Q 2 Please highlight the limitations and strengths.

Strength:
- the manuscript is very well written and clearly pinpoints the problem: 1/ current models focus on affinity but are not predictive regarding the onset and decay of germinal centers 2/ the biological mechanisms behind are still unknown
- the statements of Q1 supports a quantitative understanding of different processes in the GC
- it pushes efforts to develop good ODE models and to delineate more realistic parameter values
- the synergy between parameters is an interesting way to describe the system, and the paper is mathematicaly sound.

Limitations:

1- affinity maturation is completely ignored. B cells are simulated irrespective of having a good or bad affinity, although it is the first mechanism that could contribute to GC kinetics.

Answer: To assume that the first mechanism that could contribute to GC kinetics is whether B cells have good or bad affinity is a strong, preconceived idea currently lacking experimental evidence. As a working hypothesis it is intriguing, but needs to be put to test. Nevertheless, as we show in the Appendix below, competition between B cells of different affinites is very unlikely to conspicuously impact the global GC dynamics.

For instance, when cells reach a high affinity, the dynamics of selection or the proliferation do not show 'super clones' anymore (mirrored by the fact that clonal dominance happens early, see Tas 2016 science).

Answer: We do not agree entirely with this comment. Tas et al showed unequivocally that clonal dominance is quite relative, and that clonal diversity is much higher than previously generally thought, even at later stages of GCs. This has been confirmed more recently by M Carroll and col (eLIFE (2018) 7, e33051) who showed reversion of an apparent clonal dominance to earlier clones at later stages of GCs.

Also, even if naive or low affinity cells would constantly enter the GC, only in the first days they would have a chance to compete with higher affinity GC cells recycling from the light zone. I can understand that previous paper focus on the dynamics of affinity maturation and here the authors focus on GC dynamics, but I am afraid that ignoring affinity can impact the final statements. I think, before looking at complex mechanisms, a fair representation of affinity would be necessary.

Answer: As pointed out above (and argued in detail in the Appendix below) introducing affinity selection in our models is unnecesary at this stage because not only would it have little impact in the global GC dynamics of the different cell categories but it would also complicate the models.

2- a maximum carrying capacity is manually added to the model (eq 1.9). What is the rationale behind this choice ? I reckon previous papers like Kesmir's had such mechanism. As the present focus is the biphasic GC dynamics I believe the peak of the GC should rather be a consequence of GC selection or dynamics than created artificially. Can you justify why the carrying capacity is required in the model ? Especially in the case of Tfh selection, it is known that Tfh and B cells supports each-other (lanzavecchia immunity 2014), so one would expect that Tfh numbers contribute to define the peak value of the GC.

Answer: This is a very interesting question. We added a maximum carrying capacity to account for the fact that the required growth factors are supplied in limited amounts and that they may not be replenished immediately after consumption. GC B cells require, for instance, IL-21 for proliferation (Keith et al (2012) Autoimmunity, 45:333). Nevertheless, we showed in the sensitivity analysis that parameter Kb has only a marginal impact on GC dynamics.

Similarly, with respect to parameter Kt the sensitivity analysis shows that its impact on the GC dynamics is marginal in model 3 for variations of Kt within a four-fold range centered at Kt = 300.

Following the referee's question, we performed many new calculations sistematically increasing Kt several orders of magnitude. The results show that the maximum of total T and B cells increase considerably, and plateau for very long times, so that their critical times are also greatly increased. Yet, in agreement with our fixed point analysis, those plateaus and critical times increase only up to a threshold point, reached at Kt -> infinity, that is, for $\alpha T = 1$, with most cases in the range, respectively, $1\text{-}5 \times 10^5$ total T cells and 100-1500 days (depending on the values of the other parameters).

This strongly supports the importance of taking into account the fact that growth resources are supplied in relatively limited amounts and are not replenished immediately after consumption. Actually, if the

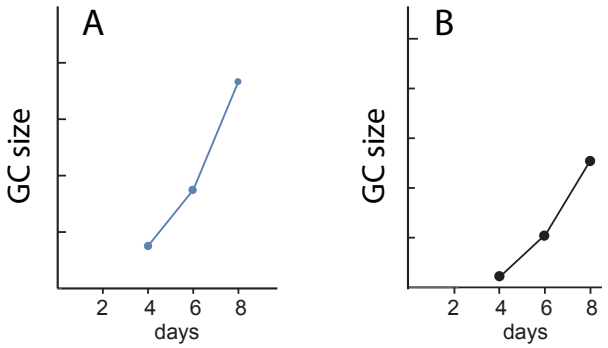
scheme of model 3 were to describe the main GC interactions closer to reality compared to models 1 and 2, the observed model behavior should lead naturally to the postulate that Tfh cells compete in GCs for limited growth resources.
The GC dynamics in models 1 and 2 with $\alpha T = 1$ are qualitatively different from that in model 3. For instance, while free Tfh cells alone still dominate by and large, they peak at much lower levels ($0.5\text{-}1.3 \times 10^5$ cells) and their critical time change little with respect to the value for $\alpha T = Kt/(Kt + Ttotal)$.
In any event, as anticipated by our fixed point analysis, GCs peak as a consequence of GC dynamics and not due to an artifical behavior imposed by considering a maximum carrying capacity.

In the absence of a maximum carrying capacity, adding any kind of competition among B cells (for instance, affinity based) could only lead to a dominance of one or a few B cell clones but the kinetics of T cells would remain the same, so that a very high and long plateau dominated by free T cells would still be reached. Thus, this kind of models highlights the importance of taking into account the changing availability of growth resources (due to the dynamics of the cells consuming them), even if only in a crude way as done in this work.

3- The most critical parameter seems to be n, the number of divisions B cells would pursue. As they compare the case with n=1 and n=3. This parameter is also interesting because likely modulated by adjuvants or Tregs. Why not adding n as a parameter in the sensitivity analyses?
Answer: See answer in the Appendix below.
Also, we have modified the text to include the following brief explanation (see first paragraph of subsection 3.1):
"Recently, a theoretical reassessment … approach [55]. On the other hand, using an experimental system in which B cells are artificially forced to present to Tfh cells levels of membrane peptide-MHCII (p-MHCII) well above those of mid/high affinity B cells, it has been recently estimated that the number of cellular divisions by GC centroblasts range from 1 to 6 before they return to the centrocyte stage, with a majority of cells following 3 divisions [56]. In contrast, under physiologic conditions mid/high affinity B cells follow less cell divisions [56]."

Minor points/suggestions

4- It would be sensible to compare the dynamics of the model with real data. The cited paper of Wittenbrink [53] contains beautiful GC dynamics data. Further, I am not sure the dynamics of the three models are accurate, as an exponential increase of GC size is normally observed, and was also obtained from model 1 in the previous JI paper of the authors. Why in the present manuscript the increase in GC size seems linear?
Answer: That is an intuitive and optical impression that can be misleading. In the following figure the early phase of the GC dynamics in Wittenbrink [53] is compared with the early phase of one random in silico experiment of model 3 (note that a similar comparison can be done with models 1 and 2).



A) From Wittenbrink et al, J Immunol (2011), 187:6185. GC size measured as area of a GC transversal cut.
B) From one random in silico experiment of model 3. GC size measured as number of total lymphocytes.

The initial phase of the total B and total T cell dynamics is exponential growth in all the three models. But sooner or later (depending on the model and the parameter values) the pace of growth is slowed due to the differences in the kinetics of different cell populations, some decreasing and some increasing (see point 9 below) so that for some time their difference is nearly constant, making therefore the net growth nearly linear. After some time the populations that increase are compensated by those that decrease, and eventually all cell populations decrease.
We hope to have now dissipated any doubt about the accuracy of the dynamics of the three models.

5- It would be interesting to discuss which parameters can be manipulated in vivo,
Answer: We understand the practical interest in understanding which parameters can be manipulated in vivo. But our present work does not allow to speculate beyond the point of suggesting that modifying externally p2 (and to a lesser extent Kt) could have an important impact in GC dynamics. However, even that speculation has to be taken with caution because, as we suggest, *it could be that there are unknown control mechanisms* regulating in vivo the effective p2.

and for example can we relate different properties of adjuvant of vaccines are linked with the persistence of GC according to the model. For instance, could you explain that slow delivery of antigens lead to persistent GCs (recent works of Shane Crotty) ?
Answer: The GC life-span considered in our manuscript as the reference against which we compare the calculated GC life-spans in the different in silico experiments of the three models is the normally observed life-span of experimentally induced GC reactions, and hence is a maximum for individual GCs. However, the distribution of life-spans of individual GCs in a primary immune response is currently a big unknown due to essential limitations of in vivo techniques. Nevertheless, it seems they can be overcome, at least partially, thanks to a technique borrowed from neurobiology (see M Carroll and col, eLIFE (2018) 7, e33051). Yet, for the time being there is only information about the global GC reaction, during which different GCs can arise and wane in a non synchronized way. Therefore, by "persistent GCs" one can only mean "persistent GC reaction", in other words, a particular case in which GCs can be detected for longer times than in a typical GC reaction.
In our manuscript we already discuss GC dynamics in the three models being highly sensitive only to parameters p2 and Kt and hence these are the most obvious targets of regulation. By the same token they are also natural candidates to be manipulated in vivo. However, since we do not know yet the role played by follicular Treg (Tfr) cells in GCs and the mechanism by which they affect the intensity and duration of GCs we cannot at present offer reasonable speculations about how to manipulate parameters to modify GC life-span.

6- it would be interesting to further discuss which other mechanisms can account for the decay of GC. Tregs ? CTLA4 ? Existence of negative regulations (they are never considered in the field ...).
Answer: We discuss Tfr cells in the Discussion section (toward the end of the paragraph before the last one).

It is already interested that this manuscript proposes mechanisms not yet fully observed, such as FDC maturation or Tfh maturation, but they are positive mechanisms. What about proposing negative mechanisms ? The Tregs are already discussed, maybe just more ideas.
Answer: FDC or Tfh maturation mechanisms can actually be considered as negative mechanisms, because they make a fraction of B cells to become output cells. Nevertheless, we take note of the reviewer's suggestion for further developments of this work.

7- why taking a predefined dynamics for the model with FDC maturation (F:Ad -> Fm:Afm). Couldn't it be a consequence of the dynamics of the GC ?
Answer: This question wasn't clear to us. The F:Af -> Fm:Afm maturation process does not follow a predefined dynamics in our model, at least in the sense of a predefined time function. Its dynamics is a consequence of the GC dynamics, accelerating or slowing depending on the ammount of accumulated interactions of F:Af with free B cells.

8- the evaluation of the amount of antigen captured by one B cell follow an interesting reasoning but is based on in vitro experiments, likely on artificial membranes, maybe good to discuss at the end how it impacts the result

Answer: Our reasoning is based on both in vitro (P Tolar experiments) and in vivo experiments (namely, those of Cyster and col in JEM 2009).

When considering the parameter reference values (in Models and methods) we argued that the fraction of antigen not depleted per Ba cell is at least $\partial = 0.9998$. We based this estimation on data from recent in vitro experiments with high-affinity antigen-specific B cells interacting with plasma membrane sheets decorated with antigens, and from in vivo experiments analyzing anti-OVA immune responses from mice transferred with high-affinity, anti-OVA transgenic B cells. In addition, we assumed that: (1) B cells cover, in FDC-B cell interactions, a dendrite area that is at most 1/10 of that covered on a planar membrane, (2) each FDC is a depot of a minimum of 10^3 antigen molecules in form of immunocomplexes, and (3) there are about 300 FDCs per GC. We consider assumption 2 to be already a strong underestimation. Relaxing assumptions 1 and 3 and considering instead a 10-fold higher area and a 3-fold lower number of FDCs per GC, makes the fraction of Ag not depleted per Ba cell to be at least $\partial = 0.994$. This is still higher than the value $\partial = 0.99$ used in our simulations. Therefore, not considering such a conservative value of ∂ would impact the kinetic behavior of model 1 by slowing it and leading to higher peaks, thus making it less realistic, without changing the issue that toward the end of the GC reaction there would remain practically no Ag in the follicle.

9- I would suggest to separate the top plots of figure 3 and 4 into two such that the curves can be more clearly separated.

Answer: We welcome this good suggestion.

Maybe explain more intuitively what happens along the simulation around the peak ? What makes it go down ?

Answer: We have now included a brief, more intuitive explanation in the text (second paragraph in subsection 3.2):

"All models share the same characteristic kinetics of Be cells: this is always the first cell population to peak, then it decreases to a value near zero within a relatively short period of time. This triggers a cascade of events in the kinetics of the other cell populations. In model 1, Be and Ba peak when Af is near zero, while at this time free B cells start to increase exponentially because they have no free Ag to combine with. Nevertheless, irrespective of whether Ag is consumed or not, in all models, Ba is limited by the amount of total Ag, and therefore when Ba is close to its maximum the combined rates of Be death plus conjugation to T cells (Tb formation) starts to dominate the kinetics of Be cells causing them to decline. As a consequence, Be cells engage less T cells (form less Tb) and so free T cells increase slightly, which makes Be cells to further decline. When Be cells fall below a certain level, most free T cells (and free Tm cells in model 3) cannot engage with them and, hence, they become visible, with an exponential increase. Consequently, Tb conjugates decrease. In turn, and because of this, Bd and Td start also to decline, which leads to a decrease in the generation of new free B and T cells. In models 2 and 3, an increasing fraction of Be cells become output B cells (Bm). However, they follow different pathways in each model, thus contributing differently to the kinetics of the other cell populations."

Q 3 Are there objective errors in the methods or results, and are the conclusions supported by the presented data?

10- There are many different ways the model could be designed. Concluding that Tfh and Ag consumption can not explain the desired dynamics based on a chosen parameter set and its neighborhood is a bit of an overstatement. Maybe another model or parameter set could do the job. So I don't think it is shown here in a convinving manner enough that model 3 can not explain the data.

Answer: There may be a misunderstanding. We do not conclude that Tfh cells cannot explain the data (in fact, all the three models are based on Tfh cells). What we do say is that "our model 3" explains the GC dynamics poorer than our model 2. We agree that one can design another model that could also do the job, but, as shown in our manuscript, it would not be our model 3. We insist in this point: the alternative model could well be an extension or a variant of our model 3, but clearly such model would not be our model 3.

In general I am afraid of a misinterpretation of modelling here. It is fine to develop models that are not consistent yet and showing an incremental improvement of their predictive capacity. But using it to conclude on the biological mechanism are not suitable in this case here seems a bit too strong.
Answer: This could also be a misunderstanding of our conclusions. We do not conclude that a biological mechanism is or is not suitable. What we do conclude is that a given mechanism *as modeled in our manuscript* is or is not sufficient to explain GC dynamics. If one believes, for instance, that a mechanism like that of model 3 is indeed at work in GCs, then the conclusion is that there must be at least one additional mechanism, still to be uncovered, working in concert with the model 3 mechanism. That is, one should find a suitable and biologically supported extension of model 3. However, the same can be done with respect to model 2 if one were to believe that the mechanism in it is the main one driving the GC dynamics.
Nevertheless, at the end of the third paragraph in the Discussion we have included a brief text, similar to the explanation above, where we have tried to be clearer in order to avoid misunderstandings.

I think this manuscript is not mature enough to accurately say which mechanisms are actually possible or not. For instance the dynamics do not mirror real data but rather a peak and decay. However, I think the interest and strength of this study is to describe the quantitative interplay between mechanisms and to lay steps for further ODE models. It is already a great achievement.
Answer: We thak the referee for this nice comment. We hope to have clarified above that we do not intend to say "which mechanisms are actually possible or not".

Why not instead discussing how the model could be extended to be more realistic, which other mechanisms could explain the dynamics, or which parameters are critical to know ? For instance, I agree in the view of literature that Tfh are very important for GC selection (see paper GC without T cells from Vinuesa 2000), likely more than antigens themselves (Hammer 2000), so I agree with the statements but I don't think they can be supported by the model.
Answer: Before going into an affinity maturation model, we intend to determine an appropriate framework model for the GC dynamics that is not biased from the start towards one or another view of the possible GC selection mechanisms. Then we believe one can try to ponder through modeling analysis the merits and/or shortcomings of the different existing hypotheses of GC selection.

Appendix - How much the presence of different affinity-classes of B cells can affect the GC dynamics?

1. Binding rate constants c_1 and c_2 of B and Be cells, respectively

Rate constants c_1 and c_2 correspond to binding processes that are consequences of random interactions. Hence, they can be assumed to be essentially independent of B cell affinity for antigen (Ag), provided they have a minimum affinity for Ag. Therefore, it is expected that the free B cell and Be cell subsets interact with FDC-Ag and Tfh cells with average rate constants c_1 and c_2, regardless of the antibody (Ab)-affinity distribution within that free B cell and Be cell subsets.

2. Death rate constant d_B of B and Be cells

The fraction of B and Be cells that do not bind —and hence die—, respectively, FDC-Ag and Tfh cells, could comprise mostly the lowest affinity B cells. However, the actual clonal composition of that fraction of B cells is likely irrelevant for the GC dynamics as discussed below.

With respect to unbound, not activated B cells, there is no reason to think they do not die with average death rate d_B, irrespective of the affinity class they belong to.

With respect to Be cells, if they die with constant rate d_B, their death rate is described by the equation

$$\mathrm{Be}' = -d_B\,\mathrm{Be}. \tag{1}$$

Let us assume now that B cells can be grouped in 3 affinity classes, and denote $\mathrm{Be}^{(1)}$, $\mathrm{Be}^{(2)}$, and $\mathrm{Be}^{(3)}$ the Be cells belonging to each class, so that, $\mathrm{Be} = \mathrm{Be}^{(1)} + \mathrm{Be}^{(2)} + \mathrm{Be}^{(3)}$.

If each class of the Be cells die with its own death rate constant, denoted d_B^i for $i = 1,\ 2,\ 3$, then their decay due to death is described by the equations:

$$\mathrm{Be}^{(1)\prime} = -d_B^1\,\mathrm{Be}^{(1)}, \qquad \mathrm{Be}^{(2)\prime} = -d_B^2\,\mathrm{Be}^{(2)}, \qquad \mathrm{Be}^{(3)\prime} = -d_B^3\,\mathrm{Be}^{(3)}.$$

Therefore, the death rate of total Be cells is given by the equation

$$\mathrm{Be}' = -\sum_i d_B^i\,\mathrm{Be}^{(i)} = -\Big(\sum_i d_B^i\,f_i\Big)\mathrm{Be} \tag{2}$$

where $f_i = \frac{\mathrm{Be}^{(i)}}{\mathrm{Be}}$ for $i = 1, 2, 3$. Since $\sum_i f_i = 1$ the coefficient $\sum_i d_B^i\,f_i$ in equation (2) is a weighted average of the three coefficients d_B^i, that is, $\sum_i d_B^i\,f_i = \overline{d_B}$, and hence:

$$\mathrm{Be}' = -\overline{d_B}\,\mathrm{Be} \tag{3}$$

We can see that even in this case the death rate of total Be cells follows an equation formally identical to that in the former case. The only difference between equations (1) and (3) is that in equation (1) d_B is constant, while in (3) $\overline{d_B}$ is time-dependent. However, its value is bounded at any time by the minimum and the maximum values of d_B^i:

$$\min_i d_B^i \leqslant \overline{d_B} \leqslant \max_i d_B^i.$$

Given that, according to our global system sensitivity analysis of the three models, the GC dynamics is quite robust with respect to d_B, it is justified to assume that $\overline{d_B}$ is constant.

Finally, we find worth to recall that in mice with transgenic *bcl*-2 or *bcl-x*L constitutively expressed in B cells, so that apoptosis of GC B cells is reduced by 10-fold, and as consequence affinity maturation is disrupted, GCs number and size are not increased and they have normal biphasic dynamics (Takahashi et al, J Exp Med (1999) 190:399; Smith et al, J Exp Med (2000) 191:475). In addition, several other reports have shown that GCs seeded by B cells with low or high affinity do not display gross differences in kinetics or size (Dal Porto et al, J Immunol (1998) 161, 5373; Vora et al, J Exp Med (1995) 181, 271).

3. Number of consecutive B cell divisions, *n*

The Bd cells generated at any time after de-conjugation from Tfh cells could divide with different proliferation rates and for different number of consecutive cell cycles to become free B cells. The distribution of proliferation rates, or what is equivalent, of the time it takes to an activated B cell to follow a full cell division cycle, is likely rather constant, irrespective of the B cell affinity class B cells belong to (Nussenzweig and col have reported a 1.5-2-fold decrease in division time in B cells artificially forced to increase the levels of membrane peptide-MHCII (p-MHCII) well above those of mid/high affinity B cells [Science, 349:643(2015)]). Therefore, it is justified to assume that the average of that distribution, p_1, is constant.

Considering the proliferation rate constant and the number of consecutive cell divisions followed by Bd cells, in the manuscript we assume free B cells increase in number with rate:

$$\mathrm{B}' = p_1\big(1+\alpha_B\big)\mathrm{Bd} \tag{4}$$

where $\alpha_B = (2^n - 1)K$ depends on n the number of consecutive cell divisions followed by Bd cells. As before, let us now assume that n vary among Bd cells belonging to 3 different affinity classes $\mathrm{Bd}^{(1)}$, $\mathrm{Bd}^{(2)}$, and $\mathrm{Bd}^{(3)}$ so that $\mathrm{Bd} = \mathrm{Bd}^{(1)} + \mathrm{Bd}^{(2)} + \mathrm{Bd}^{(3)}$, and that Bd cells in each class divide with average number of cell divisions n_1, n_2 and n_3. In this case, the growth rate of free B cells is described by the equation:

$$\mathrm{B}' = p_1\big(1+\alpha_B^{(1)}\big)\mathrm{Bd}^{(1)} + p_1\big(1+\alpha_B^{(2)}\big)\mathrm{Bd}^{(2)} + p_1\big(1+\alpha_B^{(3)}\big)\mathrm{Bd}^{(3)} = p_1\Big(\sum_i \big(1+\alpha_B^{(i)}\big)f_i\Big)\mathrm{Bd} \tag{5}$$

where $\alpha_B^{(i)} = (2^{n_i} - 1)K$ for $i = 1,\ 2,\ 3$, and $f_i = \frac{\mathrm{Bd}^{(i)}}{\mathrm{Bd}}$.

Again, $\sum_i f_i = 1$, and hence the coefficient within parenthesis in the right hand side of (5) is the following weighted average:

$$\sum_i \big(1+\alpha_B^{(i)}\big)f_i = \sum_i \big(f_i + \alpha_B^{(i)} f_i\big) = 1 + \sum_i \alpha_B^{(i)} f_i = 1 + \overline{\alpha_B}$$

Therefore,

$$\mathrm{B}' = p_1\big(1+\overline{\alpha_B}\big)\mathrm{Bd} \tag{6}$$

where $\overline{\alpha_B}$ is the weighted average of the three coefficients $\alpha_B^{(i)}$ so that equation (6) is formally identical to (4).

From the definition of $\alpha_B^{(i)}$ we have:

$$\overline{\alpha_B} = \sum_i \alpha_B^{(i)} f_i = \sum_i (2^{n_i} - 1)K f_i = \Big(\sum_i (2^{n_i} f_i - f_i)\Big)K = \Big(\Big(\sum_i 2^{n_i} f_i\Big) - 1\Big)K = \big(2^{\bar{n}} - 1\big)\,K$$

where $\bar{n}$ is defined by $\bar{n} = \log_2\big(\sum_i 2^{n_i} f_i\big)$.

Clearly, $2^{\bar{n}}$ is a weighted average of positive values 2^{n_1}, 2^{n_2}, 2^{n_3} and therefore it is bounded by the minimum and the maximum values of 2^{n_i}, that is, $\min_i 2^{n_i} \leqslant 2^{\bar{n}} \leqslant \max_i 2^{n_i}$. But $\min 2^{n_i} = 2^{\min n_i}$ and $\max 2^{n_i} = 2^{\max n_i}$. Consequently, $2^{\min_i n_i} \leqslant 2^{\bar{n}} \leqslant 2^{\max_i n_i}$, and as a result,

$$\min n_i \leqslant \bar{n} \leqslant \max n_i.$$

From the report of Nussenzweig and col (Nature, 509:637(2014)), the distribution of the number of consecutive cell cycles followed by Bd cells could vary between cells belonging to different affinity classes. Nevertheless, their estimation that Bd cells could go from 1 to 6 cell divisions was based on the same system mentioned above to force B cells to increase considerably the normal levels of membrane p-MHCII. In contrast, under normal conditions mid/high affinity B cells follow less cell divisions. In our simulations we compared the cell kinetics and sensitivity analysis results for $\bar{n} = 1$ and $\bar{n} = 3$. At the suggestion of the referee, we have now performed similar analysis for $\bar{n} = 5$. The results show that model 3 performs considerably worse for $\bar{n} = 5$ than for $\bar{n} = 3$. Thus, the cell kinetics in model 3 is considerably slowed and attains considerably larger peaks for $\bar{n} = 5$ than for $\bar{n} = 3$, particularly with respect to total B cells, while the sensitivities and synergies are comparable to those obtained with $\bar{n} = 3$. In contrast, in model 2 the pace of the cell kinetics is not markedly changed and the peaks increase at most to double for $\bar{n} = 5$ compared to $\bar{n} = 3$. This reinforces our previous conclusion that model 3 performs worse than the other two models with respect to cell kinetics.

4. Parameter a_2

Finally, parameter a_2 corresponds to the distribution of conjugation durations of B and Tfh cells. This distribution has been analyzed by the group of Nussenzweig (Science, 345:1058(2014)). In that paper the authors used, once again, the artificial system mentioned above to force B cells to increase considerably the normal levels of membrane p-MHCII. These B cells are considered to represent very high affinity B cells. When those B cells under enforced selection were compared to B cells under physiologic conditions, their distribution of conjugation durations were similar, with means that differed less than 1.6-fold. Considering that in normal GCs there is a less extreme difference of affinities, we conclude that it is justified the assumption that parameter a_2 is constant.

In summary, we believe our modeling approach provides a general framework on top of which one can add hypothetical selecting mechanisms.