Science Advances
AAAS

advances.sciencemag.org/cgi/content/full/5/12/eaax9586/DC1

Supplementary Materials for

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

L. J. Blumberg, J. E. Humphries, S. D. Jones, L. B. Pearce, R. Holgate, A. Hearn, J. Cheung, A. Mahmood, B. Del Tito, J. S. Graydon, L. E. Stolz, A. Bitonti, S. Purohit, D. de Graaf, K. Kacena, J. T. Andersen, G. J. Christianson, D. C. Roopenian, J. J. Hubbard, A. K. Gandhi, K. Lasseter, M. Pyzik, R. S. Blumberg*

*Corresponding author. Email: rblumberg@bwh.harvard.edu

Published 18 December 2019, *Sci. Adv.* **5**, eaax9586 (2019)
DOI: 10.1126/sciadv.aax9586

This PDF file includes:

Supplementary Materials:

Development of SYNT001

Humanized variants of a murine anti-human FcRn antibody were generated using Composite Human Antibody™ technology, a technology designed to minimize potential immunogenicity through the avoidance of human T cell epitopes. Four heavy chains and four light chains were designed, and the combinations were expressed as human IgG4 (S241P) containing a C_H3-domain C-terminal lysine deletion (ΔK478)(*48*). Purified humanized variants were tested in a competition ELISA in which variants were titrated with a fixed concentration of biotinylated murine anti-human FcRn antibody for binding to immobilized hFcRn. The ELISA was performed at both physiological pH and at acidic pH (pH 6.0), and the combination VH1/Vk2 was selected as the lead antibody. To improve binding affinity at both physiological and acidic pH conditions, the antibody was affinity matured using phage display. Heavy and light chain variable domain CDR3 regions were semi-randomized using oligonucleotide mutagenesis and separate heavy and light chain phage libraries were screened using decreasing concentrations of biotinylated hFcRn to select the highest affinity binders from each library. Selected scFv variants were prepared and screened in a competition ELISA to select lead candidates. Candidate affinity matured heavy and light chain variants were subsequently combined and expressed as full-length IgG4 (S214P, ΔK478) and re-tested by competitive ELISA. The binding of selected antibodies to hFcRn was then assessed in a competition ELISA assay with full-length human IgG at pH 6.0 where purified variants competed with biotinylated human serum IgG for binding to immobilized hFcRn. Based upon the combined data together with the iTope™ in silico analysis scores, SYNT001 was proposed as the lead.

Cell Line Development

SYNT001 is produced using a CHO DG44 parental cell line transfected with a SYNT001 Chinese Hamster Elongation Factor-1 (CHEF-1) expression vector developed by CMC Biologics(*49*). The expression vector contained the coding sequences for both the SYNT001 heavy chain and the SYNT001 light chain, each under the control of the CHEF-1 promoter. In addition, the dihydrofolate reductase (DHFR) gene was present as a selectable marker. Clone AL14 was selected as the production cell line based on growth, product quality, and genetic stability. SYNT001 clinical trial material was produced under cGMP by serial expansion of one vial of Clone AL14 Master Cell Bank to generate sufficient cell count to inoculate a 500 L Single Use Bioreactor (SUB) for production. Cells were cultured in the 500 L SUB for 14 days prior to harvest. The clarified harvest was purified using Protein A chromatography and additional chromatography and filtration steps, resulting in a product concentration of 50 ± 5 g/L in a buffer containing 20 mM histidine, 40 mM glycine, 4% sorbitol, pH 6.5.

Surface plasmon resonance (SPR)

SPR was conducted using a Biacore 3000 instrument (GE Healthcare) with CM5 sensor chips coupled with the antibodies (~550 resonance units [RU]) using amine-coupling chemistry as described by the manufacturer. The coupling was performed by injecting 2.5 μg/ml of each protein into 10 mM sodium acetate buffer, pH 4.5 (GE Healthcare), using the amine coupling kit (GE Healthcare). HBS-P buffer pH 7.4 (0.01 M HEPES, 0.15 M NaCl, 0.005% P20) or phosphate buffer pH 6.0 (67 mM phosphate buffer, 0.15 M NaCl, 0.005% Tween 20) were used as running and dilution buffers. Binding kinetics were determined by injecting titrated amounts (400.0-12.5 nM) of monomeric FcRn over immobilized antibody at pH 7.4 or pH 6.0. Monomeric His-tagged hFcRn and cynomolgus FcRn (pFcRn) were obtained from SINO Biological Inc. All SPR experiments were conducted at 25°C with a flow rate of 40 μl/min.

Binding data were zero-adjusted, and the reference cell value subtracted. The Langmuir 1:1 ligand binding model provided by the BIAevaluation software (version 4.1) was used to determine the binding kinetics.

Crystallization Characterization of SYNT001

Cloning

The extracellular region of the human FcRn α-chain (full length numbering residues 1 to 296, N125A non-glycosylated mutant (or accordingly N102A excluding signal peptide sequence of 23 amino acids) and the human β_2m were separately inserted into the mammalian cell expression vector pJTI Fast DEST (Jump-In Fast Gateway Targeted Integration System, ThermoFisher Scientific) using the MultiSite Gateway Pro cloning kit (ThermoFisher Scientific) to create pJTI FcRn-α and pJTI FcRn-β. The FcRn α-chain was fused at the C-terminus to a TEV-protease site followed by an octahistidine nickel-affinity tag to aid in purification. The coding sequences for FcRn-α and β_2m were also coupled to a green and red fluorescent protein, respectively, by an internal ribosome entry site (IRES) to expedite selection of a high expressing stable mammalian cell lines(*50*). In each vector, these expression cassettes were flanked by an upstream cytomegalovirus (CMV) promoter and a downstream herpes simplex virus thymidine kinase polyadenylation (TKpolyA) signal for expression control.

Expression and purification of FcRn

Adherent HEK 293 cells were co-transfected with equal weight amounts of pJTI FcRn-α, pJTI FcRn-β, and pJTI PhiC31 (ThermoFisher Scientific), and stable colonies were isolated by hygromycin B selection. A double-fluorescent red/green high-expressing colony was manually picked, expanded, and adapted to suspension culture in FreeStyle 293 serum-free media supplemented with 1% fetal bovine serum(*51*). FcRn was purified from cell media by two-

column nickel affinity and gel filtration chromatography followed by with affinity tag removal, using established methods(*52*). Purified FcRn was dialyzed into buffer containing 20 mM *tris*(hydroxymethyl)aminomethane (Tris) pH 7.5 and 150 mM NaCl prior to crystallization.

Purification of SYNT001 F(ab')$_2$

SYNT001 F(ab')$_2$ was cleaved from the intact SYNT001 antibody by overnight incubation with immobilized papain agarose resin (ThermoFisher Scientific). Fc and residual intact antibody were removed by binding to Pierce Protein A Plus UltraLink Resin (ThermoFisher Scientific) and the remaining F(ab')$_2$ was further purified using a Superdex 200 10/300 GL gel filtration column (GE Healthcare) equilibrated with 20 mM Tris pH 7.5 and 150 mM NaCl.

Crystallization, data collection, and structure determination

FcRn and SYNT001 Fab were mixed in a 1:1 stoichiometric ratio and concentrated to 8 mg/ml. Crystals of the SYNT001 Fab:FcRn complex were grown by sitting drop vapor diffusion at 4°C against crystallization buffer containing 0.1M 2-(cyclohexylamino)ethanesulfonic acid (CHES) pH 9.5 and 50% polyethylene glycol (PEG) 200. Crystals were flash frozen in liquid nitrogen directly from the crystallization drop and X-ray diffraction data were collected at beamline NE-CAT 24-ID-E of the Advanced Photon Source at Argonne National Laboratory. X-ray data were indexed, merged, and converted to structure factors using HKL2000(*53*) and CCP4(*54*). A test set for refinement was created from a random 5% of reflections. The structure of SYNT001 Fab:FcRn was solved by molecular replacement in Phaser(*55*) using PDB IDs 3SQO (chain H), 5K59 (chain L), and 4N0F (chains E and F) as search models. COOT(*56*) was used for manual model rebuilding and Phenix(*57*) was used during refinement. The atomic coordinates and structure factors were deposited with RCSB accession PDB ID 6NHA.

To compare albumin and IgG1 Fc binding site of FcRn, ternary crystal complex of FcRn, albumin, and IgG-Fc (PDB code 4N0U) was superimposed on FcRn:SYNT001 Fab crystal structure. All the structural figures and comparisons were drawn using PyMOL (The PyMOL Molecular Graphics System, Schrödinger, and LLC) and labels were added using Adobe Photoshop.

Blockade of IgG and IgG IC protection in humanized mice

Mice

B6.129X1-*Fcgrt*^{tm1Dcr}/DcrJ mice (*58*) carry a null allele of the α-chain of *FcRn* (designated as *Fcgrt*$^{-/-}$). B6.Cg-Fcgrt^{tm1Dcr}Tg(FCGRT)32Dcr/DcrJ mice (*31, 58*) are hemizygous for the hFcRn transgene (*FCGRT*$^{TG/-}$) , deficient in mouse orthologue and express hFcRn cDNA transgene under the control of its the natural human promoter (designated as *FCGRT*$^{TG/-}$*Fcgrt*$^{-/-}$). Homozygous hFcRn mice (*FCGRT*$^{TG/TG}$*Fcgrt*$^{-/-}$) also carrying the transgene for human β_2m (*B2M*$^{TG/TG}$) (*B2M*$^{TG/TG}$*FCGRT*$^{TG/TG}$*Fcgrt*$^{-/-}$) were previously described(*11, 58*). All mice were maintained under specific pathogen-free conditions and the procedures were approved by the Institutional Animal Care and Use Committee of Harvard Medical School and Brigham and Women's Hospital or Jackson Laboratory Animal Care and Use Committee.

Inhibition of IgG protection

Pharmacodynamic (PD) blockade of IgG catabolism was assessed in 14 week old *FCGRT*$^{TG/-}$ *Fcgrt*$^{-/-}$ preloaded by *i.v.* injection with hIgG (245 mg/kg) and hIgG1 (HuLys11, 5 mg/kg) anti-hen egg lysozyme (HEL, Sigma-Aldrich) at time 0, blood sampled at 48, 56, 72, 80, 96, 120, and 144 hours, and treated by *i.v.* injection with vehicle (n=10) or SYNT001 (n=9, 20 mg/kg) at 49 hours. HEL was used to capture HuLys11 from diluted plasma samples by ELISA, and this activity was quantified using goat anti-human kappa-alkaline phosphatase (Southern Biotech).

Inhibition of CIC protection

PD blockade of multimeric IgG IC catabolism was evaluated in 11-week-old *FCGRT*$^{TG/-}$*Fcgrt*$^{-/-}$ mice(*22*). Multimeric IC was formed *in vitro* with 750 µg/ml of a chimeric antibody consisting of a mouse Fab specific for the hapten 4-Hydroxy-3-iodo-5- nitrophenylacetyl (NIP) and human IgG1 Fc (^{NIP}hIgG) incubated with 75 µg/ml NIP conjugated-ovalbumin (NIP-OVA, Biosearch Technologies) for 20 minutes at room temperature in PBS as previously described(*22*, *24*). Mice were preloaded by *i.v.* injection with IgG IC equivalent to 7.5 mg/kg ^{NIP}hIgG + 0.75 mg/kg NIP-OVA at time 0, blood sampled at 8, 24, 32, 48, 56, 72, and 96 hours, and treated by *i.v.* injection with vehicle (n=10) or SYNT001 (n=10, 20 mg/kg) at 25 hours. NIP-OVA was used to capture ^{NIP}IgG/NIP-OVA IC from diluted plasma samples by ELISA, and this activity was quantified using mouse anti-human Fc-biotin (Southern Biotech) and streptavidin-HRP (Southern Biotech).

Immunohistochemistry

SYNT001 was biotinylated and applied to acetone and formalin fixed cryosections of normal human tissues from at least three separate donors in comparison to a biotinylated human IgG4 antibody control by Charles River Laboratories (Frederick, MD). Staining was performed by established methods and detected by an immunoperoxidase procedure. Tissues were obtained from human adrenal, bladder, blood cells, blood vessels, bone marrow, brain, breast, colon, eye, fallopian tube, gastrointestinal tract, heart, kidney, liver, lung, lymph node, ovary, pancreas, parathyroid, peripheral nerve, pituitary, placenta, prostate, salivary gland, skin, spinal cord, spleen, skeletal muscle, testis, thymus, thyroid, tonsil, ureter, uterine cervix and endometrium. All primary antibodies were diluted identically for all tissues and cell preparations. Recombinant hFcRn and tissues from mice transgenic for the human *FCGRT* heavy chain (*FCGRT*$^{TG/-}$*Fcgrt*$^{-/-}$

)(*58*) were used as positive controls and human hypercalcemia malignancy peptide and *Fcgrt*$^{-/-}$ mouse tissues were used as negative controls.

In vitro assessment of FcRn innate and adaptive immune function

IgG IC presentation and cross-presentation by murine Ag presenting cells

For primary APC studies, ICs were formed in serum-free RPMI-1640 medium by one hour incubation of 100 µg/ml of anti-NIP-OVA hIgG1^{WT} and hIgG1^{IHH} and NIP-OVA at a concentration of 0.5 µg/mL at 37 °C, 5% CO_2. Each OVA molecule was conjugated to 11 molecules of NIP allowing for binding of approximately five chimeric anti-NIP IgG antibodies per OVA molecule which generated IC concentrations that approximated those found *in vivo*(*35*). CD11c^{+} APC were MACS purified with CD11c MicroBeads UltraPure (Miltenyi, 130-108-338) from the spleens of *B2M*$^{TG/TG}$*FCGRT*$^{TG/TG}$*Fcgrt*$^{-/-}$(*58, 59*) that had been inoculated subcutaneously with 5x10^{6} GM-CSF-secreting B16-F10 melanomas two weeks prior to spleen harvest, as described previously(*24*). 5x10^{4} APCs were pre-treated for 30 minutes with the indicated concentrations of SYNT001 or the IgG4 isotype control. IgG IC variants were then added and incubated for three hours. APCs were washed thrice to remove unbound ICs and then co-cultured for 48 hours with 1x10^{5} OT-I or OT-II cells. More specifically, CD8^{+} T cells recognizing $OVA_{257-264}$ peptide in the context of MHCI H-2^{b} were MACS purified using CD8α^{+} T cell Isolation kit (Miltenyi Biotec, 130-104-075) from spleens and peripheral LN of OT-I mice. CD4^{+} T cells recognizing $OVA_{323-339}$ peptide in the context of MHCII H-2^{b} were MACS purified using CD4^{+} T cell Isolation kit (Miltenyi Biotec, 130-104-454) from spleens or peripheral LN from OT-II mice.

Whole blood assay and blockade of IgG IC stimulation of cytokine release

This human study was approved by Brigham and Women's Hospital Institutional Review Board (Protocol #: 2017P000996/PHS). Heparinized human blood was collected from healthy volunteers according to a protocol approved by the Institutional Review Board of Brigham and Women's Hospital. Whole blood was aliquoted 100 µl/well into 96 well plates. IgG IC were formed in serum free RPMI-1640 by one-hour incubation of 50 µg/ml of anti-NIP-OVA $hIgG1^{WT}$, $hIgG1^{IHH}$, $hIgG1^{N297A}$ or $hIgG1^{IHH/N297A}$ and NIP-OVA at a concentration of 0.5 or 1 µg/mL at 37 °C, 5% CO_2. 100 µl of IgG IC variants or NIP-OVA alone was added to aliquoted blood and incubated for 2 days at 37 °C, 5% CO_2. At 24 and 48 hours after initial addition of IgG IC, 60 µl of supernatant was collected and assayed for different cytokine levels by cytokine bead assay as indicated. To assess the effect of SYNT001 on FcRn-mediated innate immune response, whole blood was aliquoted and incubated with $IgG1^{WT}$ IC or $IgG1^{IHH}$ IC as above. 60 minutes before the addition of IC the samples were pretreated with 10, 50 or 100 nM SYNT001 or control IgG4, whole or as $F(ab')_2$ fragments derived from monoclonal antibody with identical S241P hinge mutation. At 24 hours after initial addition of IgG IC, 60 µl of supernatant was collected, diluted fivefold and assayed for TNF levels by ELISA (BD Biosciences, 555212), BD Human Inflammatory cytokine Cytometric Bead Array (BD Biosciences, 551811) or LEGENDplex Human Inflammation Panel (BioLegend, 740118) according to manufacturer's instructions.

Staining of human peripheral blood leukocytes

Heparinized human blood was collected from healthy volunteers, overlaid on Mono-Poly Resolving Medium (MP Biomedicals) and mononuclear and polynuclear cells were isolated according to a manufacturer's instructions. The cells were counted and $1x10^6$ cells were stained with Fixable Viability Dye eFluor™506 (ThermoFisher, 65-0866-14) in PBS according to a

manufacturer's instructions. The cells were then resuspended in FACS buffer (PBS, 2% FCS, 2 mM EDTA) and stained for 30 min at 4˚C with PE-conjugated mouse anti-human FcRn antibody, ADM31 (custom conjugated by Biolegend) or PE-conjugated IgG2b (Biolegend, 402204) isotype control (diluted 1 in 800) as well as AF700 or APC/Cy7 CD3 (317341, 317339), BV605 CD19 (302243), BV421 or PerCP/Cy5.5 CD1c (331525, 331513), APC DC-SIGN (330107), APC/Cy7 CD14 (367107), PE/Cy7 or APC CD15 (323029, 323007), , AF488 CD16 (302019), PE/Cy5 CD56 (362515)(all from Biolegend) and FITC CD66 (BD Pharmingen, 551479), diluted accordingly to manufacturer's instructions, then washed, fixed for 30 min at 4˚C in 2% paraformaldehyde solution, washed again and finally resuspended in FACS buffer. The cells were acquired on CytoFLEX flow cytometer (Beckman Coulter, Brea, CA) and analyzed using FlowJo software (TreeStar, Ashland, OR).

Studies in NHP

Five-week i.v. repeated dose toxicity study in Cynomolgus Monkeys

Twenty male and twenty female (1:1) cynomolgus monkeys were randomized into six groups (n= 4 or 6) and received weekly (day 1, 8, 15, 22, and 29) bolus intravenous infusions of PBS (Vehicle), 10 mg/kg, 30 mg/kg or 100 mg/kg SYNT001. Two males and two females in the vehicle and 100 mg/kg dose groups, respectively were followed through day 58 and comprised the recovery animals. The remaining animals were euthanized on day 31.

Main study group animals were sampled prior to dosing on days 1, 8, 15, 22, and 29 and recovery animals on day 58 for anti-SYNT001 ADA. The following parameters and end-points were evaluated in this study: clinical signs, body weights, body weight changes, clinical pathology (hematology and clinical chemistry), inflammatory cytokine levels,

immunophenotyping, PK evaluation, PD markers (immunoglobulin and albumin), ADA, effect on TDAR with KLH and macroscopic and microscopic findings at necropsy.

PK Analysis of SYNT001 in Cynomolgus Monkeys

Twenty male and female cynomolgus monkeys were randomized into four groups (n=4-6/sex) and received weekly bolus intravenous infusions of PBS (Vehicle), 10 mg/kg, 30 mg/kg and 100 mg/kg SYNT001. Samples for toxicokinetic analysis were collected at 0, 0.83, 2, 4, 6, 8, 12, 16, 20, 24, and 48 hours post dose on day 1 and on day 29 at 0.83, 2, 4, 6, 8, 12, 16, 20 and 24 hours. A single additional sample was taken at 144 hours post dose at day 29 in recovery group (PBS and 100 mg/kg) animals only.

Individual SYNT001 serum concentration-time profiles from SYNT001-treated animals were analyzed using model-independent methods. Toxicokinetic parameters were obtained for each animal on days 1 and 29. Concentrations less than the lower limit of quantization (LLOQ, < 15.7 ng/mL) were reported as such and set to zero in the calculations. For each animal, the following toxicokinetic parameters were determined: estimated concentration at time zero (C_0), maximum observed serum concentration (C_{max}) and time of maximum observed serum concentration (T_{max}) and area under the serum concentration-time curve (AUC). The AUC from time 0 to 144 hr ($AUC_{(0\text{-}144h)}$) was calculated by the linear trapezoidal method for all animals with at least three consecutive quantifiable concentrations, $T_{1/2}$ were reported for each serum concentration-time profile that had sufficient serum concentrations in the terminal elimination phase (at least three samples not including T_{max}) and an adjusted R^2 of ≥ 0.9.

$$R = AUC_{(0\text{-}144h)} \text{ day } 29 \div AUC_{(0\text{-}144h)} \text{ day } 1$$

When final quantifiable sample (T_{last}) did not equal the last collection interval, the % AUC extrapolated (% AUC_{Extrap}) was calculated as

$$\%AUC_{Extrap} = [(AUC_{(0\text{-}144h)} - AUC_{(0\text{-}Tlast)}) / AUC_{(0\text{-}144h)}] \times 100$$

$$\%AUC_{Extrap} = [(AUC_{(0\text{-}inf)} - AUC_{(0\text{-}Tlast)}) / AUC_{INF}] \times 100$$

All $AUC_{(0\text{-}144h)}$ values were calculated with less than 25% extrapolation. AUC from time 0 to the time of the final quantifiable sample ($AUC_{(0\text{-}Tlast)}$), and AUC from time 0 to Infinity ($AUC_{(0\text{-}inf)}$) were calculated by the linear trapezoidal method.

Detection of SYNT001 in cynomolgus monkey serum

An ELISA was developed for total human IgG determination that was used to measure SYNT001 in monkey serum. This assay captured SYNT001 using a goat anti-human F(ab')2 antibody (AffiniPure F(ab')2 Fragment Goat Anti-Human IgG: Jackson ImmunoResearch Laboratories, Inc) immobilized on a clear Immulon 4 HBX ELISA plate. Detection is achieved through a mouse anti-human lgG antibody conjugated with horse-radish peroxidase (Mouse Anti-Human IgG4 Fc-HRP, Southern Biotech). The assay was qualified for SYNT001 with the lower limit of quantitation (LLOQ) = 10 ng/mL and upper limit of quantitation (ULOQ) = 500 ng/mL.

Immuno-phenotyping Analysis

Approximately 0.5 mL of blood samples were collected from all animals twice pretest, and from all main study animals on days 8, 15, 22, 29, and prior to the recovery necropsy. Samples were collected via the femoral vessel. The animals were not fasted prior to blood collection with the exception of the intervals that coincided with fasting for clinical pathology collections. The specimens were accessioned and processed on the day of collection. Aliquots of the whole blood specimens were stained with predetermined volumes of previously tested and titered monoclonal antibodies specific for each phenotype marker. For detection of surface and total primate FcRn expression, mouse anti-human FcRn antibody, ADM31, was utilized(*60*). ADM31 has been

shown to bind macaque FcRn(*33*), to block FcRn-albumin interactions(*11*) and is thought to interact with FcRn via a region that is not overlapping with IgG binding site and would thus not be predicted to interfere with SYNT001 binding. This is further supported by *in vitro* recycling assays whereby ADM31 did not block IgG recycling(*34*). The red blood cells in each tube were lysed and the remaining cells fixed with IO Test™ 3 Fixative. After fixation samples were stored refrigerated (2 to 8°C) for up to 48 hours. Flow count fluorospheres were added to each sample prior to being analyzed on a Beckman-Coulter FC 500 MPL flow cytometer with MXP software. During analysis, at least 1,000 lymphocyte events were collected per each sample. For each specimen, absolute cell count and cell percentage values were calculated. The single platform method was used to determine absolute counts. This procedure employed an internal standard of fluorescent microbeads. The number of cells bearing each phenotype was determined independently. In addition to determining the absolute leukocyte count, the panel of tests contained monoclonal antibodies to identify leukocytes ($CD45^+$), helper T cells ($CD45^+CD3^+CD4^+$), cytotoxic T cells ($CD45^+CD3^+CD8^+$), NK cells ($CD45^+$ $CD3^-CD159a^+$), B cells ($CD45^+CD20^+$) and monocytes ($CD45^+CD14^+$). Platelets were analyzed on Siemens Advia 2120 and Advia 2120i Hematology Systems (Siemens) according to standard manufacturer's protocol.

Immunoglobulin (IgG, IgA, IgM) and albumin assessments in cynomolgus monkeys

The IgA, IgG, IgM and albumin were measured on the Beckman Coulter AU2700 (Beckman Coulter, Brea, CA) using IgA (Beckman Coulter, OSR61171), IgG (Beckman Coulter, OSR61172), IgM (Beckman Coulter, OSR61173) and albumin (Beckman Coulter, OSR6102/OSR6202/OSR6602) detection reagents according to manufacturer's instructions at Charles River Laboratories. The methodology of each assay utilized an immune-turbidimetric

method to form immune-complexes between the immunoglobulin in the serum and a goat anti-immunoglobulin antiserum. The formed complex then caused light scatter which was measured by a turbidimeter on the analyzer. The reduction in light scatter due to size of the IC was directly proportional to the amount of immunoglobulin in the serum sample. This automated turbidimetric immunoassay assay was fully validated and the method and the collection of data and analysis were in compliance with FDA's good laboratory practice (GLP) guidelines.

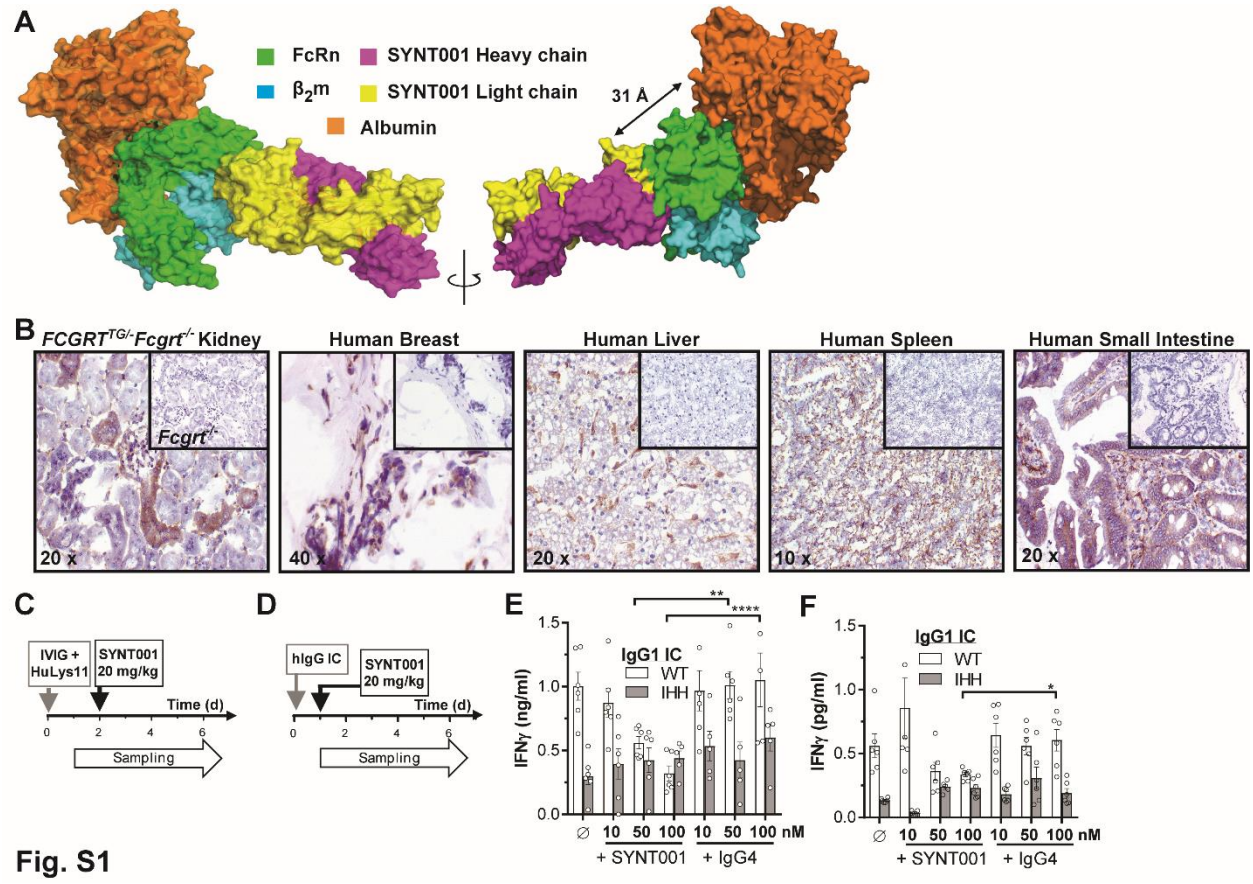

Fig. S1

Fig. S1. Characterization of SYNT001. (**A**) hFcRn:β_2m-SYNT001 binding interface is distant and non-overlapping with hFcRn:β_2m-albumin binding site. SYNT001 light and heavy chains (yellow and magenta, respectively) make interactions with hFcRn α-chain (green) and β_2m (cyan) that differ from Albumin (orange) binding interface. (**B**) Representative IHC images of FcRn staining from kidney of *FCGRT*$^{TG/-}$/*Fcgrt*$^{-/-}$ mice as well as human breast, liver, spleen and small intestine. Insets show negative controls with *Fcgrt*$^{-/-}$ mice or isotype antibody staining. (**C**) Schematic representation of SYNT001 blockade of monomeric IgG recycling. (**D**) Schematic representation of SYNT001 blockade of multimeric IgG IC recycling. (**E**) Effect of SYNT001 on IgG IC cross-presentation. CD11c^{+} APC from *B2M*$^{TG/TG}$*FCGRT*$^{TG/TG}$*Fcgrt*$^{-/-}$ mice were pre-treated with hIgG1^{WT} or hIgG1^{IHH} IC variants in presence of SYNT001 F(ab)$_2$ fragments or IgG4 isotype control F(ab)$_2$ fragments followed by co-culture with MHC class I-restricted CD8^{+} T

cells. 48 hours later IFNγ production was measured by ELISA. Mean ± SEM with actual data points is depicted. (**F**) Effect of SYNT001 on IgG IC presentation. $CD11c^+$ APC treated as in (E) were co-cultured with MHC class II-restricted $CD4^+$ T cells. 48 hours later IFNγ production was measured by ELISA. Mean ± SEM with actual data points is depicted. (E,F) * P<0.05, **** P<0.0001 by 2-way ANOVA with Fisher's LSD post hoc test. All experiments were repeated at least twice.

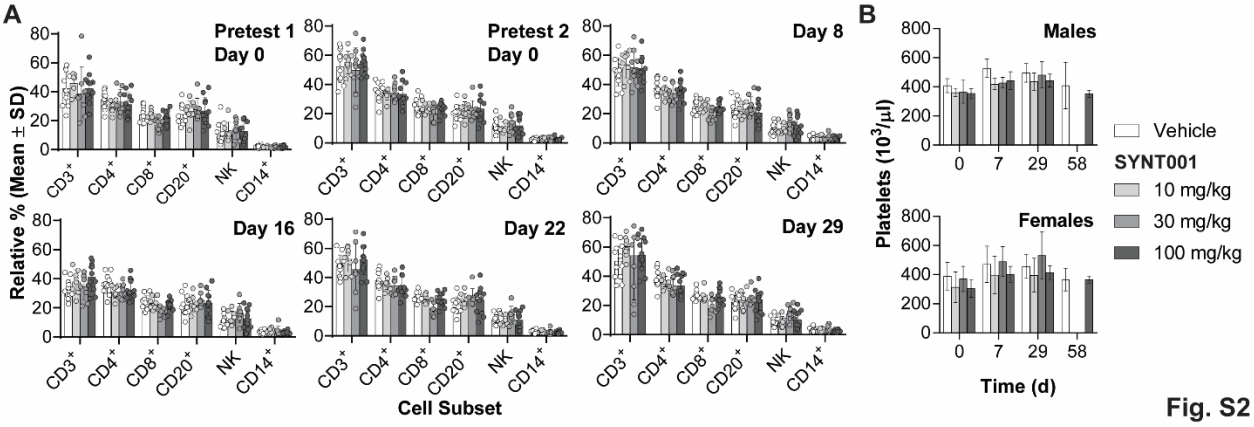

Fig. S2. The immunologic effects of repeated-dose SYNT001 administration in cynomolgus monkeys. (**A**) Relative frequencies of different lymphocyte subsets in cynomolgus monkeys upon repeated-administration of SYNT001. Blood samples from vehicle, 10 mg/kg, 30 mg/kg and 100 mg/kg SYNT001 cohorts were collected at various indicated time points (day 0, 8, 16, 22 and 29), stained with antibodies for markers identifying the indicated cell subsets and analyzed by flow cytometry. (**B**) Platelet counts from vehicle, 10 mg/kg, 30 mg/kg and 100 mg/kg SYNT001 male (top) and female (bottom) cohorts were collected at various indicated time points (day 0, 7, 29 and 58). (vehicle, 100 mg/kg, n=12; 10 mg/kg, 30 mg/kg, n=8). Bar graphs with mean ± SD are displayed. Symbols represent actual data points from each individual animal.

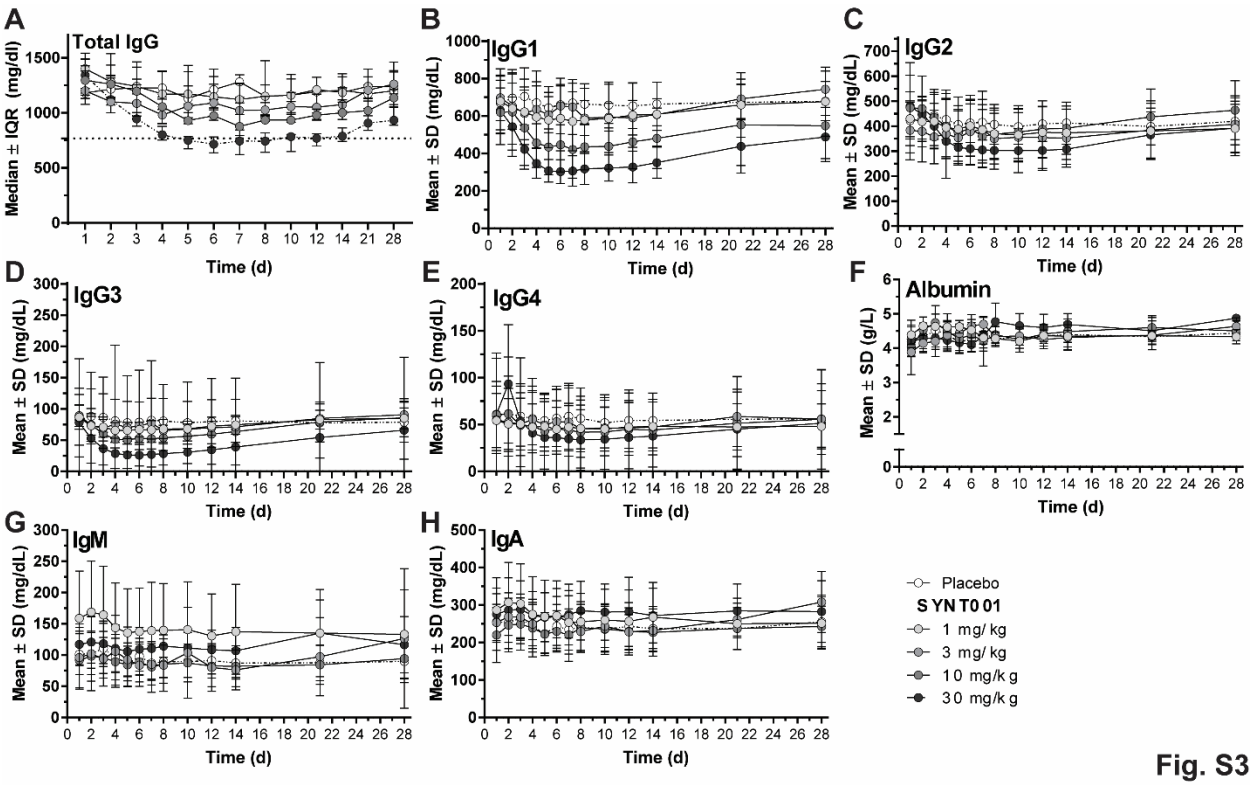

Fig. S3. PD of single-dose SYNT001 administration in healthy human individuals. Change in (**A**) total serum IgG concentrations in all subjects enrolled in the Phase I study. Dotted line represents LLN of 768 mg/dL. Changes in circulating (**B**) IgG1, (**C**) IgG2, (**D**) IgG3, (**E**) IgG4, (**F**) albumin, (**G**) IgA and (**H**) IgM concentrations in all subjects enrolled in the Phase 1 study. Median ± IQR is shown in (A) Mean ± SD are shown for (B-H) (n=8 for Placebo, n=6 for 1, 3 and 10 mg/kg, n=5 for 30 mg/kg SYNT001 cohorts). MMRM modeling indicated statistically significant (P<0.05) differences of IgG levels compared to placebo in the 1 mg/kg (days 2-14), 3 mg/kg (days 2-10), 10 mg/kg (days 2-14) and 30 mg/kg (days 2-21) dose groups.

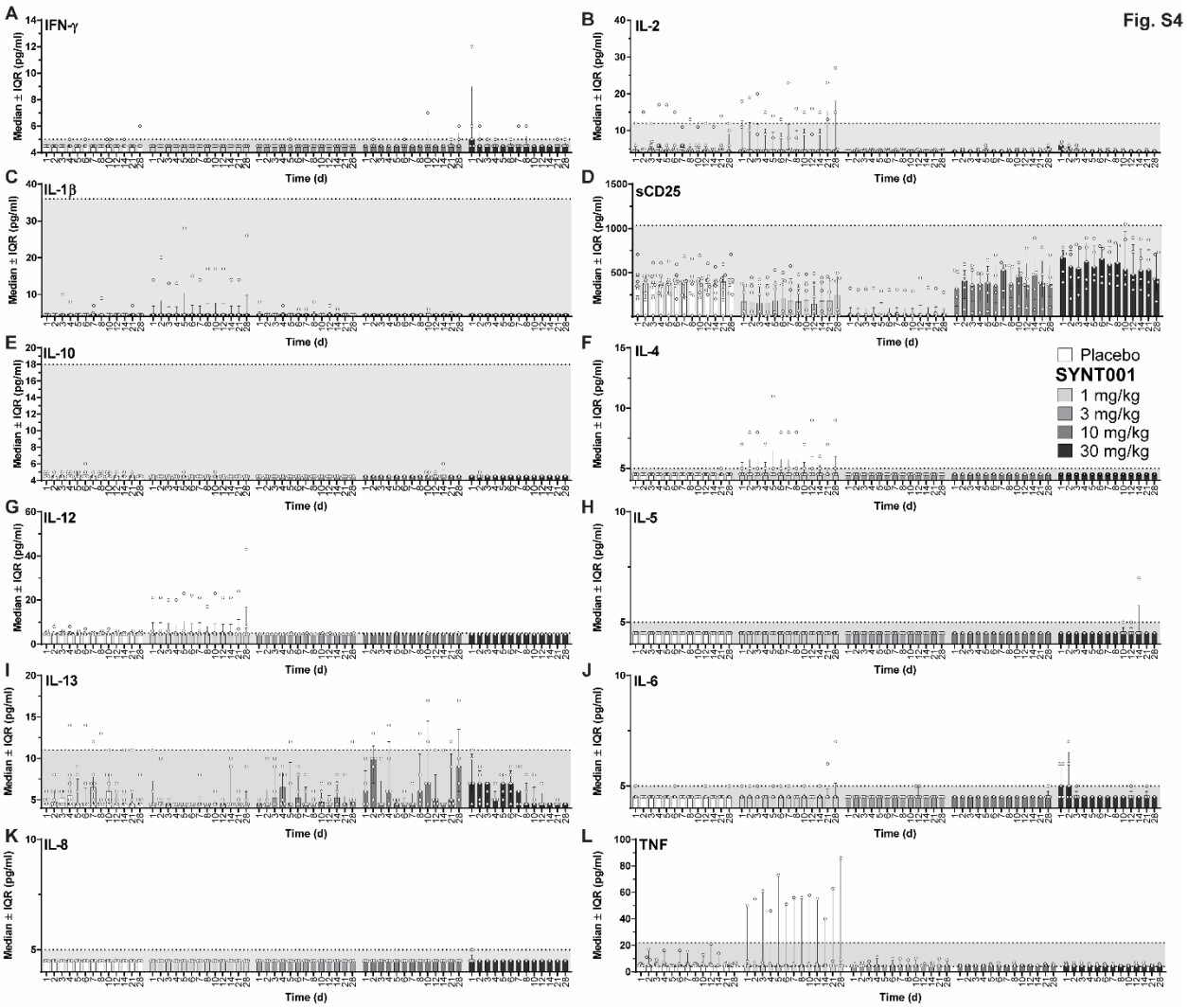

Fig. S4. Effect of FcRn-blockade by SYNT001 on immune responses in healthy individuals. Levels of (**A**) IFN-γ, (**B**) IL-2, (**C**) IL-1β, (**D**) soluble IL-2Rα (sCD25), (**E**) IL-10, (**F**) IL-4, (**G**) IL-12, (**H**) IL-5, (**I**) IL-13, (**J**) IL-6, (**K**) IL-8, (**L**) TNFα for all subjects enrolled in Phase I study. Bar graphs with median ± IQR are displayed. Symbols represent actual data points from each individual research volunteer (n=8 for Placebo, n=6 for 1, 3 and 10 mg/kg, n=5 for 30 mg/kg SYNT001 cohorts).

Table S1. SYNT001-FcRn:β_2m crystal structure data collection and refinement statistics (Protein Data Bank ID: 6NHA).

Data collection	
Beamline	APS NE-CAT 24-ID-E
d_{min} (Å)	2.4
Wavelength (Å)	0.979
No. of observations	517896
Multiplicity[a]	12.5 (11.0)
Average $(I)/(\sigma_I)$[a]	19.73 (1.16)
Completeness[a] (%)	93.9 (87.4)
R_{merge}[a,b] (%)	0.116 (2.385)
$CC_{1/2}$[a]	0.999 (0.404)
CC^*[a,c]	1 (0.759)
Refinement	
Bragg spacings (Å)	49.8 - 2.38
Space group	$P2_12_12_1$
Cell parameters: *a,b,c* (Å)	64.83, 77.66, 215.60
R[d] / R_{free}[e] (%)	19.0 / 24.5
No. of unique reflections	41525
No. of total atoms (non-H)	6263
No. of protein atoms (non-H)	6058
No. of hetero atoms (non-H)	39
No. of waters	166
Average B-factor (Å^2)	58.3

Rmsd bond length (Å)	0.005
Rmsd bond angle (°)	0.83
Ramachandran favored / allowed[f] (%)	95.7 / 99.2

[a.] Values in outermost shell are given in parentheses.

[b.] $R_{merge} = (\sum | I_i - \langle I_i \rangle |)/\sum | I_i |$, where I_i is the integrated intensity of a given reflection.

[c.] $CC^* = (2CC_{1/2}/1+ CC_{1/2})^{1/2}$ Ref. [101].

[d.] $R = \sum | |F_o| - |F_c| | / \sum | |F_o| |$, where F_o and F_c denote observe and calculated structure factors, respectively.

[e.] R_{free} was calculated using 5% of data excluded from refinement.

[f.] Calculated using Molprobity[102].