Antigen-tethering proteins on follicular dendritic cells influence the affinity and diversity of germinal-center B cells

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To optimally protect a host, the adaptive immune system must generate antibodies that bind the pathogen with high affinity, yet are diverse enough to protect against potential escape mutations. To generate these antibodies, B cells inside so-called germinal centers (GCs) compete for survival based on their ability to extract antigen from the surface of specialized presenting cells called follicular dendritic cells (FDCs). It is an open question whether a mechanism exists to actively regulate the selection pressure for increased affinity or diversity among GC B cells. Curiously, FDCs tether antigen via not one, but two classes of receptors, complement receptors (CRs) and an Fcγ receptor (FcγR), that differ qualitatively in their ligands, affinities, and expression kinetics. Here, we present a minimal biophysical model of this two-receptor antigen-presentation system which suggests that the presence of FcγR may act as a biophysical control knob to steer the relative affinity and diversity of GC B cells. Our model is consistent with experimental observations reporting low affinity but high diversity in the absence of FcγRs on FDCs.

One of the key protective features of the adaptive immune system is the generation of novel antibodies (Abs). Mechanistically, the process of affinity maturation occurs in microanatomical environments known as germinal centers (GCs) [1, 2] (Fig. 1a). GCs are organized into two compartments, the Light Zone (LZ) and the Dark Zone (DZ), which are responsible for selection and diversity generation, respectively. In the LZ, antigen (Ag) is presented on the surface of follicular dendritic cells (FDCs) [3–5], which the B cells explore for cognate Ag. Upon detection, a B cell attempts to extract Ag using its B cell receptor (BCR) [6–13]. If extracted, the Ag is endocytosed, digested into peptides, and presented on MHC class II molecules to follicular helper T cells (T_{FH}s). If sufficient T-cell help is received, the B cell migrates to the DZ, where it undergoes rapid divisions and somatic hypermutation. The number of divisions is set by the amount of the T-cell help [14]. The mutated daughters then return to the LZ and repeat this process. Crucially, GC B cells will undergo apoptosis if they fail to either find cognate Ag or receive T-cell help. Hence, B cells that are able to acquire more Ag and therefore receive more T-cell help will produce more progeny and thus form a larger fraction of the future GC population. During this process, a small fraction of B cells are exported to effector fates, including plasma cells (responsible for immediately secreting Abs) and memory cells (poised for reactivation during future infections).

To optimally protect the host, the GC reaction must achieve two competing objectives: the evolved Abs must bind with high enough affinity to clear the pathogen, but the Ab repertoire must be diverse enough to anticipate future escape mutations. Relevant to these ends, a key contributor to the GC reaction is an effect known as "Ab feedback" [15–22]. Briefly, Ag is generally presented on the membrane of an FDC in the form of an immune complex (IC), consisting of an Ag or Ags coated in complement (proteins of the innate immune system) and Abs (see Fig. 1b). As the immune response progresses, weaker Abs in ICs are displaced by stronger Abs. This turnover has been proposed to influence the selection pressure in the GC. For instance, IC Abs can block BCRs that compete for the same or overlapping epitopes (Ab binding sites). This so-called "epitope masking" has alternately been proposed to drive GC selection to higher affinity (by incrementally increasing the difficulty of BCRs to bind to Ag [15]) and/or to drive diversification of GCs (by giving a competitive edge to B cells cognate to alternative epitopes [18]). Whether there is an active mechanism to steer between these two fates—increasing affinity or diversification—is an open question.

In addition to masking, Ab feedback has also been proposed to influence the GC reaction by modulating how strongly ICs bind to FDCs. Jiang and Wang proposed a conceptual model in which ICs are tethered to the FDC via a receptor that binds to Abs [22]. In this picture, Ab feedback drives GC selection to higher affinity by gradually increasing the BCR affinity required to extract ICs from the FDCs. Intriguingly, in physiological GCs, ICs are bound to the FDC via not one, but two classes of receptors. First, the complement receptors (CRs) are independent of IC Abs: they bind directly to complement fragments, which are themselves covalently bound to Ags [23, 24]. The second receptor is FcγR (specifically

FcγRIIB), which binds to ICs via the conserved tail of IgG Abs [25–29]. The mechanical role of FcγR, if any, is often assumed to be negligible as its affinity for the Ab tail ($K_A \sim 10^4 - 10^7$ M^{-1}) [30, 31] is orders of magnitude lower than that of CRs ($K_A \sim 10^8 - 10^9$ M^{-1}) [23, 24, 32] for complement. Why the immune system nevertheless evolved to use two classes of receptors has not been addressed.

Here, we provide a minimal biophysical model of affinity maturation in light of the FDC's two-receptor tethering system. Using our model, we first note that the FcγRs, though individually weak, can have a substantial effect when multivalently bound to an IC via other FcγRs and CRs. We arrive at the following potential implications for affinity maturation: without FcγR, our analysis suggests that epitope masking would limit affinity maturation and instead promote diversification for Ags with multiple epitopes. By contrast, the presence of FcγR increases the difficulty of Ag extraction for all B cells, regardless of epitope specificity. This egalitarian threshold limits diversification, and thereby favors continued affinity maturation of the high-affinity dominant B cell lineages. While further experimental investigation is needed, our model suggests the conceptual implication that FcγR expression is a biophysical knob that FDCs can use to actively regulate the affinity-diversity effects of Ab feedback.

Results

Without FcγR, epitope masking promotes epitope diversity and stalls affinity maturation. To motivate the role of FDC FcγRs, we first examine B cell competition when FcγR is absent. First consider the simplest situation in which the Ag has only one epitope, as shown in Fig. 2a. Using a simple phenomenological model for Ag extraction based on binding and unbinding rates of the relevant noncovalent bonds, one finds that the probability that the B cell successfully extracts this Ag is

$$P_{\text{primary}} = \left(\frac{1}{1+\tau_{\text{IgG}}/\tau_{\text{BCR}}}\right)\left(\frac{1}{1+\tau_{\text{CR}}/\tau_{\text{BCR}}}\right), \quad (1)$$

where τ_{IgG} is the average IgG-Ag bond lifetime, τ_{CR} is the CR-complement bond lifetime, and τ_{BCR} is the BCR-Ag bond lifetime, see Supplemental Materials (SM) for derivations. The first term in Eq. (1) arises from the competition between the BCR and IgG for binding to the antigen (i.e., epitope masking), while the second term captures the relative binding strengths of the BCR and CR. A contour plot of Eq. (1) is shown in Fig. 2b, with contours at 5%, 50%, and 95% probability for the B cell to extract the Ag. The shaded area is the region where the gradient in probability with respect to τ_{BCR} is the strongest. Note that when the IgG is weak, the competition for Ag extraction is entirely between the BCR and the CR; however, when the IgG is stronger than the CR, the main limit to Ag extraction is whether or not the BCR is bound to the Ag in the first place. This model is admittedly based on a highly simplified picture of Ag extraction (see Discussion); yet, the qualitatively important feature is the presence of the elbow in Fig. 2b, which will be present in any model for epitope masking because there must be a crossover between the relative difficulty for the BCR to overcome tethering versus masking.

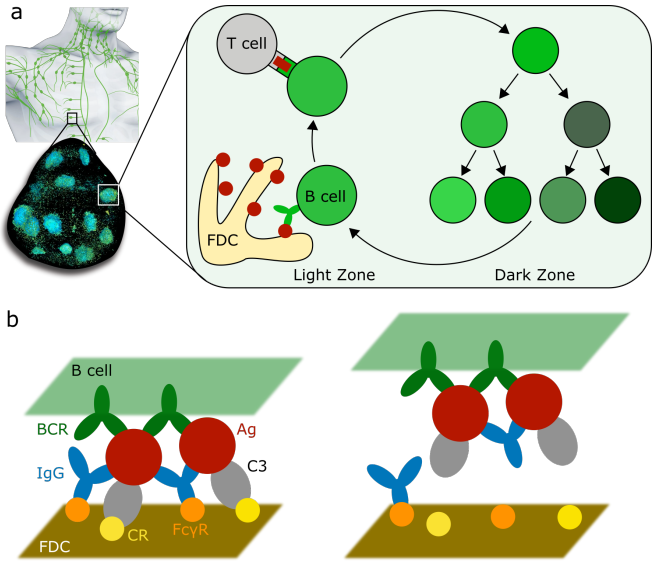

FIG. 1. **The germinal center reaction and extraction of immune complexes.** (a) Left: schematic of the human lymphatic system with a zoom-in confocal microscopy image of a single lymph node (adapted from [33]). Individual GCs are labeled by expression of activation-induced cytidine deaminase (AID). Right: basic steps of a GC reaction include the B cell extracting Ag (red) with its BCR from the surface of an FDC, presenting pMHC to a T_{FH}, then migrating to the DZ to undergo proliferation and somatic hypermutation (represented by color change). (b) Schematic of the B cell-FDC junction. The IC consists of Ag (red), Abs (e.g. IgG, blue) and complement proteins (e.g. C3, gray). The B cell binds directly to the Ag (red) via its BCR (green). The FDC binds to the IC via CRs (yellow) and FcγRs (orange). The B cell attempts to extract Ag by physically retracting its cell membrane. (Schematic of lymphic system in (a) adapted from Stocktreck Images)

The solid red curve in Fig. 2b is a qualitative sketch of the typical immunological trajectory in the space of IgG and BCR affinities for the subgroup of B cells targeting the primary epitope. Initially, the IC IgGs are very weak, but the BCR and IgG affinities progressively increase throughout the immune response. Figure 2c shows qualitative sketches of the population (expressed as a fraction of the GC) and affinity (expressed in terms of τ_{BCR}) of the primary epitope-targeting B cells as functions of time. Notably, the affinity increases indefinitely in this idealized scenario. This follows because in the presence of a single epitope, epitope masking continuously challenges B cells to create higher and higher affinity recep-

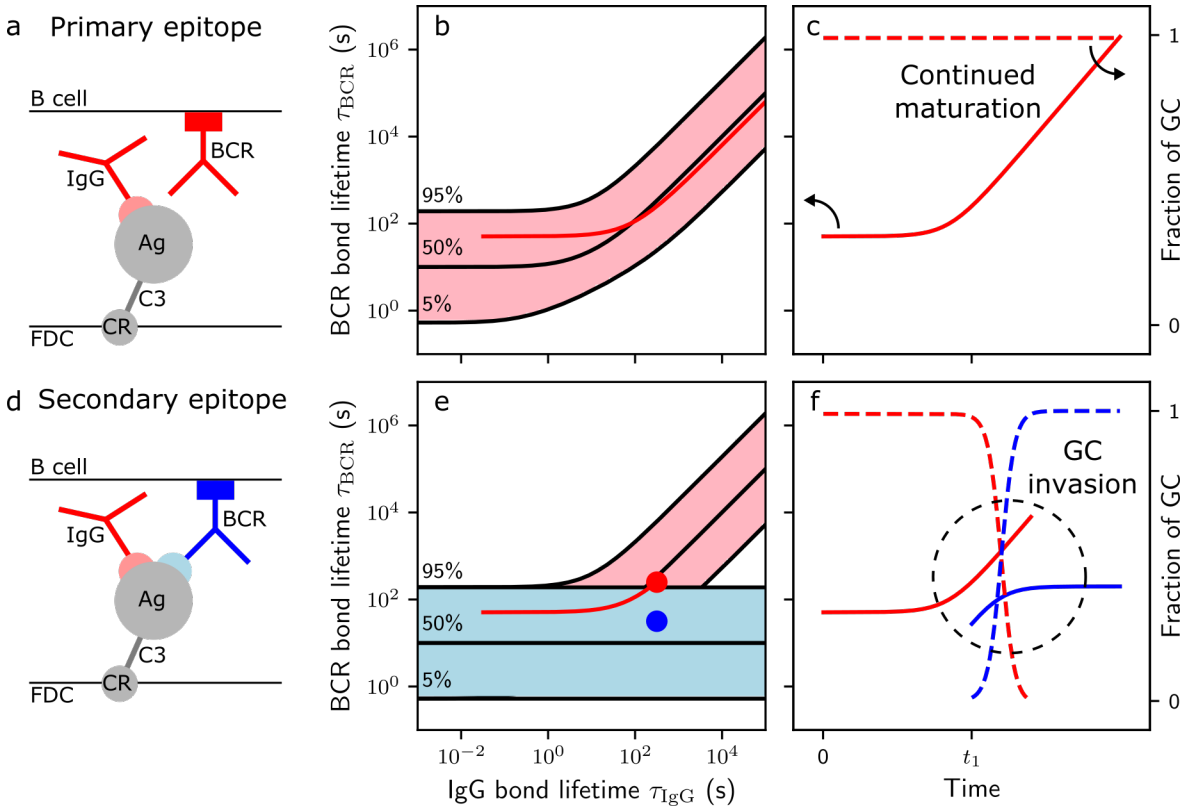

FIG. 2. **Without FcγR, epitope masking on multi-epitope antigens limits affinity maturation.** (a) Junction between a B cell and an FDC lacking FcγR. In this panel, the Ag only has single epitope (red dot). The competition between the BCR and the ambient IgG for the same epitope reduces the probability that the BCR binds. (b) Contour plot (level curves at 5%, 50%, and 95%) of the probability that the B cell extracts the Ag, see Eq. (1). The light red filling indicates the region where the gradient is strongest, enabling hill climbing of B cell affinity. The solid red curve is a qualitative sketch of the evolution of the strongest bond lifetimes of the IgGs and of the BCRs from the primary epitope-targeting B cells as parameterized by time. (c) Corresponding schematic sketches of the fraction of the GC population comprising the primary epitope-targeting B cells (dashed line), along with their maximum affinity (solid curve) expressed in terms of bond lifetime. (d) Schematic of the B cell-FDC junction when the Ag has a second, independent epitope (blue dot). (e) Superimposed contour plots of the Ag-extraction probability for BCRs specific for the primary (red) and secondary (blue) epitope are plotted as a function of the bond lifetime of the IgG specific for the primary epitope, see Eq. (2). The highlighted red and blue points indicate that B cells targeting a secondary epitope with lower affinity (blue), can have a higher extraction probability than those targeting the primary epitope with higher affinity (red), whose ability to extract Ag is limited by epitope masking. (f) Resulting time trace of the population fraction (dashed curves) and epitope affinity (solid curves) of the primary (red) and secondary (blue) epitope-targeting B cells. The secondary-epitope targeters, arriving at time t_1, can acquire Ag more effectively than the existing primary targeters due to epitope masking. The contour plots in b,e are produced with $\tau_{CR} = 10$ s.

tors to bind to the Ag. This scenario is consistent with Ref. [15], which suggests an effective comoving equilibrium between soluble IgG and GC BCR affinity.

However, the implications of masking are more subtle if the Ag has additional epitopes, as shown in Fig. 2d for two epitopes. In the illustrated case that the IgG for the primary epitope (red dots) does not block binding to the secondary epitope (blue dot), the probability of extraction via the secondary epitope is

$$P_{\text{secondary}} = \frac{1}{1 + \tau_{CR}/\tau_{BCR}}, \tag{2}$$

which is independent of the bond lifetime (τ_{IgG}) of the IC IgG targeting the primary epitope. As shown in Fig. 2e, once the IgGs produced by the primary-epitope B cells exceeds the strength of the CR, the following situation arises: B cells targeting the secondary epitope can have a weaker affinity than those targeting the primary epitope, but because the primary targeters must also compete with their own IgGs, the primary targeters have a lower probability of extracting Ag. Thus, despite having lower affinity, the secondary epitope-targeting B cells will outcompete the primary epitope-targeting B cells. This takeover is depicted qualitatively in Fig. 2f. Consequently, epitope masking stalls affinity maturation and instead drives epitope diversification.

FcγR enables continued affinity maturation. To enable continued affinity maturation in the typical case

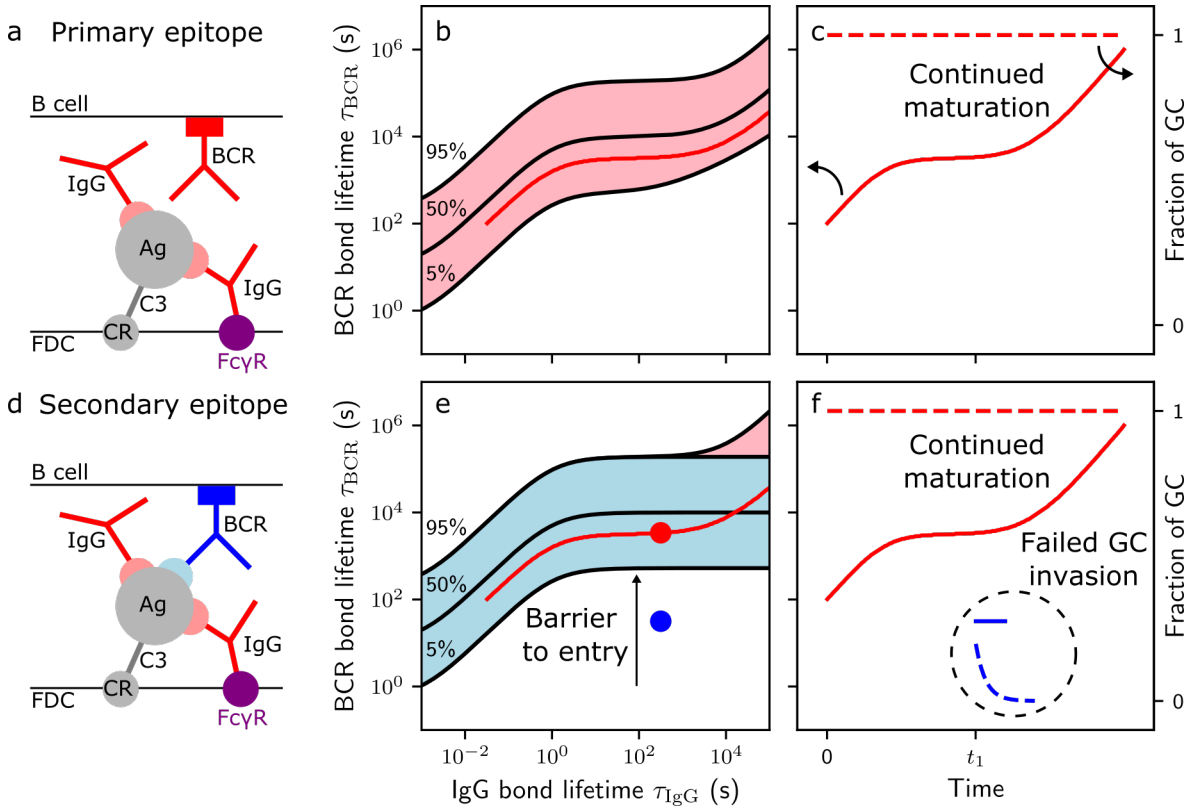

FIG. 3. **The FcγR on the FDC protects affinity maturation of dominant B cell lineages.** (a) Schematic of an IC anchored to an FDC via both a CR-C3 bond and an IgG-FcγR bond. (b) Contour map of the resulting probability of Ag extraction, *cf.* Fig. 2b. (c) Schematic time traces of the primary epitope-targeting B cells population and affinity in the presence of a single epitope. (d) Schematic as in panel (a), but now with a secondary epitope. (e) Extraction probability landscapes for the primary (red) and secondary (blue) epitopes. Unlike Fig. 2e, the extraction probability landscapes for both the primary and secondary epitopes experience an increasing ramp with the primary IgG bond lifetime. This limits the ability of low-affinity B cells targeting alternative epitopes to compete in the GC, thereby protecting B cells cognate to primary epitopes from competition. (f) Schematic time trace of the population dynamics showing the failure of a secondary B cell lineage to compete in the GC. The blue dashed curve is shown on an expanded scale for visibility. Parameters for the contour plots in b,e are $\tau_{\rm off} = 10^{-3}$ s, $\tau_{\rm CR} = 10$ s, and $\tau_{\rm Fc\gamma R} = 1$ s.

when Ags possess multiple epitopes, a mechanism is required such that both the primary and secondary epitope-targeting B cells experience an increasing barrier to extracting Ag as the ambient IgG affinity increases. We propose that expression of the FcγR on the FDC may provide such a mechanism. At first glance, it may seem counterintuitive that FcγR could play any role at all in the mechanics of extraction—its affinity for Fcγ ($10^4 - 10^7$ M^{-1}) [30, 31] is orders of magnitude weaker than that of CR1 or 2 for complement ($10^8 - 10^9$ M^{-1}) [23, 24, 32]. Yet, a closer biophysical examination suggests that the effect of FcγR binding to ICs can be quite dramatic. Consider the case shown schematically in Fig. 3a,d where an Ag is bound to an FDC by both a CR bond and an FcγR bond. For the B cell to extract the Ag from the FDC, both the CR bond and the FcγR bond must simultaneously break. However, at relevant temperatures, the bonds will reform on a timescale $\tau_{\rm off} \lesssim 10^{-3}$ s (see SM) that is much shorter than either the CR lifetime $\tau_{\rm CR} \sim 10$ s [32] or the FcγR lifetime $\tau_{\rm Fc\gamma R} \sim 1$ s [31]. Hence, the redundancy of having a second bond, even if weak, dramatically decreases the rate of detachment, because simultaneous breakage is rare.

For a concrete illustration, consider the minimal ICs in Fig. 3a,d. In terms of binding rates, the probability of Ag extraction via the secondary epitope is

$$P_{\rm secondary,Fc\gamma R} = \frac{1}{1 + \tau_*/\tau_{\rm BCR}}, \tag{3}$$

where

$$\tau_* = \tau_{\rm CR} \frac{1 + \tau_{\rm IgG}/\tau_{\rm off}}{1 + \tau_{\rm IgG}/\tau_{\rm Fc\gamma R}}, \tag{4}$$

and $\tau_{\rm Fc\gamma R}$ is the average lifetime of the FcγR bond. For the primary epitope, there is an additional factor associated with masking:

$$P_{\rm primary,Fc\gamma R} = \left(\frac{1}{1 + \tau_{\rm IgG}/\tau_{\rm BCR}}\right) P_{\rm secondary,Fc\gamma R}. \tag{5}$$

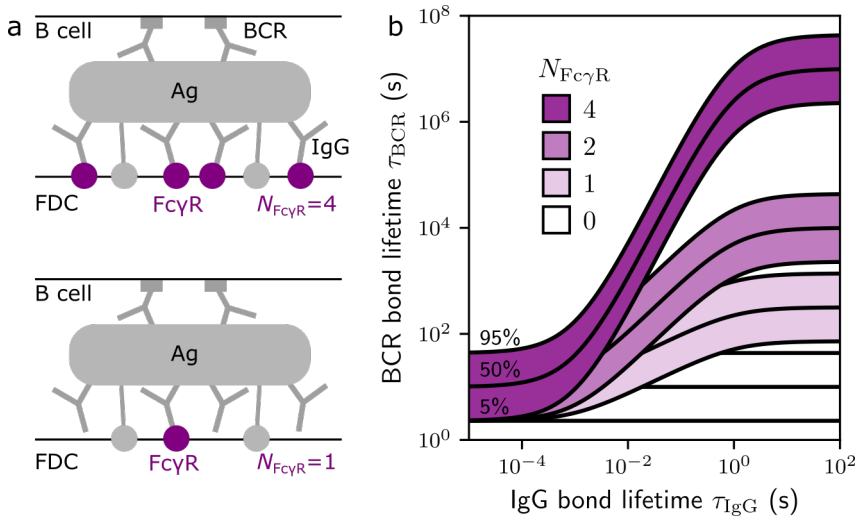

FIG. 4. **FcγR expression steers competition landscape of GC B cells.** (a) Schematics of FDCs with high ($N_{\mathrm{Fc\gamma R}} = 4$) and low ($N_{\mathrm{Fc\gamma R}} = 1$) expression of FcγR. (b) Ag-extraction probability landscape as a function of the number of FcγR bonds, see Eqs. (6-7). Higher expression of $N_{\mathrm{Fc\gamma R}}$ results in a higher upper plateau of the purple contours. This corresponds to a higher barrier for B cells, particularly those weakly binding to alternative epitopes, to extract Ag. Parameters are $N_{\mathrm{CR}} = N_{\mathrm{BCR}} = 2$, $\tau_{\mathrm{off}} = 10^{-3}$ s, $\tau_{\mathrm{CR}} = 10$ s, and $\tau_{\mathrm{Fc\gamma R}} = 1$ s.

The two landscapes in Eqs. (3,5) are shown in Fig. 3e,b, respectively. The key qualitative feature is that the FcγR bond increases the extraction threshold for both the primary and secondary epitope-targeting B cells. This increase is felt by both epitope subgroups because the FcγR, unlike masking, does not affect the ability of a specific BCR to bind to the Ag, but rather affects the way the Ag binds to the FDC. Interestingly, if only a single epitope is present, the effect of FcγR on the affinity maturation dynamics is qualitatively unnoticeable: the single B cell lineage increases affinity monotonically in Fig. 3c, just as in Fig. 2c. However, when a second epitope is available, FcγR has a dramatic qualitative effect: BCRs with weak affinity to secondary epitopes are no longer able to extract Ag and compete (see Fig. 3e). Invasion by these B cells is avoided because the extraction probability landscape comoves for both the primary and secondary epitopes. This analysis suggests that FcγR may act to suppress epitope diversification and allow primary epitope-targeting lineages to continue affinity maturation.

Cells often regulate receptor expression to control behavior. This raises the question of how the number of receptors bonded to an IC influence the probability of extraction by a B cell. Consider the IC in Fig. 4a, which is bound to the B cell by N_{BCR} BCRs, and to the FDC by N_{CR} CRs and $N_{\mathrm{Fc\gamma R}}$ FcγRs. In the limit that $\tau_{\mathrm{off}} \ll \tau_{\mathrm{Fc\gamma R}}, \tau_{\mathrm{CR}}$, the probability that a given B cell extracts an Ag is given by:

$$P_{\mathrm{extract}} = \frac{1}{1 + (\tau_*/\tau_{\mathrm{BCR}})^{N_{\mathrm{BCR}}}}, \tag{6}$$

which is a Hill function with crossover τ_* and cooperativity N_{BCR}. The crossover τ_* depends on the number and type of the receptors bound to the FDC, as well as on the affinity of the IgGs:

$$\tau_* \approx \tau_{\mathrm{off}} \left(\frac{\tau_{\mathrm{CR}}}{\tau_{\mathrm{off}}}\right)^{\frac{N_{\mathrm{CR}}}{N_{\mathrm{BCR}}}} \left(\frac{1 + \tau_{\mathrm{IgG}}/\tau_{\mathrm{off}}}{1 + \tau_{\mathrm{IgG}}/\tau_{\mathrm{Fc\gamma R}}}\right)^{\frac{N_{\mathrm{Fc\gamma R}}}{N_{\mathrm{BCR}}}}. \tag{7}$$

As illustrated by the exponents in Eq. (7), the numbers of bonds of each type exponentially alters the value of τ_{BCR} required to extract Ag.

Fig. 4b illustrates the probability of Ag extraction for different numbers of FcγR bonds. A larger number of FcγR bonds makes it more difficult for low-affinity B cells (targeting any epitope) to extract Ag. This prevents low-affinity B cells that target alternative epitopes from outcompeting higher-affinity B cells that target the primary epitope and so face the obstacle of masking. Reducing the number of FcγR bonds may remove the Ag-extraction barriers for B cells targeting alternative epitopes, allowing them to compete in and potentially take over the GC. Because the FDC can regulate the expression of FcγR, this raises an intriguing possibility that FDCs may actively influence the resulting affinity and epitope diversity of the GC.

Discussion

In brief, we propose a biophysical picture in which the two classes of Ag tethering proteins on FDCs collaborate to regulate the B cell epitope diversity generated by the GC reaction. An implication of this model is that in the absence of FcγR on FDCs, GCs should generate relatively low affinity but highly diverse B cell repertoires.

This is consistent with experiments [34] in which mice lacking FcγRs on FDCs show reduced affinity but increased diversity. Interestingly, the magnitude of the effect in these experiments was Ag-dependent. Based on our results (cf. Eq. (7)), additional investigation into the relative Ab and complement valency of the Ags would be informative.

These FcγR knockout experiments could be complemented by additional, more nuanced, probes. For instance, consider immunization with an Ag featuring two well-separated epitopes, E_1 and E_2. If IgG with high-affinity for E_1 is administered before and during immunization, FcγR bonds will suppress the immune response for both E_1 and E_2. Suppose that instead of IgG, one administers $(\text{Fab})_2$ which lacks an Fcγ tail. In this case, our mechanism would imply that development of Abs for E_1 may be suppressed due to masking by the external $(\text{Fab})_2$, but proper affinity maturation for E_2 should proceed. Versions of this experiment could also be done with other non-IgG immunoglobulins.

In addition to their mechanical roles in Ag extraction, other functional roles for CRs and FcγRs on FDCs have been demonstrated or proposed. The expression level of CRs on FDCs is relatively constant over time, and these receptors have been implicated in defining the spatial organization of the LZ [32], retaining Ag for long periods of time [4], and receiving Ag from noncognate B cell escorts [35]. The precise roles of FcγR are a subject of debate. FcγR is also expressed by GC B cells and has an inhibitory effect in that context. It has therefore been suggested that one role of the FcγR on FDCs is to sequester Fc tails away from B cells, thereby making ICs on the surface of FDCs more immunopotent [28]. Moreover, FcγR expressed on FDCs appears to support FDC activation, including chemokyne release and further upregulation of FcγR [29]. More generally, the mechanics of Ag extraction considered here needs to be disentangled from several other factors influencing B cell selection, including time-evolving follicular helper and regulatory T cell populations [36–38], changes in Ag levels [39], and re-entrance of circulating memory B cells [40].

One can envision elaborations to our simplified biophysical model. For tractability, we consider an unforced disassociation scenario. When extracting Ag, GC B cells form a specialized immunological synapse in which they exert pico-Newtons of force on localized contacts [6, 9]. It has been proposed, for example, that the amount of force applied is tuned to optimize affinity discrimination [12]. Additional features worth investigating are that complement can bind to Abs in addition to Ag, the polymeric nature of the ICs [18], the possibility that whole sections of the FDC membrane are extracted [8], and the role of receptor crowding and receptor height [41]. Another potentially relevant ingredient is class-switching: initially, the majority of IC Abs are of the IgM subtype, and only later are IgGs produced, which can engage FcγRs. Class switching may result in a relatively abrupt rather than continuous change in the Ag extraction landscape. Even in light of these more complex considerations, we expect our qualitative arguments to still be relevant. The crucial ingredient is that FcγR uses IgG as a dynamic, indirect link to the IC. After the early stages of the GC, IgGs' engagement to FcγRs increases the difficulty of extracting Ag for all B cells, regardless of epitope specificity. This limits the competitiveness of weaker B cells that target secondary epitopes, even though these might otherwise have an advantage due to masking of the dominant epitope(s).

Finally, our model suggests an intriguing possibility: FDCs could dynamically regulate the affinity-diversity trade-off over time. While Ab feedback is to a large extent unavoidable, it is plausible that evolution may have found ways to actively steer its influence. Unlike the CRs, whose expression is roughly constant over time, FcγR is upgregulated roughly 50-fold upon FDC activation [28]. This early upregulation may initially focus the GC reaction on nurturing one immunodominant clone. A later putative downregulation would then allow epitope diversification over longer time scales. A crossover from affinity increase early in the GC reaction to increased diversity later is indeed generally observed; however, direct connection to temporal modulation of FcγR expression on FDCs has not, to our knowledge, been systematically studied. We hope our work inspires further investigation into the biophysical mechanisms regulating affinity and diversity in the GC reaction.

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