeric IgG2κ in mice.\textsuperscript{66} Besides native IgG2κ, we tested as well the half-life of structural isomers IgG2κA and κB, which were synthesized by redox treatment as described by Dillon et al.\textsuperscript{68} Balb/c mice (as used by Montano et al.) were compared to C57Bl/6 WT and FcRn\textsuperscript{−/−} mice, and in none of the mouse strains did we witness a difference between the half-lives of the respective light chain isotypes of either the IgG1 or IgG2 subclass, or between any structural isomere of IgG2κ. Naturally, in the FcRn\textsuperscript{−/−} mice, concentration of human IgG dropped quickly, and fell below detection level at around day 10. Clearance of light chain isotypes of IgG1 and IgG2 was measured in humans suffering from hypogammaglobulinemia; a type of primary immune deficiency disease characterized by a very low concentration of IgG. These patients receive regular transfusions of IVIg, a pool of IgG isolated from thousands of individuals, to compensate for their compromised humoral immune system. By comparing the relative serum concentrations of each IgG type in patients four weeks after treatment, to the concentration in the original IVIg preparation, we were able to monitor the clearance of IgG in a human \textit{in vivo} system. We found the subclass composition of respective light chains to be very similar in both IVIg and in serum, meaning that the half-lives of the two light chain isotypes of IgG1 and IgG2 were the same. The discrepancy between our results and those of Montano and Morrison is not easy to explain, but it must be kept in mind that the circumstances of our respective mouse experiments were not exactly the same. While we used a human IgG ELISA to measure the concentrations of unlabelled antibody in the mice’s serum, Montano and Morrison detected radiation levels emitted by antibodies directly labelled with a radioactive isotope. Perhaps the joining of an isotope to Montano’s antibodies could affect affinity to FcRn or the stability of the antibody, both of which could conceivably influence half-life. This has, for example, been shown to be the case for biotinylation of IgG, which can reduce its placental transfer by a factor 10 compared to unlabelled antibody – probably because it affects its binding to FcRn. On the other hand, in chapter 5 we were able to confirm the results of Gurbaxani et al. using Biacore, which showed that FcRn affinity for IgG2κ is even lower than for IgG2λ (whereas Montano and Morrison found a longer half-life for IgG2κ), while no such light chain preference was detected for IgG1.\textsuperscript{67} However, a different affinity for FcRn does not necessarily have to translate into differential pharmacological clearance, as affinity to FcRn does not necessarily predict half-life, and binding to FcRn within cells is fundamentally different than on biosensors.\textsuperscript{67} This has for example been the case for several monoclonal antibodies with mutations affecting their binding to FcRn; some of which display increased but others decreased half-life with increased binding affinity to FcRn on biosensors, probably because of differential binding at low and neutral pH.\textsuperscript{69–72}
In summary, our data suggest that it is unlikely that the structural isoforms characteristic to IgG2\(\kappa\) are the reason for the strangely inefficient feto-maternal transport of IgG2. Our theory was that the ability of IgG2\(\kappa\) to form different structures might compromise its affinity to FcRn and therefore impede its transport across the placenta. But as we detected no tendency for differential transport of either IgG2 light chain, we can only assume that the cause for lower placental transport of IgG2 has to lie elsewhere, as discussed in more details in preceding section. Suggestions for how to research that particular problem have already been discussed in the previous paragraph on FcRn mediated feto-maternal antibody transport.

While it is conceivable that the short hinge of IgG2 could cause the requirements for FcRn-binding to differ compared to the other subclasses, there is no clear reason for why FcRn-mediated recycling and transcytosis of IgG2 should be so disparate. There is some indication that the two pathways are regulated by different proteins, with the actin motor myosin Vb and the GTPase Rab25 initiating transcytosis from the recycling endosome while Rab11a regulates recycling to the basolateral membrane. 73 Furthermore there are also some tryptophan-based basolateral-targeting signal in the FcRn cytoplasmic tail, which direct IgG taken up at the apical side into a transport pathway across the cell-layer. 74 But exactly how the regulation by these proteins could vary depending on which antibody subclass is bound to FcRn is unclear and was not addressed in this thesis.

**Phagocytosis**

FcRn is highly expressed in phagocytes, particularly neutrophils, and has been shown to be involved in phagocytosis. 75 In chapter 5 we examined whether the difference in FcRn affinity for IgG2\(\kappa\) and IgG2\(\lambda\) might be affected by this difference.

We found that when phagocytosis was performed with wild type (WT) antibodies, IgG2\(\lambda\) performed significantly worse at around 67.8±5.7% of IgG2\(\kappa\)'s efficiency (i.e. when IgG2\(\kappa\) phagocytosis levels were set as 100%). When using mutated antibodies unable to bind to FcRn, the \(\kappa\) \(\lambda\) difference was not only still present, but appeared even greater; with the mutated IgG2\(\kappa\) and IgG2\(\lambda\) variants performing phagocytosis at 75.4±3.2% and 31.5±5% of IgG2\(\kappa\) WT levels, respectively. The significantly reduced phagocytosis of the mutants compared to WT confirms a supporting role for FcRn in phagocytosis, while the trend towards a greater light-chain isotypic difference in the absence of FcRn suggests that rather than mediating the observed light-chain effect, FcRn actually counteracts it. This makes perfect sense when one considers that we demonstrated that FcRn's light chain preference was the opposite of that for FcyRIIa and FcRn has been previously been shown to participate in neutrophil phagocytosis in tandem with FcyR. We therefore also tested the binding affinity to the only reported IgG2 -binding FcyR present on neutrophils, FcyRIIa. We detected an opposite trend for FcyRIIa, which showed a preference for IgG2\(\kappa\) rather than IgG\(\lambda\). This affinity difference is thus likely to explain the more efficient neutrophil
phagocytosis mediated by IgG2κ. Exactly how a light chain influences the affinity of a receptor binding to the Fc tail remains unclear. One possible cause might be the unique 3D conformation of IgG2, which has the shortest hinge region of all the subclasses and whose κ isotype can form at least 3 different structural isomers based on differential arrangement of its disulphide bonds connecting the Fab’s to the Fc (predominantly IgG2κA, κB and intermediate structure, κAB). 68,76,77 It has been shown that the asymmetrical IgG2κAB, which is the most prevalent structural isomer present in serum, displays a rigid tripod-like structure with the Fab arms folded back quite close to the Fc tail. 68,76,78-81 This configuration, which should differ between the three most common isomers, could thus conceivably influence availability of the upper part of the Fc in a light chain isotype dependent way, as the FcγR bind the CH2 domain and the lower hinge Fc between the two Fab arms. 78 However, our results do not suggest that the unique IgG2κ structural isoforms contribute to the heightened affinity of the κ isotype to FcγRIIa, since we saw no difference between IgG2κA, IgG2κB and native untreated IgG2κ (predominantly κAB)-mediated phagocytosis by neutrophils.

In summary, based on our results, we conclude that the IgG2 light chain does not influence FcRn mediated transport, in spite of differential affinity of the two IgG2 light chain isotypes to the receptor. While it does have a slight effect on FcRn-mediated phagocytosis, it opposes a more powerful difference mediated by FcγRIIa, resulting in higher degree of phagocytosis through IgG2κ.

**FcRn IN THE UPTAKE AND ANTIGEN PRESENTATION OF OPSONIZED VIRUS**

In the last few years, a role for FcRn in antigen presenting cell (APC) antigen uptake (and subsequent expression on MHC molecules I and II) has been gradually revealed by various groups, primarily in OVA antigen-restricted OT-I or OT-II mice. 82–84 For instance, Quiao et al discovered that FcRn was vital for efficient DC presentation of IgG complexed OVA to CD4 T cells in OT-II mice, and also for the presentation of gliadin by human monocyte derived DCs to a CD4 T cell clone generated from a celiac patient. 83 Furthermore, FcRn is also needed for cross-presentation of low concentrations of OVA by CD8-CD11b+ DCs to CD8 T cells in mice. 84 Transgenic Balb/c mice which overexpress FcRn have more efficient humoral immune responses following OVA vaccination than their WT counterparts, due to more efficient APC endocytosis, antigen expression and production of more Ag specific plasma cells. 85,86 FcRn also facilitates efficient mucosal vaccination by transporting IgG over the mucosal epithelium into the lumen of for instance lung, intestine or vagina, and then transporting the immune complexed antigen back over the barrier, where it can be taken up by the underlying APCs. 87-89 In addition, while human monocytes express some FcRn, the expression is highly up-regulated upon differentiation into immature
DCs and down-regulated when they become mature, directly correlating with the cell’s ability to endocytose and phagocytose antigens (unpublished observations).

In chapter 6, we investigated whether the same holds true when the antigen in question is a virus, known for evading immunological memory and causing recurring infections, which could engage the innate immune system as well as the adaptive one. This was done by comparing the immune responses of C57BL/6 WT and FcRn<sup>−/−</sup> mice following immunization with RSV-Palivizumab immune complexes, and by comparing T cell activation by DCs loaded with RSV immune complexes containing either WT or mutated IgGs which were unable to bind to FcRn. In short, we found no benefit from FcRn participation in either DC antigen presentation in vitro or in vivo priming of immune responses in mice following intranasal administration of RSV immune complexes.

While this seems inconsistent with studies showing FcRn participation, we suggest that this could be explained by the many differences in the experimental setup of our study. For instance, RSV immune complexes would be subject to phagocytosis, rather than to the endocytosis route reserved for small soluble proteins such as OVA. As Liu et al. demonstrated in 2011, FcRn cannot always contribute to cross-presentation of phagocytosed opsonized beads because the phagosomes never reach low enough pH necessary for FcRn binding in some DC. 82 Furthermore, it is conceivable that the resident DC population involved in RSV uptake in the lung processes antigen in a different manner than the antigen-presenting cells involved in the immune response against a protein antigen in the circulation.

While this seems inconsistent with studies showing FcRn participation, we suggest that this could be explained by the many differences in the experimental setup of our study. For instance, RSV immune complexes would be subject to phagocytosis, rather than to the endocytosis route reserved for small soluble proteins such as OVA. As Liu et al. demonstrated in 2011, FcRn cannot always contribute to cross-presentation of phagocytosed opsonized beads because the phagosomes never reach low enough pH necessary for FcRn binding in some DC. 83 Furthermore, it is conceivable that the resident DC population involved in RSV uptake in the lung processes antigen in a different manner than the antigen-presenting cells involved in the immune response against a protein antigen in the circulation.

Although no vaccine against RSV exists to date, prophylaxis can be administered to those deemed at risk in the form of a neutralizing therapeutic antibody called Palivizumab. Since the purpose of a prophylactically administered antibody is to neutralize a pathogen before it is able to attack the host, the lack of exposure to the immune system might compromise the host’s ability to form an immunological memory against the invading pathogen (AMIS; antibody mediated immune suppression). 90 Therefore, the prophylactic treatment of an at-risk individual against RSV could simply delay the infection until the next exposure occurs. The most important results of this research was that intranasally administered RSV is actually more efficient in eliciting a
memory immune response when it is given in the form of an immune complex with IgG than just as a weakened un-opsonized virus. Thus, treating patients with a therapeutic antibody against RSV does not hinder their ability to develop an immunological memory against the pathogen; on the contrary, it boosts it. However, this effect was not due to FcRn mediated transport of immune complexes over the mucosal endothelium or FcRn controlled routing within the antigen presenting cell. Rather, the credit goes to activating FcγRs, quite likely expressed on dendritic cells in the airways.

An adjuvant-free, weakened RSV virus vaccine tested in the 60’s failed catastrophically, resulting in the death of two infants and causing the scientific community to be hesitant when it comes to testing new RSV vaccines on humans. Much of the research today aimed at discovering a working vaccine focuses on creating a genetically modified, harmless virus which shares enough epitopes with the original virus to create a memory response against it. After decades of frustrating results, new hope awakens following a recent paper in Science by McLellan et al. There they reveal the crystal structure of the pre-fusion conformation of an RSV fusion glycoprotein RSV F, which might just be the epitope needed for successful vaccinations in the future. Nevertheless, it might be interesting to test as well an RSV vaccine comprising of a weakened, IgG-complexed whole virus formulation together with appropriate adjuvants.

Furthermore, we would like to see more research done on wild type mice or humans in order to fully elucidate the role of FcRn in transport across mucosal endothelia as well as intracellular routing of complexed antigens inside antigen presenting cells such as dendritic cells. To our knowledge, only one experiment in 2008 has shown any contribution of FcRn on antigen presentation to CD4 T cells in humans, where monocyte derived dendritic cells presented complexed gliadin to a T cell clone derived from a celiac patient. Furthermore, no results have shown the same for wild type, non-transgenic mice with intact non-antigen restricted immune systems and a normal expression of FcRn, meaning that there is limited evidence that the FcRn effect is relevant when the entire immune system, both innate and adaptive, is involved during an actual pathogen challenge under physiological circumstances. It is imperative to develop in vitro human models to ascertain whether this FcRn effect is meaningful or indeed present in the human immune system. A more complete knowledge of the factors involved in mounting and/or boosting good immune responses is crucial for development of new treatments ranging from vaccines to immune therapies such as cancer, where antibody treatment can not only be used for direct tumor eradication, but also to boost the patient’s own immune responses. In contrast, this knowledge is also extremely important to prevent such unwanted immunization in autoimmune diseases, but also in pregnancy, where the anti-D treatment has been used since the 1960’s to prevent immune responses of the mother to the fetal red cells. These two seemingly paradoxical mechanisms – how antibodies can either activate or inhibit immune responses, and can moreover be
either beneficial or detrimental to a person’s health depending on the circumstances, serve as examples on how antibodies can act as double-edged swords and show us how much there is still to learn in this field.

CONCLUDING REMARKS

The aim of our research was to further clarify some uncertainties regarding the nuances of FcRn function in the body. Of special interest was the significance of FcRn participation in antigen uptake and presentation of virus and in phagocytosis, as well as the scope of FcRn involvement in antibody half-life extension and feto-maternal transport.

Recently, the field of immunology has been increasingly focused on the instrumental role of dendritic cells as conveyors of information in the form of antigenic peptides and modulators of the subsequent immune response, and the factors that influence them. While FcRn has been comprehensively shown to participate in the uptake, and to influence the fate of, immune-complexed soluble protein antigen in a murine setting, the same has not been demonstrated for viruses. While neutralizing antibodies did facilitate a more efficient immune response against the virus, we found no contribution of FcRn to this process. Based on these results, the most important characteristic of RSV neutralizing antibodies would be, aside from high affinity to the virus, to be able to bind classical FcγRs efficiently. Following that logic, IgG3, which has strong FcγR and complement mediated effector functions but poor binding to FcRn under physiological circumstances, could be an excellent candidate for a neutralizing α-RSV antibody.

Based on available research it is generally assumed that FcRn is the only receptor involved in both IgG serum half-life extension and feto-maternal transport. However, the exclusion of all other receptors in the process has not yet been conclusively demonstrated. The “alternative receptor” theory resurfaces with regular intervals, prompted by observations of discrepancies between two FcRn-mediated mechanisms of intracellular transport of monomeric IgG, namely half-life extension by recycling the IgG to the same surface, and maternal transport by cellular transcytosis. In order to address these issues we focused on factors which are either known to, or might theoretically, influence binding to FcRn or FcγRs, to isolate the contribution of each receptor to these processes. In short, we found no valid indication that either half-life extension or feto-maternal transport could be influenced by any other known Fcγ receptor. While IgG2 light chain isotypes were found to affect phagocytosis, this effect was mediated through (and correlated with) different affinity to FcγRIIa, and was not a result of any particular structural isomers distinctive to IgG2κ. Even though the two IgG2 isotypes also had different affinities to FcRn, any effect this might have on phagocytosis by neutrophils was completely overshadowed by the more dominant, and opposite, light chain preference of FcγRIIa. It is an intriguing
result that variability in the Fab region of IgG, far removed from the binding site of either receptor, could influence the affinity to and interaction with those receptors. Thus affinity seems, in this case, not to be just a direct result of the amino acid composition at the binding site, but is affected by changes in the overall structure which can be influenced by substitution located far away in the sequence, or indeed in another protein subunit.

Perhaps counter-intuitively, in spite of this differential affinity to FcRn, we observed no correlating isotypic difference in serum persistence and feto-maternal transport of IgG2k and IgG2λ. Upon closer inspection this is perhaps not surprising as it has already been demonstrated that antibody half-life, and therefore by deduction, conceivably also placental transport, does not correlate with affinity to FcRn. 67 One must bear in mind that the function of FcRn during transport of this monovalent ligand depends on a balancing act of binding and releasing at acidic and neutral pH, and since we only measured affinity in an acidic environment, we cannot truly predict what will happen at the site of release. 97

Much work is still to be done to fully elucidate the complex roles FcRn and other Fcγ receptors play in the humoral immune system. A more detailed understanding of the factors that influence these receptors, and as a result the effector functions of IgG and their serum persistence, will help us create more specialized, efficient and cost-effective therapeutic antibodies, specifically tailored to their particular purpose. Furthermore it will help us appreciate which characteristics of naturally occurring antibodies, in a normal or an auto-immune setting, confer maximum effectiveness or pathogenicity, respectively, and how each situation can influenced to our advantage.

REFERENCE LIST


44. Tesar DB, Tiangco NE, Bjorkman PJ. Ligand valency affects transcytosis, recycling and intracellular trafficking mediated by the neonatal Fc receptor. Traffic. 2006 Sep;7(9):1127-42.


46. Burmeister WP, Huber AH, Bjorkman PJ. Crystal structure of the complex of rat


65. Poulsen MS, Rytting E, Mose T, Knudsen LE. Modeling placental transport: correlation of in vitro BeWo cell permeability and ex vivo human


