Modeling the Binding Kinetics of Antibody, Antigen and FcγRs

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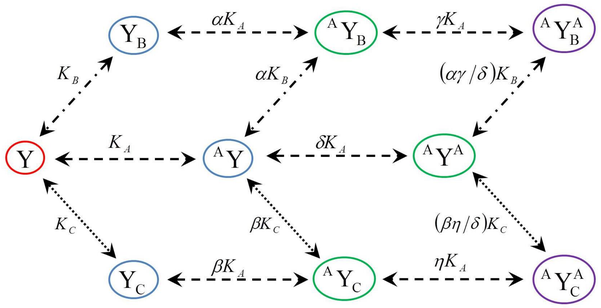
OBJECTIVES Fcγ receptors (FcγR) play a dominant role in the *in vivo* activity of anti-tumor antibodies. The *in vivo* activity of different IgG sub-classes correlates with the affinity ratio of their binding to activating and inhibitory FcγR (termed A/I ratio) [1], and clinical trials are being conducted with Fc-engineered antibodies with enhanced binding to activating FcγRs [2].

Cross-linking of FcγR and antibody-antigen complex is a pre-requisite for FcγR-mediated cell-killing or immuno-modulatory effects and thus the concentrations of antibody, antigen and FcγRs and the affinity with which these components bind to each other determines the strength and duration of the initial signal for downstream biological activity. The objective of this study is to mechanistically model the complex interaction of FcγR, antibody and antigen .

METHODS IgG has two identical binding sites for antigen in the Fab domain and one binding site for FcγRs in the Fc domain. In this model competitive binding of two classes of FcγRs to the Fc domain binding site is considered (B and C in figure 1). B and C can be taken to represent two classes of FcγRs, say high affinity FcγRI ($K_D = 10^{-8}$M) and low affinity FcγRII ($K_D = 10^{-6}$M) [3], respectively; or they could also represent activating FcγRIIa and inhibitory FcγRIIb, respectively, depending on the context of study. Binding is assumed at equilibrium and thus 12 equilibrium dissociation constants exist within the model. Thermodynamic consideration reduces these 12 constants to 8, i.e., K_A, K_B, K_C,α,β,γ,δ,η. Employing equilibrium constraints and conservation relationships of antibody, antigen, and FcγR species, a set of six nonlinear equations can be derived (shown in Box 1 and the associated variables and parameters are defined in Box 2). These six equations govern the fractional concentrations of free antibody, and ternary and quaternary complexes relative to the total antibody concentration. For any given set of affinities and total concentrations of antibody, antigen, and FcγRs, the concentrations of all species can be numerically calculated by solving this set of equations.



Red circle – free antibody
Blue circle – binary complex
Green circle – ternary complex
Purple circle – quaternary complex

---- binding to antigen A
-·-·- binding to FcγR B
····· binding to FcγR C

Figure 1 Equilibrium binding scheme for the interaction between antibody Y, antigen A, and FcγRs B and C. The binding of FcγRs B and C to the Fc site is assumed to be competitive. $\boldsymbol{K_A}$, $\boldsymbol{K_B}$, and $\boldsymbol{K_C}$ are equilibrium dissociation constants for binding between Y and A, Y and FcγR B, and Y and FcγR C, respectively. $\boldsymbol{\alpha}$ is the affinity ratio between Y and ^{A}Y for FcγR B (which is equal to the ratio between Y and Y_B for antigen A due to equilibrium); $\boldsymbol{\beta}$ is the affinity ratio between Y and ^{A}Y for FcγR C (which is equal to the ratio between Y and Y_C for antigen A due to equilibrium); $\boldsymbol{\gamma}$,$\boldsymbol{\delta}$, and $\boldsymbol{\eta}$ are defined in the text.

Box 1

$$x_1\left(1+\frac{w_A}{d_1}+\frac{w_B}{d_2}+\frac{w_C}{d_3}\right)+x_2+x_3+x_4+x_5+x_6=1,$$

$$\alpha x_2 = x_1\frac{w_A}{d_1}\frac{w_B}{d_2},\quad \beta x_3 = x_1\frac{w_A}{d_1}\frac{w_C}{d_3},\quad \delta x_4 = x_1\left(\frac{w_A}{d_1}\right)^2,$$

$$\alpha\gamma x_5 = x_1\left(\frac{w_A}{d_1}\right)^2\frac{w_B}{d_2},\quad \beta\eta x_6 = x_1\left(\frac{w_A}{d_1}\right)^2\frac{w_C}{d_3}.$$

Box 2

$$x_1=\frac{[Y]}{[Y]_{total}},\ x_2=\frac{[^{A}Y_B]}{[Y]_{total}},\ x_3=\frac{[^{A}Y_C]}{[Y]_{total}},\ x_4=\frac{[^{A}Y^{A}]}{[Y]_{total}},\ x_5=\frac{[^{A}Y_B^{A}]}{[Y]_{total}},\ x_6=\frac{[^{A}Y_C^{A}]}{[Y]_{total}}.$$

$$g_A=\frac{[A]_{total}}{[Y]_{total}},\ g_B=\frac{[B]_{total}}{[Y]_{total}},\ g_C=\frac{[C]_{total}}{[Y]_{total}},\ b_1=\frac{K_A}{[Y]_{total}},\ b_2=\frac{K_B}{[Y]_{total}},\ b_3=\frac{K_C}{[Y]_{total}}.$$

$$w_A=g_A-x_2-x_3-x_4-x_5-x_6,\quad w_B=g_B-x_2-x_5,\quad w_C=g_C-x_3-x_6.$$

$$d_1=b_1+x_1,\quad d_2=b_2+x_1,\quad d_3=b_3+x_1.$$

The receptor occupancy can be defined in terms of receptor species being occupied by Ab and Ab-Ag complexes. Here we are mainly concerned with the cross-linking of FcγR and antibody-antigen complex (immune complex), and thus the occupancies in this context are the fractional concentrations of ternary and quaternary species relative to their total FcγR concentrations and these occupancies are defined in Box 3.

Box 3

$$RO_{B1}=\frac{[^{A}Y_B]}{[B]_{total}},\quad RO_{B2}=\frac{[^{A}Y_B^{A}]}{[B]_{total}},$$

$$RO_{C1}=\frac{[^{A}Y_C]}{[C]_{total}},\quad RO_{C2}=\frac{[^{A}Y_C^{A}]}{[C]_{total}}.$$

Here we use $\boldsymbol{K}$ to represent the *intrinsic equilibrium dissociation constant* whose inverse is intrinsic affinity measuring the strength of individual Ab binding site to an epitope of Ag. If it is assumed that there is no co-operativity between the two antigen binding sites, then $K_A = K/2$ and $\gamma = \delta = \eta = 4$. FcγRII and FcγRIII have relatively weak intrinsic affinities for their IgG Fc binding site and thus their functions are critically dependent on the binding to antibody and multivalent antigen complex (immune complexes). The multivalent binding is measured by *functional affinity*, which is defined as the inverse of equilibrium dissociation constant [4]. Thus parameters α and β can be used to characterize enhanced binding affinity due to multivalent interaction.

RESULTS Figure 2 shows the simulation for the scenario of two classes of FcγRs defined by a high affinity and a low affinity. For the low affinity FcγR the cross-linking between antibody-antigen and FcγR can only happen when its functional affinity towards the immune complex increases. This simulation result is consistent with the general believe that a functional IgG ligand of low binding affinity is exclusively in the form of an immune complex [3]. Figure 3 simulates the competitive binding of an activating FcγR and an inhibitory FcγR for the FcγR binding site of an antibody. Increasing the affinity of activating FcγR can dramatically increase the cross-linking of FcγR with Ab-Ag complex. If a direct correlation between the cross-linking and the *in vivo* ADCC (antibody-dependent cellular cytotoxicity) activity is assumed then the simulated result is consistent with current *in vivo* data [5] and is in support of the paradigm of bioengineering strategy proposed in [1].

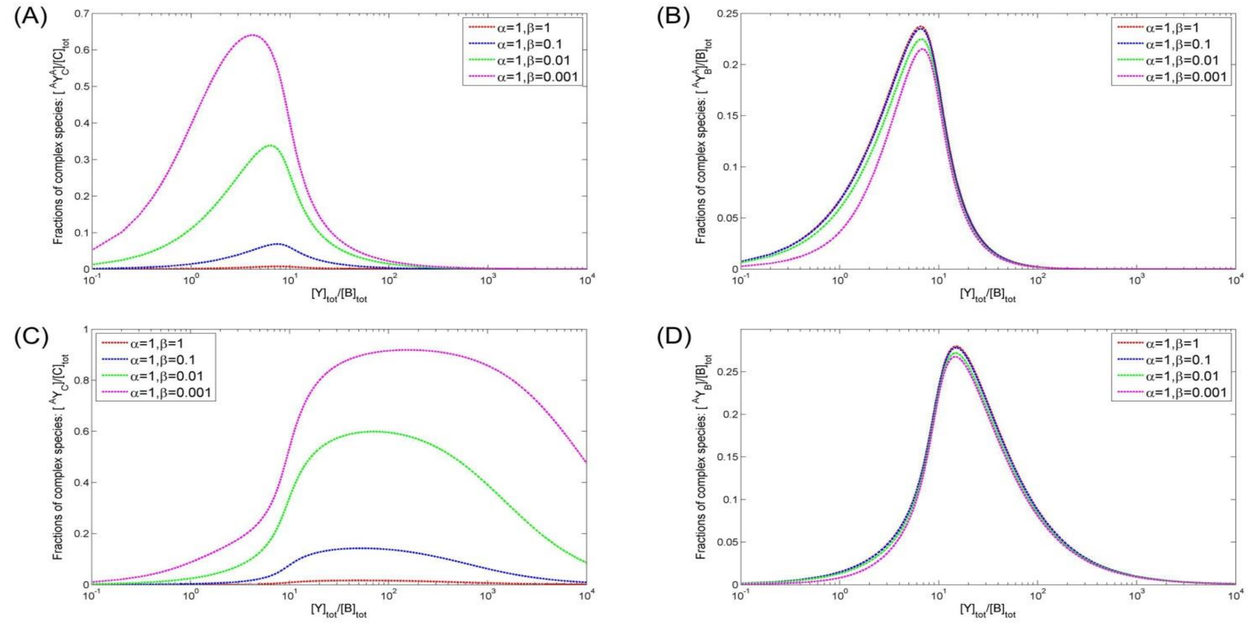


Figure 2 Simulation of competitive binding between two classes of Fc$\boldsymbol{\gamma}$Rs (B and C) for the FcγR binding site of an antibody, one with a high affinity represented by B ($\boldsymbol{K_B = 10^{-8}}$M) and the other with a low affinity represented by C ($\boldsymbol{K_C = 0.5\times10^{-6}}$M). Total antigen concentration is $\boldsymbol{[A]_{tot}= 10^{-8}}$M, and total Fc$\boldsymbol{\gamma}$R concentrations are $\boldsymbol{[B]_{tot}= [C]_{tot}= 10^{-9}}$ M. The intrinsic equilibrium dissociation constant for Ab-Ag binding, $\boldsymbol{K = 10^{-9}}$ M, and $\boldsymbol{K_A = K/2}$, $\boldsymbol{\gamma = \delta = \eta = 4}$. Panels (A) & (C) show the fractions of cross-linked species with low affinity Fc$\boldsymbol{\gamma}$R. Panels (B) & (D) show the fractions of cross-linked species with high affinity Fc$\boldsymbol{\gamma}$R. Varying $\boldsymbol{\beta}$ from 1 to 0.001 represents an increased functional affinity of the low affinity Fc$\boldsymbol{\gamma}$R towards multivalent immune complexes.

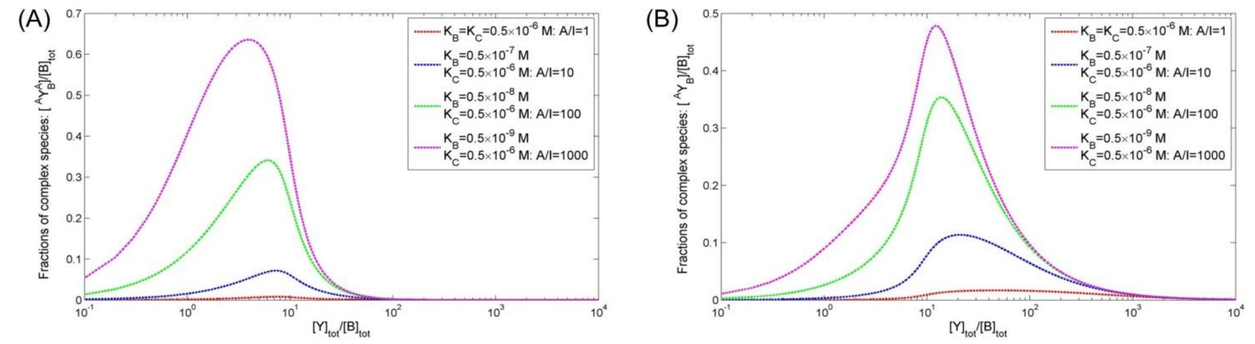


Figure 3 Simulation of competition between activating Fc$\boldsymbol{\gamma}$R B and inhibitory Fc$\boldsymbol{\gamma}$R C for varying A/I ratio modelled by increasing the intrinsic affinity of Fc$\boldsymbol{\gamma}$R B. Total antigen concentration $\boldsymbol{[A]_{tot}= 10^{-8}}$M, total Fc$\boldsymbol{\gamma}$R concentrations: $\boldsymbol{[B]_{tot}= [C]_{tot}= 10^{-9}}$ M. The intrinsic equilibrium dissociation constant for Ab-Ag binding, $\boldsymbol{K = 10^{-9}}$ M, and $\boldsymbol{K_A = K/2}$, $\boldsymbol{\gamma = \delta = \eta = 4}$, $\boldsymbol{\alpha = \beta = 1}$. Panel (A) the fraction of cross-linked species through quaternary complex for Fc$\boldsymbol{\gamma}$R B; Panel (B) the fraction of cross-linked species through ternary complex for Fc$\boldsymbol{\gamma}$R B.

CONCLUSION A mechanistic binding model has been developed to quantify the interplay of antibody, antigen, and FcγRs. The model describes the interaction between these species and can be useful to guide Fc engineering efforts to optimize immune system activation with therapeutic antibodies. Further studies are underway to incorporate the binding model into a PBPK framework to simulate the *in vivo* consequences of these interactions.

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