P-1054 From affinity maturation to kinetics maturation in GC modeling

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Summary

Germinal centres (GC) are microanatomical structures, in which B lymphocytes proliferate, undergo somatic hypermutations (SHM) and positive selection to produce high-affinity antibodies (Ab). Ab affinity (KA) is defined as the ratio of kinetic constants kon and koff which determine the kinetics of bond between a B-cell receptor (BcR) and antigen (Ag). Earlier research suggest that GC may not only select based on affinity but may preferentially select clones with specific kinetic properties (kinetic maturation [1-3]). We, therefore, hypothesized that under specific modes of selection the GC will preferentially select clones with specific kinetic properties. Mechanisms underlying such selection process are unclear but we propose two putative mechanisms that explicitly include both kinetic constants and compare this to the mechanism in which B-cell selection is solely based on affinity. We use a pre-existing agent-based model of the GC that comprises primary cellular mechanisms of the GC reaction, and which implements affinity-based selection [4]. We extended this model to allow for two modes of kinetic maturation. We show that if binding and unbinding of the BcR to the FDC-presented Ag is made dependent on kon and koff respectively, there is no competitive advantage for specific clones. However, considering the extraction of the Ag from the FDC also depends on koff and, consequently, can be interrupted before completion, a selective advantage for B cells with low association/dissociation rates is the result. Although this finding needs experimental validation, it shows that selection mechanisms for binding kinetics might operate as part of the GC reaction.

INTRODUCTION / PURPOSE

One of our immune system's mechanisms to fight against some pathogens is producing high-affinity antibodies. Antibodies are Y-shaped proteins that bind to specific molecules on the surface of pathogens called Antigens. The affinity of antibodies that the immune system produces increases through an affinity maturation process. Affinity maturation takes place in the Germinal Centers (GCs). GCs are microanatomical structures within secondary lymphoid organs in which B lymphocytes proliferate and mutate their B-cell receptor (BCR) genes, therefore gaining new affinities to the antigen. BCR complexes include membrane bond antibodies that enable B cells to bind to interact and bind to antigens. Further, these B cells get positively selected for their affinities. In order to get positively selected, B cells collect antigens presented by follicular dendritic cells (FDCs), internalize the antigen, process, and present it through major histocompatibility complex II (MHCII) molecules in the form of peptide-MHCII. These B cells interact with T follicular helper cells through which they receive signals that determine their fate. B cells that can not collect any antigen and are not selected die, and selected cells differentiate to Memory B cells, Plasma B cells, and or remain in GC for more rounds of proliferation and mutation. Memory B cells are used to lunch a faster and more efficient immune response in case of future encounters of the same antigen by the immune system. Plasma cells produce significant amounts of high-affinity antibodies that bind to antigens and neutralize pathogens or mark them for elimination and removal from the body. It has been shown that positive selection in GC is dependent on the concentration of peptide-MHCII molecules that B cells present to T follicular helper cells [Victora et al 2010] and, therefore, on the amount of antigens B cells collect. It is assumed that positive selection in GC depends on the affinity, therefore, B cells with higher affinities have a higher chance of getting selected and survive. Hence, selected cells with higher BCR affinities later produce higher affinity antibodies. The antibody-antigen affinity is usually measured and reported using equilibrium dissociation constant (KD) that is equal to the ratio of dissociation rate constant (koff) to association rate constant (kon) (KD=koff/kon). Affinity, by definition, depends on both how fast antibodies bind to antigens (kon) and how slowly they dissociate back to their free form (koff). In theory, two B cells with equal affinities for the antigen can have different association and dissociation rates (e.g., one B-cell associate fast but also dissociates fast (high kon, high koff) while the other B-cell with the same affinity associates slowly but also dissociates at a much slower rate (low kon, low koff)). However, it is not fully known how these two play a role in the positive selection, whether a fast association is more beneficial for B cells or a slow dissociation. This work aims to study the polarity of selection in GC towards fast association and or slow dissociation with a 3D agent-based model of GC.

DISCUSSION

It has not been studied before whether the GC selection is biased towards the fast association or slow dissociation. Here we show that there could be a significant preference for having lower dissociation rates (low koff) compared to high association rates (high kon) in GC due to, for example, the use of mechanical forces for extraction of antigen in GC. However, it should be noted that experiments must validate these results. We hope that this work inspires experiments that could shed light on this subject. Understanding more about GC selection potentially could help to optimize the immune response for better neutralization.

METHODS and EQUIPMENTS

We simulate three scenarios and run each 30 times with different random seeds. Each simulation starts with three founder B cell clones with equal affinities but different association dissociation rates implemented as probabilities (Table 1). In this design, at equal affinity, cells from Clone-L have a relatively lower association and dissociation rates compared to the other two clones. Cells from Clone-M have moderate association and dissociation rates, and cells from Clone-H have high association and dissociation rates compared to the other two clones. The effect of SHM is fixed for each clone. This way, cells cannot change their clone by SHM, and the evolution of clones can be compared throughout time. Scenario-0 is the reference scenario. In this scenario, positive selection depends solely on the affinity, in a process in which association and dissociation rates are not directly involved. This scenario is used to produce the output of a typical 21-day GC reaction [Meryer-Hermann et al., 2012] compared with other scenarios. In Scenario-2, the association and dissociation rates are involved in the process of Ag collection. B cells associate with antigens with different association rates and dissociate similarly with different rates implemented by probabilities. Therefore, some B cells could dissociate before initiating antigen extraction due to very high dissociation rates. In addition, interruptions are simulated during the Ag extraction process based on the assumption that B cells preferentially use mechanical forces to extract antigen from a presenting surface. These mechanical forces are suggested to cause the rupture of weak BCR-antigen bonds during extraction and eliminate the collection process. Scenario-1 is similar to scenario-2 but without interruptions during the antigen extraction process. This is to investigate the sole effect of interruptions on the evolution of clones with different association/dissociation rates.

RESOURCES

Victora GD, Schwickert TA, Fooksman DR, Kamphorst AO, Meyer-Hermann M, Dustin ML, Nussenzweig MC. Germinal center dynamics revealed by multiphoton microscopy with a photoactivatable fluorescent reporter. Cell. 2010;143(4):592-605. doi: 10.1016/j.cell.2010.10.032. Meyer-Hermann M, Mohr E, Pelletier N, Zhang Y, Victora GD, Toellner KM. A theory of germinal center B cell selection, division, and exit. Cell Rep. 2012;2(1):162-174. doi: 10.1016/j.celrep.2012.05.010. Tolar P, Spillane KM. Force generation in B-cell synapses: mechanisms coupling B-cell receptor binding to antigen internalization and affinity discrimination. Adv Immunol. 2014;123:69-100. doi: 10.1016/B978-0-12-800266-7.00002-9. Nowosad CR, Spillane KM, Tolar P. Germinal center B cells recognize antigen through a specialized immune synapse architecture. Nat Immunol. 2016;17(7):870-877. doi: 10.1038/ni.3458.

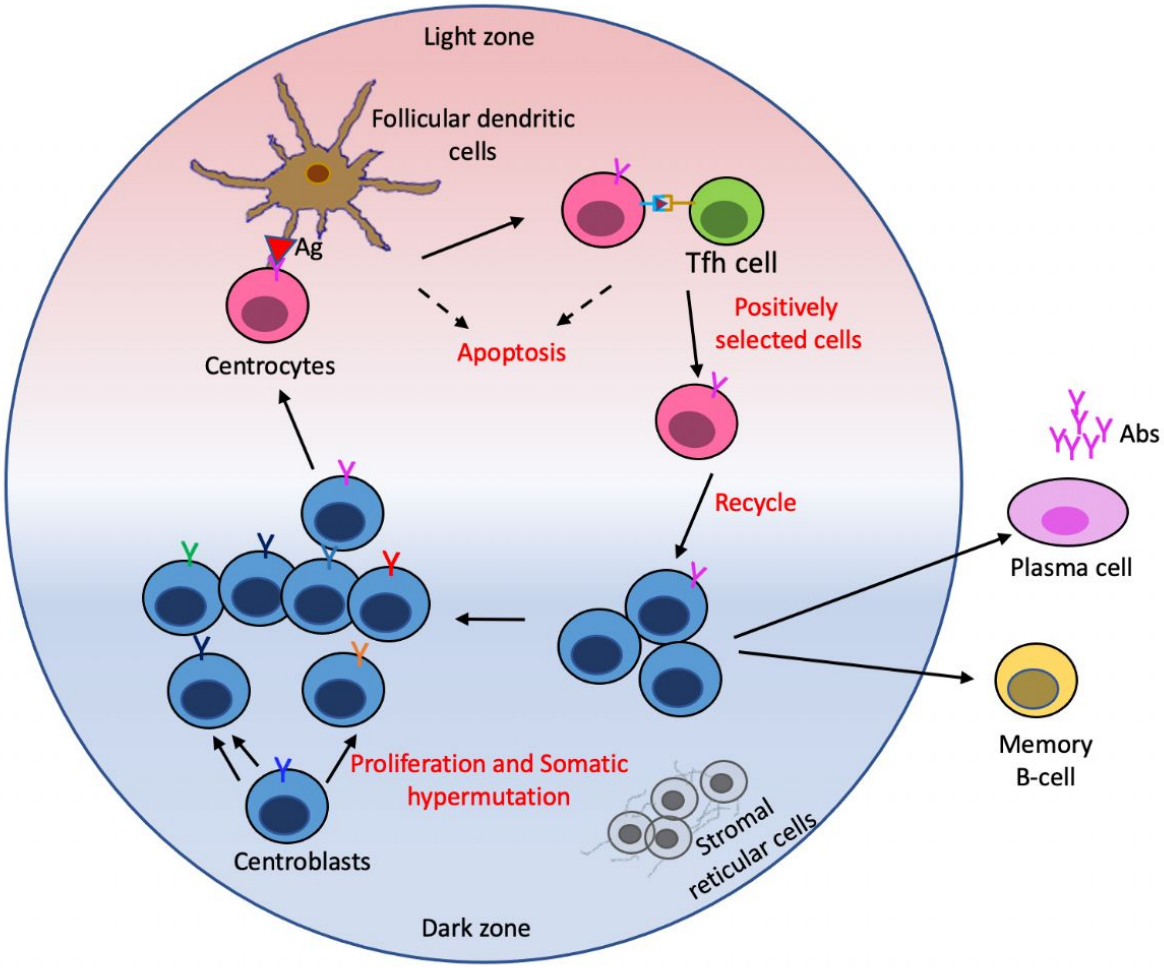
TABLES

Clone	Initial association	Initial dissociation	Affinity	Effect of SHM
Clone-L	Low(P=0.04)	Low(P=0.0)	Low(P=0.04)	Association
Clone-M	Moderate(P=0.2)	Moderate(P=0.8)	Low(P=0.04)	Association/Dissociation
Clone-H	High(P=1.0)	High(P=0.96)	Low(P=0.04)	Dissociation

IMAGES

Schematic representation of a GC reaction The GC comprises a DZ and an LZ in which different processes take place. Reticular stromal cells express CXCL12 chemokine in the DZ (blue gradient), and FDCs express CXCL13 chemokine in the LZ (red gradient). CBs which are more sensitive to CXCL12 proliferate and change their affinity for the Ag through SHM in the DZ. Subsequently, CBs differentiate to CCs that move to the LZ because of their sensitivity to CXCL13 and collect Ag from FDCs. CCs internalize, process, and present the Ag peptides on their surface to interact with Tfh cells. CCs that cannot collect Ag and or cannot receive Tfh help due to competition die by

apoptosis. Positively selected CCs recycle back to DZ for further proliferation and mutation or differentiate to MBCs or PCs.



Schematic of Ag collection scenarios Three mechanisms of Ag collection based on affinity and kinetic selection. The green rows denote the steps that the scenarios have in common. (A) Scenario-0: Competition for Ag depends on affinity directly. (B) Scenario-1: Competition for Ag collection depends on association (Pa) and dissociation (Pd) probabilities. (C) Scenario-2: similar to Scenario-1, but during the Ag extraction, at each time-step, the bound between CC and Ag gets interrupted (red arrow) that may lead to disruption of Ag extraction before it is fully complete.

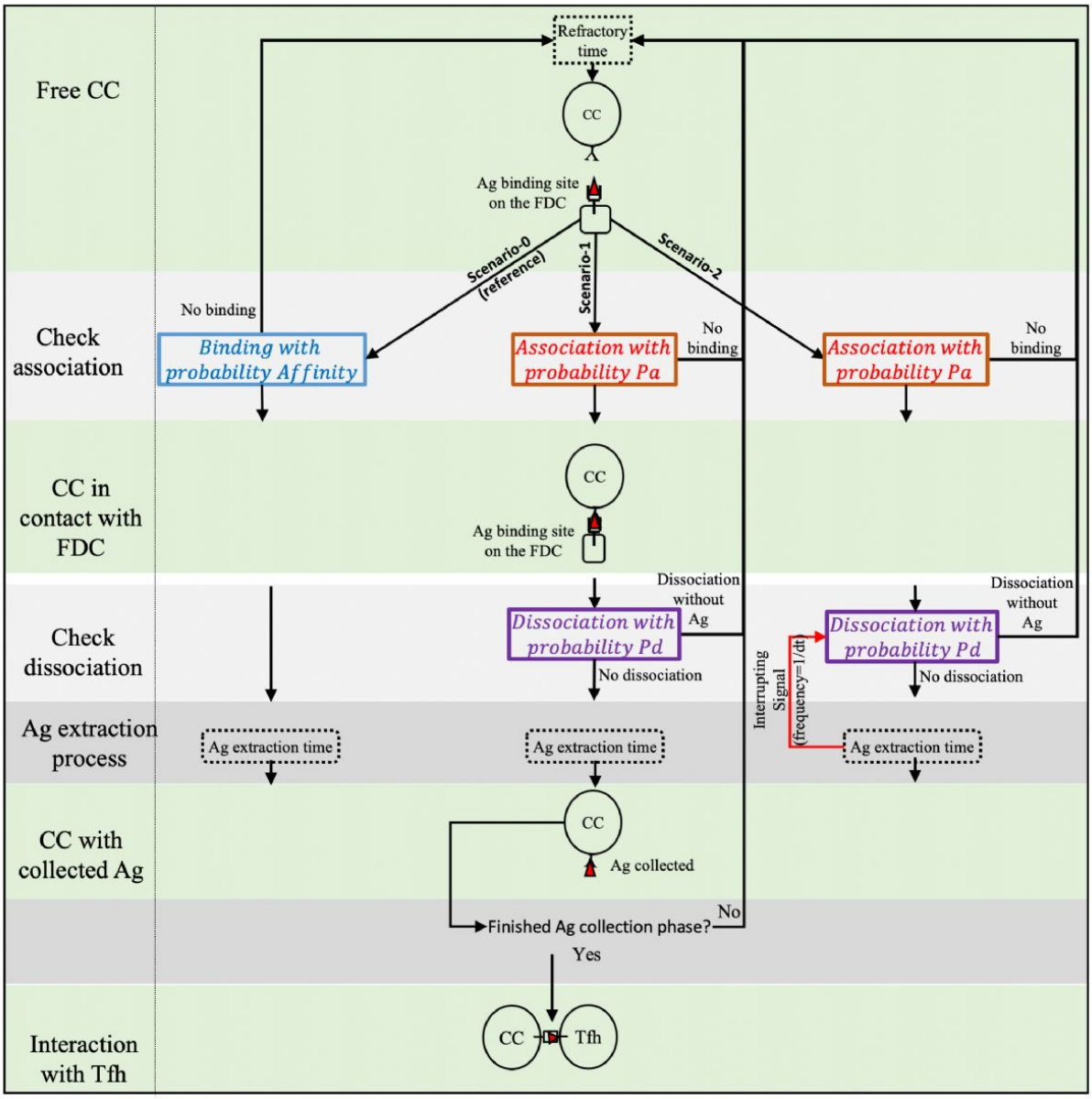


Figure 1, population dynamics in the reference scenario Fig 1A: The population of Centroblasts (proliferating B cells) and Centrocytes (B cells competing for positive selection) in three clones. | Fig 1B: The amount of collected Ag by each clone of cells in time. | Fig 1C: The events during the antigen collection process for B cells. Only cells that finish the ag collection phase by collecting at least one antigen portion are shown. | This figure shows that there is no biased toward fast association and or slow dissociation, since clones with initially equal affinities but different association/dissociation rates show a similar population size (Fig 1A), collect similar amounts of Ag (Fig 1B), and show similar dynamics during antigen collection phase (Fig 1C). No difference is observed between clones because association/dissociation rates were not directly involved in the antigen collection process, and clones had similar initial affinities.

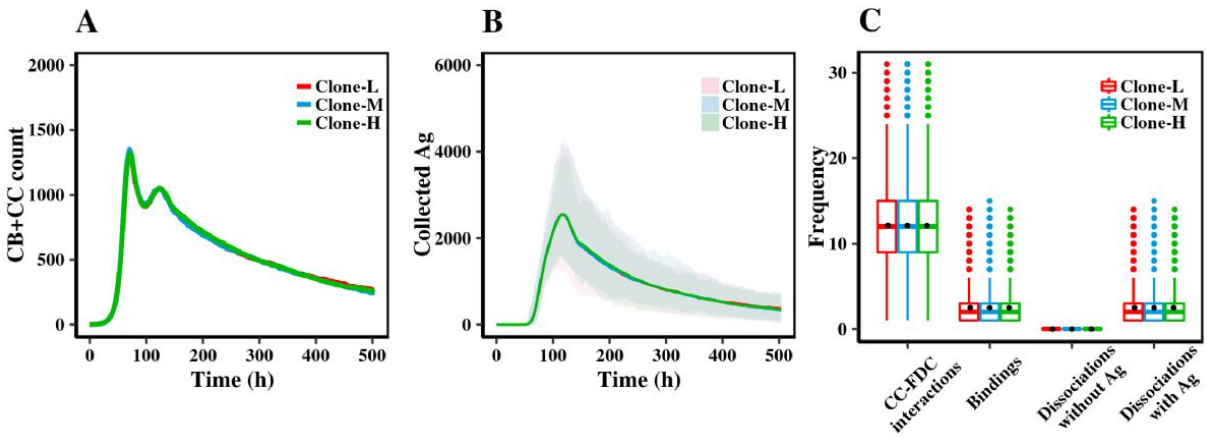
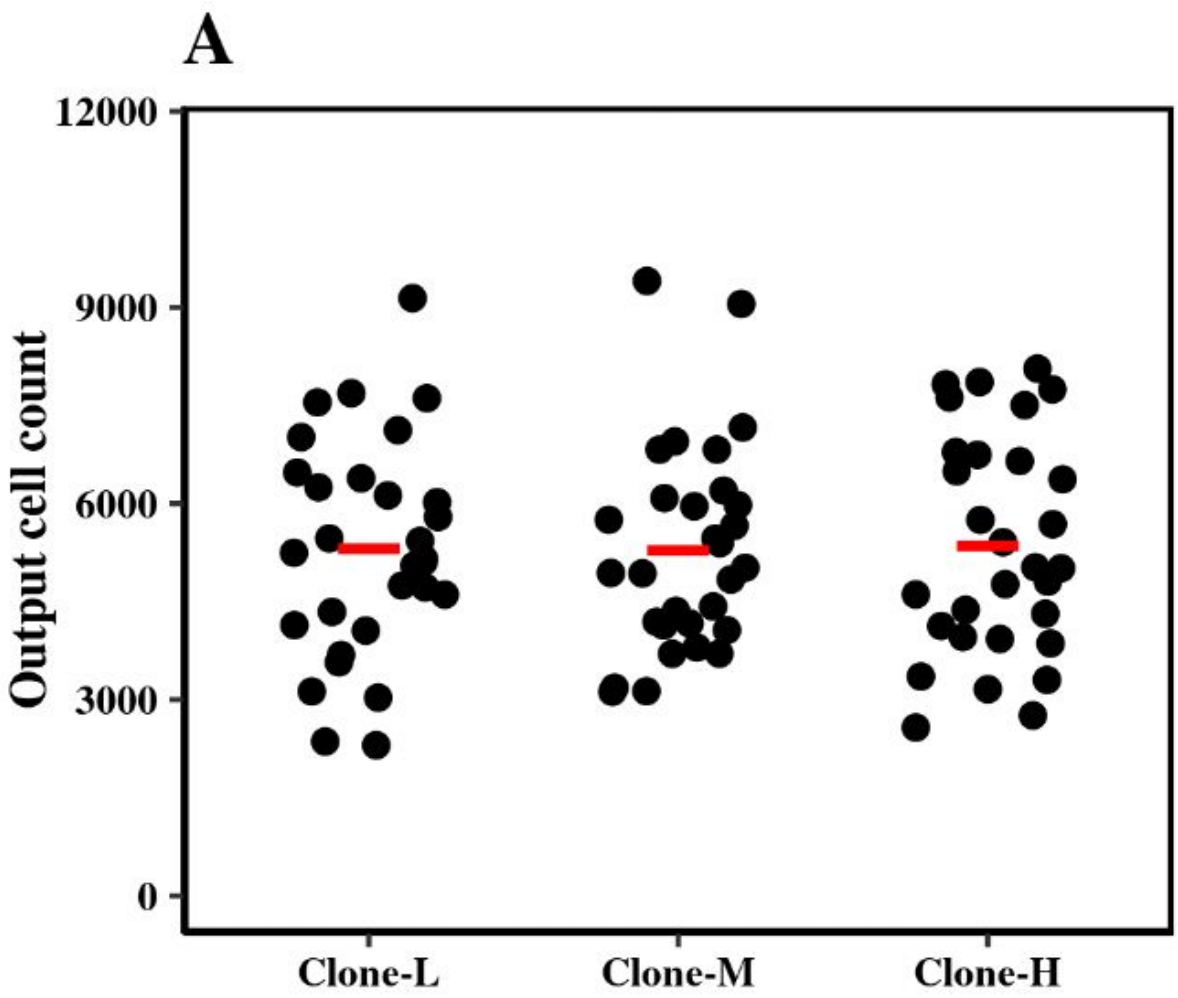
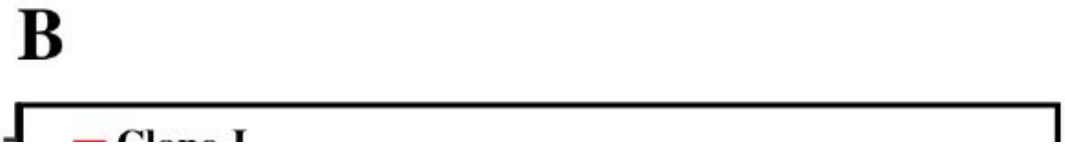


Figure 2 Affinity maturation and output cell production in the reference scenario Fig 2A: Produced output cells consisting of Memory and Plasma cells in 30 repeats of the reference scenario (red lines show the average) | Fig 2B: Average affinity of B cells in GC (dashed lines) and Average affinity of accumulated output cells (solid lines) | This figure shows no difference between the average produced output cells from three clones and no difference between the level of affinity maturation.





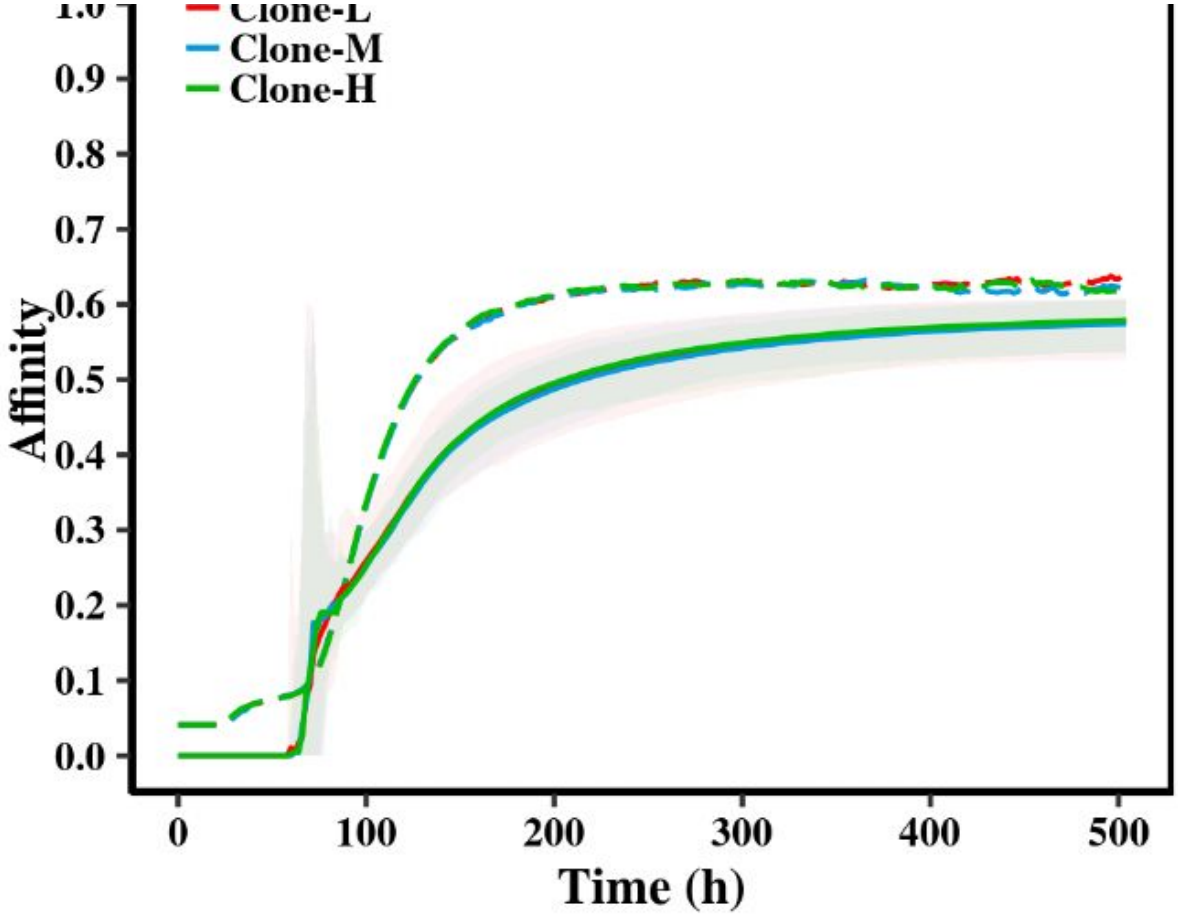


Figure 3 Population dynamics in Scenario-2 Fig 3A: The population of Centroblasts (proliferating B cells) and Centrocytes (B cells competing for positive selection) in three clones. | Fig 3B: The amount of collected Ag by each clone of cells in time. | Fig 3C: The events during the antigen collection process for B cells. Only cells that finish the ag collection phase by collecting at least one antigen portion are shown. | | This figure shows a bias towards a slow dissociation rate compared to a fast association rate in scenario-2. Clone with a slow dissociation rate (Clone-L) shows a higher population size (Fig 3A) and higher amounts of collected antigen (Fig 3B) compared to the other two clones. Fig 3C shows that Clone-H had a higher association frequency. However, most of these bonds were dissociated without collecting antigens. In contrast, clone-M and clone-L had a lower frequency of associations, but fewer of these bonds were disrupted without antigen extraction. Even though the average amount of collected antigen per cell is similar between cells from three clones (Fig 3C black dots in the last group), more cells from Clone-L collected antigens than cells from Clon-M or Clone-H, and therefore the antigen collected by Clone-L in total is higher compared to other two clones (Fig 3B).

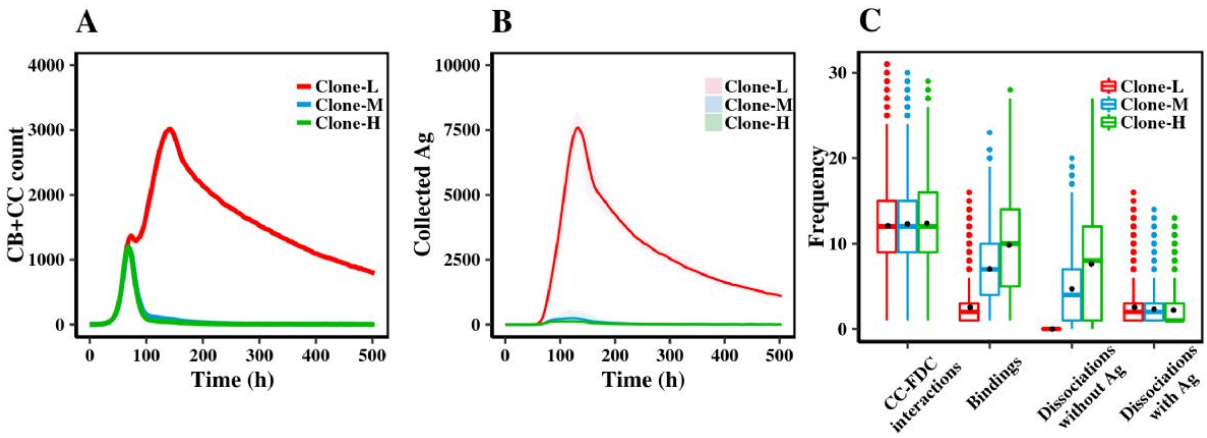


Figure 4 Output cell production and affinity maturation in scenario-2 Fig 4A: Produced output cells from each clone in 30 repeats of scenario-Fig 4B: The average affinity of GCC B cells (dashed lines) and accumulated output cells (solid lines) in scenario-2. | Fig 4C: The distribution of produced output cells according to association/dissociation probability and affinity. Percentage of output cells with each association/dissociation probability overall output cells are shown. The curves show iso-affinity lines and points on the same carve have the same affinities but different association/dissociation probabilities and consequently different rates. | | This figure shows that in scenario-2 compared to the reference scenario, most of the output cells are produced from Clone-L, which shows a preference towards clones with low dissociation rates. Also, Clone-M and Clone-H show a higher level of maturation compared to Clone-L. This elevated level of maturation is also visible in comparison to results from the reference scenario. This increase in maturation was expected because of the interruptions during the antigen extraction and has been suggested in the literature [Tolar et al., 2014, Nowosad et al., 2016]. Also, the distribution of output cells (Fig 4C) shows that there was a bias toward the production of cells with slow dissociation rates compared to cells with high association rates at equal affinities (iso-affinity curves).

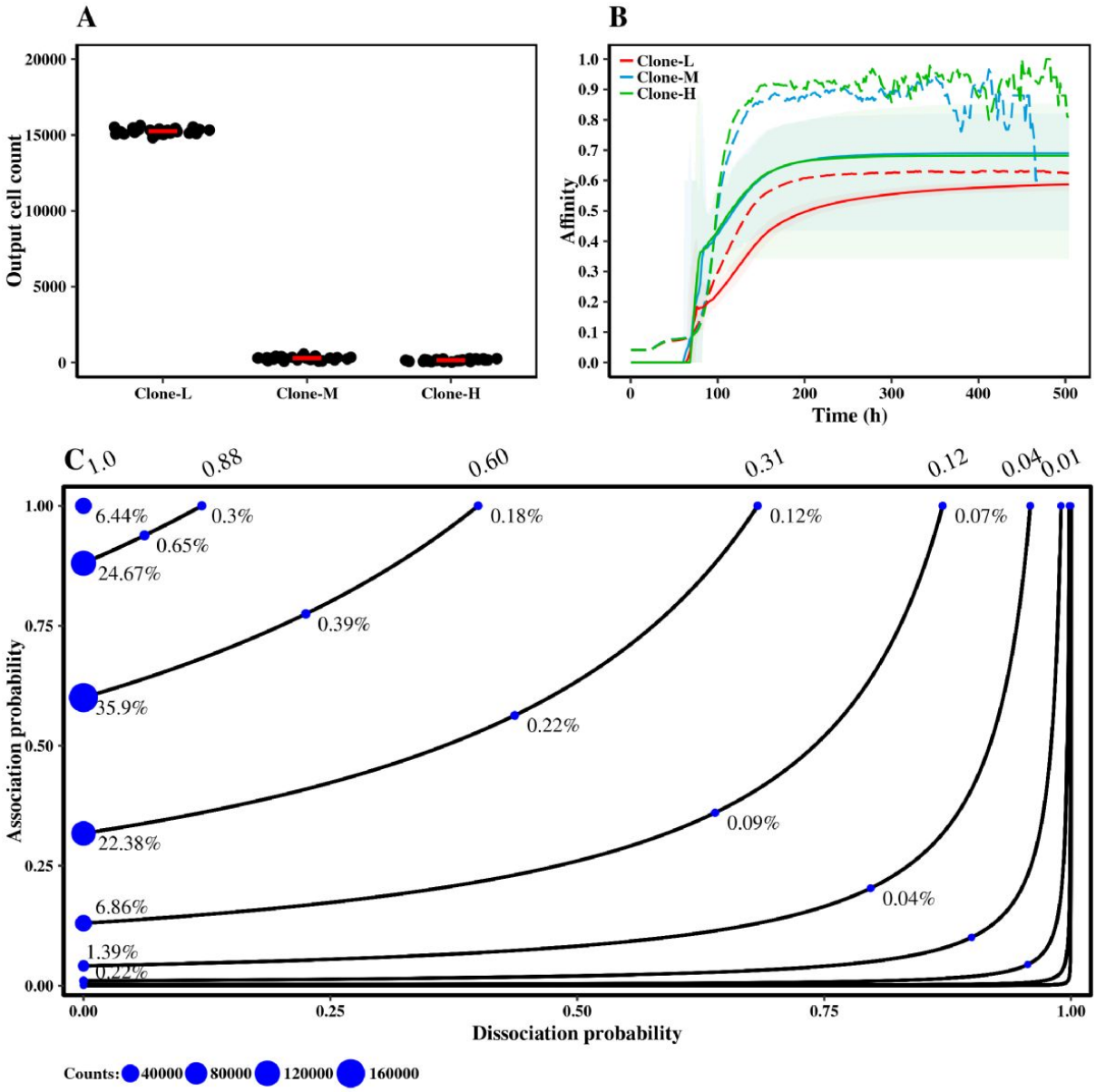


Figure 5 Population dynamics in Scenario-1 Fig 5A: Population dynamics of clones in scenario-1. | Fig 5B: Collected Ag by each clone in scenario-1. | Fig 5C: Dynamics of B cells during the antigen collection phase. | This figure shows the result for scenario-1, in which scenario-2 is replicated but without interruptions during antigen extraction. It is visible that three clones have a similar population size and collected similar amounts of antigens (Fig 5B). The 5C shows that similar to scenario-2, Clone-L and Clone-M had fewer associations and fewer dissociations without collecting antigen than Clone-H.

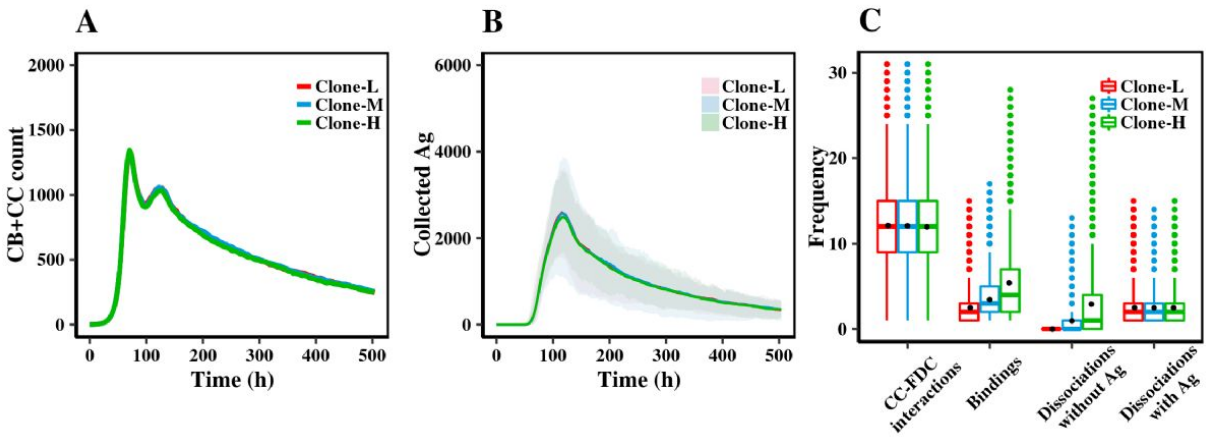
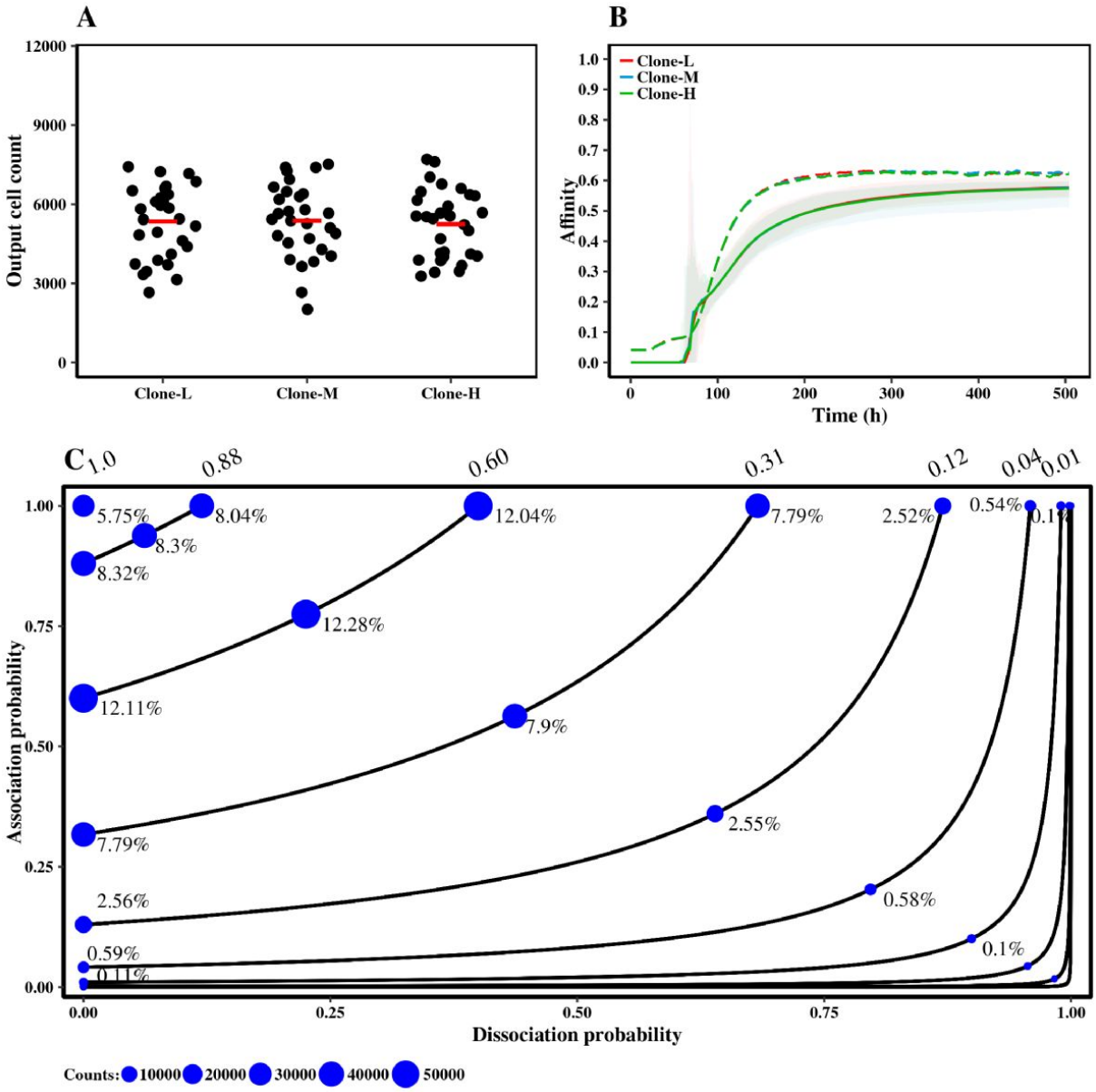


Figure 6 Output cell production and affinity maturation in scenario-1 This Figure shows that the number of produced output cells from three clones are similar on average and affinity maturation of three clones is in similar orders. Also, there is no preference in producing output cells with respect to association/dissociation rates. This shows that the preference for clones with low dissociation rates that existed in scenario-2 resulted from interruptions during the extraction of antigen. The model shows no bias toward the fast association of slow dissociation without such interruptions, similar to the reference scenario.


A
Output cell count
12000
9000
6000
3000
0
Clone-L
Clone-M
Clone-H
B
Affinity
Clone-L
Clone-M
Clone-H
Time (h)
C
Association probability
Dissociation probability
1.0
0.88
0.60
0.31
0.12
0.04
0.01
5.75%
8.04%
8.3%
8.32%
12.04%
12.28%
12.11%
7.79%
2.52%
0.54%
7.9%
2.55%
7.79%
2.56%
0.58%
0.59%
0.1%
Counts: 10000 20000 30000 40000 50000