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Research Article

Pharmacokinetic Properties of Humanized IgG1 and IgG4 Antibodies in Preclinical Species: *Translational Evaluation*

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ABSTRACT. Assessment of the factors that regulate antibody exposure–response relationships in the relevant animal models is critical for the design of successful translational strategies from discovery to the clinic. Depending on the specific clinical indication, preclinical development paradigms may require that the efficacy or dosing-related attributes for the existing antibody be assessed in various species when cross-reactivity of the lead antibody to the intended species is justified. Additionally, with the success of monoclonal antibodies for management of various human conditions, a parallel interest in therapeutic use of these novel modalities in various veterinary species has followed. The protective role of neonatal Fc receptor (FcRn) in regulation of IgG homeostasis and clearance is now well recognized and the "nonspecific clearance" of antibodies through bone marrow-derived phagocytic and vascular endothelial cells (via lysosomal processes) is modulated by interactions with FcRn receptors. In this study, we have attempted to examine the PK properties of human IgG antibodies in dog and monkey. These studies establish a translational framework for evaluation of IgG antibody PK properties across species.

KEY WORDS: Antibody; Pharmacokinetics; Translation.

INTRODUCTION

Similar to small-molecule drugs, translation of the exposure–response relationships during development of monoclonal antibodies can be a major challenge hindering effective development of this class of biologics (1,2). Assessment of the factors that regulate antibody exposure–response relationships in relevant animal models is critical for the design of successful translational strategies from discovery to the clinic (3,4). Additionally, evaluation of the pharmacodynamic (PD) system efficiency and stimulus–response mechanisms that convert receptor occupancy into the pharmacological response(s) along with effective application of quantitative pharmacology are among the key translational considerations throughout the antibody development process. Depending on the specific clinical indication, preclinical development paradigms may require that the efficacy or dosing-related attributes for the existing antibody be evaluated in various species when cross-reactivity of the lead antibody to the intended species is justified (3–5).

IgG is the most abundant serum immunoglobulin in humans, and serum IgG homeostasis is of particular importance in mediating humoral immunity. The protective role of neonatal Fc receptor (FcRn) in regulation of IgG homeostasis and clearance is now well recognized (6–10). Further, it appears that normal variations in endogenous serum IgG levels or the usual therapeutic doses of antibodies do not impact the IgG clearance (11). Therefore, for intact antibodies or Fc-fusion proteins, the "nonspecific and linear clearance" through bone marrow-derived phagocytic (12) and vascular endothelial cells (13,14) (via lysosomal processes) is modulated by interactions with FcRn receptors. The elimination of antibodies can also be affected to a great extent by their interaction with their intended target (1,2,11,15). When a biologic ligand binds to a cell membrane receptor, the ligand can induce receptor internalization. Once internalized, the ligand and receptor can undergo degradation or the ligand might be recycled to the cell surface if the ligand remains bound to a recycled receptor. This "target-dependent" clearance pathway is often referred to as an "antigen-mediated sink" for therapeutic antibodies. Target-related sinks are most commonly observed for biologics targeting internalizing cell membrane receptors with high normal tissue expression.

With the rise in interest for application of antibody therapeutics in companion animals, effort has been focused to

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further understand the structure-function properties of canine IgGs (16,17). Like human, canine IgGs consist of four subclasses referred to as A, B, C, and D isotypes (16). All canine subclasses bind the neonatal Fc receptor except subclass C. Functional similarity for canine IgGs to their human analogs have also been established[1] (16). Comparison of the human system with canine immunology allows for building a more effective translational bridge for application of human antibodies in veterinary medicine. In this study, we have attempted to examine the PK properties of human IgG antibodies in dog and monkey. Tissue distribution studies were also conducted in mice. These studies establish a translational framework for application of human IgG antibodies across species.

METHODS

Reagent and Test Articles

Humanized anti-RSV (respiratory syncytial virus) antibody in IgG4 isotype (Lot 60AGK) and in IgG1 isotype (PK study in dog and monkey: lot 42AQI and 53AFY; respectively, and Lot 88AXM for tissue distribution study in mice) were produced from stable engineered CHO-K1SV cell line at Merck & Co., Inc., Palo Alto, CA, USA. Briefly, a humanized monoclonal antibody with an IgG1 backbone (G1 m17 allotype) with kappa light chain (km3 allotype) or with an IgG4 backbone with the S228P mutation to prevent Fab arm exchange (18) was engineered. Antibodies were produced by recombinant DNA technology in a CHO stable cell line and purified via protein A (MabSelect™ SuRe™, GE Healthcare) followed by a Q-anion exchange step (Q-FF) and cation-exchange step (Poros HF). Purified antibodies were formulated in sodium acetate, sucrose buffer at pH 5.5. Purity was assessed by size-exclusion chromatography (SEC), reverse-phase HPLC (RP), and endotoxin levels tested using the LAL (limulus amoebocyte lysate) assay with an Endosafe device (Charles River Laboratories). Purity for the IgG1 antibodies by SEC and RP was determined to be ~ 98.6% and > 97%, respectively and endotoxin levels were below the limit of detection (< 0.01 EU/mg). Purity for the IgG4 antibodies by SEC and RP was determined to be > 99% and 100%, respectively and endotoxin levels were below the limit of detection (< 0.01 EU/mg). Additionally, a human IgG4 antibody (Lot 77AOO; non cross-reactive to mouse antigen) was expressed via recombinant DNA technology by transient transfection using Expifectamine transfection reagent in CHO-Expi cell line (Thermo Fisher Scientific). Supernatant was purified (~ 95.2% purity) via protein A (MabSelect™ SuRe™, GE Healthcare) followed by a Q-anion exchange step (Q-FF). This IgG4 antibody was used in mouse tissue distribution study (Fig. 4). *FcRn Sequence Alignment.* Amino acid sequences of the neonatal Fc receptor (FcRn) from human (NM_001136019.2), rhesus macaque (NM_001257520.1), cynomolgus monkey (NM_001284551.1), dog (XM_005616309.3), and mouse (NM_010189.3), were obtained from NCBI (https://www.ncbi.nlm.nih.gov). The amino acid sequences were aligned using AlignX, based on the Clustal W algorithm (19), in Vector NTI Advance® 11.5. Calculation of the percent identity and percent similarity by AlignX was conducted by comparing aligned sequences between individual species. A pairwise alignment is produced using dynamic programming guided by a table of scores for matches and mismatches and penalties for insertions or deletions. A higher score is given to amino acids that are identical or similar in alignment. To generate a multiple sequence alignment, a progressive alignment method is used. Similarity between all pairs of sequences is first calculated, scored, and converted into a distance, then a guide tree is constructed from the distance matrix and sequences are progressively added to the pairwise alignment according to the branching order in the guide tree.

In Vivo Studies. Animal experiments were conducted in full compliance with local, national, ethical and regulatory principles, and local licensing regulations, per the spirit of Association for Assessment and Accreditation of Laboratory animal Care (AAALAC) international expectations (http://www.aaalac.org/accreditation/index.cfm) for animal care and use/ethics committees at University of Louisiana at Lafayette, and Merck & Co., Inc., Palo Alto, CA, USA. Procedures involving the care and use of animals in the study were reviewed and approved by the Institutional Animal Care and Use Committees as listed above. Throughout the study, animals were observed twice daily, and at each blood collection time point, and were evaluated for potential adverse clinical observations or adverse reactions to the test article. No adverse clinical observations or reactions were observed.

Pharmacokinetic properties of anti-RSV antibodies were evaluated in beagle dogs (9–11 kg) randomized into six groups ($n = 3$/group). A single dose of each antibody was administered intravenously at 0.3, 1, and 3 mg/kg. Blood samples were collected via venous catheters at: pre-dose, day 0 at 15 min, 30 min, 1 h, 2 h, 4 h, 7 h, and days 1, 2, 3, 6, 7, 8, and 9 post-dose. Blood samples were processed for serum and samples were harvested and stored at − 70 °C until analysis. Additionally, a single dose of the IgG1 or IgG4 antibodies were administered intravenously to six biologics-naïve cynomolgus monkeys (4.0–6.0 kg) at 3 mg/kg via a cephalic vein. Blood samples were collected via a peripheral vessel at: pre-dose, day 0: 2 h, and days 1, 2, 7, 9, and 14 post-dose. Blood samples were processed for serum and samples were harvested and stored at − 70 °C until analysis.

For tissue distribution studies, healthy BALB/c or C57BL/6 female mice were given a single IV bolus dose of 3.0 mg/kg DyLight 650–labeled IgG1 or IgG4 antibodies. Animals were euthanized at predetermined time points at days 1, 2, and 5. The concentrations of test articles in plasma, whole blood, and selected tissues were quantified by a fluorescence emission-linked assay (20,21).

Analytical Methods. Levels of IgG1 and IgG4 antibodies in dog and monkey serum were measured by a qualified electrochemiluminescence (ECL)-based immunoassay on Meso Scale Discovery platform (Meso Scale Discovery, Rockville, MD, USA; https://www.mesoscale.com). The

[1] Analogous human IgGs were described based upon Fc gamma, FcRn and complement binding: Human IgG1, 2, 3, and 4 are comparable to canine IgG B, A, C, and D, respectively (from reference (16)).

capture reagent was immobilized biotinylated mouse anti-human immunoglobulin kappa light chains (BD Biosciences, cat# 555790). The detection reagent was ruthenylated mouse anti-human IgG (CH2 domain) (Thermo Scientific, cat#MA5-16929). The lower limit of quantitation (LLOQ) of IgG1 or IgG4 antibodies was 9.14 ng/mL in both dog and monkey serum.

Pharmacokinetic Analysis

Non-Compartmental Analysis. GraphPad Prism version 7.02 was used for plotting concentration-time profiles. Individual animal serum concentration-time data were analyzed using non-compartmental analysis (NCA) methods. All PK parameters were calculated using Phoenix 64 (Certara L.P.; https://www.certara.com). Non-compartmental analyses utilized Model 201 (IV).

Antibody Labeling and Sample Collection. DyLight™ 650 labeling kit (Thermo Fisher Scientific, Waltham, MA) was used to conjugate N-hydroxysuccinimide ester fluorescence dye (excitation at 652 nm and emission at 672 nm) to IgG1 and IgG4 using the method detailed in a previous publication (21). Whole blood samples were collected via cardiac puncture in a K_2-EDTA CapiJect microcollection tubes (Terumo Medical Corporation, Somerset, NJ) and placed on ice. For drug analysis in blood, whole blood samples were aliquoted into polypropylene vials (Corning Glassworks, Corning, NY), and stored at − 80°C. Plasma was separated from whole blood (centrifugation for 6 min at 6000×*g* at 4°C, and stored at − 80°C). For tissue lysates, organ samples were collected and immediately placed into 2-ml Precellys Lysing Tubes (Bertin Technologies, Rockville, MD), weighed, and frozen by placing on dry ice. To prepare lysate samples upon partial thawing, a 1:5 dilution of Dulbecco's PBS containing 1% Triton X-100 lysis buffer (MP Biomedicals, Solon, OH) and 1 X Halt Protease Inhibitor single use cocktail (ThermoFisher Scientific, Waltham, MA) were added to the samples. Tissue slurries were prepared using a Precellys Evolution Homogenizer (Bertin Technologies). The slurry was centrifuged (10,000×*g* at 4°C for 10 min) and the tissue lysate supernatants were collected and either processed immediately or stored at − 80°C until analysis.

Fluorescence Emission-Linked Assay. For assessment of drug concentration in whole blood, plasma, and tissue lysate samples, from treated mice or a set of corresponding blank tissue samples from naïve animals, samples were diluted (1:10 total) in the lysis buffer. The diluted samples (150 μL) were transferred to 96-well polystyrene plates with low fluorescence background, and the net fluorescence intensity was measured using the Glomax® Multi-Detection system microplate reader (Promega; https://www.promega.com) equipped with a fluorescence optical filter with excitation/emission wavelengths of 625/660–720. Concentrations of fluorophore-labeled antibody were calculated by generating calibration curves in naive BALB/c or C57BL/6 mouse liver tissue, whole blood, and liver lysate. A linear regression analysis was performed on the fluorescence data for the calibration curves using GraphPad Prism 7 (GraphPad Software Inc., La Jolla, CA). Concentrations of DyLight™ 650-labeled antibodies were calculated as microgram equivalents per gram of wet tissue and microgram equivalents per milliliter of blood or plasma as described previously (21). Subsequent tissue/blood ratios were calculated by dividing concentration in the respective tissues with the concentration in blood at the corresponding time point. It should be noted that for tissue-to-blood ratio determination, tissues collected were not perfused, and mathematical correction of residual blood from tissues was not performed.

RESULTS

Evaluation of FcRn Sequence Homology Across Species. Previous studies have demonstrated that human FcRn binds to human, rabbit, and guinea pig IgGs, but not significantly to rat, bovine, sheep, or mouse IgGs (22). In contrast, mouse FcRn binds to all human IgG isotypes. Mature human FcRn consists of a 274 amino acid (*aa*) extracellular domain (ECD) with two N-terminal alpha domains, one α3/immunoglobulin like domain, a 23 *aa* transmembrane segment, and a 44 *aa* cytoplasmic domain (9,10). Within the ECD, human FcRn shares 67% *aa* sequence identity and 76% sequence similarity with mouse FcRn (Fig. 1). A higher percent identity and similarity between the extracellular domain of human FcRn and nonhuman primate FcRn was observed (~ 97 to 98%; Fig. 1). Sequence alignment for human and canine FcRn indicated an approximately 81 to 84% sequence identity and similarity, respectively (Fig. 1).

Pharmacokinetic Properties of Human IgG Antibody Isotypes in Dogs and Nonhuman Primates. PK properties of canine IgG antibodies in dogs have been reflective of a long serum half-life and limited volume of distribution confined to blood compartment (23). In this study, we evaluated the PK properties of human antibodies and IgG1 and IgG4 isotypes, in dogs. Anti-RSV antibody (IgG1 and IgG4 isotypes) was selected so that the "nonspecific" clearance properties to be evaluated as target antigen is not present in healthy animals (i.e., to exclude the target-related impact on antibody PK properties). Intravenous dosing of each isotype in a separate group of animals reflected a linear pharmacokinetics for each isotype (Table I and Fig. 2a) and no apparent difference in CL or Vss was observed across the different dose groups for each antibody over a tenfold dose range (Table I). A prolonged serum exposure with half-lives ranging from ~ 7 to 12 days was observed for these antibody isotype in dogs (Fig. 2a, and Table I). Similarly, as linear pharmacokinetics was anticipated in the absent of target, pharmacokinetic studies in nonhuman primates only included the dose of 3 mg/kg for IgG1 and IgG4 isotypes (Fig. 2b, Table II). Due to potential immunogenicity of human antibodies in preclinical species, longer study duration generally may not allow collection of meaningful data. We acknowledge that estimation of the PK parameters may not represent an accurate estimate due to this limitation.

a

	Rhesus	Cyno	Dog	Mouse
% identity	97.1	97.1	81.3	66.6
% similarity	98.0	98.0	83.9	75.9

b

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                10        20        30        40        50        60
    human  MGVPRPQPWALGLLLFLLPGSLGAESHLSLLYHLTAVSSPAPGTPAFWVSGWLGPQQYLS
      cyno  MRVPRPQPWALGLLLFLLPGSLGAESHLSLLYHLTAVSSPAPGTPAFWVSGWLGPQQYLS
    rhesus  MRVPRPQPWALGLLLFLLPGSLGAESHLSLLYHLTAVSSPAPGTPAFWVSGWLGPQQYLS
       dog  MGVPRPRSWGLGFLLFLLPTLRAADSHLSLLYHLTAVSAPPGTPAFWASGWLGPQQYLS
     mouse  --MGMPLPWALSLLLVLLPQTWGSETRPPLMYHLTAVSNPSTGLPSFWATGWLGPQQYLT

                70        80        90       100       110       120
    human  YNSLRGEAEPCGAWVWENQVSWYWEKETTDLRIKEKLFLEAFKALG--GKGPYTLQGLLG
      cyno  YDSLRGQAEPCGAWVWENQVSWYWEKETTDLRIKEKLFLEAFKALG--GKGPYTLQGLLG
    rhesus  YDSLRGQAEPCGAWVWENQVSWYWEKETTDLRIKEKLFLEAFKALG--GKGPYTLQGLLG
       dog  YNNLRAQAEPYGAWVWENQVSWYWEKETTDLRTKEGLFLEALKALG--DGGPYTLQGLLG
     mouse  YNSLRQEADPCGAWMWENQVSWYWEKETTDLKSKEQLFLEALKTLEKILNGTYTLQGLLG

                130       140       150       160       170       180
    human  CELGPDNTSVPTAKFALNGEEFMNFDLKQGTWGGDWPEALAISQRWQQQDKAANKELTFL
      cyno  CELSPDNTSVPTAKFALNGEEFMNFDLKQGTWGGDWPEALAISQRWQQQDKAANKELTFL
    rhesus  CELSPDNTSVPTAKFALNGEEFMNFDLKQGTWGGDWPEALAISQRWQQQDKAANKELTFL
       dog  CELGPDNTSVPVAKFALNGEDFMTFDPKLGTWNGDWPETETVSKRWMQQAGAVSKERTFL
     mouse  CELASDNSSVPTAVFALNGEEFMKFNPRIGNWTGEWPETEIVANLWMKQPDAARKESEFL

                190       200       210       220       230       240
    human  LFSCPHRLREHLERGRGNLEWKEPPSMRLKARPSSPGFSVLTCSAFSFYPPELQLRFLRN
      cyno  LFSCPHRLREHLERGRGNLEWKEPPSMRLKARPGNPGFSVLTCSAFSFYPPELQLRFLRN
    rhesus  LFSCPHRLREHLERGRGNLEWKEPPSMRLKARPGNPGFSVLTCSAFSFYPPELQLRFLRN
       dog  LYSCPQRLLGHLERGRGNLEWKEPPSMRLKARPGSPGFSVLTCSAFSFYPPELQLRFLRN
     mouse  LNSCPERLLGHLERGRRNLEWKEPPSMRLKARPGNSGSSVLTCAAFSFYPPELKFRFLRN

                250       260       270       280       290       300
    human  GLAAGTGQGDFGPNSDGSFHASSSLTVKSGDEHHYCCIVQHAGLAQPLRVELESPAKSSV
      cyno  GMAAGTGQGDFGPNSDGSFHASSSLTVKSGDEHHYCCIVQHAGLAQPLRVELETPAKSSV
    rhesus  GMAAGTGQGDFGPNSDGSFHASSSLTVKSGDEHHYCCIVQHAGLAQPLRVELETPAKSSV
       dog  GLAAGSGEGDFGPNGDGSFHAWSSLTVKSGDEHHYRCLVQHAGLPQPLTVELESPAKSSV
     mouse  GLASGSGNCSTGPNGDGSFHAWSLLEVKRGDEHHYQCQVEHEGLAQPLTVDLDSSARSSV

                310       320       330       340       350       360
    human  LVVGIVIGVLLLTAAAVGGALLWRRMRSGLPAPWISLRGDDTGVLLPTPGEAQDADLKDV
      cyno  LVVGIVIGVLLLTAAAVGGALLWRRMRSGLPAPWISLRGDDTGSLLPTPGEAQDADSKDI
    rhesus  LVVGIVIGVLLLTAAAVGGALLWRRMRSGLPAPWISLRGDDTGSLLPTPGEAQDADSKDI
       dog  PVVGIVIGFLLLTAVAVGGALLWRRMRKGLPAPWMSLRGDDVGALLPTPGVPKDADS---
     mouse  PVVGIVLGLLLVVVAIAGGVLLWGRMRSGLPAPWLSLSGDDSGDLLPGGNLPPEAEPQGA

                367
    human  NVIPATA
      cyno  NVIPATA
    rhesus  NVIPATA
       dog  -------
     mouse  NAFPATS
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Fig. 1. Sequence conservation of FcRn. **a** Sequence of FcRn is highly conserved in primates. Calculation of the percent identity and percent similarity was conducted by comparing aligned sequences between individual species using AlignX in Vector NTI Advance® 11.5. The percent identity and percent similarity between the extracellular domain of human FcRn and FcRn from each species is presented in the Table. **b** Sequence alignment of FcRn from five mammalian species. AlignX was used to generate the sequence alignment from the five mammalian species shown. Sequence differences are shown as black lettering on gray background

Comparisons of Body Weight-Adjusted PK Parameters. Characterization of antibody PK properties in appropriate animal models can greatly enhance the translation of exposure information across species. In general, the nonspecific clearance processes in nonhuman primates that

Table I. Pharmacokinetic Parameter Estimates in Dogs Following Administration of Anti-RSV Antibodies (IgG1, and IgG 4; Non-compartmental analysis; Intravenous dose; 0.3 to 3.0 mg/kg

Dose (mg/kg)	Isotype	$AUC_{(0-\infty)}$ (day*μg/mL)	CL (mL/day/kg)	V_{ss} (mL/kg)	$t_{1/2}$ (day)
0.3	IgG1	62.3 ± 10.6	4.91 ± 0.796	71.4 ± 3.04	10.2 ± 2.24
1.0		244 ± 13.4	4.11 ± 0.219	69.9 ± 1.60	12.0 ± 0.335
3.0		708 ± 273	4.66 ± 1.71	63.0 ± 11.1	10.7 ± 6.33
0.3	IgG4	65.3 ± 39.3	6.14 ± 4.10	58.5 ± 14.1	8.85 ± 8.11
1.0		202 ± 28.3	5.03 ± 0.744	71.1 ± 7.53	9.62 ± 0.421
3.0		496 ± 127	6.33 ± 1.66	61.5 ± 4.48	6.53 ± 2.70

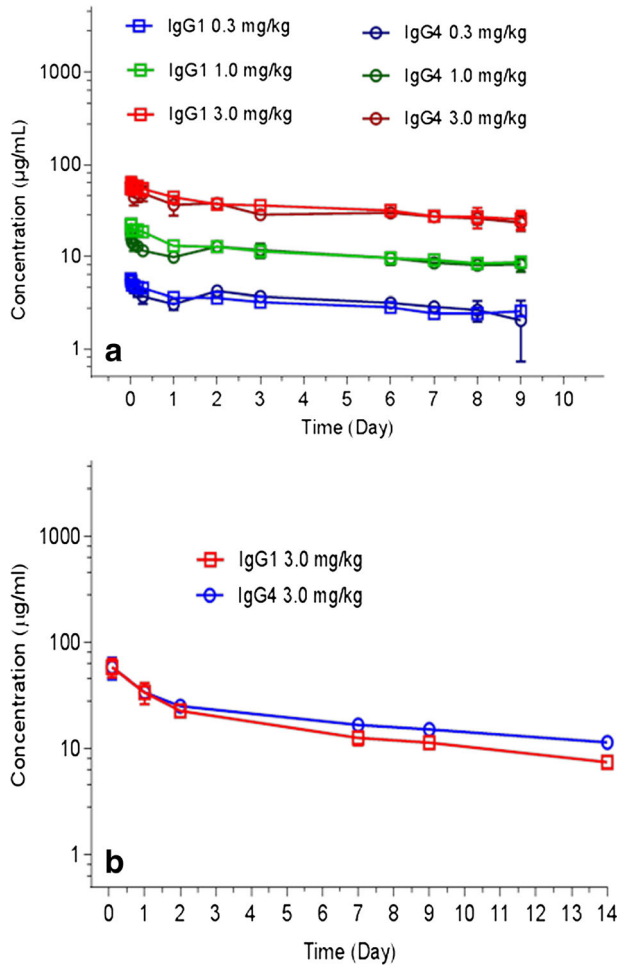

Fig. 2. Serum concentration-time profiles in dogs and monkeys. Animals were given single dose intravenously. Serum concentrations of antibodies were determined using an electrochemiluminescence (ECL)-based immunoassay with an LLOQ of 9.14 ng/mL. **a** Serum concentration-time profiles (mean+/-SD) following single-dose administrations of anti-RSV, IgG1, or IgG4, antibodies in dogs ($n = 3$/group). **b** Serum concentration-time profiles (mean+/-SD) following single-dose administrations of anti-RSV, IgG1 in monkeys ($n = 3$/group)

reflect the FcRn interaction is predictive of human linear clearance and pharmacokinetics (see the discussion section). Using the standard allometric scaling equation shown below (24), the clearance across species could be scaled up and down by adjusting for species body weight (BW; Tables III and IV).

$$CL_{Human} = CL_{Animal}{}^{*} \left(BW_{Human}/BW_{Animal}\right)^{0.85}$$

Body weight adjusted clearance and Vss parameters for the individual animals are shown in Fig. 3a and b. Again, both antibody isotypes were comparable with respect to their PK properties in each species and between the species. Comparable weight-adjusted PK properties in dogs and monkeys reflect a similarity in nonspecific clearance mechanisms that govern elimination of these IgG isotypes.

Tissue Distribution Studies in Mouse. In this study, we also examined biodistributional properties of IgG antibody isotypes in mice using Dylight 650 label antibody and according to procedure described previously (21). Comparable plasma concentrations were achieved for both isotypes in mice (Fig. 4a). Additionally, exposure in mice following administration of the 3 mg/kg dose was comparable to that observed in dogs and monkeys (~ 20–30 µg/ml) at selected time points. Tissue distribution for the two IgG isotypes was comparable as indicated by tissue-to-blood ratios of the antibody in key organs (Fig. 4b- IgG1 and 4C-IgG4). No evidence of organ uptake was observed as the tissue-to-blood ratio over time (days 1 to 5) was lower than "1" in all tissues examined.

DISCUSSION

In veterinary medicine, monoclonal antibodies are expected to play an important role in treatment of companion animals. For example, positive results have been observed with application of anti-CD20 antibodies in dogs with canine lymphoma (25). Similarly, therapeutic modulation of pain by anti-nerve growth factor (anti-NGF) monoclonal antibody in dogs has proved promising (23,26). As the diversity in therapeutic indications addressed by monoclonal antibodies expands, effective application of these modalities in relevant canine models is becoming more prevalent. For example, canine and human hearts are reported to share many characteristics at both the organ and cellular levels (27). Additionally, due to their size, almost all in vivo techniques used to assess contractility of human hearts can be utilized in canines. Therefore, preclinical development of antibodies in this and other relevant indications will require an understanding of the human or humanized antibody pharmacokinetics in these

Table II. Pharmacokinetic Parameter Estimates in Dogs and Monkeys (Non-compartmental analysis; Intravenous dose; 3.0 mg/kg)

Species	Isotype dose (mg/kg)	AUC $_{(0-\infty)}$ (day*µg/mL)	CL (mL/day/kg)	V_{ss} (mL/kg)	$t_{1/2}$ (day)
Dog ($n = 3$)	IgG1 (3.0)	708 ± 273	4.66 ± 1.71	63.0 ± 11.1	10.7 ± 6.33
Monkey ($n = 3$)		318 ± 37.4	9.52 ± 1.05	93.0 ± 8.63	7.64 ± 0.996
Dog ($n = 3$)	IgG4 (3.0)	496 ± 127	6.33 ± 1.66	61.5 ± 4.48	6.53 ± 2.70
Monkey ($n = 3$)		471 ± 26.6	6.38 ± 0.358	97.8 ± 17.6	11.5 ± 2.57

Table III. Prediction of Human Clearance Based on Reported Clearance in Monkey and Human for Antibodies with Reported Linear Clearance (Human clearance was predicted based on equation: $CL_{Human} = CL_{Animal} (BW_{Human}/BW_{Animal})^{085}$ Reproduced from Reference 23)

Antibody	CL_{Monkey} reported (mL/day/kg)	CL_{Human} reported (mL/day/kg)	CL_{Human} predicted (mL/day/kg)	CL_{Human} (ratio of predicted to reported)
Infliximab	4.86	3.55	3.27	0.92
Bevacizumab	4.81	3.55	3.24	0.91
Daclizumab	5.30	4.5	3.57	0.79
Golimumab	9.82	5.39	6.61	1.23
CNTO328 *Siltuximab*	5.76	4.39	3.88	0.88
CNTO136 Sirukumab	7.22	6.10	4.86	0.80
Median	5.76	4.39	3.88	0.91

Body weight of "Monkey = 5 kg" and "Human = 70 kg" was used for prediction of human clearance
Median for the reported human CL = 4.39 mL/day/kg

models. To date, little information is available on this topic; here, we evaluated the pharmacokinetics of humanized antibodies, of IgG1 and IgG4 isotypes, in dogs. Additionally, to build a translational framework for evaluation of the exposure data, we compared the PK information in dogs to that observed in nonhuman primates where translational value to human have been established previously (Tables III and IV, and reference (24)).

FcRn is a heterodimeric molecule consisting of a heavy chain with the three extracellular domains that noncovalently associate with β2-microglobulin as a light chain. The crystal structure of rat FcRn confirms its similarity to class I MHC molecules (9,10,28). Recent molecular and cellular studies indicate that FcRn salvages bound IgG or albumin in endosomes from lysosomal degradation following a nonspecific fluid pinocytosis and internalization process in a pH-dependent fashion (29). The pH-dependent binding process plays a critical role in regulating IgG homeostasis. At pH 6.5, FcRn binds to IgG with nM affinity which becomes progressively weaker at pH 7.5. Alterations in affinity to FcRn can have direct impact on the nonspecific clearance mechanisms that regulate antibody (or albumin) elimination. Recent studies have further highlighted the stringent interaction of human FcRn with IgG from heterologous species (22). For example, while mouse FcRn binds human IgG, its human counterpart does not interact with mouse IgG significantly (22). This preferential binding parallels the lower amino acid sequence identity observed between mouse and human (Fig. 1) and supports the rapid elimination of mouse antibodies from human circulation (11,15). Sequence alignment for human and canine FcRn indicated an approximately 81 to 84% identity and similarity which was lower than that observed for nonhuman primates (Fig. 1). In this article, we explored

Table IV. Prediction of Human Clearance Based on Anti-RSV Clearance in Dogs

Dose (mg/kg)	Isotype	[a]CL_{Dog} (mL/day/kg)	[b]CL_{Human} predicted (mL/day/kg)
0.3	IgG1	4.91	3.67
1.0		4.11	3.07
3.0		4.66	3.48
Median		4.66	3.48
0.3	IgG4	6.14	4.59
1.0		5.03	3.76
3.0		6.33	4.73
Median		6.14	4.59

[a] Mean values from Table I; Human clearance was predicted based on equation:
$CL_{Human} = CL_{Animal} * (BW_{Human}/BW_{Animal})^{085}$
[b] Body Weight of "Dog = 10 kg" and "Human = 70 kg" was used for prediction of human clearance

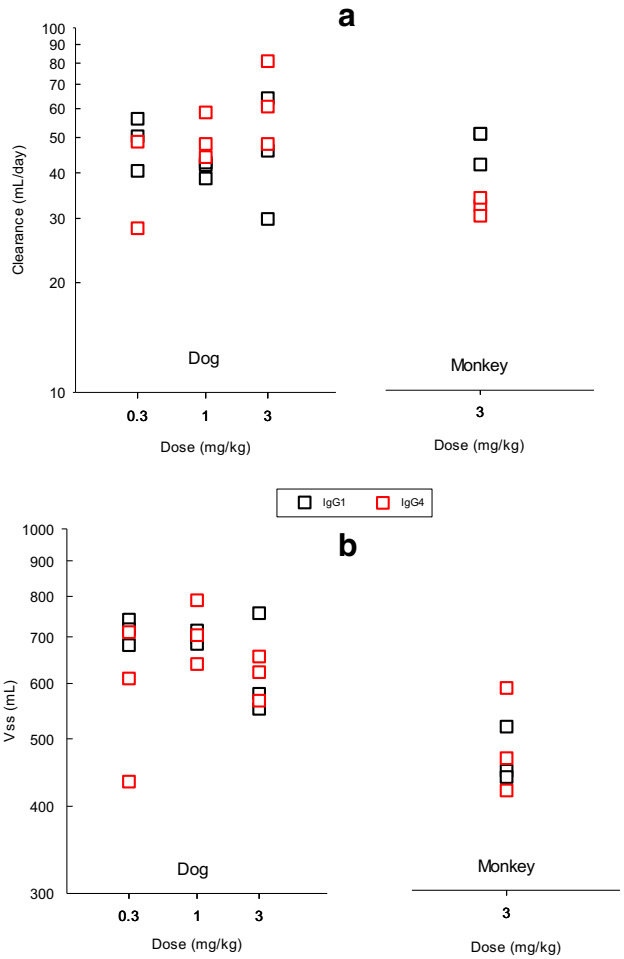

Fig. 3. Body weight-adjusted clearance and Vss in dogs and monkeys pharmacokinetic parameters obtained from individual animals were adjusted for their respective body weights (BW used for dog = 10 kg, for monkey = 5 kg). **a** Body-weight adjusted clearance parameter and **b** Vss are shown in dogs and monkeys. Parameters were obtained from non-compartmental analysis as discussed in method section. No statistically significant differences were observed between IgG1 and IgG4 in either dogs or monkeys (one-way ANOVA, $p > 0.05$)

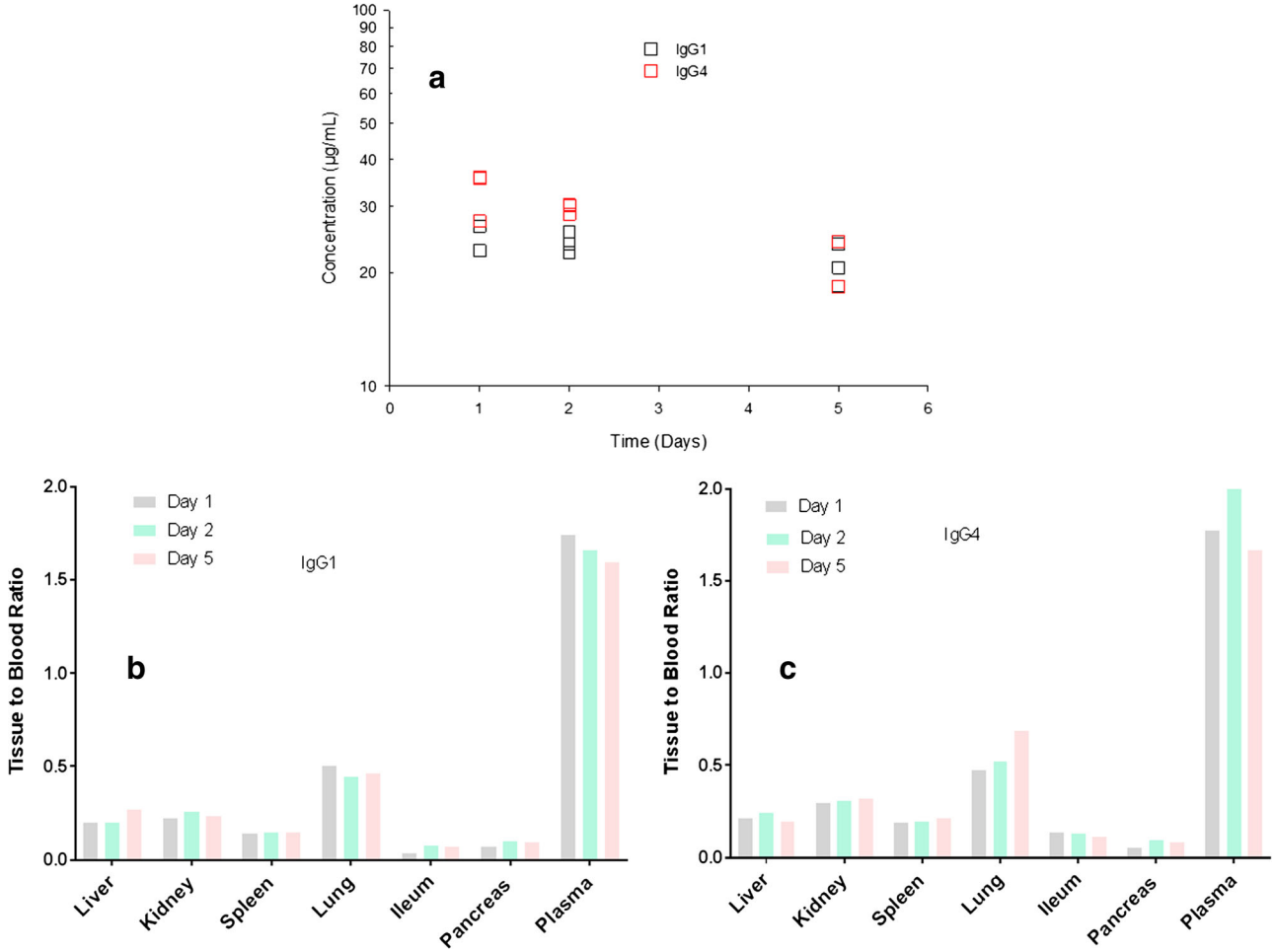

Fig. 4. Plasma concentration-time profiles and tissue-to-blood ratios for DyLight™ 650-labeled IgG1 and IgG4 antibodies. The plasma concentration of the antibodies (panel **a**) were evaluated on day 1 (2 h after dosing), day 2 and day 5. Data plotted are from 3 individual mice per time point. The tissue distribution of DyLight™ 650-labeled IgG1and IgG4 antibodies were evaluated on day 1, day 2, and day 5 in the liver, kidney, spleen, lung, ileum, and pancreas lysates (panel **b**, **c**). The tissue-to-blood ratios were based on the concentration of DyLight™ 650-labeled antibody present in the tested tissues compared to blood for IgG1 (**b**) and IgG4 (**c**) isotypes, respectively. Tissue/blood ratios greater than 1 are indicative of tissue uptake. Data plotted are mean from two mice per time point. Samples were collected from BALB/c or C57BL/6 mice dosed with a single 3 mg/kg IV dose of DyLight™ 650-labeled IgG1 or IgG4 antibody, respectively

the *in vivo* pharmacokinetics properties of fully human IgG1 and IgG4 antibodies in dogs in order to examine the impact of FcRn-dependent clearance pathways on the behavior of these antibodies *in vivo*. The antibodies used in this study did not recognize any specific targets in dogs (or any preclinical species) and hence the focus for this work mainly explored the "nonspecific and linear clearance" processes in the selected species.

Successful translation of antibody exposure profiles across species requires a mechanistic understanding of the processes that regulate antibody pharmacokinetics (11,15). For antibodies, and in the absence of target-mediated mechanisms, identity and similarity of species FcRn sequence can inform on potential translatability of exposure data to human. Ling *et al.* (24) evaluated interspecies scaling for prediction of human antibody clearance where the linear nonspecific processes were the prevalent clearance mechanism. Table III provides a comprehensive summary reproduced from the publication by these authors (24). As shown, predicted human clearance using the nonhuman primate data agrees closely with the reported human clearance (Table III: ratio of the predicted to the actual clearance ranges from 0.8 to 1.2). Using a similar approach and the experimental canine clearance data discussed in this article, prediction of human clearance data paralleled that obtained from nonhuman primates (Tables III and IV). The predicted human clearance from dog data is summarized in Table IV. The median value for predicted human clearance ranged from 3.48 to 4.59 mL/day/kg for IgG1 and IgG4 isotypes, respectively (Table IV); these predictions were comparable to the median predicted clearance obtained from translation of data from nonhuman primates (Table III, Median predicted CL = 3.88). These results highlight the translational value of linear clearance processes for IgG1 and IgG4 antibodies in studies conducted in dogs.

Biodistribution defines the reversible transfer of molecules from one location to another within the body. Movement of molecules from blood to body tissues depends on various factors as related to drug size, polarity,

lipophilicity, charge, membrane porosity and structure, blood flow characteristics, as well as concentration and pressure gradients (30,31). For IgG antibodies with similar physical and structural properties (charge, polarity), the trans-capillary transport across the blood capillary beds occurs mainly via diffusion and/or convection and, hence, will mainly depend on the capillary endothelium and the underlying basement membrane structure. Additionally, FcRn influences biodistribution of IgG antibodies. FcRn KO mice, tissue-to-plasma ratio was comparable to that observed in wild-type animals; this observation suggested that tissue distribution was primarily due to other transport processes such as convection or diffusion and various tissue structural properties (27). In this study, we did not observe any difference in distribution profiles between IgG1 and IgG4 antibody isotypes (Fig. 4). Additionally, a comparable serum exposure profiles was observed in mice following administration of comparable doses in dogs or monkeys (*i.e.,* 3.0 mg/kg; Figs. 2a, b and 4a). This comparable exposure highlights similar biodistributional processes between the species examined. However, it is important to point out that at the tissue level, the involvement of FcRn dependent biodistribution or clearance might be different. Yip *et al.* have shown a small increase in tissue to plasma ratios in mouse liver and spleen for experimental IgG1 with no FcRn binding when labeled with a residualizing radioisotope indium-111 (32). Similarly, Eigenmann *et al.*, applying a whole body PBPK model, were able to show differences in tissue specific intrinsic clearance and FcRn salvage capacity (14).

Immunogenicity of biologic products can be a limiting factor in therapeutic use and can adversely affect the product pharmacokinetics, safety, and efficacy (4,33). Many factors can influence the generation of anti-drug antibody responses (4). Immunogenicity can alter pharmacokinetics by affecting clearance and biodistribution; it can reduce efficacy and also introduce safety concerns. Administration of human proteins, such as fully human antibodies, to preclinical species generally results in development of anti-drug-antibody (ADA) response. However, in general, immunogenicity in animal models is not predictive of a potential comparable response in humans. This study was not designed to address the immunogenicity profiles following administration of the antibodies examined. However, the appearance of ADA responses against therapeutic antibodies usually results in noticeable decreases in drug concentrations from sample to sample at later time points. This was not observed in any of the studies described here. Additionally, selection of the appropriate species to conduct preclinical studies is a critical consideration. To that end, the primary requirements are that the species should express the antigen, and the antibody should bind with sufficient affinity to modulate the target in a manner comparable to that in human. Data supporting species relevance will also include cross-species comparison of antigen tissue distribution, sequence similarity, epitope binding, and functional potency assays. Therefore, preclinical efficacy studies in dogs should include these additional considerations for effective translation of exposure and target interaction, along with relevant pathological considerations into the anticipated clinical studies.

CONCLUSIONS

Assessment of the factors that regulate antibody exposure–response relationships in the relevant animal models is critical for the design of successful translational strategies from discovery to the clinic. In addition to the popularity of monoclonal antibodies for treatment of companion animals, preclinical development of antibodies in canine preclinical models will require an understanding of fully human antibody pharmacokinetics. In this study, we explored the in vivo pharmacokinetics properties of fully human IgG1 and IgG4 antibodies in dogs in order to examine the impact of FcRn-dependent clearance pathways on the behavior of these antibodies *in vivo*. Results presented here established a translational framework for evaluation of IgG antibody PK properties across species.

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