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Gestation age dependent transfer of human immunoglobulins across placenta in timed-pregnant guinea pigs

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ABSTRACT

Introduction: When administered during pregnancy, antibodies and other biologic drugs that contain the Fc part of the IgG molecule can traverse the placenta. Although it is generally accepted that the FcRn receptor mediates this process, gaps remain in our understanding of underlying details in humans and in common laboratory animal species.

Methods: We expanded our previous studies in timed-pregnant guinea pigs to both measure the transport of human (h) IgG at earlier gestation ages *in vivo* and evaluate FcRn function *in vitro* using Surface Plasmon Resonance (SPR) and Madin–Darby canine kidney cells (MDCK) that express guinea pig (gp) FcRn.

Results: In timed-pregnant guinea pigs both the average concentration of hIgG in the fetus and its ratio to maternal hIgG concentration increase exponentially with gestation age. Thus, hIgG fetal:maternal concentration ratios increase from an average of 1% to 3%, 17%, and 76% on GD ~26, 35, 46, and 54, respectively. *In vitro*, gpFcRn immobilized on a solid surface can bind hIgG and gpIgG preparations in a similar manner. All engineered human Fc isotype-specific constructs were internalized by MDCK-gpFcRn cells at significant levels. While not significant, their recycling and hIgG transcytosis by this cell line also trend higher than background controls.

Discussion: Pregnant guinea pigs exhibit similarities with humans in the degree and timing of transplacental transfer as well as the ability of their FcRn to bind and internalize hIgG *in vitro*. Further studies are needed to guide building appropriate systems for the evaluation of FcRn mediated function of human immunoglobulin therapies.

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1. Introduction

Finding appropriate *in vivo* and ideally *in vitro* models for assessing safety and efficacy of protein therapeutics during pregnancy is not trivial. For many such biologics, the placenta provides an effective barrier that prevents fetal exposure to a biologic drug. This is not the case for antibody therapeutics consisting of intact antibodies and engineered biologics that contain the Fc part of IgG. An active, receptor mediated process ensures the transfer of these products from maternal to fetal blood [1]. This process is believed to start in the second trimester of human pregnancy [2], with 17–22 weeks fetuses having IgG concentrations that are at least ten times lower than maternal levels [3]. As human pregnancy progresses, fetal IgG levels continuously increase to reach and even surpass maternal levels at the end of the third trimester [4–7], resulting in fetal:maternal ratios higher than one. The same receptor, neonatal Fc receptor (FcRn) also plays a major role in rescuing IgG from catabolism, thus prolonging the half-life of IgG in the circulation [8–11].

Information on trans-placental transfer of human antibody preparations in common laboratory animal species is incomplete. At the end of gestation many species, including rabbits and monkeys exhibit trans-placental transfer of human antibodies [12,13]. Others, such as newborn rats and mice obtain most of their antibodies from their dam through lactation [13–15], although it has been shown that maternally administered human IgG can also be transferred to the fetus to achieve fetal:maternal ratios higher than one [16].

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We and others have shown that the pregnant guinea pig is a good model for studying antibody transfer during pregnancy [17–21] and can transfer all human IgG subclasses at the end of gestation [18]. Based on these findings, we set out to test the hypothesis that, like in humans, fetal concentration of the human IgG in the pregnant guinea pig increases with gestation. By providing data on fetal exposure to hIgG at different stages of the development and on species specific FcRn activity, our studies may help guide choosing appropriate models to assess safety and efficacy of antibody therapies during pregnancy.

2. Materials and methods

2.1. Animal studies

All animal procedures were performed in accordance with protocols approved by the CBER Animal Care and Use Committee. Hartley Albino (Crl:HA) guinea pigs were purchased from commercial sources and mated to produce timed pregnancies as previously described [20]. Four groups of pregnant sows, one for each gestational age, n = 3–4/group, were used. On gestation day (GD) ~22, ~30, ~41 and ~49, approximately corresponding to the end of first trimester, middle and end of second trimester and middle of the third trimester, the animals were weighed and a polyclonal commercial human IgG purified from pooled plasma of healthy donors with high titers of antibodies against Hepatitis B, HepaGam® (Emergent Biosolutions, 549 IU/mL and 41 mg/mL) was administered intravenously at a dose 100 IU/kg (0.182 ml/kg or ~7 mg/kg). Dose was chosen to correspond with the approved dose for infants born to mothers testing positive for Hepatitis B [22]. Five days after injection, i.e. GD ~35, ~46, and ~54, blood samples were collected from all dams, two of the litters on GD ~46, and all the litters of GD ~54 via cardio- or cordo-centesis; whole fetuses were collected from all the remaining animals (Table 1, italics). Due to a technical error, terminal samples in the group that received injections on GD ~22 were collected on GD ~26, four days after injection. Five days post-injection was used as the sampling point because previously we observed a plateau in the fetal concentration of maternally administered hIgG at 4–5 days post-administration [18], but also because it is short enough to allow for the assessment of administered product free from the interference of anti-drug antibodies to the foreign biologic. The GD designations were approximate because the breeding procedure allowed for a maximum of three days of co-habitation for the mating pair. Thus, the litter designated GD ~22 was obtained from sows that could be 22 ± 1 days pregnant.

Fetuses were carefully separated from the placenta, cleaned with cold PBS, weighed, flash frozen individually, and then homogenized by placing 50% tissue:PBS w:v mixture on ice with an OMNI TH apparatus (Omni International, Kennesaw, GA). The mixture was centrifuged at 10,000x *g* for 10 min at 4 °C and the supernate frozen at −80 °C for storage until use. hIgG in the serum and tissue homogenates was determined with a human IgG ELISA kit (Assaypro, St. Charles, MO). All samples were measured in duplicates and data points out of data fitting range or with CV > 15% were excluded from analysis and repeated measurements taken, if possible. The kit did not cross-react with guinea pig serum or homogenates from controls that did not receive hIgG.

2.2. Plasmids and cell culture

2.2.1. Gaussia Luciferase-hFc1, 2, 3, and 4 chimeric proteins

Gaussia luciferase gene (GLuc, New England Biolabs, Ipswich, MA) was inserted by restriction digest onto each of four plasmids for mammalian expression, pFUSE-hIgG(n)-Fc1 (n = 1,2, 3, or 4,

Table 1
Gestation age dependent transfer of human IgG in the pregnant guinea pig. Maternal and fetal hIgG concentrations were determined by fitting absorbance data measured in ELISA to a standard curve. Italics signify data from measurements in total body homogenates. Concentrations from all litter-mates were averaged to obtain the mean litter concentration and the fetal:maternal ratio was calculated by dividing this value by the hIgG concentration from the respective dam. The assumption was made that in the fetus IgG was distributed equally in tissue and serum, and no adjustment was made for the concentration measured in total body homogenates versus serum. One way ANOVA with Bonferroni post hoc analysis was used to compare gestation dependent hIgG fetal concentration or concentration ratios.

Gestation day	GD26			GD35			GD46			GD54		
hIgG Concentration	Maternal Concentration, µg/mL	Mean Litter Concentration (n), µg/mL	Fetal:Maternal Concentration Ratio (%)	Maternal Concentration, µg/mL	Mean Litter Concentration (n), µg/mL	Fetal:Maternal Concentration Ratio (%)	Maternal Concentration, µg/mL	Mean Litter Concentration (n), µg/mL	Fetal:Maternal Concentration Ratio (%)	Maternal Concentration, µg/mL	Mean Litter Concentration (n), µg/mL	Fetal:Maternal Concentration Ratio (%)
Individual Dams/Litters	77.6	*1.6* (2)	2.1	12.2	*0.5* (2)	4.1	74.3	15.1 (3)	20.3	59.1	26.6 (4)	45.0
	107.3	*1.1* (3)	1.0	73.4	*2.6* (3)	3.5	97.9	*11.6* (4)	11.8	76.8	72.0 (3)	93.8
	136.4	*1.4* (4)	1.0	110.3	*4.1* (4)	3.7	93.1	*3.8* (2)	4.1	99.1	88.4 (3)	89.2
				91.6	*1.2* (3)	1.3	103	34.4 (3)	33.4			
Group Mean	107.1	1.4	1.4	71.9	2.1	3.2	92.1	16.2	17.4	78.3	62.3*	76.0#
Std. Deviation	29.4	0.2	0.6	42.6	1.6	1.3	12.5	13.0	12.6	20.0	32.0	26.9
N (n)	3	3 (9)	3	4	4 (12)	4	4	4 (12)	4	3	3 (10)	3

*hIgG concentration or # its fetal:maternal ratio is statistically different from all the other gestation ages sampled.