OBSTETRICS

M281, an anti-FcRn antibody, inhibits IgG transfer in a human ex vivo placental perfusion model

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BACKGROUND: The transfer of pathogenic immunoglobulin G antibodies from mother to fetus is a critical step in the pathophysiology of alloimmune and autoimmune diseases of the fetus and neonate. Immunoglobulin G transfer across the human placenta to the fetus is mediated by the neonatal Fc receptor, and blockade of the neonatal Fc receptor may provide a therapeutic strategy to prevent or minimize pathological events associated with immune-mediated diseases of pregnancy. M281 is a fully human, aglycosylated monoclonal immunoglobulin G1 antineonatal Fc receptor antibody that has been shown to block the neonatal Fc receptor with high affinity in nonclinical studies and in a phase 1 study in healthy volunteers.

OBJECTIVE: The objective of the study was to determine the transplacental transfer of M281 and its potential to inhibit transfer of immunoglobulin G from maternal to fetal circulation.

STUDY DESIGN: To determine the concentration of M281 required for rapid cellular uptake and complete saturation of the neonatal Fc receptor in placental trophoblasts, primary human villous trophoblasts were incubated with various concentrations of M281 in a receptor occupancy assay. The placental transfer of M281, immunoglobulin G, and immunoglobulin G in the presence of M281 was studied using the dually perfused human placental lobule model. Immunoglobulin G transfer was established using a representative immunoglobulin G molecule, adalimumab, a human immunoglobulin G1 monoclonal antibody, at a concentration of 270 μg/mL. Inhibition of immunoglobulin G transfer by M281 was determined by cotransfusing 270 μg/mL of adalimumab with 10 μg/mL or 300 μg/mL of M281. Concentrations of adalimumab and M281 in sample aliquots from maternal and fetal circuits were analyzed using a sandwich enzyme-linked immunosorbent assay and Meso Scale Discovery assay, respectively.

RESULTS: In primary human villous trophoblasts, the saturation of the neonatal Fc receptor by M281 was observed within 30–60 minutes at 0.15–5.0 μg/mL, suggesting rapid blockade of neonatal Fc receptor in placental cells. The transfer rate of adalimumab (0.23% $\pm$ 0.21%) across dually perfused human placental lobule was significantly decreased by 10 μg/mL and 300 μg/mL of M281 to 0.07 $\pm$ 0.01% and 0.06 $\pm$ 0.01%, respectively. Furthermore, the transfer rate of M281 was 0.002% $\pm$ 0.02%, approximately 100-fold lower than that of adalimumab.

CONCLUSION: The significant inhibition of immunoglobulin G transfer across the human placental lobule by M281 and the minimal transfer of M281 supports the development of M281 as a novel agent for the treatment of fetal and neonatal diseases caused by transplacental transfer of alloimmune and autoimmune pathogenic immunoglobulin G antibodies.

Key words: autoimmune disease, fetal transfer, monoclonal antibodies, M281, neonate, placental perfusion model

Alloimmune and autoimmune diseases of the fetus and newborn result from maternal development of potent pathogenic immunoglobulin G (IgG) antibodies that are transferred from mother to fetus.[1] For example, hemolytic disease of the fetus and newborn, fetal neonatal autoimmune thrombocytopenia, and autoimmune congenital heart block result from development of maternal IgG antibodies against fetal red blood cell antigens, platelets, or the developing fetal AV node, respectively.[1–3] With elevated maternal titers and, importantly, pathogenic IgG potency, the exposure of the fetus to these alloantibodies or autoantibodies through the increasing placental transfer of IgG from the second to third trimester results in damage to fetal tissues and disease development.[4,5]

The neonatal Fc receptor (FcRn) mediates the passage of IgG from mother to fetus in addition to maintaining the long half-life of IgG by IgG recycling in vascular endothelial cells.[6] These mechanisms suggest that an inhibitor of FcRn-IgG interaction may prevent or minimize gestational transfer of pathogenic IgG from mother to fetus as well as decrease pathogenic antibody titers in maternal circulation. M281 is a monoclonal anti-FcRn antibody that binds with high affinity to the IgG binding site on FcRn and blocks IgG binding to FcRn. In a phase 1 normal healthy volunteer study, M281 was observed to rapidly saturate systemic FcRn upon intravenous administration and decrease circulating IgG consistent with inhibition of IgG recycling.[7]

The aims of the current study were to determine the potential of M281 to inhibit transplacental IgG transfer using adalimumab as a representative IgG molecule and to determine whether M281 itself is transferred across the human placenta using the dually perfused human placental model.

Materials and Methods

Test agents and reagents

Adalimumab (Humira; AbbVie Inc, North Chicago, IL) and IVIg (Carimmune, lyophilized preparation; CSL Behring, Bern, Switzerland) were obtained from commercial sources. M281 was produced and manufactured by Momenta Pharmaceuticals Inc (Cambridge, MA). Dextran 40, gentamicin sulfate, heparin, sodium bicarbonate,

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AJOG at a Glance

Why was this study conducted?

This study was conducted to evaluate the transplacental transfer of M281, a monoclonal anti-FcRn antibody and its potential to inhibit the transfer of immunoglobulin G from maternal to fetal circulation.

Key findings

M281 significantly inhibits the maternal-to-fetal transfer of a representative immuoglobulin G molecule (adalimumab) in the ex vivo dually perfused human placental lobule model. However, M281 itself shows insignificant transfer from maternal to fetal circulation.

What does this add to what is known?

M281, a novel anti-FcRn antibody, may reduce the transfer of pathogenic immunoglobulin G from maternal to fetal circulation. These data support further investigation of M281 in the management of alloimmune or autoimmune diseases of the fetus and newborn.

antipyrine, phenacetin, and methanol were purchased from Sigma-Aldrich (St Louis, MO). Gibco M199 media was obtained from ThermoFisher Scientific (Waltham, MA).

Clinical material

Placentas were collected immediately following cesarean-sectioned abdominal deliveries from the labor and delivery ward of the John Sealy Hospital, the teaching hospital of the University of Texas Medical Branch (Galveston, TX) according to a protocol approved by the institutional review board. Only placentas determined by macroscopic examination to be of normal morphology and from uncomplicated term (38−40 weeks) singleton pregnancies were included to the study. Placentas from women with multiple gestation, hypertension, evidence of infection, systemic disease, drug or alcohol abuse, intrauterine growth restriction, and known fetal congenital abnormalities were excluded.

FcRn receptor occupancy assay

A receptor occupancy assay (detailed methods in Supplementary Material) was used to determine the concentration and time required for M281 to fully occupy FcRn in primary human villous trophoblasts (HVTs). M281 was tested over a range of concentrations (0.015−5.0 μg/mL) and incubation times (5−120 minutes). Receptor occupancy was assessed as the percentage of unoccupied (free) FcRn per cell after incubation with unlabeled M281 for various periods of time. Briefly, primary HVT monolayers were incubated with media alone, M281, or isotype-matched (IgG1) control antibody (Southern Biotech, Birmingham, AL) for the indicated times. At each time point, cells were detached, subjected to ice-cold fixation, and permeabilized at pH 7.4 (Cytofix/Cytoperm; BD Biosciences, San Jose, CA) and then incubated for 30 minutes on ice in the dark with a VT645-labeled M281 for binding to cell-associated, unoccupied FcRn. Cells were washed in ice-cold buffer and analyzed for geometric mean fluorescence index of cell-associated VT645-labeled M281. M281 label in cells incubated with media alone represented the measure of 100% unoccupied receptors. Full FcRn saturation was defined as <10% unoccupied receptors.

Placental perfusion studies

The transfer of test compounds M281 and adalimumab from the maternal-to-fetal circulation was studied using the ex vivo technique of dual perfusion of placental lobule as previously described by Nanovskaya et al.[8-10] Briefly, each placenta was examined for tears, and 2 chorionic vessels (1 artery and 1 vein) supplying a single intact peripheral cotyledon were cannulated with 3F and 5F umbilical catheters, respectively. The cotyledon was trimmed and placed in the perfusion chamber with the maternal surface upward. The intervillous space on the maternal side was perfused by 2 catheters piercing the basal plate. The flow rate of medium in the fetal and maternal circuits was maintained at 3.0 and 12 mL/min, respectively. The maternal perfusate was equilibrated with a gas mixture made of 95% oxygen/5% carbon dioxide and the fetal perfusate with a mixture of 95% nitrogen/5% carbon dioxide.

Each placental cotyledon was perfused for an initial period of 1 hour in the absence of test compounds (control period) to evaluate the physical integrity of the tissue. During the control period, the perfusion system was used in open-open configuration (ie, without recirculation of the perfusate). The perfusion was terminated, and experiment was not initiated if 1 or more of the following criteria were observed during the control period: fetal volume loss of >2 mL/h and/or a difference between partial pressure of oxygen in fetal vein and artery < 60 mm Hg.

Following the control period, maternal and fetal perfusates were replaced with fresh medium containing 3 mg/mL of bovine serum albumin. The experimental period was performed using closed-closed configuration of the system (ie, with recirculation of the perfusates). The experimental period was initiated after the addition to the maternal reservoir of tests substances. The nonionizable, lipophilic marker compound antipyrine (100 μg/mL) was cotransfused with test substance(s) to assure that all studies maintained an adequate level of perfusion overlap (ie, transfer of antipyrine to the fetal circuit within 120 minutes) was >35%.[11,12]

In a first set of experiments, the extent of M281 transfer (concentrations between 300 and 20,000 μg/μL) across dually perfused human placental lobule was carried out for 4 or 6 hours. In a second set of

experiments, the extent of transplacental transfer of adalimumab (270 μg/mL) during 6 hours of perfusion[13] was established. A third set of experiments was then performed wherein the inhibitory effect of M281 on the transplacental transfer of adalimumab (270 μg/mL) was studied. In these experiments, M281 concentrations of 10 and 300 μg/mL were investigated. These concentrations of M281 represent serum levels of M281 observed in the phase 1 study of M281.[7]

Samples from maternal artery and fetal vein (in 0.5 mL aliquots) were taken at 0, 30, 60, 120, 180, 240, 270, 300, 330, and 360 minutes during the experimental period and frozen at −80°C until analysis.

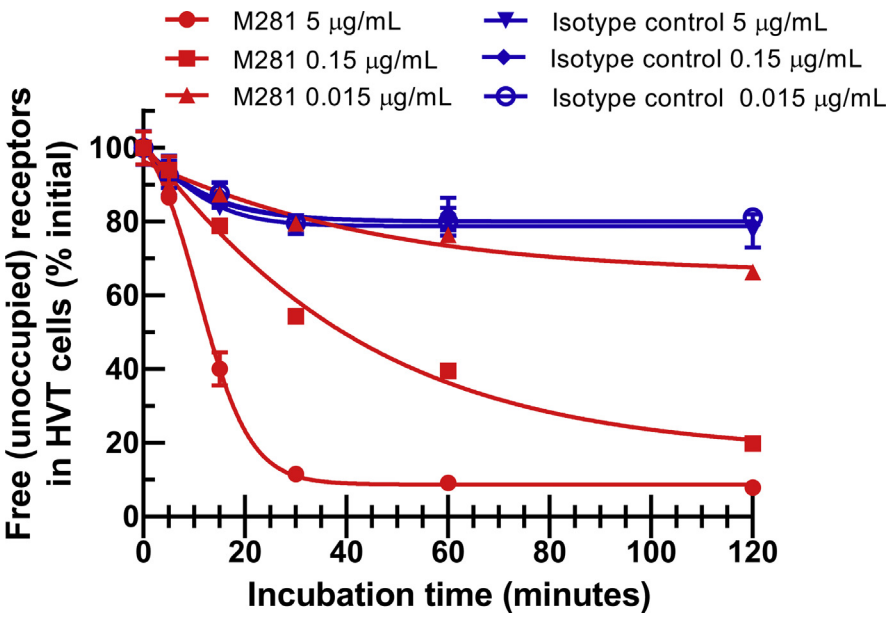
Sample and data analysis

Quantitation of the antipyrine

Concentrations of antipyrine in maternal and fetal aliquots were measured using a modified high-performance liquid chromatography (HPLC) method previously described by Morck et al.[14] Briefly, protein was precipitated by the addition of 200 μL of ice-cold acetonitrile containing 10 μL/mL of internal standard phenacetin to each 200 μL sample. Samples were centrifuged at 8000 rpm, and supernatants were run on an Agilent 1200 HPLC system equipped with a G1315D DAD detector (Agilent Technologies, Santa Clara, CA).

Antipyrine was analyzed using an HPLC method with a lower limit of quantification of 5 μg/mL. The HPLC analysis was performed at room temperature (22−25°C) using a reverse-phase, C18-based column (Waters Atlantis T3, Atlantis T3 Column, 100Å, 3 μm, 3 mm × 150 mm [Waters Corporation, Milford, MA]) fitted with a guard cartridge (Waters T3, 3 μm, 2.1 mm × 150 mm [Waters Corporation]). The samples were run with a linear gradient ranging from 25% to 95% methanol water over 14 minutes at a flow rate of 0.3 mL/min; the injection volume was 10 μL and detection was performed using absorbance at 260 nm.

FIGURE 1
Rapid uptake and saturation of FcRn by M281



FcRn saturation is indicated by a decrease in unoccupied receptors detectable following cell permeabilization and incubation with fluor-labeled M281. Primary HVTs were incubated for different periods of time with various concentrations of unlabeled M281 or isotype-matched control antibody. At each time point, cells were detached, washed, fixed/permeabilized, and incubated with VT645-labeled M281. Flow cytometry was used to quantitate cell-associated mean fluorescence index. Values are mean ± standard deviation, representative graph of n = 2 experiments.

HVT, human villous trophoblast.

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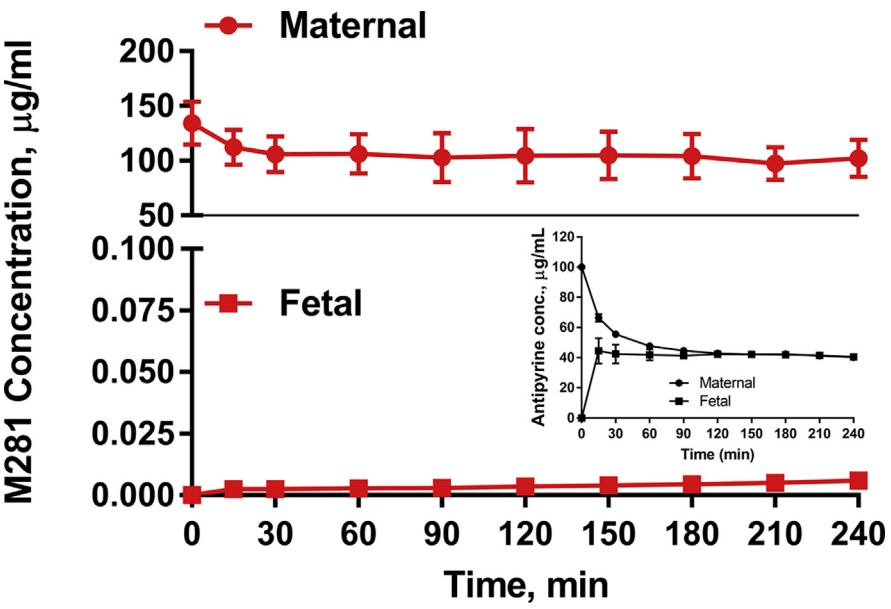
Quantitation of adalimumab

The concentrations of adalimumab in all maternal and fetal sample aliquots were measured using a sandwich enzyme-linked immunosorbent assay (Momenta Pharmaceuticals) with a lower limit of quantitation of approximately 1 ng/mL. Briefly, 96-well plates were coated with recombinant human tumor necrosis factor alpha overnight, followed by incubation with adalimumab-containing samples and standards at room temperature. Adalimumab was detected by a colorimetric method using peroxidase-conjugated donkey antihuman IgG secondary antibody in the presence of chromogenic substrate TMB (ThermoFisher Scientific, Waltham, MA), which allowed an absorbance readout at 450 nm. Test substance levels were interpolated from the calibration standards plotted using a 4-parameter logistic curve fit.

Quantitation of M281

M281 concentrations in all the maternal and fetal perfusate sample aliquots were determined using a Meso Scale Discovery electrochemiluminescence assay (Bio-Agilytix, Durham, NC) with a lower limit of quantification of 5 ng/mL. Briefly, plates coated with a mouse anti-M281 idiotype capture antibody and blocked with enzyme-linked immunosorbent assay blocking buffer E104 (Bethyl Laboratories, Montgomery, TX) were incubated with test samples for 1 hour at room temperature. After washing, plates were incubated for 1 hour with a second biotinylated mouse anti-M281 idiotype detection antibody, followed by detection with Sulfo-Tag Streptavidin, and analyzed on a Meso QuickPlex SQ 120 (Meso Scale Discovery, Rockville, MD). Data were analyzed using MSD Workbench 4.0 (Meso Scale Discovery).

FIGURE 2
Low placental transfer of M281



M281 (300 μg/mL) and control antipyrine (100 μg/mL) were added to the maternal circuit at $t = 0$ and measured in both maternal and fetal circuits. Fetal transfer of M281 was extremely low, while antipyrine (*inset*) rapidly achieved fetal-maternal circuit equilibrium, indicating successful and consistent perfusion overlap. Data are shown as mean ± standard deviation (n = 3).

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Fetal transfer rate

The fetal transfer rate (FTR) for each test substance (adalimumab, M281, or antipyrine) was calculated as 100 × concentration of the test substance in the fetal circuit at the end of the experimental period/concentration of the test substance in the maternal circuit at the start of the experimental period.

Statistical analysis

A linear mixed-effects model was used for the analysis of all the experimental data with random terms for both slope and intercept for each donor identification to account for the correlation among measurements from the same donor/placental perfusion experiment. Two main experimental groups were considered for this analysis: adalimumab alone and adalimumab plus M281. All concentrations in aliquots from fetal samples were divided by the concentrations in aliquots from maternal samples at corresponding time points and then log transformed. The sensitivity of the results to possible outliers was also assessed and *P* values were adjusted for multiple comparisons.

Results

Time and concentration dependence of FcRn binding by M281 in HVTs

A receptor occupancy assay was used to determine the time and concentration of M281 required to saturate all available FcRn in HVTs. M281 exhibited a rapid onset of complete receptor occupancy in <60 minutes in HVTs at a concentration of 5 μg/mL as demonstrated by the decrease in unoccupied FcRn to background levels (≤10%) (Figure 1). M281 at 0.15 μg/mL resulted in nearly full occupancy by 120 minutes, whereas insignificant occupancy was observed up to 120 minutes at 0.015 μg/mL. As expected, an isotype control antibody that does not bind FcRn with high affinity at both intracellular and extracellular pH exhibited no occupancy of FcRn in HVT in this assay.

TABLE 1
Transplacental transfer rates of M281

Study type	Maternal circuit, adalimumab, μg/mL[a]	Maternal circuit, M281, μg/mL [a]	Fetal circuit M281 at study end, mean (SD), μg/mL	Fetal transfer rate of M281, mean (SD), %	Experimental period, hours	Number of studies
M281 alone	—	300	0.006 (0.004)	0.005 (0.003)	4	3
M281 alone	—	3000	0.08 (0.07)	0.002 (0.001)	4[b]	6
M281 alone	—	3000	0.08 (0.04)	0.002 (0.001)	6	3
M281 alone	—	20,000	0.4 (0.3)	0.003 (0.001)	4	5
M281 plus adalimumab	270	10	ND	ND	6	3
M281 plus adalimumab	270	300	0.02 (0.02)	0.006 (0.009)	6	5

Mean antipyrine fetal transfer rates for each group ranged from 40.6% to 41.9%. Fetal transfer rate = 100 × concentration of the test substance in the fetal circuit at the end of the experimental period/concentration of the test substance in the maternal circuit at the start of the experimental period.

ND, not detectable, below limit of quantitation; *SD*, standard deviation.

[a] Concentration of test compounds at initiation of the experimental period; [b] One study was terminated early at 3 hours.

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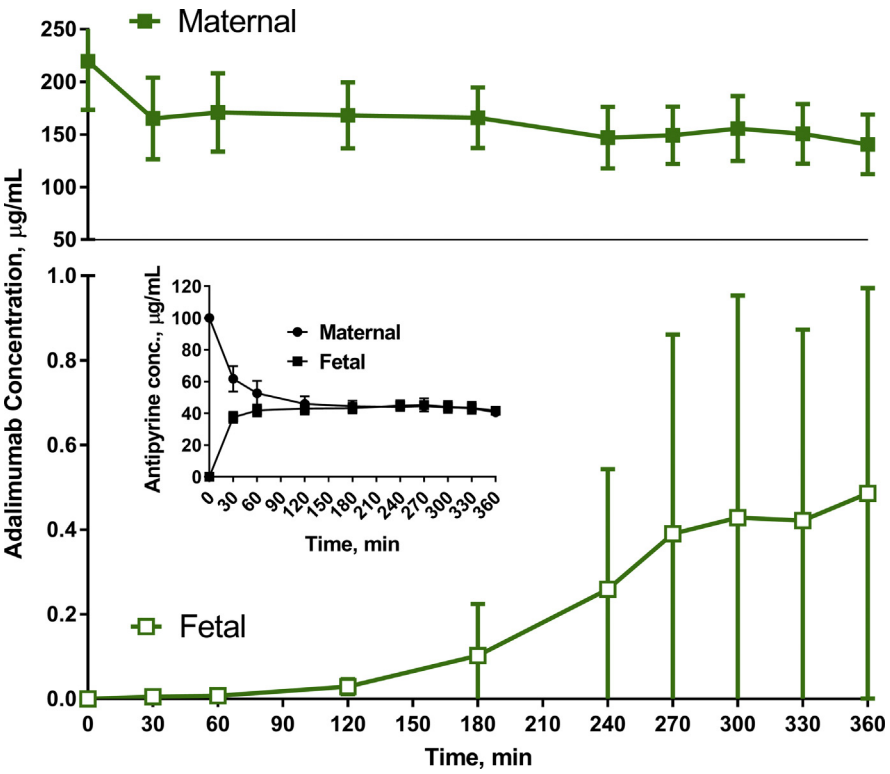
FIGURE 3
Placental transfer of adalimumab



Adalimumab (270 μg/mL) and control antipyrine (100 μg/mL) were added to the maternal circuit at $t = 0$ and measured in both maternal and fetal circuits during the experimental period. Fetal transfer of adalimumab increased significantly after 60 minutes. Successful, consistent perfusion overlap was achieved in these studies because antipyrine (*inset*) transfer rapidly reached equilibrium between maternal and fetal circuits. Data are shown as mean ± standard deviation (n = 8).

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Transfer of control compound, antipyrine, across the dually perfused human placental lobule

Antipyrine, a widely used control molecule in perfusion studies, is expected to transfer rapidly and equilibrate between the fetal and maternal circuits in a successful perfusion.[11] In all experiments reported here, maternal-fetal circuit equilibration was achieved within the expected timeframe. The FTR of antipyrine coperfused with M281 was 41% ± 0.5%, with adalimumab, 42% ± 2.7%, and with a combination of M281 and adalimumab, 43% ± 2.8%, indicating a similar degree of perfusion overlap among different sets of experiments and thus enabling comparability between studies reported.

Transfer of test compounds across the dually perfused human placental lobule

Very low concentrations of M281 were detected in the fetal circuit throughout the experimental period (Figure 2 and Table 1). The low amount of M281 transferred to the fetal circuit increased steadily and in proportion with the concentration added to the maternal circuit, suggesting transfer by a nonsaturable process. The average FTR of M281 over the concentrations tested (300 μg/mL, 3000 μg/mL, 20,000 μg/mL) ranged from 0.002−0.006% (Table 1) indicated an extremely low transfer rate, even at the highest concentration tested.

The transfer of the representative IgG (adalimumab) showed an initial decline in concentration in the maternal circulation during the initial 30 minutes, which can be attributed to the distribution of the adalimumab in the perfused lobule. Adalimumab appeared in the fetal circuit after 60 minutes, and its transfer increased substantially through the end of the experimental period (Figure 3). At the end of 6 hours of perfusion, the average FTR of adalimumab was 0.23% ± 0.21% (range, 0.05− 0.65%, n = 8, Table 2).

The effect of M281 on transplacental transfer of adalimumab

In the presence of M281, the detectable transfer of adalimumab across the placental lobule was delayed (>120 minutes vs >60 minutes), and fetal transfer rates were decreased, irrespective of the concentrations of M281 tested (Figure 4 and Table 2). However, the FTR of M281 was not affected by the presence of adalimumab (Table 1).

Comment

The ability of M281 to inhibit IgG1 transfer across the human placenta was evaluated using the dual-perfusion term placental lobule model under conditions intended to represent the highest efficiency transfer of pathogenic IgG. A human IgG1 monoclonal antibody, adalimumab, was used as a representative IgG molecule. IgG1 and IgG3 comprise the 2 subclasses of pathogenic IgG in the majority of alloimmune and autoimmune diseases of the fetus and newborn, with IgG1 being the predominant subclass.[13,15−18] IgG1 is also the most efficiently transferred IgG subclass in both human pregnancy and the placental perfusion model.[4,13] Because the transfer of IgG increases with the progression of gestational age,[4,5] we used human term placentas representing the period for the highest IgG transfer. Additionally, the adalimumab concentration in the maternal circuit is within the range of concentrations observed for pathogenic alloantibodies and autoantibodies.[19−22]

The FTR of the representative IgG, adalimumab (0.23% ± 0.21%), in the absence of M281 was similar to previously reported IgG transfer rates

TABLE 2
M281 inhibition of IgG transfer from maternal to fetal circulation

Maternal circuit M281, μg/mL[a]	Maternal circuit adalimumab, μg/mL[a]	Fetal circuit adalimumab at study end, mean (SD), μg/mL	Fetal transfer rate Adalimumab, mean (SD), %	*P* value[b]	Number of studies	Experimental period, hours
0	270	0.50 (0.5)	0.23 (0.21)	NA	8	6
10	270	0.12 (0.02)	0.07 (0.01)	< .001	3	6
300	270	0.12 (0.01)	0.06 (0.01)	< .001	5	6

Mean antipyrine fetal transfer rate for these studies was 41.7% ± 2.7% for adalimumab alone and 43.8% ± 4.2% for all adalimumab plus M281 studies. Fetal transfer rate = 100 × concentration of the test substance in the fetal circuit at the end of the experimental period/concentration of the test substance in the maternal circuit at the start of the experimental period.

NA, not applicable.

[a] Concentration of test compounds in the maternal perfusate at initiation of the experimental period; [b] *P* values were calculated compared with no M281 using a linear mixed-effects model with random slope and intercept.

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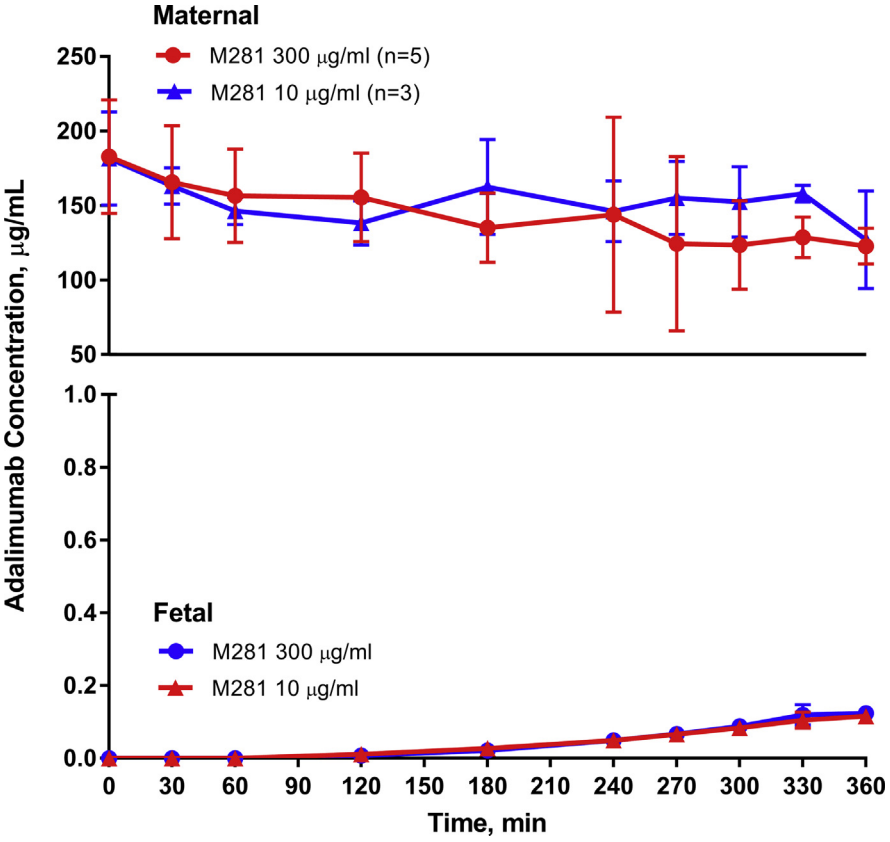
(0.08−0.5%) in this model.[13,23−26] The high variability in the extent of adalimumab transfer across the placenta lobule may be explained in part by factors such as individual variability in IgG uptake by pinocytosis, expression of IgG-binding Fc gamma receptors, or vesicular trafficking. No association was found between FcRn expression and adalimumab FTR in these studies (data not shown).

FIGURE 4
Placental transfer of adalimumab is inhibited by M281



Adalimumab (270 μg/mL), M281 (10 μg/mL, n = 3, or 300 μg/mL, n = 5) and control antipyrine (100 μg/mL) were added to the maternal circuit at $t = 0$ and measured in both maternal and fetal circuits over the experimental period. M281, irrespective of concentration tested, significantly decreased adalimumab transfer compared with its transfer in the absence of M281 (Figure 3). Data are shown as mean ± standard deviation.

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In the presence of M281, the adalimumab transfer rate decreased by approximately 3- to 4-fold (Table 2) and remained at 0.06−0.07%, irrespective of the M281 concentration tested (10 or 300 μg/mL). It should be noted that the diminished transfer rate of adalimumab in the presence of M281 is similar to the previously reported transfer rate for immunoglobulin A,[26] an immunoglobulin isotype known to transfer extremely poorly across the human placenta in pregnancy. Furthermore, IVIg, which at high concentrations acts as a competitive inhibitor at the FcRn IgG binding site, was used as a positive control. The inhibitory effect of 6700 μg/mL of IVIg on adalimumab transfer to the fetal circuit (FTR 0.07% ± 0.03%; Supplemental Table 1) was similar to the inhibitory effect of M281 at 10 ug/mL (FTR 0.07% ± 0.01%, Table 2).

These data suggest that the transfer of IgG in the presence of M281 may be negligible in vivo. Both 10 and 300 μg/mL of M281 resulted in similar decreases in adalimumab transfer rates, suggesting that the inhibitory activity of M281 on IgG transport was saturated. These ex vivo model results are consistent with the rapid uptake and receptor occupancy of FcRn by M281 in HVTs at concentrations as low as 5 μg/mL.

Because concentrations of M281 greater than 10 μg/mL were maintained along with FcRn receptor occupancy with weekly 30 mg/kg dosing of healthy volunteers in the phase 1 study, a sustained blockade of IgG transfer across the human placenta may be achievable in clinical studies.[7]

The transfer rate of M281 itself across the perfused placenta lobule was 0.002% $\pm$ 0.002%, which is nearly 100-fold lower than the transfer rate for the representative IgG, adalimumab alone. This low transfer rate of M281 is unlikely to maintain active drug concentrations in the fetal circulation in vivo because concentrations in the fetal circuit were substantially below active M281 concentrations observed in HVTs in vitro.

The extremely poor placental transfer of M281 may be explained by the high-affinity binding of M281 to FcRn at both intracellular and extracellular pH, which prevents its release from FcRn during any step of the transfer process.[6] The low transfer of M281 across the human placenta is significant because the potential for a low or negligent exposure of the developing fetus and neonate is an important safety consideration in the future clinical development of M281.

The results of this investigation demonstrate that M281, a direct and potent inhibitor of FcRn, decreased transfer of IgG across term placenta. These data together with the rapid FcRn occupancy and reduction in circulating IgG observed on M281 administration in nonpregnant healthy volunteers in the phase 1 study[7] and in chronic, reproductive, and immunological toxicology studies in nonhuman primates (data not shown) suggest that maternal administration of M281 could potentially lower alloantibody or autoantibody titers and prevent their passage into the fetus to potentially delay, minimize, or prevent fetal and neonatal disease development.

While M281 is expected to inhibit transplacental transfer and increase maternal clearance of both pathogenic and beneficial IgG, a single maternal IVIg infusion just prior to birth would provide passive immunity to the neonate and recovery of maternal circulating IgG in clinical settings. A favorable risk-benefit may be achievable for initial evaluation of M281 safety and efficacy in an indication such as severe early-onset antenatal hemolytic disease of the fetus, an indication with predictably poor outcomes, noninvasive disease monitoring, and potential for standard-of-care rescue therapy.[27-29] Other potential indications include pregnancies with a history of autoimmune congenital heart block,[3] fetal neonatal alloimmune thrombocytopenia with intracranial hemorrhage,[30] or neonatal thyrotoxicosis.[31]

In summary, these human placental perfusion studies together with the additional M281 nonclinical and clinical studies support further clinical research to evaluate the safety and efficacy of M281 in severe pregnancy-associated alloimmune and autoimmune diseases of the fetus and newborn. ■

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Data requests from any qualified researchers who engage in rigorous, independent scientific research will be considered if the trials are not part of an ongoing or planned regulatory submission (this includes requests for data on unlicensed products and indications). Data will be provided following review and approval of a research proposal, statistical analysis plan, confirmation that the requested data can be shared under applicable privacy laws, and execution of a data-sharing agreement. Data requests can be submitted at any time, and the data will be accessible for 12 months, with possible extensions considered. Requests can be sent to MedInfo@momentapharma.com.

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Supplementary Materials

FcRn receptor occupancy assay

Primary human villous trophoblasts (HVTs) (ScienCell, Carlsbad, CA) were cultured in complete trophoblast media (ScienCell) on plates coated with poly-L-lysine (ScienCell). For receptor occupancy studies, HVTs were seeded in poly-L-lysine-coated, 6-well plates and cultured until 80% confluence. Media alone, M281, or an immunoglobulin G1 isotype-matched control antibody (Southern Biotech, Birmingham, AL) in trophoblast media media alone was added to HVT at time 0 and incubated at 37°C in a tissue culture incubator for various times. At indicated times, plates were placed on ice. The media was aspirated, and cell monolayers were rinsed once with ice-cold Dulbecco's phosphate-buffered saline (Millipore Sigma, St Louis, MO) and then detached with room-temperature HyQTase (ScienCell). After centrifugation at 400 × g, cells were resuspended in Cytofix/Cytoperm solution (BD Biosciences, San Jose, CA) at 4°C for 20 minutes, washed twice with ice-cold 1 × Perm buffer (BD Biosciences), and resuspended in Perm buffer with 10% fetal bovine serum (ScienCell) with 7.5 μg/mL VT645-labeled M281 for 30 minutes. Following incubation, cells were washed twice with ice-cold Perm buffer, resuspended in FACS buffer (BD Biosciences), and filtered prior to FACS analysis (FACSCanto, BD Biosciences).

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SUPPLEMENTAL TABLE 1
IVIg inhibition of IgG transfer from maternal to fetal circulation

Maternal circuit IVIg, μg/mL [a]	Maternal circuit adalimumab, μg/mL [a]	Fetal circuit adalimumab at study end, mean (SD), μg/mL	Fetal transfer rate adalimumab, mean (SD), %	*P* value [b]	Number of studies	Experi-mental period, hours
6700	270	0.12 (0.05)	0.07 (0.03)	< .001	5	6 [c]

Mean antipyrine fetal transfer rate for these studies was 41.7% ± 2.7% for adalimumab alone and 43.8% ± 4.2% for all adalimumab plus M281 studies. Fetal transfer rate = 100 × concentration of the test substance in the fetal circuit at the end of the experimental period/concentration of the test substance in the maternal circuit at the start of the experimental period.

[a] Concentration of test articles in the maternal perfusate at the initiation of the experimental period; [b] *P* values were calculated compared with no M281 using a linear mixed-effects model with random slope and intercept; [c] One study was terminated early at 5.5 hours.

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