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Control systems and decision making for antibody production

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This paper synthesizes recent progress toward understanding the integrated control systems and fail-safes that guide the quality and quantity of antibody produced by B cells. We focus on four key decisions: (1) the choice between proliferation or death in perifollicular B cells in the first 3 days after antigen encounter; (2) differentiation of proliferating perifollicular B cells into extrafollicular plasma cells or germinal center B cells; (3) positive selection of B cell antigen receptor (BCR) affinity for foreign antigen versus negative selection of BCR affinity for self antigen in germinal center B cells; and (4) survival versus death of antibody-secreting plasma cells. Understanding the engineering of these control systems represents a challenging future step for treating disorders of antibody production in autoimmunity, allergy and immunodeficiency.

The quality and quantity of antibodies that circulate in our body are the end result of a control system that is much more elaborate than could have been imagined by the first students of humoral immunity. Beginning a century ago, Ehrlich and colleagues recognized that the system had a "horror autotoxicus" for avoiding antibody production against self antigens and that antibody affinity and isotype against foreign antigens matured over time and with repeated exposure[1]. The challenge since has been to understand how the right kinds and amounts of antibody are made.

Early concepts to explain the above characteristics were elegantly simple and remain at the core of our current understanding. A binary cell decision switch was conceived by Burnet[2]: antigen binds to the BCR and triggers B lymphocytes to undergo either clonal deletion (cell death) to prevent autoantibody production or clonal proliferation followed by a program of differentiation yielding antibody-secreting plasma cells and quiescent memory B cells. Burnet and Lederberg[3] envisaged these alternative responses as programmed in newly formed versus mature B cells, respectively, so that no B cell would ever 'decide' between two fates at a single point in its development.

The current understanding of these growth–death decisions is that they in fact take place at each step of B cell development and are not automatic and programmed but instead integrate diverse inputs[4]. The simplest concept involves the integration of two signals[5]. Antigen binding to the BCR delivers signal 1 and triggers B cell paralysis (death or anergy) unless the bound antigen can also be recognized by a T cell to deliver signal 2 that diverts the B cell from paralysis to proliferation. The T cell antigen receptor (TCR)-induced membrane protein CD40 ligand (CD40L) is the most important T cell–derived signal 2 and activates CD40 on B cells to signal their survival and proliferation[6]. Alternatively, immunogenic antigens can include a second moiety such as lipopolysaccharide (LPS) or other ligands for Toll-like receptors (TLRs) that directly deliver signal 2 (ref. 7). Signal integration in this way exemplifies a fundamental principle for fail-safe engineering: two different keys or codes must be congruently received before a potentially damaging response can be executed, and when one of the codes is not supplied the system defaults to a 'safe' mode. Many more than two inputs are used *in vivo*, as discussed below.

Decision 1: activation of B cell proliferation

B cells and T cells are organized within secondary lymphoid organs (lymph nodes, spleen, Peyer's patches, tonsils) into follicles and T cell zones, respectively, on the basis of differential migration toward chemoattractants[8] (**Fig. 1**). B cells express CXCR5, a G protein–coupled receptor that mediates chemotaxis toward the follicular stroma- and follicular dendritic cell (FDC)-derived chemokine CXCL13. They express CCR7, a receptor for chemokines CCL19 and CCL21 made by stroma in the T cell zone but at lower levels than do T cells. By contrast, unstimulated T cells have a complementary phenotype ($CXCR5^{lo}CCR7^{hi}$) ensuring that these two lymphocyte subsets patrol different zones during their 1-day stopovers in secondary lymphoid organs as they circulate through the body.

Antigen engagement by the BCR signals, within 1–6 hours, a series of changes in gene expression that move the cell from G0 to G1 phases of the cell cycle and facilitate antigen presentation to T cells[9]. Increased CCR7 expression signals movement of antigen-binding B cells to the follicle–T cell zone border[10–12]. Induction of CD86 on the B cell provides ligands for CD28 on the interacting T cells, enhancing T cell synthesis of prosurvival cytokines that protect the B cell from apoptosis induced by Fas ligand (FasL) on the T cell, and instead FasL delivers a weak proliferative signal[13,14]. In self-reactive B cells, continuous antigen exposure depresses CD86 induction and decreases CXCR5 expression, so that they die at the follicle–T zone junction[15–17].

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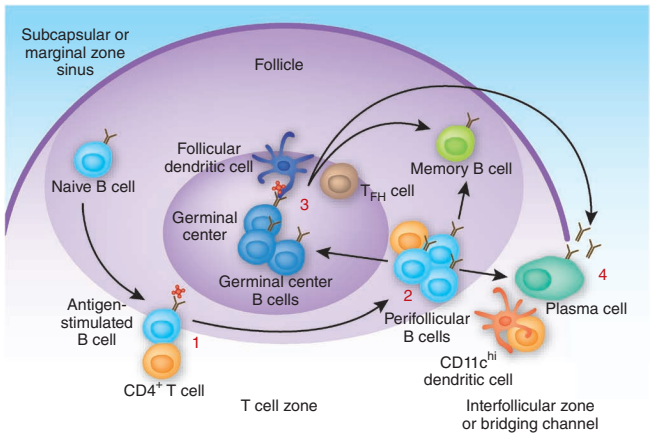

Figure 1 Key B cell decisions after antigen exposure. (1) Within hours, binding of antigen to the BCR triggers B cell movement to the interface between the follicle and T cell zone, where B cells present antigen peptides to $CD4^+$ T cells. At this point the B cells (and T cells) integrate inputs to decide between death or proliferation. During the next 2–3 days, the B cells migrate actively, form repeated stable conjugates with $CD4^+$ T cells and proliferate at the periphery of the follicle, particularly near the interfollicular zones and bridging channels rich in $CD11c^{hi}$ dendritic cells. (2) Three to four days after antigen exposure, the B cells integrate inputs to decide between differentiation into plasma cells, GC B cells or memory B cells. GC B cells continue dividing rapidly, hypermutate their BCR V-regions and interact with follicular dendritic cells bearing antigen and T follicular helper cells (T_{FH} cells). (3) GC B cells integrate inputs to decide between survival or death depending on their BCR affinity for foreign and self antigens. (4) Plasma cells integrate inputs to decide between survival or death to determine the quantity and duration of antibody in the circulation.

B cell proliferation begins within 1–2 days at the T zone–follicle junction with the activated B cells redistributing in a perifollicular pattern, concentrating particularly near the subcapsular interfollicular areas of lymph nodes or bridging channels in spleen[10,16,18,19] (**Fig. 1**). These sites are particularly rich in mature, $CD11c^{hi}$ dendritic cells[20,21] that can present antigen to B cells[22,23] and provide BAFF or APRIL survival signals for B cells and plasma cells (see below). At this stage, activated B cells are highly motile while making sequential conjugates with antigen-recognizing $CD4^+$ T cells lasting 10–40 min (ref. 12). The cumulative effects of signals received by the B cell during the 1.5-day lead-up to the first cell division can shape the fate of progeny B cells many divisions later[24,25].

An orphan G protein–coupled receptor, Epstein-Barr virus–induced molecule 2 (EBI-2, or GPR183), redistributes activated B cells to the perifollicular regions and dendritic cell–rich interfollicular zones and bridging channels[26,27]. EBI-2 expression increases after BCR stimulation by antigen in an NF-κB-dependent manner that is abolished in self-reactive B cells[9]. In response to antigen and T cell help, EBI-2-deficient B cells fail to redistribute to perifollicular regions and bridging channels and instead localize at the center and base of follicles near the FDC networks[26,27]. The chemoattractive ligand for EBI-2 is unknown.

Decision 2: three alternative differentiation fates

Beginning 3 days after antigen exposure and T cell help, proliferating B cells begin to follow one of three alternate fates by differentiating into extrafollicular plasma cells, germinal center (GC) B cells or recirculating early memory B cells that have not yet isotype-switched or undergone hypermutation[18]. B cells carrying identical BCRs can proceed down any of the three differentiation pathways[18,28], indicating that their destiny is not predetermined.

Changes in chemoattractant receptors channel B cells along these alternate fates. Plasma cells retain EBI-2, lose CXCR5 and express high levels of CXCR4—a receptor for CXCL12, which is made by many stromal cells—whereas GC B cells downregulate EBI-2 but retain CXCR5 and high CXCR4 (refs. 18,26,27,29). The effects of these receptors on cell positioning influences survival and proliferation of differentiated progeny. EBI-2 is needed for extrafollicular migration, and its genetic deficiency diminishes the numbers of extrafollicular plasma cells but not GC cells, whereas retroviral expression of EBI-2 to prevent its downregulation on GC B cells diminishes their numbers while increasing plasma cells[26,27]. Correct migration of differentiating GC B cells will increase their exposure to survival and/or growth signals from FDCs, whereas migration toward $CD11c^+$ dendritic cells within the bridging channels will enhance delivery of survival signals for differentiating plasma cells.

Differences in transcription factor expression underpin the plasma cell–GC cell decision. Plasma cell differentiation depends upon high expression of Blimp-1 and interferon regulatory factor 4 (IRF4), whereas GC differentiation depends upon high expression of Bcl-6. Blimp-1 and Bcl-6 repress one another, providing a bistable state[30–35], while IRF4 also represses Bcl-6 (ref. 36). This transcriptional switch directly determines B cell tissue positioning. Bcl-6 represses EBI-2 (ref. 31) for correct positioning of GC cells, whereas Blimp-1 represses CXCR5 (ref. 30) and induces CXCR4 (ref. 37) for correct positioning of plasma cells.

Is the decision between Blimp-1 and Bcl-6 intrinsically programmed, stochastic or induced by particular inputs? Plasma cell differentiation seems to be the default fate, as it can be triggered by a wide range of stimuli through a probabilistic process linked to the number of cell divisions[38,39]. It is augmented by T cell–derived cytokines that signal through the transcription factor STAT3 to promote Blimp-1 expression, including IL-2, IL-6 and IL-21 (refs. 33,40,41) and opposed by continuous antigen exposure in self-reactive B cells[42].

By contrast, GC differentiation is difficult to induce *in vitro*. B cells stimulated by CD40L and the T cell–derived cytokine IL-21 can be diverted from a plasma cell fate into continuously proliferating GC cells in culture if they are forced to express Bcl-6 combined with forced expression of the antiapoptotic protein Bcl-x_L (ref. 43) or with deficiency of the proapoptotic protein p53 (ref. 44). Bcl-x_L is expressed in GC B cells in the apical zone and is induced by CD40 (refs. 44,45), but the physiological signals for inducing a combination of Bcl-6 and Bcl-x_L and suppressing p53 are unclear, as strong BCR and CD40 signaling represses Bcl-6 through NF-κB-mediated induction of IRF4 (ref. 36). TLR signaling is also a potent inducer of NF-κB and IRF4, and inhibition of Bcl-6 by this pathway might explain the suppression of GCs during *in vivo* B cell responses to particles or bacteria bearing TLR ligands[46,47].

The quality and duration of B cell interactions with $CD4^+$ T cells seems to be a critical determinant for the decision to differentiate into GC cells. Deficiency of SAP (signaling lymphocytic activation molecule–associated protein), encoded by the *SH2D1A* gene mutated in X-linked lymphoproliferative disease[48], shortens the duration of B–T cell conjugates in the first 2 days after antigen exposure and profoundly decreases GC B cell numbers despite little loss of extrafollicular plasma cells[49–51]. Two homotypic adhesion receptors that require SAP in T cells to function, CD84 and Ly108, cooperate to form these durable cell conjugates[52]. SAP-deficient $CD4^+$ T cells fail to differentiate[49–51] into a distinct transcriptional state of $CXCR5^{hi}$ T follicular helper (T_{FH}) cells that reside in GCs[53]. Like GC B cells,

T_{FH} cells also depend on Bcl-6 expression[54–56]. T_{FH} cells make abundant IL-21, and IL-21 acts on GC B cells to sustain normal Bcl-6 expression, proliferation and persistence[57–60]. T_{FH} cells also make the cytokine IL-4, and combined deficiency of IL-21 receptor and IL-4 prevents normal GCs from forming[57].

Another key issue is how BCR affinity for antigen affects the decision between plasma cell or GC cell differentiation. Several *in vivo* studies have compared the fate of B cells responding to antigen bound with differing affinities. In one set of studies, there was little difference between low- and intermediate-affinity B cells in the initial rate of B cell proliferation and accumulation of GC B cells or plasmablasts during the first 4 days, but antigen recognized with low affinity supported less accumulation and persistence of IgG-switched extrafollicular plasma cells[18]. In another study, low-affinity B cells accumulated as GC cells unless they competed with B cells binding the same antigen with higher affinity, in which case they were outcompeted[61]. Lesser accumulation of low-affinity GC B cells was found in a third study to be due to equivalent proliferation but increased apoptosis[62]. Consistent with the latter, enforced Bcl-x_L expression allows low-affinity B cells to persist in the post-GC antibody response[45]. Thus, BCR affinity for antigen seems to have less effect on the initial steps of B cell activation or differentiation *in vivo* but profound selective effects for survival of the plasma cell or GC progeny. Selection by differences in survival rates rather than in growth rates maximizes diversity of the antibody response[62].

Independent from the plasma cell or GC cell decision, isotype switching from IgM to IgG occurs during the perifollicular B cell proliferation phase, yielding isotype-switched plasma cells and early memory cells[18,21]. Switching is probabilistically linked to the number of cell divisions[39] and requires intermediate levels of IRF4 that induce activation-induced cytidine deaminase (AID)[34,35]. Switching to IgG does not alter the initial phase of perifollicular B cell proliferation, but the extended cytoplasmic tail of the IgG BCR markedly enhances extrafollicular plasma cell accumulation[63]. An important unresolved issue is whether the increased plasma cell accumulation promoted by the IgG BCR tail and by higher BCR affinity[18] results from antigen recognition by plasmablasts or from signals that have been 'stored up' before the first cell division[24,25].

Decision 3: antibody selection in germinal centers

Perhaps the most enigmatic, yet important, step in antibody selection occurs after differentiation into GC B cells. Point mutations in antibody V-regions are induced in GC cells, and those that improve affinity for the inciting foreign antigen are positively selected to dominate antibody production over time. The control system must also deal with the fact that V-region mutations create or increase antibody affinity for self antigens to produce autoantibodies that are pathogenic if unchecked[64]. These autoantibodies are normally absent from high-affinity antibody responses even after immunization with foreign antigens that closely resemble self antigens[65]. Understanding antibody selection in GC B cells must therefore explain how, at a single stage of B cell differentiation, binding to foreign antigen promotes positive affinity selection, whereas binding to self antigen elicits negative affinity selection.

The evidence that GC B cells are positively affinity selected by preferential survival rates was summarized above[45,62]. Foreign antigens are rapidly transported to the meshwork of FDCs within follicles, where they are displayed for months in intimate contact with GC B cells[66]. Secreted antibody, including natural pre-existing antibody present at the beginning of the response[67], positively affects this process in two ways: antibody bound to antigen both enhances its localization to the FDCs by means of complement receptor CR2 and the IgG Fc region receptor FcγR2 (refs. 67,68) (**Fig. 2**) and, through FcγR2, stimulates FDCs to express integrin ligands for GC B cells[69]. Recent progress has elegantly documented the cellular relay systems that bring antigen to FDCs and the dynamic migration of GC and follicular

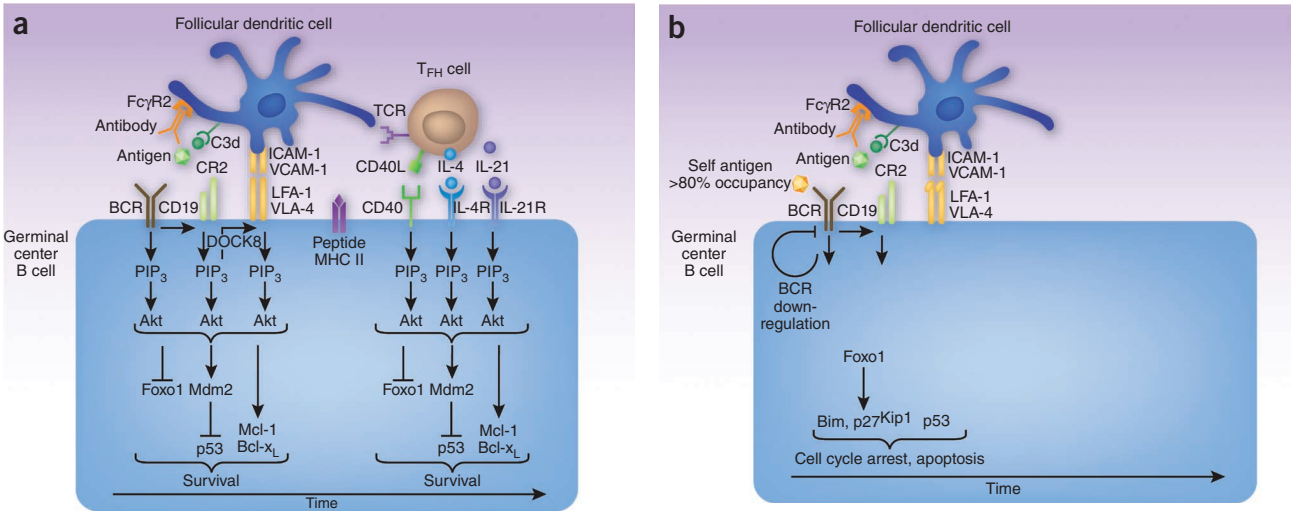

Figure 2 Proposed mechanism for concurrent positive and negative affinity selection of GC B cells. (**a**) Positive affinity selection. Foreign antigen is displayed in small amounts on FDCs bound with complement C3d or as immune complexes bound to FcγR2. A small percentage of the high-affinity BCRs on the GC B cell bind antigen and activate PI(3)K—directly and through CD19, the latter augmented by complement receptor 2 (CR2) binding to C3d. PIP_3 recruits DOCK8 for inside-out signaling, increasing avidity of integrins LFA-1 and VLA-4 for ICAM-1 and VCAM-1. Integrin outside-in signaling cooperates with BCR and CD19 signaling to sustain PIP_3 production and Akt activation, preventing accumulation of the proapoptotic and antiproliferative proteins p53, Bim and $p27^{Kip1}$; inducing expression of the antiapoptotic proteins Mcl-1 and Bcl-x_L; and promoting short-term GC B cell survival. Signaling will be cut short if antigen is displaced from the BCR by competing antibodies or other B cells, or by mutations in CD19 or DOCK8. Subsequent presentation of peptide–MHC class II to T_{FH} cells elicits CD40L, IL-4 and IL-21, promoting longer-term survival of the GC B cell. (**b**) Negative affinity selection. The majority of BCRs are engaged simultaneously by self antigen, causing intracellular signaling events that downregulate surface BCRs. Without sufficient surface BCRs, PIP_3 production ceases, resulting in the accumulation of growth-arrest and proapoptotic proteins.

B cells through the FDC meshwork, where antigen-specific B cells can be seen tearing off 'packets' of antigen and FDC membrane[70].

The cellular basis for negative affinity selection has been experimentally modeled by eliciting GCs against a defined foreign antigen and tracking the fate of the GC B cells when they acutely encounter a cross-reacting antigen that has been introduced into the circulation in tolerogenic form to mimic a normal self protein[71–73]. The antigen-reactive GC B cells undergo apoptosis within 4–8 h of encountering the tolerogenic antigen, preceded by a marked downregulation of surface BCRs and by movement of the GC B cells away from the apical light zone where FDC networks are concentrated and toward the basal part of the GC and T cell zones[71]. Apoptosis and elimination of the GC B cells is partly inhibited by a Bcl-2 transgene, but not by diminished Fas expression in *lpr* mice, and occurs regardless of whether the soluble antigen can or cannot be recognized by the resident T_{FH} cells. In unpublished studies (C.C.G. and R.B.), we have extended this approach to track negative affinity selection of GC B cells when the tolerogenic antigen is constitutively expressed as a self antigen. The presence of the cross-reacting self antigen did not inhibit activation and differentiation into GC B cells, but it selectively decreased the accumulation of GC B cells and markedly decreased their surface BCR levels. A similar selective decrease in the accumulation of GC B cells has been shown to result from an arginine mutation in the V-region of hapten-specific B cells that confers cross-reactivity with self DNA[74]. These experiments illuminate an efficient negative affinity selection mechanism for eliminating self-reactive antibodies that arise during somatic hypermutation in GCs.

How do B cells choose between death and survival in the GC depending on affinity for foreign or self antigens? Extending Bretscher and Cohn's two-signal concept[5], competition for T_{FH} cells is certain to provide part of the decision algorithm. *In vitro* and *in vivo* imaging studies predict that positive affinity selection could be achieved by translating differences in B cell spreading and capture of membrane-displayed antigens from FDCs into differences in amounts of peptide–major histocompatibility complex (MHC) presented to T_{FH} cells, resulting in varying transmission of survival signals such as CD40L, IL-4 and IL-21 (refs. 75,76). GC B cell persistence needs repeated CD40 stimulation, as indicated by the dissolution of GCs *in vivo* upon injecting a blocking antibody to CD40L[77] and by the need for CD40 restimulation every several days to maintain growth of B cells that have adopted a GC-like state through retroviral expression of Bcl-6 *in vitro*[44]. Differential receipt of signal 2 from T_{FH} cells cannot easily account, however, for the examples above of negative affinity selection of antibodies in which GC death is induced despite the binding of antigens that are recognized by the resident T_{FH} cells. Binding to tolerogenic self antigens, by inducing GC B cell migration away from the apical light zone[71], might starve GC B cells of contact with T_{FH} cells, primarily located there. Evidence that limiting availability of T_{FH} cells is important comes from the observation that pathogenic lupus autoantibodies result from the greatly increased T_{FH} cells within GCs of mice with a mutation in the Roquin T cell regulatory protein[51,78].

The speed of GC B cell apoptosis triggered by soluble tolerogenic antigens (hours)[71–73] is nevertheless much faster than the loss of GC B cells that follows the blocking of CD40L with an injected antibody (days)[77]. As opposed to varying access to signal 2 from T_{FH} cells, concurrent negative and positive affinity selection in the GC might equally be achieved by differences in the quality and dynamics of BCR signal 1. When BCR signaling is diminished in CD45-deficient B cells, they can still differentiate into GC cells, but their persistence is markedly truncated unless rescued by forced expression of Bcl-x_L[79]. A key BCR-elicited signal for GC B cells is phosphatidylinositol-3-OH kinase (PI(3)K), which generates phosphatidylinositol-3,4,5-trisphosphate (PIP_3) on the inner leaflet of the plasma membrane. BCR-induced phosphorylation of the B cell surface protein CD19 creates docking sites for PI(3)K recruitment and activation[80]. When CD19 is absent or the docking sites mutated, extrafollicular plasma cells and GC B cells differentiate, but the latter fail to persist or be positively affinity-selected against foreign antigens[81–83]. Humans with CD19 mutations develop common variable immunodeficiency (CVID), with compromised production of high-affinity antibodies to rabies vaccine and few $CD27^+$ post-GC memory B cells[84]. The defect in GC accumulation in CD19-deficient mice is corrected when combined with B cell deficiency of the phosphatidylinositol-3-phosphatase, PTEN, pinpointing PIP_3 amount as the critical signal for GC B cell persistence[85]. Mutations in the PI(3)K catalytic subunit, PI(3)K-δ, have a similar effect in crippling GC B cell accumulation[86–88].

PIP_3 in the inner membrane serves as a docking site to recruit and activate a plethora of intracellular signaling proteins, including critical initiators of BCR signaling such as Btk and Vav[89,90]. A recently discovered PIP_3-binding domain, the DOCK homology region 1 (DHR1), recruits members of the DOCK family of proteins to plasma membranes and stimulates their GTP-exchange factor activity for Rho GTPases[91]. Mutations in a lymphoid-specific member of this family, DOCK8, mirror the consequences of CD19 deficiency by crippling persistence of GC B cells and IgG antibody responses[92]. Like CD19 mutations, DOCK8 mutations in humans cause a variable deficiency in production of antibodies, although this is accompanied by intrinsic T cell defects that lead to uncontrolled viral skin infections and an unexplained hyper-IgE syndrome[93,94]. When DOCK8-deficient B cells are introduced into immunized wild-type mice that provide normal T cell help, they differentiate into GC B cells and accumulate V-region mutations at a normal rate, but the latter fail to be enriched for affinity-enhancing amino acid replacements as occurs in wild-type controls[92]. DOCK8 thus seems to be an essential mediator of CD19-PI(3)K signaling for GC B cell persistence and positive affinity selection.

What is downstream from DOCK8? DOCK8 is required for recruitment of the integrin LFA-1 around clustered BCRs in the peripheral zone of the immunological synapse formed between B cells and membranes displaying antigen and the LFA-1 ligand, ICAM-1 (ref. 92). This process depends on inside-out activation of LFA-1 to increase its avidity for ICAM-1, initiated by BCR signals through CD19, PI(3)K and Rac2 (refs. 95,96). Other DOCK proteins also promote inside-out activation of integrins[91]. Outside-in signals from integrins that have bound their ligands also activate PI(3)K[97,98]. In T cells, this feed-forward loop between antigen receptor and integrin activation of PI(3)K at the immunological synapse sustains PIP_3 production for many hours[99,100]. LFA-1 and VLA-4 integrins are expressed on GC B cells, and their respective ligands ICAM-1 and VCAM-1 increase on FDCs during GC formation in response to immune complexes binding to FcγR2b[69] and NF-κB signaling within the FDC[101]. When this FDC pathway for ICAM-1 and VCAM-1 induction is selectively crippled, there is poor accumulation and persistence of GC B cells[101]. *In vitro*, ICAM-1 and VCAM-1 promote GC B cell survival[102]. Mutations in CD19 or DOCK8 would therefore be predicted to shorten the duration of PIP_3 production during B cell recognition of antigen displayed on FDCs.

A key mechanism by which sustained PIP_3 production promotes cell survival is by activating the serine-threonine kinase Akt (also called protein kinase B). Akt has pleiotropic effects promoting cell survival and growth, including inhibitory phosphorylation of the proapoptotic protein Bad and inhibitory phosphorylation of Foxo transcription factors[103]. Proteins whose mRNAs are induced by Foxo

transcription factors and repressed by Akt include the proapoptotic protein Bim and the cell cycle inhibitor $p27^{Kip1}$ (ref. 103). Akt also activates MDM2 to degrade p53, thus relieving a critical inhibitor of Bcl-6–induced GC B cell growth[44]. Akt induces expression of the antiapoptotic proteins Bcl-x_L and Mcl-1, which are expressed strongly in GC B cells[104]. Thus, sustained PIP_3 activation of Akt would be expected to promote many of the key features of GC B cells undergoing positive affinity selection.

Bringing together the above evidence leads us to hypothesize an integrated decision system for concurrent positive and negative affinity selection in GC B cells (**Fig. 2**). PIP_3 will be induced for a longer duration in GC cells bearing BCRs with higher affinity for foreign antigens displayed on FDCs or ripped from FDCs, leading to more sustained Akt activation and preferential survival (**Fig. 2**). Competition with secreted antibody, or with other B cells, would abbreviate PIP_3 production in lower-affinity B cells, much as occurs in T cells competing with antibodies to peptide–MHC class II[100]. By interfering with inside-out signaling to integrins, mutations in CD19 or DOCK8 diminish the amount and duration of PIP_3 activity so that GC cells do not persist and are not positively selected despite bearing higher-affinity BCRs. Because CD40L, IL-4 and IL-21 also activate PIP_3 formation and Akt, it follows that BCR and integrin-induced PIP_3 might be reinforced in those GC B cells that also present the most foreign peptide and MHC class II to T_{FH} cells.

Rapid negative affinity selection of self-reactive BCRs can be explained in this model by cessation of BCR-induced PIP_3 production owing to most of the BCRs being engaged and downregulated by the tolerogenic antigen. Before the onset of apoptosis during GC negative selection, surface BCR levels rapidly decrease more than 90% (ref. 71), which resembles the BCR downregulation that precedes clonal deletion of immature self-reactive B cells in the bone marrow[105]. As BCR downregulation is caused by high receptor occupancy and cross-linking, and B cells recognizing small packets of immunogenic antigens on FDCs do not show extensive BCR downregulation, this mechanism can integrate inputs concerning the distribution of antigen. If it is limited to small, focal packets from FDCs, it is likely to be foreign, but if it is widely available in sufficient amounts to engage most of the BCRs on the GC cell, it is likely to be self. In bone marrow or follicular B cells, loss or downregulation of surface BCR expression extinguishes tonic PI(3)K activation of Akt to cause accumulation of Foxo1 and induction of Bim, $p27^{Kip1}$ and RAG-1 (refs. 106–110). Negative affinity selection of self-reactive GC B cells might recapitulate the process occurring in the bone marrow: downregulation of surface BCRs leads to a drop in PI(3)K and PIP_3, activation of Foxo1, induction of $p27^{Kip1}$ and exit from cell cycle, and induction of apoptosis through Bim and p53.

Decision 4: plasma cell longevity

GC B cells can differentiate into memory B cells or plasma cells that persist in the absence of antigen re-exposure for years[111]. Although the affinity constraints on memory B cell differentiation seem relatively relaxed[112,113], a recent study has confirmed that only those GC B cells that have acquired a sufficient increase in antigen affinity are capable of plasma cell differentiation[114]. It is likely that this decision is regulated by the signals that mediate positive selection in the GC (**Fig. 2**), although the specific mechanism remains undefined. Most of the GC-derived plasma cells migrate to the bone marrow or local mucosa-associated lymphoid tissues[112,113]. Migration of post-GC plasma cells to the bone marrow depends on CXCR4 on the plasma cells and CXCL12 produced by bone marrow stroma[29,115]. The bone marrow niche supports plasma cell survival through cooperative survival signals from stroma-derived cytokines (see below), stroma-displayed ligands for the integrins LFA-1 and VLA-4 and stromal ligands for the adhesion receptor CD44 (refs. 116,117). A proportion of GC-derived plasma cells remain in the spleen, accounting for up to 25% of plasma cells in extrafollicular foci[118]. Long-term plasma cell survival in the spleen also depends on colonization of limited niches, initially in close association to $CD11c^{hi}$ mature DCs[119] but later dispersing to the red pulp[118].

$CD11c^{hi}$ dendritic cells in the spleen and stromal cells in the bone marrow secrete BAFF and APRIL, two TNF-related cytokines with a key role in plasma cell survival[111]. BAFF binds to three receptors, BAFF-R, TACI and BCMA, whereas APRIL only binds to TACI and BCMA. BAFF-R is critical for survival of B cells at many maturation stages; whether BAFF-R is important for plasma cell survival is unclear owing to lack of mature B cells in BAFF-R-deficient mice, but it seems unlikely as BAFF-R expression decreases as B cells differentiate into plasma cells[120–122]. In humans, BAFF-R is expressed on all B cell subsets except plasma cells in the bone marrow[120]. TACI is expressed on human plasma cells[121], and TACI that has been activated by oligomeric forms of BAFF or APRIL but not by trimeric ligands supports survival of mouse plasmablasts *in vitro*[123]. BCMA is expressed on both mouse and human plasma cells[120], and studies using BCMA-deficient mice show that this receptor is important for the survival of long-lived plasma cells in the bone marrow[124].

In the bone marrow, both BAFF and APRIL support the survival of long-lived plasma cells in mice[125], and blockade of both BAFF and APRIL is required to decrease plasma cell numbers in the spleen[126]. Human BAFF enhances survival of plasmablasts generated from memory B cells[122]. Establishment of a bone marrow reservoir of plasma cells in early life requires APRIL, which is itself poorly expressed by stromal cells in the bone marrow compartment early in life[127]. APRIL secreted by neutrophils in the gut binds to heparan sulfate proteoglycans to promote plasma cell survival in the human mucosa[128]. *In vitro*, APRIL complexed with heparan sulfate proteoglycans, but not trimeric APRIL, promotes plasmablast survival[123]. Inhibitors of BAFF tested in the clinic eliminate B cells but do not affect natural and acquired humoral immunity[129], whereas the TACI-immunoglobulin fusion protein atacicept blocks both BAFF and APRIL and seems to be a more promising treatment for antibody-mediated diseases[130].

Other cytokines also seem important for plasma cell survival. These include IL-5, IL-6 and TNF[117]. Inflammation creates new niches that support long-term plasma cell survival in both secondary lymphoid tissues and nonimmune organs[131]. T cell–derived signals are required to maintain plasma cells in human tonsil grafted into immunodeficient mice[132]. This, together with the recent demonstration of interactions between antigen-specific T cells and plasma cells in extrafollicular foci of mouse spleen[18], suggests that T cells might have an unappreciated role in the support of plasma cell longevity.

Concluding remarks

Much has been learned recently about the control system governing the quality and quantity of circulating antibody. Far from being a single binary switch, it comprises a series of decision points where B cells integrate many inputs influencing their fate. Just as the two-signal integration principle represents a form of fail-safe engineering, emerging evidence from dynamics and integration of signals and cells suggests that more complex principles of control will also operate. Much more sophisticated methodological and mathematical tools will be needed by future students of humoral immunity to explain abnormal antibody states and predict the system's response to new interventions.

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