Blocking FcRn in humans reduces circulating IgG levels and inhibits IgG immune complex–mediated immune responses

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The neonatal crystallizable fragment receptor (FcRn) functions as an intracellular protection receptor for immunoglobulin G (IgG). Recently, several clinical studies have reported the lowering of circulating monomeric IgG levels through FcRn blockade for the potential treatment of autoimmune diseases. Many autoimmune diseases, however, are derived from the effects of IgG immune complexes (ICs). We generated, characterized, and assessed the effects of SYNT001, a FcRn-blocking monoclonal antibody, in mice, nonhuman primates (NHPs), and humans. SYNT001 decreased all IgG subtypes and IgG ICs in the circulation of humans, as we show in a first-in-human phase 1, single ascending dose study. In addition, IgG IC induction of inflammatory pathways was dependent on FcRn and inhibited by SYNT001. These studies expand the role of FcRn in humans by showing that it controls not only IgG protection from catabolism but also inflammatory pathways associated with IgG ICs involved in a variety of autoimmune diseases.

INTRODUCTION

The recognition that immunoglobulin G (IgG) autoantibodies can have pathogenic effects has stimulated the development of numerous strategies aimed at removing or decreasing the effects of IgG and its associated immune complexes (ICs) (1, 2). Preclinical data support the notion that blockade of the neonatal Fc receptor (FcRn) may also serve as a unique treatment for IgG-mediated autoimmune diseases (3–7). FcRn is a major histocompatibility complex (MHC) class I–related molecule that associates noncovalently with β2-microglobulin (B2m) (8). Despite its name, it is now well recognized that FcRn is widely expressed throughout human life in multiple types of parenchymal (9–11) and hematopoietic cells (12–15). FcRn functions within intracellular endosomes, where it binds IgG and albumin at distinct, nonoverlapping sites under acidic but not neutral pH conditions (16). Thus, FcRn is considered a saturable “protection” receptor that serves to prevent the catabolism of IgG and albumin (8). It does so through its ability to bind these two proteins within early and recycling acidic endosomes whereby it diverts them from destruction in lysosomes, lastly returning these cargo molecules to the neutral pH of the cell surface where they are released (17–19). This process accounts for the unusually long half-life of circulating IgG and albumin (8) and is likely the basis for many of the therapeutic benefits of intravenous immunoglobulin (IVIG) as exogenously administered antibodies have been suggested to compete for FcRn binding resulting in its saturation and lowering of endogenous IgG levels (6).

Mouse studies illustrate that FcRn expression in cells of bone marrow origin contributes to the protection of not only circulating monomeric IgG (13, 20, 21) but also circulating ICs (CICs) such that when FcRn is absent from hematopoietic cells, CICs are eliminated more rapidly from the bloodstream (22). In addition to regulating CIC levels, FcRn in Ag-presenting cells (APCs) also determines the ability of IgG ICs to promote the secretion of inflammatory cytokines (23) and their intracellular routing to compartments critical to Ag processing and presentation via MHC class II to CD4+ T cells and cross-presentation via MHC class I to CD8+ T cells (22, 24). Consistent with this, genetic deletion or pharmacologic inhibition of FcRn protects from autoimmune diseases in mouse models (4, 6, 7). This suggests that FcRn functions broadly in inflammatory pathways by preventing the degradation of IgG and IgG ICs and enabling IgG ICs to mediate innate and adaptive immune functions.

Although several approaches to directly blocking FcRn for the potential treatment of autoimmunity have recently emerged (25–27), they have exclusively focused on the reduction of circulating monomeric IgG levels and have not considered the possible impact that FcRn has on immune response to IgG ICs. Here, we report a humanized, affinity-matured IgG4-k monoclonal antibody, SYNT001, that specifically interferes with human FcRn (hFcRn) binding to IgG and describe its pharmacokinetic (PK), pharmacodynamic (PD), and safety profile in cynomolgus monkeys and healthy human volunteers. In doing so, we reveal that blockade of FcRn rapidly lowers the levels of CICs in humans and the ability of IgG ICs to induce inflammatory responses by hematopoietic cells.
RESULTS
Development and characterization of SYNT001 in mice

We developed a humanized, affinity-matured, deimmunized IgG4-κ monoclonal antibody (SYNT001) containing a S241P mutation that binds hFcRn at neutral and acidic pH (Table 1). The cocrystal structure of hFcRn in complex with a SYNT001-derived Fab fragment was solved to a resolution of 2.4 Å (table S1) and showed binding in a 1:1 stoichiometric ratio by engaging residues 85 to 88, 112 to 117, and 130 to 133 on the hFcRn α heavy chain and residues 1 to 3 and 58 to 60 on the βm light chain (Fig. 1A and fig. S1A). This binding surface completely overlaps with that of IgG1 Fc binding to the hFcRn α chain and βm (Fig. 1, B and C) and is distinct from the albumin binding site (fig. S1A).

Immunohistochemistry showed that SYNT001 bound to the expected hematopoietic, endothelial, and epithelial cells in a wide variety of tissues consistent with previous observations (fig. S1B) (8, 28, 29) and identified several cells and tissues previously unrecognized to express FcRn. The latter included the epithelium of the bladder, parathyroid gland, pituitary gland, and platelets (Fig. 1D).

SYNT001 promoted clearance of human IgG (hIgG) antibodies and IgG ICs in vivo as shown in mice expressing hFcRn (TG/TG) (31). Treatment with SYNT001 (20 mg/kg) significantly reduced the plasma concentration of HuLys11 compared with the vehicle-treated control group [area under curve of vehicle (AUC Vehicle), 256.6 ± 17.72; AUC SYNT001, 116 ± 21.01%] (Table 1), which represent of HuLys11 remaining (time) (Fig. 1E).

ICs were examined using hapten, 4-hydroxy-3-iodo-5-nitrophenylacetic acid (NIP), conjugated ovalbumin (OVA NIP) as a model Ag and an anti-NIP hlgG1 antibody (hIgG1 WT) (24). hIgG1 WT ICs were administered to FCGRT TGF/Fcgrt−/− mice as previously described (fig. S1D) (22). SYNT001 (20 mg/kg) significantly accelerated CIC removal (AUC Vehicle, 345.5 ± 43.05; AUC SYNT001, 117 ± 5.424% of hIgG1 WT IC remaining (time)) compared with mice receiving vehicle, in which the CIC persisted (Fig. 1F).

We also treated primary CD11c+ APCs from B2M TGF/FcG RT TGF/FcGr t−/− mice expressing hFcRn/hβm in vivo as NHP serum that developed by day 15 in all monkeys with an apparent concentration of 2.2 ± 0.2, 5.1 ± 0.4, and 23.2 (Table 1). SYNT001 (20 mg/kg) significantly accelerated CIC removal (AUC Vehicle, 256.6 ± 17.72; AUC SYNT001, 116 ± 21.01%) (Table 1) and returned to baseline before subsequent doses (Fig. 2H, top), no significant changes in total FcRn staining were observed (Fig. 2H, bottom), suggesting that SYNT001 administration caused FcRn internalization but not enhanced degradation. Last, the SYNT001 PD effects were not associated with organ toxicity of study animals at all doses such that the no observed adverse effect level was the highest dose tested (100 mg/kg).

| Type of FcRn | Kd (10^-7/Ms) | Kd (10^-4/s) | K0 (nM)* |
|-------------|---------------|--------------|----------|
| pH 7.4      |               |              |          |
| hFcRn       | 35.6 ± 0.3    | 2.93 ± 0.08  | 1.19 ± 0.06 |
| pFcRn       | 4.7 ± 0.1     | 5.6 ± 0.0    | 11.9     |
| pH 6.0      |               |              |          |
| hFcRn       | 43.6 ± 1.6    | 2.71 ± 0.56  | 0.87 ± 0.2 |
| pFcRn       | 2.2 ± 0.2     | 5.1 ± 0.4    | 23.2     |

*The kinetic rate constants were obtained using a simple first-order (1:1) Langmuir biomolecular interaction model. The kinetic values represent the average of duplicates.

SYNT001 reduces IgG in cynomolgus monkeys

We used NHPs as a pharmacologically relevant model for conducting Good Laboratory Practice toxicology studies as SYNT001 demonstrated similar binding to primate and hFcRn (Table 1). Male (n = 4 or 6) and female (n = 4 or 6) cynomolgus monkeys received five weekly bolus intravenous doses of 10, 30, or 100 mg/kg of SYNT001, which caused dose-dependent and specific reduction of total circulating levels of IgG (Fig. 2A) as evidenced by a 71% decrease in exposure at 10 mg/kg but only a 32% decrease at 100 mg/kg. Repeated administration was associated with reductions in Cmax and AUC (0–144h) that were inversely proportional to the dose as evidenced by a 71% decrease in exposure at 10 mg/kg but only a 32% decrease at 100 mg/kg. This was consistent with the presence of anti-SYNT001 antidrug antibodies (ADAs) in NHP serum that developed by day 15 in all monkeys with an apparent reduction in the PD effect after administration of the third dose in the 10 and 30 mg/kg dose groups (Fig. 2E). This trend, however, was not observed in 100 mg/kg dose cohort, which maintained a maximal PD effect through the duration of the study (Fig. 2A), in accordance with the ability to dose through the ADA (32).

Immunophenotyping by flow cytometry revealed no change in the relative percentages and absolute quantities of white blood cells including total T cells, CD4+ and CD8+ T cells, B cells, natural killer cells, or monocytes (fig. S2A) as well as platelets (fig. S2B). In addition, we followed FcRn expression during SYNT001 treatment of NHPs through cell surface and intracellular staining by focusing on monocytes that have been reported to distinctively express high FcRn levels on the cell surface in humans (12). To do so, we used a mouse anti-hFcRn monoclonal antibody, ADM31, which cross-reacts with NHP FcRn (33) and blocks FcRn-albumin (11), but not FcRn-IgG (34) interactions, making it distinct from SYNT001 binding interface. ADM31 could detect the presence of FcRn on the surface of human blood monocytes, dendritic cells (DCs), and neutrophils, while lymphocytes were mostly negative (Fig. 2, F and G). In NHPs, we found that although FcRn staining of the monocyte surface decreased following SYNT001 administration and returned to baseline before subsequent doses (Fig. 2H, top), no significant changes in total FcRn staining were observed (Fig. 2H, bottom).
Clinical study of SYNT001

We conducted a single-center, double-blind, randomized (6:2), single ascending dose, first-in-human study to assess the safety, PK, and PD effects of SYNT001 in healthy human individuals. Thirty-one male individuals (Table 3) were randomized to receive a single intravenous dose of SYNT001 at 1 mg/kg (n = 6), 3 mg/kg (n = 6), 10 mg/kg (n = 6), 30 mg/kg (n = 5), or placebo (n = 8).

SYNT001 safety profile

No deaths, serious adverse events, or treatment-emergent adverse events (TEAEs) that led to discontinuation of study drug were observed. A total of nine individuals (eight pooled SYNT001 and one placebo) experienced at least one TEAE (Table 4). All TEAEs were reported in the 10 and 30 mg/kg dose groups. The most common TEAE was headache. One individual in the SYNT001 10 mg/kg dose group experienced a moderate (TEAE grade 2) headache, while the remaining seven individuals experienced mild (TEAE grade 1) headaches. One mild headache was treated with a single dose of acetaminophen; all other headaches resolved without treatment. No other TEAEs were experienced by more than one individual receiving SYNT001. There were no grade 3 or 4 TEAEs and no clinically significant changes in urinalysis, hematology, pulse oximetry, or blood chemistry results. Overall, there was no evidence of dose-limiting adverse reactions up to a maximum dose of SYNT001 (30 mg/kg), together indicating that a single intravenous infusion was well tolerated in healthy volunteers.
Pharmacokinetics

A summary of the SYNT001 mean PK parameters is shown in Table 5. The geometric mean values for AUC (0–inf) (AUC extrapolated from time 0 to infinity) increased approximately 8.7- and 41.2-fold for the SYNT001 10 and 30 mg/kg groups, respectively, compared to the 3 mg/kg group. The mean values for Cmax increased 4-, 16-, and 51-fold for the 3, 10, and 30 mg/kg groups, respectively, compared to the group given 1 mg/kg. When all dose groups were included, a nonlinear power model revealed proportionality coefficients for Cmax and AUC (0–inf) of 1.16 (90% confidence intervals, 1.10 to 1.23) and 1.65 (90% confidence intervals, 1.55 to 1.76), respectively. This provided confirmation that both Cmax and AUC increased in a greater than dose proportional manner as expected on the basis of the nonlinear kinetics (Fig. 3A).

The serum concentrations of SYNT001 peaked at 0.08 hours, and the T1/2 increased with increasing dose from 20 to 30 to 40 hours over the dose range of 1 to 30 mg/kg (Table 5). As observed for NHPs (Table 2), the mean Vd for SYNT001 approximated the expected plasma compartment volume and ranged from 15.4 to 23.0 ml/kg. Accordingly, clearance (CL) ranged from 19.0 to 1.9 ml/hour per kg and was inversely proportional to dose. The increasing T1/2 and decreasing CL with dose were consistent with the fact that semi-log plots showed evidence of nonlinearity in the lower dose groups associated with lower serum concentrations (Fig. 3A). Thus, the data indicate accelerated clearance of SYNT001 at lower serum concentrations, predicting that elimination was occurring because of target-mediated drug disposition via FcRn binding as supported by studies of monocytes in NHPs (Fig. 2H).

Pharmacodynamics

The entry criteria required individuals to have baseline total IgG levels greater than 1200 mg/dl with a study stopping rule when the level fell below the lower limit of normal (LLN; 768 mg/dl) in any individual. SYNT001 caused a dose-dependent decrease in total serum IgG concentrations in individuals who received a single dose of 1, 3, 10, and 30 mg/kg intravenously compared to individuals who received placebo (Fig. 3B and fig. S3A). The median peak effect was observed in the 30 mg/kg dose group with a −46.21% [interquartile range (IQR), −47.40 to −43.36%] change from baseline within 5 days after dosing. On day 28, the total IgG levels had mostly returned to
preadministration levels in the 1, 3, and 10 mg/kg groups. All individuals treated with SYNT001 (1, 3, or 10 mg/kg) maintained IgG levels above the LLN throughout the study. In contrast, four of five individuals in the SYNT001 30 mg/kg dose group exhibited IgG levels below the LLN by day 5. Therefore, the sixth individual was not enrolled in the SYNT001 30 mg/kg cohort. Reductions in total circulating IgG in the 30 mg/kg group were also reversible as evidenced by their median return to within $-27.33\%$ (IQR, $-29.87$ to $-27.11\%$) below baseline by day 28 after dosing.

| Day | Dose group (mg/kg) | n  | Cmax (µg/ml) | AUC0–144h (µg-hour/ml) | T1/2 (hours) | VSS (ml/kg) | CL (ml/hour/kg) | RAUC |
|-----|-------------------|----|-------------|------------------------|-------------|-------------|--------------|------|
| 1   | 10                | 8  | 289 ± 33    | 3200 ± 559             | 3.24 ± 0.18 | 28.6 ± 4.25 | 3.22 ± 0.579 | —    |
|     | 30                | 8  | 904 ± 95    | 13200 ± 1770           | 7.36 ± 1.88 | 28.8 ± 2.18 | 2.30 ± 0.327 | —    |
|     | 100               | 12 | 3530 ± 614  | 43700 ± 4650           | 9.99 ± 1.76 | 29.8 ± 4.20 | 2.31 ± 0.250 | —    |
| 29  | 10                | 8  | 186 ± 48    | 917 ± 318              | 4.14 ± 1.19 | 78.3 ± 12.4 | 11.5 ± 3.99  | 0.290 |
|     | 30                | 8  | 716 ± 326   | 4580 ± 906             | 3.96 ± 1.71 | 51.6 ± 14.5 | 6.80 ± 1.52  | 0.361 |
|     | 100               | 12 | 3260 ± 402  | 31200 ± 1518           | 6.28 ± 2.20 | 25.9 ± 6.38 | 3.28 ± 0.486 | 0.679 |

Table 2. Summary of PK parameter estimates in cynomolgus monkeys treated with SYNT001. $T_{\text{max}}$, time to reach maximum concentration; $C_L$, clearance; $R_{\text{AUC}}$, ratio of AUC(0–29) to AUC(0–1).

Table 3. Summary of demographics—PK population. Note that percentage was calculated with total number of individuals as the denominators for each cohort, respectively.

| Enrolled individuals, n (%) | Vehicle | SYNT001 (1 mg/kg) | SYNT001 (3 mg/kg) | SYNT001 (10 mg/kg) | SYNT001 (30 mg/kg) | Pooled SYNT001 |
|-----------------------------|---------|------------------|------------------|-------------------|-------------------|----------------|
| Completed study             | 8/8     | 6/6              | 6/6              | 6/6               | 5/5               | 23/23          |

| Race, n (%)                | White   | Black or African American |
|----------------------------|---------|---------------------------|
| Enrolled individuals, n (%)| 5 (62.5)| 3 (37.5)                  |

| Ethnic group, n (%)        | Hispanic or Latino | Not Hispanic or Latino |
|---------------------------|-------------------|------------------------|
| Enrolled individuals, n (%)| 8 (100)           | 0                      |

| Age (years)                | Mean (SD)         | Median (SD)            |
|---------------------------|------------------|------------------------|
| Mean (SD)                 | 35.50 (10.20)    | 33.70                  |
| Median (SD)               | 44.70 (8.10)     | 45.80                  |

| Height (cm)                | Mean (SD)         | Median (SD)            |
|----------------------------|------------------|------------------------|
| Mean (SD)                 | 172.50 (6.30)    | 173.50                 |
| Median (SD)               | 171.00 (6.30)    | 171.50                 |

| Weight (kg)                | Mean (SD)         | Median (SD)            |
|----------------------------|------------------|------------------------|
| Mean (SD)                 | 81.70 (7.50)     | 79.20                  |
| Median (SD)               | 84.30 (9.30)     | 83.50                  |

| Body mass index (kg/m²)   | Mean (SD)         | Median (SD)            |
|----------------------------|------------------|------------------------|
| Mean (SD)                 | 27.50 (1.90)     | 26.70                  |
| Median (SD)               | 28.80 (1.60)     | 29.50                  |

*Because of the IgG lower limit of normal (LLN) stopping rule, one individual was not enrolled into the 30 mg/kg cohort.
We observed reductions across all four IgG subtypes with the 30 mg/kg dose group displaying the lowest levels (Fig. 3C). Median levels remained above the LLN throughout the 28-day postdose period at all dose levels. The greatest median decreases were in the 30 mg/kg dose group for IgG3 (−68.29%; IQR, −71.83 to −61.38%) with lesser reductions of IgG1 (−51.62%; IQR, −52.62 to −49.80%), IgG2 (−36.92%; IQR, −39.81 to −33.56%), and IgG4 (−42.86%; IQR, −42.86 to −41.935%) (Fig. 3C and fig. S3, B to E). The level of IgG subtypes returned to

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**Table 4. TEAEs by preferred term—safety population.** Note that each individual was only counted once per preferred term.

| Preferred term | Vehicle (n = 8) | SYNT001 (1 mg/kg) (n = 6) | SYNT001 (3 mg/kg) (n = 6) | SYNT001 (10 mg/kg) (n = 6) | SYNT001 (30 mg/kg) (n = 5) | Pooled SYNT001 (n = 23) |
|----------------|----------------|---------------------------|---------------------------|---------------------------|---------------------------|------------------------|
| Individuals with ≥1 TEAE, n (%) | 1 (12.5) | 0 | 0 | 3 (50.0) | 5 (100) | 8 (34.8) |
| Headache | 0 | 0 | 0 | 3 (50.0) | 5 (100) | 8 (34.8) |
| Chills | 1 (12.5) | 0 | 0 | 0 | 1 (20.0) | 1 (4.3) |
| Presyncope | 0 | 0 | 0 | 1 (16.7) | 0 | 1 (4.3) |
| Fatigue | 0 | 0 | 0 | 1 (16.7) | 0 | 1 (4.3) |
| Pyrexia | 0 | 0 | 0 | 1 (16.7) | 0 | 1 (4.3) |
| Abdominal pain | 0 | 0 | 0 | 0 | 1 (20.0) | 1 (4.3) |
| Diarrhea | 0 | 0 | 0 | 0 | 1 (20.0) | 1 (4.3) |
| Nausea | 0 | 0 | 0 | 0 | 1 (20.0) | 1 (4.3) |
| Arthralgia | 0 | 0 | 0 | 1 (16.7) | 0 | 1 (4.3) |
| Muscular weakness | 0 | 0 | 0 | 1 (16.7) | 0 | 1 (4.3) |
| Myalgia | 0 | 0 | 0 | 1 (16.7) | 0 | 1 (4.3) |
| Lacrimation increased | 0 | 0 | 0 | 1 (16.7) | 0 | 1 (4.3) |
| Decreased appetite | 0 | 0 | 0 | 1 (20.0) | 0 | 1 (4.3) |
| Rhinorrhea | 0 | 0 | 0 | 1 (16.7) | 0 | 1 (4.3) |

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**Table 5. Summary of PK parameter estimates in healthy individuals treated with single dose of SYNT001.** AUC(0-24h), AUC from time 0 to the last time point with a concentration level ≥ limit of quantitation; λZ, terminal elimination rate constant; Vd, apparent volume of distribution during terminal phase after extravascular administration.

| Dose group | Parameter | Cmax (ng/ml) | Tmax (hours) | AUC(0-24h) (hours·ng/ml) | AUC(0-t) (hours·ng/ml) | AUC(0-inf) (hours·ng/ml) | λZ (1/hours) | T1/2 (hours) | Vd (ml/kg) | C(L) (ml/hour/kg) |
|------------|-----------|--------------|--------------|--------------------------|----------------------|--------------------------|--------------|-------------|-------------|-----------------|
| 1 mg/kg*   | n 6 6 6 1 1 1 1 | 18084.083 | 29794.634 | 29296.491 | 52632.931 | 1.090 | 0.636 | 17.435 | 19.000 |
| SD | 5745.119 | 11827.081 | 11903.121 |
| Median | 16119.85 | 0.080 | 25930.50 | 25279.30 |
| 3 mg/kg | n 6 6 6 6 6 6 6 | 73416.550 | 39446.202 | 39212.944 | 39458.153 | 0.529 | 1.407 | 15.395 | 15.935 | 7.877 |
| SD | 9179.148 | 80783.509 | 80447.160 | 81092.396 | 0.163 | 0.398 | 2.755 | 1.626 |
| Median | 76778.35 | 0.080 | 395961.27 | 392572.86 | 396103.65 | 0.50 | 1.39 | 15.69 | 7.60 |
| 10 mg/kg | n 6 6 6 6 6 6 6 | 299330.000 | 3286692.228 | 3283820.283 | 3462471.993 | 0.140 | 5.297 | 23.022 | 3.011 |
| SD | 65568.274 | 709349.625 | 708586.918 | 733659.101 | 0.045 | 1.441 | 8.133 | 0.708 |
| Median | 304908.00 | 0.080 | 3281142.00 | 3279597.38 | 3466938.84 | 0.13 | 5.32 | 21.47 | 2.89 |
| 30 mg/kg | n 5 5 5 5 5 5 5 | 912819.600 | 12227632.060 | 15752080.833 | 16556146.477 | 0.131 | 7.794 | 18.755 | 1.945 |
| SD | 162351.117 | 2399425.464 | 3838139.142 | 5091946.878 | 0.081 | 6.234 | 9.157 | 0.553 |
| Median | 953306.00 | 0.080 | 11977325.16 | 14730835.80 | 14805600.06 | 0.12 | 5.56 | 16.26 | 2.03 |

*In the 1 mg/kg dose group, only one individual had ≥3 sample points after Tmax; thus, AUC(0-inf), T1/2, Vd, C(L), and λZ were only computed for that one individual.
within 25% of baseline levels by day 28 in all dose groups. Three individuals in the 30 mg/kg dose group experienced IgG3 levels below the LLN between days 4 and 14. No significant changes were observed in the levels of albumin, IgA, and IgM (Fig. 3D and fig. S3, F to H).

Immunogenicity
We screened a total of 93 human serum samples from 31 enrolled individuals for binding ADAs at days 1, 14, and 28 of the study. Six of 93 samples (6.5%) that were derived from 5 of 31 individuals (16.1%) were confirmed to be positive. Two individuals in the 1 mg/kg dose group (on days 14 and 28), one individual in the 3 mg/kg dose group (on days 14 and 28), and two individuals in the 10 mg/kg dose group (both on day 14 only) tested positive. The ADA titers detected were low and were not observed to be neutralizing. Thus, we detected a low frequency of transient, low-titer, and non-neutralizing ADAs.

Immune effects
Healthy human individuals maintain low levels of CICs, which permitted assessment of SYNT001 effect on their levels (35). CICs were quantified by an enzyme-linked immunosorbent assay (ELISA), which detects C1q bound, and thus complement-fixed, IgG antibodies. At baseline, the median CIC levels for all individuals was 2.6 μg/ml (IQR, 1.90 to 3.90), which was within the normal range of the laboratory reference (0 to 3.9 μg/ml). The samples for the 1 mg/kg dose group and data beyond day 12 for the 3, 10, and 30 mg/kg dose groups were not available for evaluation due to sample integrity issues. The administration of SYNT001, but not placebo, resulted in a dose-dependent decrease in CICs (Fig. 4A). The nadir in CIC levels occurred 7 days after SYNT001 administration declining by 18.98% (IQR, 25 to 18.18%), 29.41% (IQR, 31.58 to 25.71%), and 48.39% (IQR, 55.56 to 40.91%) from baseline levels in the 3, 10, and 30 mg/kg groups, respectively. Overall, the reductions persisted for up to 10 days after a single administration of SYNT001.

Cytokine production from human monocyte-derived DC in response to IgG ICs has been shown to be dependent on FcRn (23). To further validate the effects of SYNT001 on CIC, we established a novel in vitro assay by exposing heparinized human whole blood from healthy donors to hIgG1 ICs. Although hIgG1 WT ICs induced robust secretion of tumor necrosis factor–α (TNFα) and interleukin-6 (IL-6)
within 24 hours, ICs composed of IgG engineered variants that are unable to bind FcRn (IgG1 N297A), FcγR (hIgG1 N297A), or both (hIgG1 N297A/IHH) stimulated significantly less secretion (Fig. 4B) (22, 24, 36). Thus, FcRn controls the induction of inflammatory cytokines by primary human peripheral blood cells in response to IgG ICs even in the context of FcγR engagement. We therefore next examined the ability of SYNT001 to inhibit IgG IC–induced innate immune responses. To eliminate the nonspecific effects of the human Fc fragment in this assay, we used F(ab')2 fragments and observed that SYNT001 F(ab')2 fragments, but not F(ab')2 fragments of an isotype control, inhibited TNFα and IL-6 secretion in a dose-dependent manner (Fig. 4C). SYNT001 can thus inhibit FcRn-mediated protection of CICs and impede IgG IC activation of FcRn-mediated innate cytokine production. Last, consistent with the absence of effects on circulating blood counts or evidence of significant safety findings, we did not observe increases in serum C-reactive protein (Fig. 4D) or a panel of cytokines (fig. S4, A to L) in any of the dose groups over time relative to that observed in the placebo treated individuals in this single ascending dose study.

**DISCUSSION**

This report describes the generation of SYNT001, a humanized monoclonal IgG4 antibody that blocks IgG binding to hFcRn and its characterization in humanized mice, NHPs, and in a first-in-human phase 1a clinical study. SYNT001 was well tolerated in NHPs and humans, and its administration reduced the serum concentrations of total IgG. Following a single dose of 30 mg/kg in humans, we observed decreases in total IgG and IgG1, IgG2, and IgG4 subclasses by approximately 40 to 50%, while IgG3 was uniquely susceptible with a reduction of up to 70%. The extent of IgG depletion achieved was to levels previously correlated with clinical responses observed in individuals who have undergone immunoadsorption or plasmapheresis for the treatment of autoimmune diseases caused by pathogenic autoantibodies (37–39). In addition, the level of total IgG reduction after a single dose of SYNT001 in humans was similar to that observed in NHPs. In light of the 70% decline in total circulating IgG levels observed in the 100 mg/kg SYNT001 primate study group, we expect comparable total serum IgG depletion effects in humans upon higher or repetitive SYNT001 dose administration. Together with initial results from a phase 1b study in patients with pemphigus vulgaris, this demonstrates the ability of SYNT001 to decrease anti-desmoglein antibody levels and IgG ICs in association with clinical responses (40), these studies support the potential utility of SYNT001 in the treatment of autoimmune diseases by decreasing pathogenic autoantibody levels.

As predicted by studies in radiation bone marrow chimeric mice whereby absence of FcRn expression in cells of hematopoietic origin...
resulted in faster clearance of IgG ICs (22), SYNT001 also caused a significant dose-dependent reduction of CIC in humans. Our studies therefore uniquely reveal a well-tolerated pharmacologic intervention that can remove IgG ICs from the circulation. Removal of CICs is noteworthy as it has heretofore only been achieved through invasive interventions such as immunoadsorption or plasmapheresis (41). We also observed that FcRn blockade with SYNT001 inhibited the ability of IgG ICs to induce secretion of innate inflammatory cytokines by human peripheral blood leukocytes when examined ex vivo. Together with the ability of SYNT001 to disable IgG IC engagement of Ag presentation pathways associated with activation of CD4+ and CD8+ T cells by mouse APCs expressing hFcRn as we observed, these results support the role of FcRn in enabling a wide variety of IgG IC–mediated inflammatory pathways. Hence, these results have broad implications and suggest that SYNT001 may be useful in clinical scenarios where IgG ICs are directly causing disease through deposition in tissues (e.g., IC nephritis) (42), through induction of phagocytosis and thrombosis (e.g., warm autoimmune hemolytic anemia and heparin-induced thrombocytopenia) (15, 43), or indirectly through activation of innate and adaptive immune responses (e.g., inflammatory bowel disease and rheumatoid arthritis) (7, 44).

SYNT001 reduction of circulating monomeric IgG and CIC was observed to be rapid with a nadir occurring within 5 to 6 days after dosing. Moreover, these effects were durable in that they were evident for at least a month after a single dose of drug administration yet reversible and returned to within 25% of the baseline levels by day 28 in the highest dose group. The activities of FcRn that relate to recycling and protection of IgG on the one hand or promoting the persistence and immune properties of IgG ICs on the other are likely derived from the distinct properties of the cell types that express FcRn and its functional localization within them. This suggests that SYNT001 was interacting with FcRn in multiple cell–associated compartments. The main FcRn functional fraction involved in protecting IgG and albumin from degradation is intracellular and contained primarily within early and recycling endosomes of various cell types after fluid phase endocytosis of internalized ligands (18, 19, 45). Consistent with this, cells of endothelial and epithelial origin are solely involved in the protection and transport of IgG (46) and predominantly express FcRn in intracellular organelles (29). Monomeric IgG is equivalently protected by FcRn in myeloid cells, which are also the main cell type involved in IgG IC interactions due to their expression of classical FcyR (13, 20–22). Previous studies have shown that large internalized IgG ICs are retained by FcRn in late endosomes and lysosomal–associated membrane protein 1 (LAMP-1+) organelles of APCs where they have been demonstrated to be important for FcRn–mediated immune responses (22). In addition, most of the circulating human leukocytes such as neutrophils, monocytes, and DCs uniquely express significant levels of FcRn on the cell surface as we show here. Although the functional role of FcRn in this locale is unknown, previous studies have shown that phagocytosis of IgG-opsonized particles is perturbed in absence of FcRn (15). Together, these observations suggest that inhibition of FcRn regulation of IgG levels and responses to IgG ICs involve entry of therapeutic agents into multiple cell–associated compartments within parenchymal and hematopoietic cell types.

A critical issue concerning anti-FcRn therapy is whether antibodies to FcRn cause receptor degradation. We show here that administration of SYNT001 to NHPs was associated with transient decreases in monocyte surface FcRn expression suggesting receptor internalization and a PK profile characteristic of target-mediated disposition, without major alterations in the total levels of FcRn. At any administered dose, SYNT001 did not cause a decrease in FcRn’s second ligand, albumin. These data thus argue against receptor degradation upon treatment with SYNT001.

The results of our studies are distinguished in several regards with respect to other recent phase 1 clinical studies targeting FcRn (25–27). First, using SYNT001, we demonstrate that FcRn blockade can force the destruction and inhibit the immunologic activities of IgG ICs in humans. This importantly suggests that FcRn promotes inflammation through binding IgG ICs, which SYNT001 inhibits. Second, SYNT001 blockade of FcRn also had no effect on albumin levels in the circulation, which is of interest, as transient reductions of albumin have been observed in two anti-FcRn antibody clinical trials (25, 27); in at least two cases (25, 47), it has been suggested to be due to steric hindrance by the therapeutic antibody. One possibility for the latter is that SYNT001 occupies the FcRn α chain and β2m interface in a manner that is similar to native Fc binding and may mirror physiologic interactions. In summary, the results of these studies predict that FcRn blockade using SYNT001 has the potential to treat a variety of inflammatory and autoimmune conditions, making it a promising therapeutic agent in reversing the effects of pathogenic IgG and IgG ICs.

**MATERIALS AND METHODS**

**Clinical study design**

Study of SYNT001 was performed in a phase 1a, single-center (Clinical Pharmacology of Miami, Miami, FL), double-blind, randomized (62), placebo–controlled, single ascending dose study of SYNT001 in healthy male individuals (www.clinicaltrials.gov; identifier NCT03643627). The study was approved by IntegReview IRB (Austin, TX), and all individuals provided written informed consent. This study was designed to assess the safety, tolerability, PK, and PD of SYNT001. Five sequential cohorts were to receive single doses of 1, 3, 10, 30, and 60 mg/kg of SYNT001 or placebo (dextrose 5% in water) administered as a 250-ml intravenous infusion over 1 hour. Individuals were dosed on day 1 and followed for 27 days after dosing (to day 28). Individuals remained at the clinic for 48 hours following the end of the infusion.

To avoid unnecessary hypogammaglobulinemia in healthy individuals, the study included a stopping rule: If ≥1 individual exhibited serum total IgG levels below the reference lab’s LLN (768 mg/dl) at any time following SYNT001 dosing, then no further dosing would occur. Thus, eligible individuals were required to have total IgG levels greater than or equal to the midpoint of the reference lab’s normal range (1200 mg/dl) at entry. Up to 40 individuals were planned for enrollment. As the study stopping rule regarding total IgG levels was met after enrolling seven of a planned total of eight individuals in cohort 4 (30 mg/kg), 31 total individuals were enrolled in the study. No individuals were dosed in cohort 5 at 60 mg/kg. PK, PD, and safety evaluations were performed during each individual’s stay in the clinic and upon their return to the clinic on days 4 to 8, 10, 12, 14, 21, and 28. The National Cancer Institute Common Terminology Criteria for Adverse Events (version 4.03) was used for grading clinical and laboratory toxicities.

**PK assessments**

Testing of healthy individuals sera for PK analysis consisted of the validated Competitive Binding Electrochemiluminescence (ECL) ELISA...
(MPI Research Laboratories, Mattawan, MI). Plates were coated with anti-hFcRn, followed by recombinant hFcRn to capture SYNT001. SULFO-TAG–labeled SYNT001 at a set concentration was combined with samples to allow binding of FcRn for quantification of samples in a competitive format. MSD (Meso Scale Discovery) Read Buffer T was added before analysis on an MSD Sector Imager. PK data were analyzed using a noncompartmental analysis method as implemented in Phoenix WinNonlin v6.4 (Certara, Princeton, NJ).

**PD assessments of IgG, IgG subtypes, IgA, albumin, C-reactive protein, and CICs**

Serum samples were assessed by means of immunonephelometry on the BN II nephelometric analyzer (Siemens, Munich, Germany) using Human IgG (NAS IGG, ASAS09, Siemens), IgA (NAS IGA. OSAR09, Siemens), IgM (NAS IGM, OSAT09, Siemens), Human IgG Subclass Liquid (LK001.TB, NK006.TB, NK007.TB, LK008.TB, and LK009.TB, Siemens), Albumin (NAS ALB, OSAL11, Siemens), or C-reactive protein (N CardioPhase, OQIV21, Siemens) reagents according to the manufacturer’s instructions at Charles River Laboratories (Paw Paw, MI). CICs were assessed by a standard sandwich ELISA, MicroVue CIC-C1q EIA Kit (A001, Quidel), which detects C1q-associated IgG aggregates according to the manufacturer’s instructions. Serum or plasma samples were diluted 1:50.

**Measurement of ADA**

A total of three serum samples (5 ml each; total of 15 ml) were collected for immunogenicity analyses on days 1 (before dose), 14, and 28. The ADA assay method was a sandwich MSD ECL ELISA (Rockville, MD). MSD plates were coated with streptavidin and then captured with biotin-labeled SYNT001. Serum from all individuals participating in the study or additional controls was then added, followed by detection with SULFO-TAG–labeled SYNT001. During method validation, it was determined that the screening assay cut point factor was 3.34 optical density units based on a 5% false-positive rate with a 90% confidence level. Any test articles that were greater than or equal to the screening cut point were then determined negatively by confirmatory testing. The confirmatory assay cut point was determined to be 67.57% signal inhibition. Any confirmatory assay test articles that were greater than or equal to the confirmatory assay cut point were then tested for titer to determine the level of anti-SYNT001–specific antibodies present. Titer was a numeric value calculated as reciprocal of a dilution factor.

The neutralizing antibody assay was designed to measure the ability of anti-SYNT001 antibodies to inhibit test article binding to FcRn. For neutralization steps, plates were coated with anti-FcRn and then SYNT001 and test articles or controls, followed by hFcRn. These neutralizing step incubations occurred at neutral pH. The detection steps consisted of adding biotin-labeled hIgG1, followed by streptavidin–horseradish peroxidase and 3,3′,5,5′-tetramethylbenzidine ELISA substrate. These detection step incubations occurred at pH 6.0 and were separated from capture pH to avoid competition with serum IgGs. During method validation, the neutralizing antibody assay cut point factor was determined to be 1.39 optical density units based on a 5% false-positive rate with a 90% confidence level.

**Measurement of serum cytokines**

Serum cytokines were measured by a contractor, ARUP Laboratories (Salt Lake City, UT) using the Luminex 200 analyzer (Thermo Fisher Scientific, Waltham, MA) and Luminex 200 multi-analyte profiling technology. Custom made, clinically validated, laboratory-developed test cytokine panel (0051394, ARUP Laboratories) consisted of 11 cytokines (IL-1β, IL-2, IL-4, IL-5, IL-6, IL-8, IL-10, IL-12, IL-13, TNFα, and IFN-γ) and one soluble cytokine receptor (sIL-2Rα, sCD25) with a lower detection limit of 5 pg/ml. This test was developed and its performance characteristics determined by ARUP Laboratories.

**Statistical methods**

Palm 7.04 (GraphPad Software Inc., La Jolla, CA) was used for statistical analysis. Statistical comparisons between two groups were made by Student’s t test. For three or more groups with two parameters, two-way analysis of variance (ANOVA) was used. Post hoc analysis to detect differences between treatment groups was performed by uncorrected Fisher’s least significant difference (LSD) test. In addition, mixed-effects model repeated-measures modeling analysis was used using SAS v9.4 (Cary, NC) to evaluate statistical significance in NHP and human studies. A two-sided probability (P) of < 0.05 was considered significant.

**SUPPLEMENTARY MATERIALS**

Supplementary material for this article is available at http://advances.sciencemag.org/cgi/content/full/5/12/aax9586/DC1

Fig. S1. Characterization of SYNT001.

Fig. S2. The immunologic effects of repeated-dose SYNT001 administration in cynomolgus monkeys.

Fig. S3. PD of single-dose SYNT001 administration in healthy human individuals.

Fig. S4. Effect of FcRn-blockade by SYNT001 on immune responses in healthy individuals.

Table S1. SYNT001-FcRn:ßm crystal structure data collection and refinement statistics (Protein Data Bank ID: 6NHA).

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Data and materials availability: The atomic coordinates and structure factors were deposited with RCSB accession Protein Data Bank ID 6NHA. The materials described in this publication, i.e., SYNT001, are obtainable via the methods described in WO 2016/183352 A1. Requests for further information and additional data related to this paper may be made to the authors and Alexion Pharmaceuticals Inc.

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