A Minimal Physiologically-Based Pharmacokinetic model of IgG: Impact of Inclusion of 2:1 FcRn IgG Binding Stoichiometry and a Proportion of CL That is independent of FcRn Binding

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Abstract

Purpose: FcRn protects IgG from degradation in the lysosome prolonging its half-life [1]. Some studies have shown that increasing the affinity of IgG for FcRn prolongs IgG half-life [5]. A minimal PBPK model has been used to investigate the predicted relationship between IgG binding affinity and half-life under scenarios where binding between IgG and FcRn is described by a 1:1 or 1:2 equilibrium [1,4] and a proportion of IgG clearance occurs independently of binding to FcRn [3].
Method: The structure of the PBPK model is shown in Figure 1. The assumptions of the model are 1) the recycling rates of 1:1 (FcRn-IgG) and 2:1 complex (FcRn-IgG-FcRn) are different [2]; 2) clearance that is independent of FcRn binding represents FcγR-mediated elimination of immune complexes and bone marrow-derived phagocytic cell elimination of IgG [3]; 3) FcRn binds independently to both sites on endogenous IgG, ($K_{D2}^{en} = 4K_{D1}^{en}$)[4]; while for mAbs this relationship may be different due to Fc-engineering altering the binding interaction of IgG and FcRn.
Result: The differential rate of 1:1 and 2:1 complex recycling results in a significant reduction in the sensitivity of mAb half-life to FcRn affinity when the binding between FcRn and mAb exhibits negative co-operativity (Figure 2). When 20% of the systemic clearance of endogenous IgG is independent of FcRn binding, an effective ceiling for the maximum fold increase in half-life with increased FcRn affinity is predicted by the model that is consistent with current *in vivo* observations [5].
Conclusion: This modelling study shows that accounting for 2:1 binding stoichiometry and including a component of IgG clearance that is independent of FcRn binding results in the prediction of a ceiling for the maximum fold increase in IgG half-life, achievable by increasing FcRn binding affinity that is in line with maximum increase in half-life observed in animals or humans.

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Method

The stoichiometry of the FcRn:Fc interaction has been determined to be 2:1 both in solution and in cell membranes [1], i.e., two FcRn molecules bind to two binding sites on the Fc domain of IgG. This binding under equilibrium conditions can be described using a bivalent analyte model (1)

$$\text{FcRn} + \text{IgG} \overset{K_{D1}}{\Longleftrightarrow} \text{FcRn-IgG} + \text{FcRn} \overset{K_{D2}}{\Longleftrightarrow} \text{FcRn-IgG-FcRn} \quad (1)$$

where K_{D1} and K_{D2} are equilibrium dissociation constants for the first and second binding steps, respectively. If the two binding sites are identical and independent, then

$$K_{D1} = K/2,\ K_{D2} = 2K \quad (2)$$

where K is the equilibrium dissociation constant for binding to individual sites. Simulations based on this reaction scheme for varying levels of IgG are shown in Figure 2. The impact of endogenous IgG on the interaction between FcRn and varying levels of exogenous IgG (i.e., mAbs) was also investigated (reaction scheme Fig 1 top right corner). Results are presented in Figure 3.

Finally, to mimic *in vivo* conditions the competitive binding scheme was incorporated into the structure of a minimal PBPK model (Figure 1) where the proportion of CL in the endothelial space (influenced by FcRn action) and in plasma (independent of FcRn) could be varied. The assumptions of this model are

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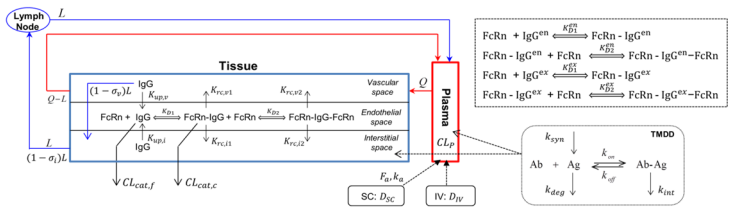


Figure 1: A minimal PBPK model incorporating 2:1 FcRn:IgG binding stoichiometry and plasma clearance (CL_P). Binding is competitive between endogenous and exogenous IgG (mAb) for FcRn. FcRn binds independently to both sites of the IgG Fc region with identical affinity [5] ($K_{D2}^{en} = 4K_{D1}^{en}$); Recycling rate for 1:1 complex is less efficient than that for 2:1 complex: $K_{rc,v1} = K_{rc,v2}/2$, $K_{rc,i1} = K_{rc,i2}/2$ and the 1:1 complex is also subject to catabolism ($CL_{cat,c}$=$CL_{cat,f}$) [3].

Results

Simulations using the bivalent analyte model show that the ratio of 1:1 to 2:1 FcRn:IgG complexes is determined by the parameter $\alpha = K_{D1}/K_{D2}$ (the smaller α, the less 2:1 complex) (Figure 2). The endogenous IgG level impacts the formation of FcRn:exogenous IgG complexes as it changes both total bound IgG and the ratio of 1:1 & 2:1 complexes (Figure 3).

When the bi-analyte model is incorporated into the minimal PBPK model, the differential rate of 1:1 and 2:1 complex recycling results in a significant reduction in the sensitivity of mAb half-life to FcRn affinity when the binding between FcRn and mAb exhibits negative co-operativity (Figure 4). When 20% of the systemic clearance of endogenous IgG is independent of FcRn binding, an effective ceiling for the maximum fold increase in half-life with increased FcRn affinity is predicted by the model that is consistent with current *in vivo* observations [5].

Using the 2:1 binding model the effect of varying the fluid phase endocytosis rate (K_{up}) was investigated. Changing this parameter can be used as a surrogate to investigate the effect of mAb charge on tissue distribution. Although the quantitative relationship between mAb isoelectric point (pI) and fluid phase uptake rate in endothelial cells is unknown, qualitatively it is anticipated that mAbs with a low pI would have a lower rate of fluid phase endocytosis due to repulsion from the negatively charged cell surface and Kup would be increased for more positively charged Abs. For a fixed plasma clearance, decreases in K_{up} result in increased half-life and increases in K_{up} result in decreased half-life (Figure 5). These findings are consistent with literature reports for mAbs engineered to have low pI [7, 8] or chemically modified to increase net positive charge (cationization) [9].

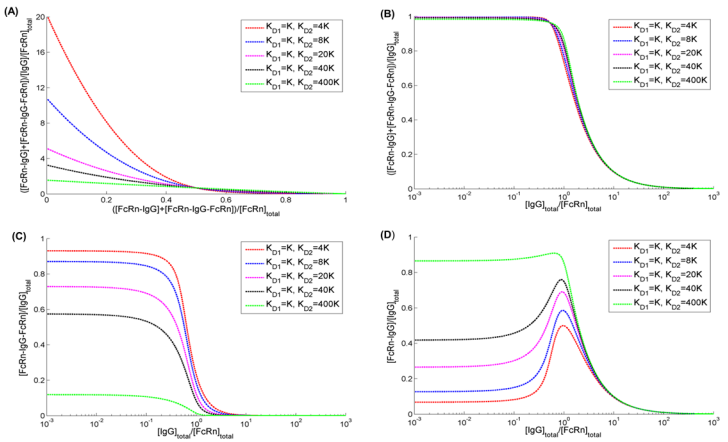


Figure 2: Bivalent analyte model for FcRn and IgG binding. (A) Scatchard plot, i.e., ' bound/free vs bound ', showing negative co-operativity; (B) Fraction of total bound IgG; (C) Fraction of 2:1 complex; (D) Fraction of 1:1 complex.. The FcRn level was 40 μM and K is 0.728 μM. Using these values in the PBPK model [6] results in a 20 day half-life for IgG.

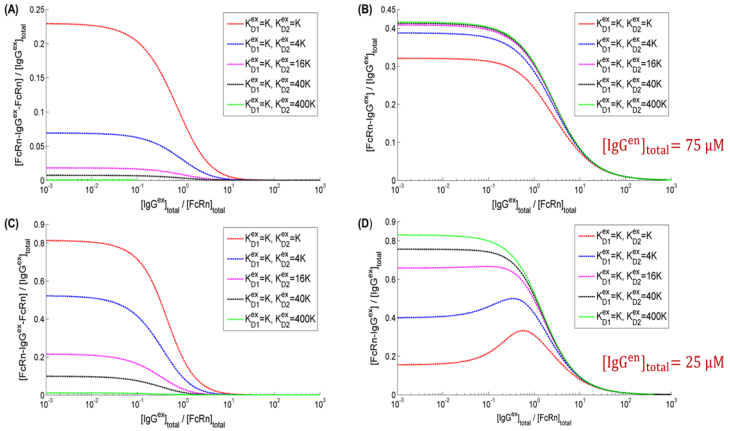


Figure 3: Competitive effect of endogenous IgG on FcRn and mAb binding using the bivalent model of FcRn and IgG binding. Simulation use the following set of parameters: $[\text{FcRn}]_{\text{total}} = 40$ μM, $K = 0.728$ μM, $K_{D1}^{en} = K$, $K_{D2}^{en} = 4K$. The endogenous IgG level was set to be 75 (panel A& B) or 25 (panel C and D) μM. (A & C) Fraction of 2:1 complex, (B & D) fraction of 1:1 complex.

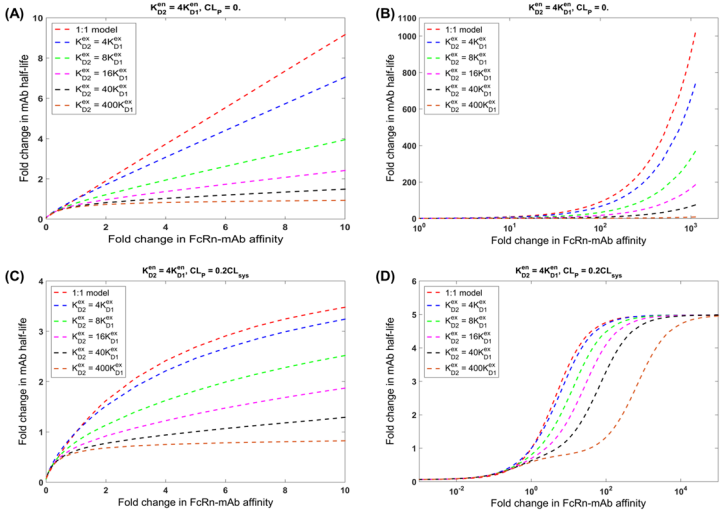
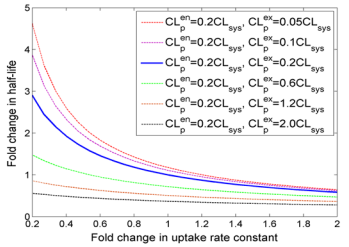


Figure 4: Simulated relationships between the fold change in mAb affinity to FcRn and the corresponding fold change in mAb half-life. The half-life and affinity are scaled by 20 days and 0.728 μM, respectively, which, along with other parameters in the model, define the profile for the wild type of IgG. (A)&(B): if all clearance is influenced by FcRn there is a reduced sensitivity of mAb half-life to changes in FcRn binding affinity with increasing negatively cooperative binding. (C)&(D): When the clearance occurring independent of FcRn is taken as 20% of the systemic clearance in addition to the reduced sensitivity to changes in FcRn affinity shown in (C), the half-life has a ceiling of 5-fold increase no matter how much increase in mAb affinity (D), and this is in consistent with in vivo observation from both animals and humans that the increase of half-life compared with IgG is less than 5 fold.



As demonstrated in figure 5, assuming a fixed percentage of plasma clearance for endogenous IgG, and allowing its counterpart for exogenous IgG to vary, both increased and decreased half-lives can be produced. This model property may offer:

(1) a potential to characterize some atypical mAb PK reported in the literature [10], where mAbs are subject to off-target binding with low affinity and high capacity; and
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Figure 5: Simulated relationship between fold change in fluid phase uptake rate and corresponding fold change in Ab half-life for varying contributions of Ab plasma clearance. Plasma clearance for endogenous IgG is fixed as 20% of the systemic clearance. The half-life and uptake rate are normalized by 20 days and 0.0298 1/h, respectively, which, along with other parameters in the model, define the profile for the wild type of IgG.

Conclusion

This modelling study shows that accounting for 2:1 binding stoichiometry and including a component of IgG clearance that is independent of FcRn binding results in the prediction of a ceiling for the maximum fold increase in IgG half-life, achievable by increasing FcRn binding affinity that is in line with maximum increase in half-life observed in animals or humans. In addition the model provides the potential for characterizing other effects on antibody tissue distribution and PK than FcRn binding affinity.

References

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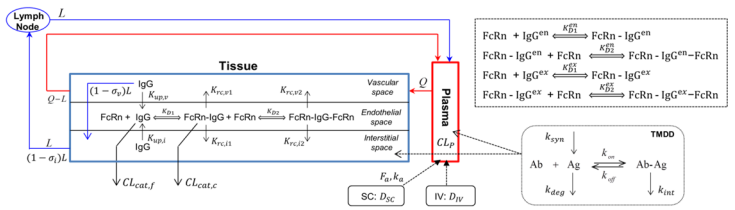

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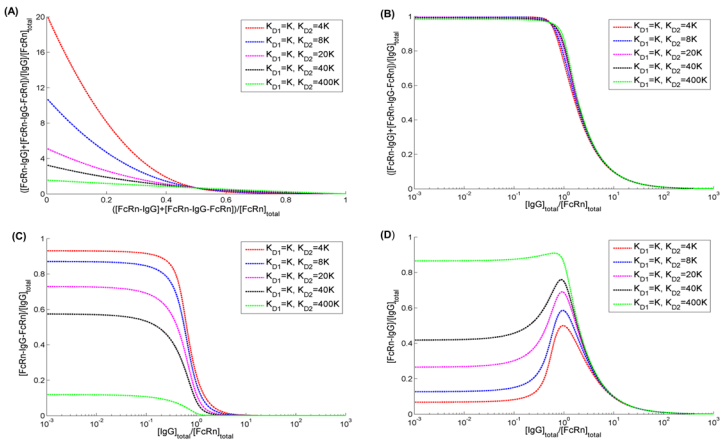

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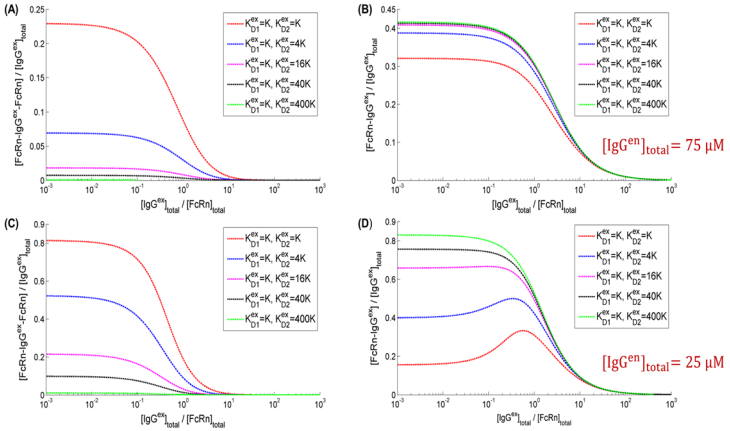

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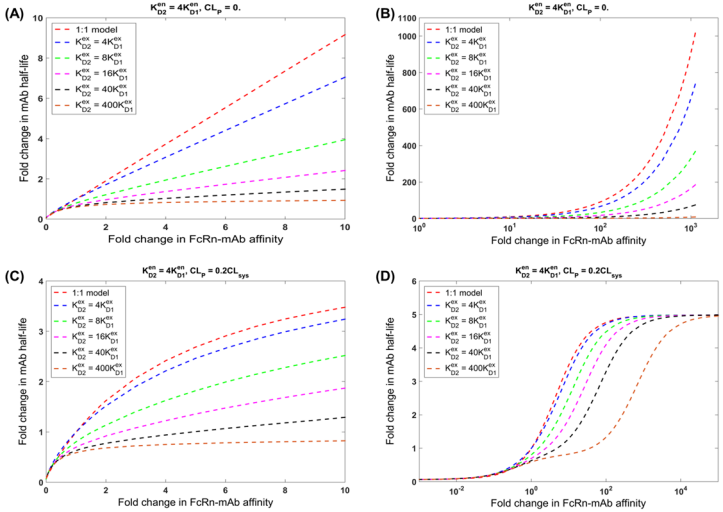

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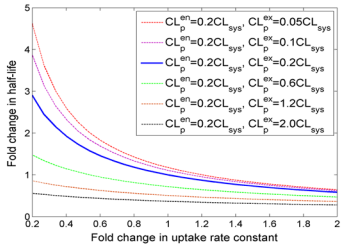

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