Determining mechanisms of generating plasma cells and memory B cells during an immune response in spleen

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Abstract

Major points:

1. we observe experimentally the dynamics of various B cell populations (plasmablasts, germinal center B cells, memory B cells, and plasma cells) after i.v. immunization of mice with radiation-attenuated sporozoites.

2. Average affinity arises fast in GC B cells, MBCs and plasma cells with similar kinetics, high affinity MBCs appear earlier than plasma cells (note that PC are measured in the bone marrow).

3. There is some correlation between average affinity of GC B cells and MBCs

4. A simple mathematical model that assumes that MBCs and PCs arise randomly during the immunization can well describe the data (with some caveats, NLL=-526).

5. A model in which low affinity GC B cells preferentially differentiate into MBCs and high affinity GC B cells differentiate into PCs does not well describe the data (NLL=-479). Visually this is subtle. This is because the model predicts rapid change in affinity (because MBCs that appear early have high affinity), but in PCs affinity arises later. This is likely to be easily corrected if we allow PCs to migrate from the spleen to BM with some delayed kinetics (this can be estimated then).

Abbreviations: PBs - plasmablasts, GC - germinal center, MCs or MBCs - memory B cells, PCs or LLPCs - long-lived plasma cells, NLL - negative log likelihood.

Introduction

Materials and Methods

Data

Cell numbers: Some of our data are previous publications [1]. As a note, plasmablasts, GC B cells, and memory B cells were measured in the spleen, while plasma cells were measured in the bone marrow. Data on plasma cell dynamics in the bone marrow are new and include recalculation of PC frequency for BM cell to the whole bone marrow assuming that one mouse femur contains 7.5% of the total BM cells [2].

Affinity: (Harry): In that particular table, anytime it says PB, those were plasma blasts from the spleen (we think that because we got them at day 4 and 7, too early to be PC). PC will all be from the bone marrow. For example I think at day 7 there are PBs and PCs. PBs are spleen and PCs are bone marrow. EM stands for early memory B cells. Thats probably confusing and should just be changed to MBC (we just assume they haven't entered a GC). But treat them as Memory Cells.

We have defined affinity of BCR as low ($A = 1$), intermediate ($A = 2$), and high ($A = 3$), and used that in modeling. These are based on mRNA sequencing from individual cells and determining CDR3 sequence of the heavy chain of the BCR gene for the cells. We have determined affinity for some of the antibodies produced by these cells.

Mathematical models

Mathematical model for the dynamics of total B cell populations (no affinity).

The pathway of B cell differentiation during an immune response has not been firmly established and may perhaps depend on specifics of immunization. We therefore developed a series of alternative mathematical models that assume different pathway of B cell differentiation (e.g, following a philosophy of previous papers [3, 4]).

The main assumption in all of such models is that antigen levels (A) determine how quickly cells divide. In one pathway of B cell differentiation, plasmablasts (B) proliferate depending on the antigen level and differentiate first into germinal center B cells (G). Germinal center B cells also proliferate and differentiate into nondividing plasma cells (P) or memory B cells (M). All cells have some death rates. Sensitivity to antigen levels for plasmablasts and germinal center B cells are determined by the half-saturation constants h_B and h_G, respectively.

In case when model parameters are independent of GC B cell affinity, i.e., $r_{G_k} = r_G = \text{const}$, affinity does not increase during GC reaction.

Mathematical model for the dynamics of GC B cells and their Ab affinity.

Here go several different versions developed.

Mathematical model for the dynamics of total B cell populations and Ab affinity.

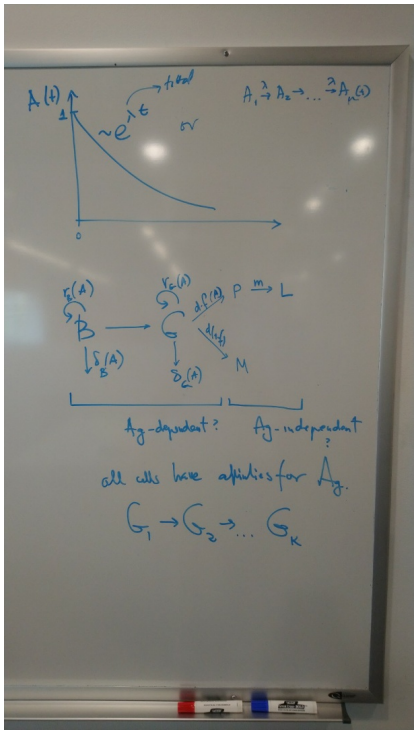

Figure 1: Schematic of a mathematical model in which differentiation of B cells depends on decaying antigen levels.

The basic assumptions of our main mathematical model are as follows:

1. **Antigen**. Dynamics of antigen is divided into two parts - antigen available to plasmablasts ($A(t)$ and antigen available to GC B cells ($A_g(t)$). Infection starts with antigen for plasmablasts which is then at rate m transported to GCs. Antigen levels in both compartments decay exponentially at rates λ and λ_g, respectively (eqns. (1)–(2)).

2. **Plasmablasts**. Dynamics of plasmablasts ($B(t)$) is only driven by the level of available antigen (denoted as $A(t)$) which decays exponentially. Plasmablasts divide and differentiate into GC B cells at a constant rate d_{BG} (eqn. (3)). We assume that plasmablasts do not change affinity of their receptors. Although we did observe that PBs had higher than germline affinity for Ag (Figure 4), there was no change in affinity over time which simplifies the model.

3. **GC B cells**. Dynamics of GC B cells ($G(t)$) is more complex (eqns. (4)–(5)). Division of GC B cells is driven by availability of antigen in GC (A_g) and is determined by the maximal division rate r_G and half-saturation constant h_G. To describe change in affinity we assume that GC B cell population consists of n sub-population denoted as $G_k(t)$ where $k = 1 \dots n$ with affinity increasing with increasing k. To model change in affinity over time we assume that GC B cells with higher affinity have a higher division rate $r_{G_k} = r\sqrt{k}$ (several other functional forms gave equivalent results). GC B cells change that affinity at the rate proportional to cellular division rate and is determined by parameter μ. GC B cells also differentiate into MBCs or PCs at the general rate d_G and die at a rate δ_G. Duration of the GC reaction is determined by the rate at which $A_g(t)$ decays over time and half-saturation constant h_G.

4. **Memory B cells and plasma cells**. Differentiating GC B cells have a probability f_M and

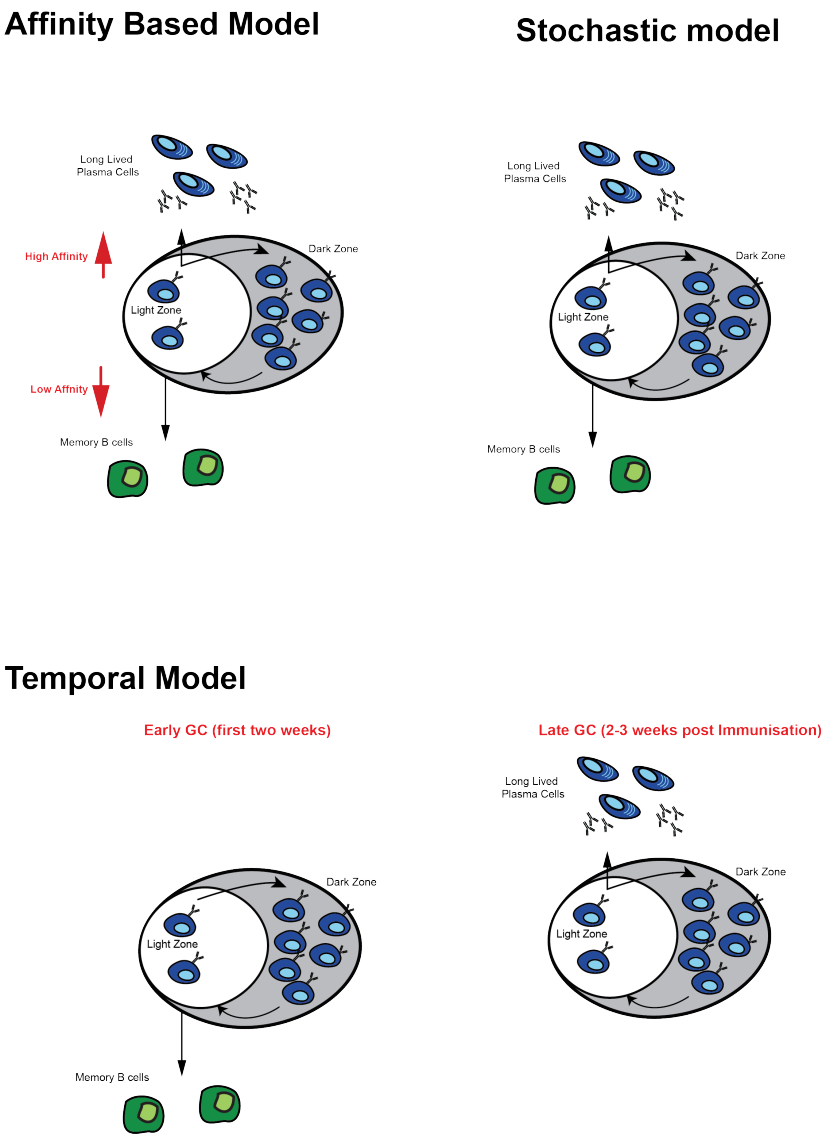

Figure 2: Alternative hypotheses for the generation of memory B cells and long-lived plasma cells during an immune response.

$1 - f_M$ to become memory B cell and plasma cell, respectively (eqns. (6)–(7)). In the simplest, random/stochastic model (Figure 2B) probability of a cell to become MBC or PC is independent of time or affinity of the GC B cell, i.e., $f_M = \text{const}$. In alternative mathematical models f_M will depend on the affinity of BCRs of GC B cells (thus, on index k) or on the time since infection. However, because affinity increases over time, both of these alternative models are nearly equivalent in our experimental settings. We assume that MBCs and PCs do divide and die at fixed rates δ_M and δ_P, respectively (eqns. (6)–(7)).

5. **Average affinity**. Average affinity of GC B cells is then given by $F_G(t) = \sum_{k=1}^{n} kG_k(t) / \sum_{k=1}^{n} G_k(t)$. Average affinity of MBCs and PCs was calculated in a similar way.

$$\frac{\mathrm{d}A(t)}{\mathrm{d}t} = -(\lambda + m)A(t), \tag{1}$$

$$\frac{\mathrm{d}A_g(t)}{\mathrm{d}t} = mA(t) - \lambda_g A_g(t), \tag{2}$$

$$\frac{\mathrm{d}B(t)}{\mathrm{d}t} = \frac{rA(t)B(t)}{h_B + A(t)} - (\delta_B + d_{BG})B(t), \tag{3}$$

$$\frac{\mathrm{d}G_1(t)}{\mathrm{d}t} = d_{BG}B(t) + \frac{r_{G_1}(1 - d_G - \mu)A_g(t)}{h_G + A_g(t)}G_1(t) - \delta_G G_1(t), \tag{4}$$

$$\frac{\mathrm{d}G_k(t)}{\mathrm{d}t} = \frac{r_{G_k}(1 - d_G - \mu)A_g(t)}{h_G + A_g(t)}G_k(t) + \frac{\mu r_{G_{k-1}}A_g(t)}{h_G + A_g(t)}G_{k-1}(t) - \delta_G G_k(t), \quad k = 2 \ldots n, \tag{5}$$

$$\frac{\mathrm{d}M_k(t)}{\mathrm{d}t} = \frac{f_M d_G r_{G_{k-1}}A_g(t)}{h_G + A_g(t)}G_k(t) - \delta_M M_k(t), \qquad k = 1 \ldots n, \tag{6}$$

$$\frac{\mathrm{d}P_k(t)}{\mathrm{d}t} = \frac{(1 - f_M) d_G r_{G_{k-1}}A_g(t)}{h_G + A_g(t)}G_k(t) - \delta_P P_k(t), \qquad k = 1 \ldots n, \tag{7}$$

where A_g is the amount of antigen in the germinal centers that regulates eventual contraction of the germinal center reaction, λ_g is the rate of antigen loss from the germinal center, r_{G_k} is the rate of GC B cell division, K is the carrying capacity, δ_G is the death rate of GC B cells when antigen levels fall below some critical level, μ is the rate at which GC B cells increase affinity.

Statistics

In our final analysis we fitted the models to both total numbers of cells in the spleen (PB, GC B cells, MBCs) or the bone marrow (PC) and their BCR/Ab affinity together. For that we used weighted least squares with two variances, σ_N^2 and σ_F^2 for cell numbers and average affinity, respectively.

To fit the models to cell number data we log-transformed the data and model predictions on the number of plasmablasts, GC B cells, MBCs and PCs. Model predictions on average affinity and the data were left on the linear scale. Then the negative log likelihood of the model given the data is given by:

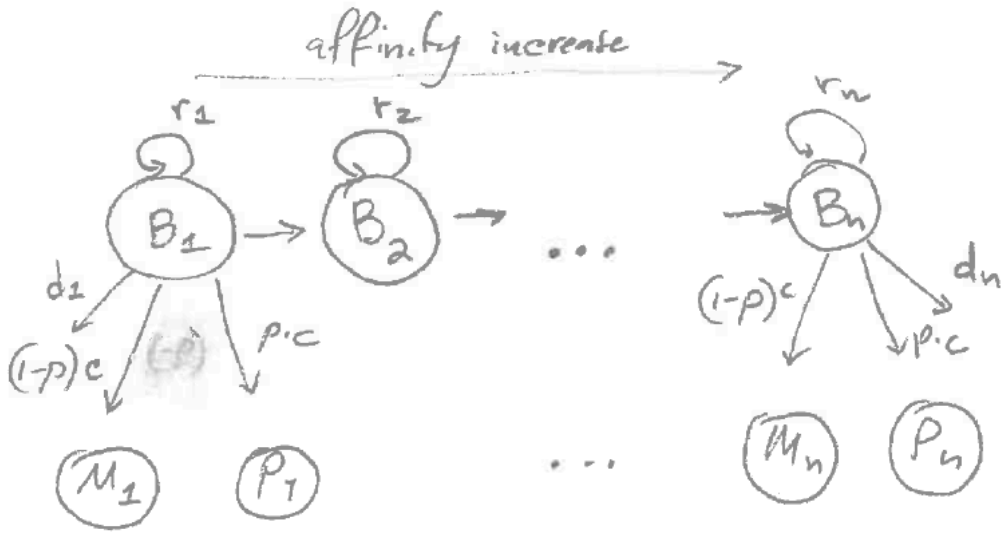

Figure 3: Mathematical models of the generation of high affinity antibodies during an immune response.

$$\mathcal{L} = \sum_{i=1}^{n_1} \sum_{j=1}^{4} \frac{(\log(\hat{X}_{ij}) - \log(X_{ij}(t_{ij})))^2}{2\sigma_N^2} + \sum_{i=1}^{n_2} \sum_{j=1}^{4} \frac{(\hat{F}_{ij} - F(t_{ij}))^2}{2\sigma_F^2} - n_1 \log(\sigma_N) - n_2 \log(\sigma_F), \quad (8)$$

where X_{ij} denotes the number of cells (denoted by j: PB, GC B cells, MBCs, or PCs) at time point i, and F_{ij} is the average affinity.

Results

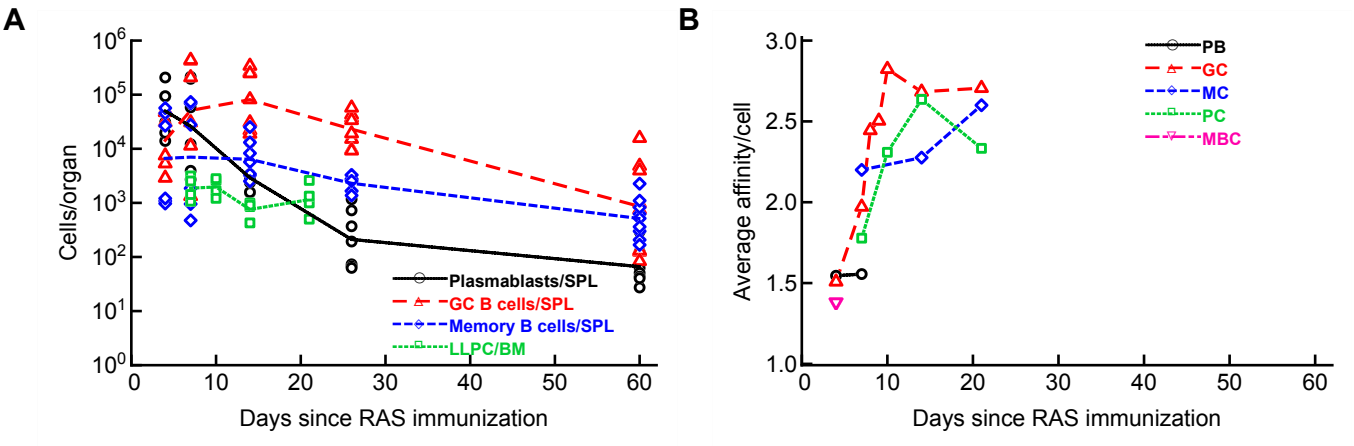

Figure 4: Kinetics of B cell response in murine spleens following RAS immunization. We followed the total number of plasmablasts, memory B cells, and germinal B cells in the spleen and long-lived plasma cells (LLPC) in the bone marrow (panel A) or the average affinity per cell as evaluated by single cell RNA sequencing (panel B).

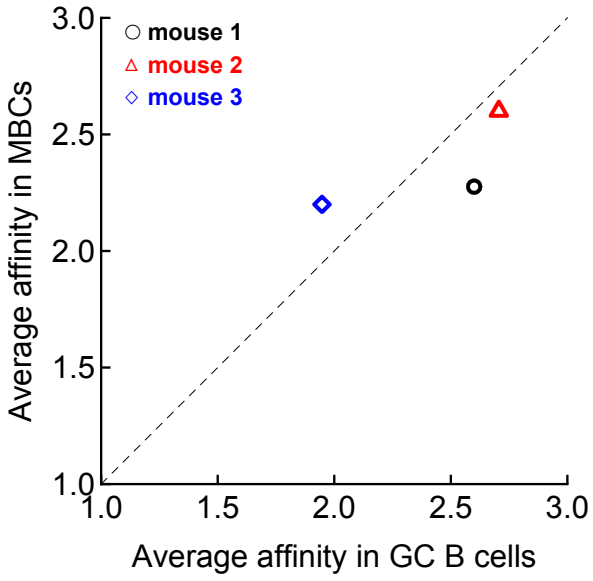

Figure 5: Strong correlation between average affinity for MBC and GC B cells. Also, MBCs display high affinity (between intermediate and high levels) suggesting that MBCs with high affinity receptors are generated during GC reaction.

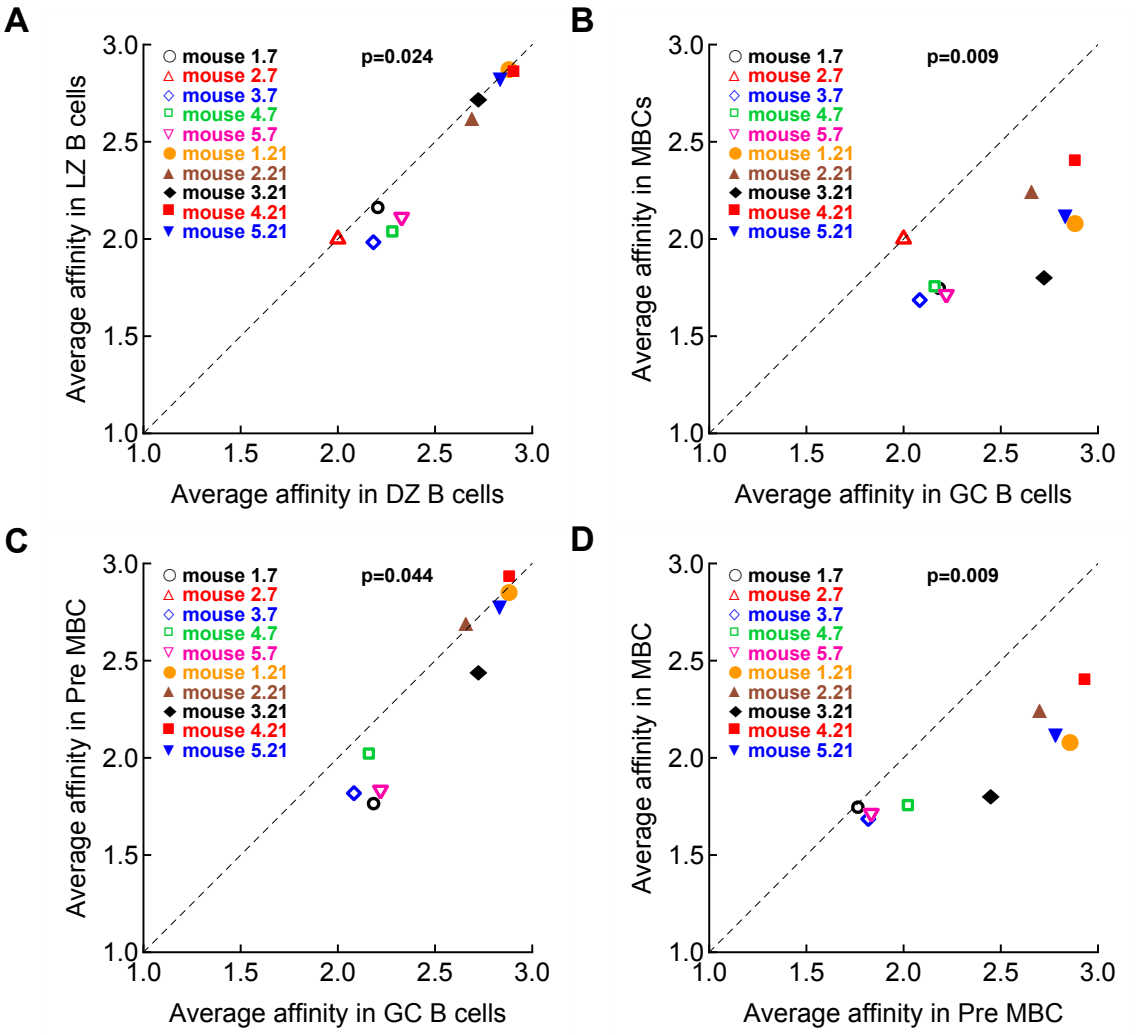

Figure 6: Average affinity of different cell types following RAS immunization as evaluated by 10x sequencing. We assign affinity as low ($A = 1$), intermediate ($A = 2$), or high ($A = 3$) to every cell in the sample and then calculate the average affinity of a given cell population such as GC B cells (GC B cells) in the light zone (LZ) or dark zone (DZ) of the GC, memory B cells (MBC) or pre-MBCs (panel C). The data are noted as a mouse ID and day since immunization, that is label mouse 1.7 denotes a mouse 1 sampled 7 days since immunization. Comparison between average affinity for a given population was done using signed-rank test with p values from the test is indicated on individual panels.

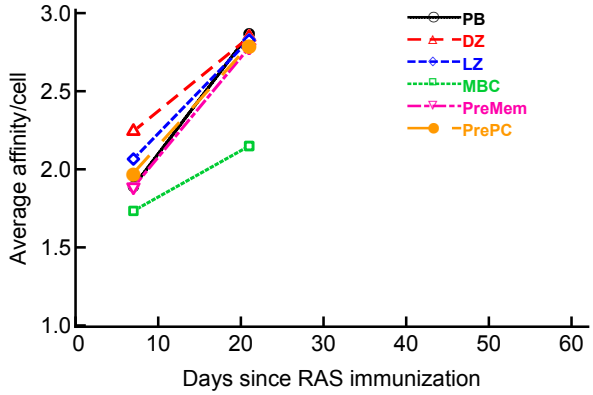

Figure 7: Change in average affinity of different cell types following RAS immunization as evaluated by 10x sequencing. Compare to Figure 4B.

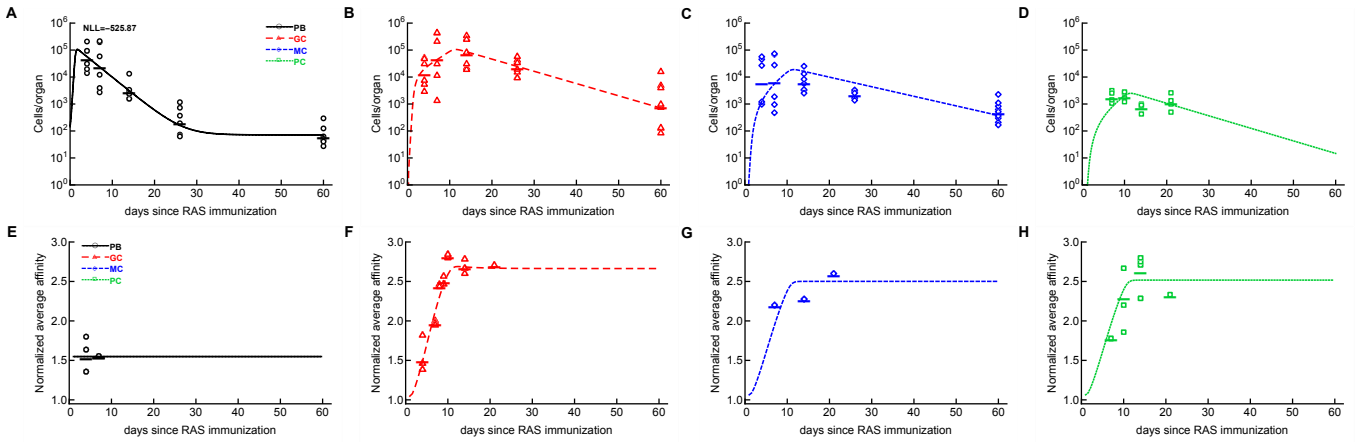

Figure 8: Predictions of the mathematical model on cell dynamics (A-D) and average affinity per cell type (E-H). This is the basic mathematical model assuming that differentiation of GC B cells occurs randomly (at a constant rate, eqns. (1)–(7)). The model was fitted to all data together using eqn. (8). The model in total has 18 parameters including variances σ_N^2 and σ_F^2.

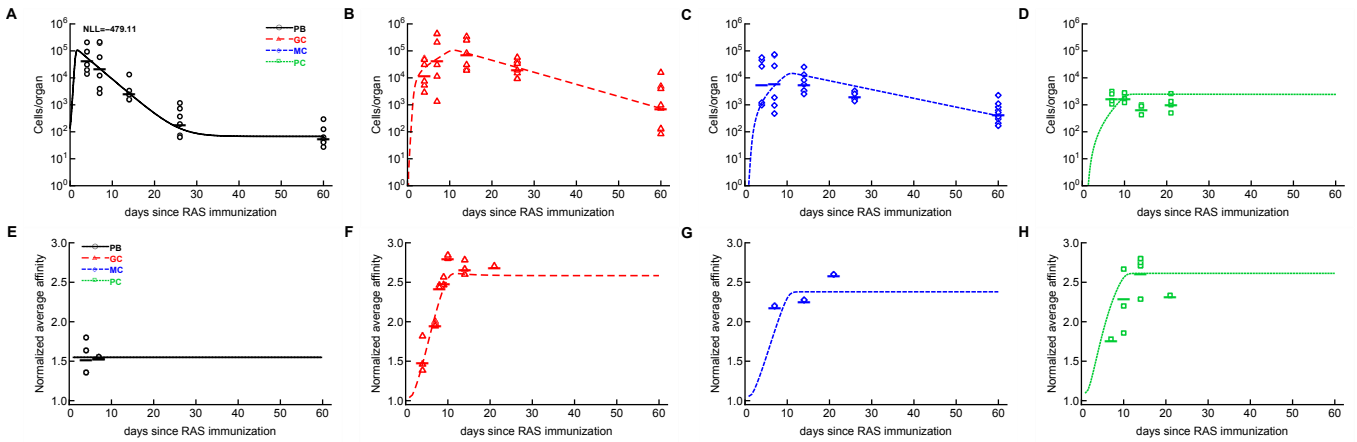

Figure 9: Predictions of the mathematical model on cell dynamics assuming that GC with low affinity become predominantly MBCs and cells with high affinity become PCs. This is modeled as $f_M = f_M e^{a_M(k-1)}$ in eqns. (6)–(7). The model does not fit the data well based on negative log-likelihood (NLL) as compared to the random model. In model fitted we fixed $a_M = 0.5$.

Discussion

Limitations so far

1. Dynamics of GC B cells is only determined by the antigen ($A_g(t)$). It is possible that other factors
2. Estimate for $r_{G_k} = 0.2$/day is small. We expect that GC B cells may be limited by other factors, so if we allow for carrying capacity, larger estimates for the growth rate are possible.
3. Estimate for transition rate $\mu = 1$ is very high. This is likely linked to low r_{G_k}.
4. Currently we only consider $n = 3$ subpopulation of B cells with different affinities. In reality, there are many more cells with different BCRs, so how restrictive it is to consider only 3 subpopulations?
5. The models do ont explain decline in affinity in GC B cells and PC after the peak at 2 weeks - why is there a decline?
6. There are likely to be several GCs after vaccination, very likely with asynchronous dynamics. How that may be modelled?
7. Because measurement of PCs was done in the bone marrow, the estimate for GC B cell differentiation into PCs includes migration rate from the spleen to the bone marrow. Should we make an extra compartment?
8. Model predictions for memory B cell and PC dynamics are not great. Should we make the model more complex to match the better? This could result in overfitting.

Data sources

Code sources

Ethics statement

Author contributions

Acknowledgments

References

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Supplementary Information

The main assumption in all of such models is that antigen levels (A) determine how quickly cells divide. In one pathway of B cell differentiation, plasmablasts (B) proliferate depending on the antigen level and differentiate first into germinal center B cells (G). Germinal center B cells also proliferate and differentiate into nondividing plasma cells (P) or memory B cells (M). All cells have some death rates. Sensitivity to antigen levels for plasmablasts and germinal center B cells are determined by the half-saturation constants h_B and h_G, respectively.

$$\begin{aligned}
\frac{\mathrm{d}A(t)}{\mathrm{d}t} &= -\lambda A(t), && \text{(S.1)}\\
\frac{\mathrm{d}B(t)}{\mathrm{d}t} &= \frac{rA(t)B(t)}{h_B + A(t)} - (\delta_B + d_{BG})B(t), && \text{(S.2)}\\
\frac{\mathrm{d}G(t)}{\mathrm{d}t} &= d_{BG}B(t) + \frac{rA(t)G(t)}{h_G + A(t)} - (\delta_G + d_G)G(t), && \text{(S.3)}\\
\frac{\mathrm{d}M(t)}{\mathrm{d}t} &= f_M d_G G(t) - \delta_M M(t), && \text{(S.4)}\\
\frac{\mathrm{d}P(t)}{\mathrm{d}t} &= (1 - f_M)d_G G(t) - \delta_P P(t), && \text{(S.5)}
\end{aligned}$$

with initial conditions $A(0) = 1$, $B(0) = B_0$, $G(0) = P(0) = M(0) = 0$.

To model change in affinity we consider n sub-populations of GC B cells, plasma cells, and memory B cells with receptors/antibodies of different affinities. One basic model we have considered tracks only the dynamics of GC B cells and their affinity:

$$\begin{aligned}
\frac{\mathrm{d}A_g(t)}{\mathrm{d}t} &= -\lambda_g A_g(t), && \text{(S.6)}\\
\frac{\mathrm{d}G_k(t)}{\mathrm{d}t} &= r_{G_k} G_k(t)\left(1 - \frac{\sum_{i=1}^{n} G_i(t)}{K}\right) - \delta_G(1 - A_g(t))G_k(t) - \mu r_{G_k} G_k(t) + \\
&+ \mu r_{G_{k-1}} G_{k-1}(t), \qquad k = 1 \ldots n, && \text{(S.7)}
\end{aligned}$$

where A_g is the amount of antigen in the germinal centers that regulates eventual contraction of the germinal center reaction, λ_g is the rate of antigen loss from the germinal center, r_{G_k} is the rate of GC B cell division, K is the carrying capacity, δ_G is the death rate of GC B cells when antigen levels fall below some critical level, μ is the rate at which GC B cells increase affinity. Note that $G_0(t) = 0$ and there is no GC B cells with highest affinity that increase their affinity further (i.e., term $\mu r_{G_n} G_n(t)$ in eqn. (S.7) is zero).

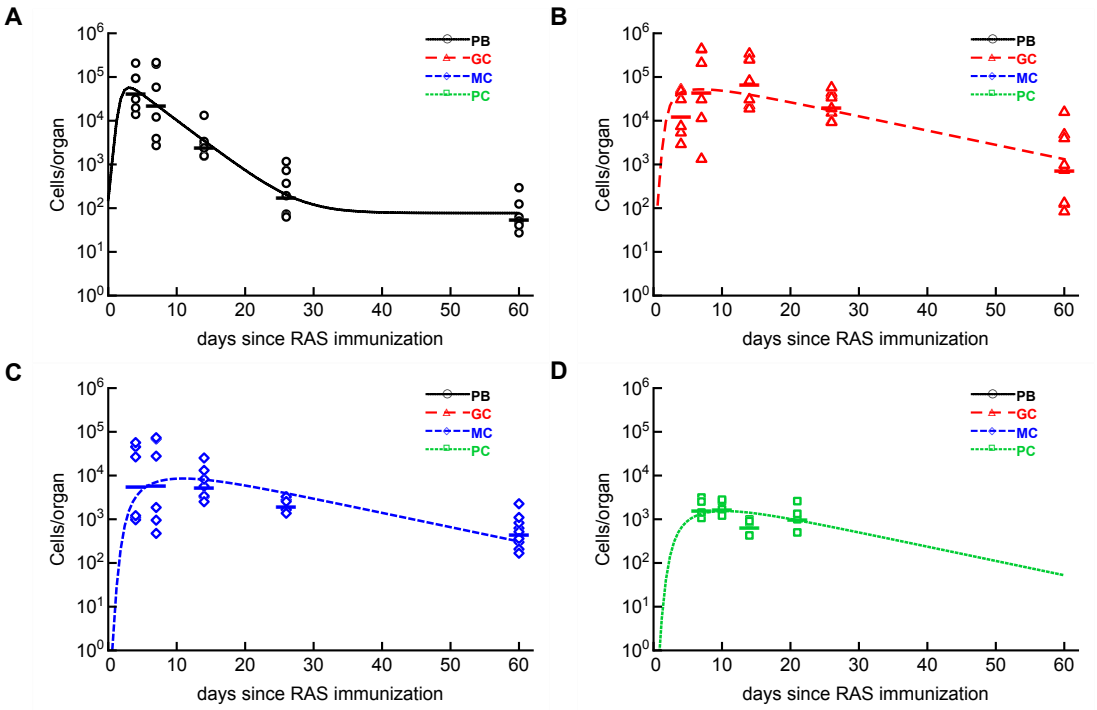

Figure S1: Basic mathematical model can relatively well describe kinetics of B cell response to RAS immunization. We fitted the model (given in eqns. (S.1)–(S.4)) to the data on B cell response to RAS immunization (Figure 4) using least squares by log10-transforming model predictions and the data. The best fit parameters are: $\lambda = 1.64$/day, $B_0 = 77.2$, $r_B = r_G = 6$/day (fixed), $h_B = 0.14$, $\delta_B = 0.11$/day, $h_G = 0.16$, $d_{BG} = 0.17$/day, $\delta_G = 0$, $d_{GP} = 0.013$/day, $d_{GM} = 0.062$/day, $\delta_P = 0.4$/day, $\delta_M = 0.34$/day. Best fit SSR = 37.3.

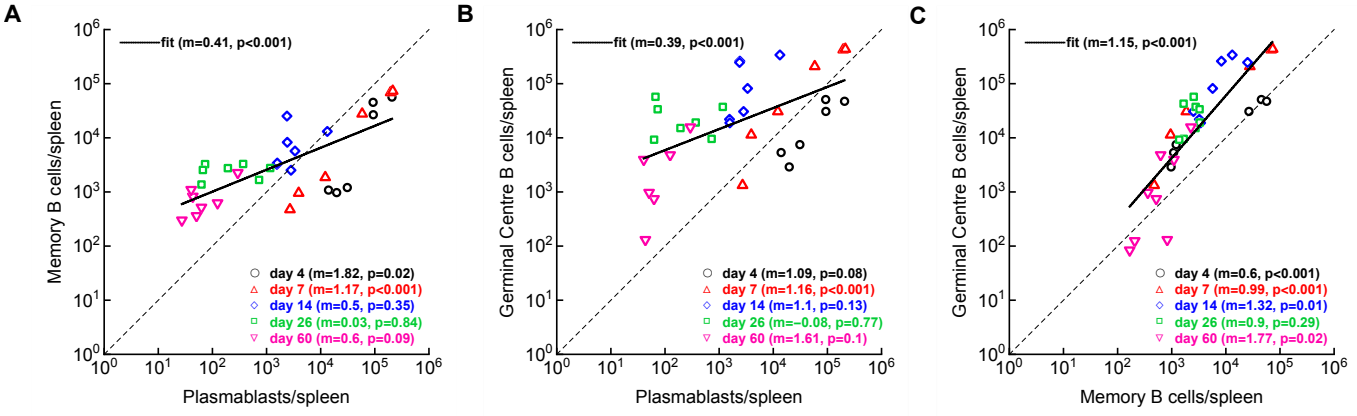

Figure S2: Correlations between different cell populations following RAS immunization. The data are sub-divided by day for individual mice; m denotes the slope of the relationship between variables.