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Affinity of antigen encounter and other early B-cell signals determine B-cell fate

Micah J Benson¹, Loren D Erickson², Michael W Gleeson¹, and Randolph J Noelle¹

¹Department of Microbiology and Immunology, Dartmouth Medical School, 1 Medical Center Drive, Lebanon, NH 03756, USA

² Department of Microbiology, University of Virginia, Charlottesville, VA 22908, USA

Abstract

Three possible effector fates await the naïve follicular B cell following antigen stimulation in thymus-dependent reactions. Short-lived plasma cells produce an initial burst of germline-encoded protective antibodies, and long-lived plasma cells and memory B cells arise from the germinal center and function to enhance and sustain the humoral immune response. The inherent B-cell receptor affinity of naïve follicular B cells and the contribution of other early B-cell signals pre-determines the pattern of transcription factor expression and the differentiation path taken by these cells. High initial B-cell receptor affinity shunts naïve follicular B-cell clones towards the short-lived plasma cell fate, whereas modest-affinity clones are skewed towards a plasma cell fate and low-affinity clones are recruited into the germinal center and are selected for both long-lived plasma cells and memory B cell pathways. In the germinal center reaction, increased levels of the transcription factor interferon regulatory factor-4 drive the molecular program that dictates differentiation into the long-lived plasma cell phenotype but has no impact on the memory B cell compartment. We hypothesize that graded interferon regulatory factor-4 levels driven by signals to B cells, including B-cell receptor signal strength, are responsible for this branch point in the B-cell terminal differentiation pathway.

Introduction

During a thymus-dependent (TD) response, the cross-linking of the B-cell receptor (BCR) with its cognate antigen initiates the transcriptional pathway that controls the development of short- and long-lived plasma cells (PCs) from resting B cells. Antigen capture by resting B cells facilitates their early activation as well as the processing and presentation of antigen to cognate CD4 T helper cells by MHC class II molecules. Subsequent triggering of B-cell growth and terminal differentiation is provided by T-cell help through the CD154–CD40 axis and is modified and enhanced by cytokines such as IL-4, IL-21 and IL-10 [1–5,6**]. The combined strength of the BCR signal and T-cell help exerts a dramatic effect on the trajectory that activated B cells follow.

TD responses are characteristic of B2 cells, also referred to as mature naïve follicular (NF) B cells, which reside within the spleen and lymph node follicles. Following encounter with antigen and T-cell help, NF B cells initially proliferate into short-lived PCs, which cluster outside the B-cell follicles; this is followed by the formation of transient structures within the B-cell follicles known as germinal centers (GCs), which are the hallmark of the TD immune response. Within GCs, class-switched somatically hypermutated high-affinity B cell clones are

generated and exit the GC to become either long-lived PCs that secrete protective high-affinity antibody or B memory (B_{mem}) cells, which function to support the PC pool. B_{mem} cells express moderate- to high-affinity surface immunoglobulin and, upon secondary encounter with antigen, provide the host with a rapid burst of both short- and long-lived PCs at a pace and quality many-fold greater than that observed during the primary encounter with antigen. GC B cells respond to a spectrum of extrinsic and intrinsic signals that drive a transcriptional program that directs long-lived B-cell fate down the PC or B_{mem} pathways.

In this article, we present the hypothesis that the total signal strength provided to the B cell through BCR and CD40, as well as other environmental cues, directs the B cell down a path of PC or B_{mem} differentiation. This review focuses on the major intrinsic quality of the quiescent NF B cell and its BCR affinity for cognate antigen, and proposes that antigen-specific oligoclonal B cells are programmed shortly after antigen encounter to differentiate to long-lived PCs, short-lived PCs or B_{mem} cells based on their intrinsic BCR affinity for antigen. Finally, we will discuss the transcriptional regulators triggered by a strong BCR signal and CD40 ligation, and will further propose that interferon regulatory factor-4 (IRF-4) is the molecular switch through which a strong signal drives the PC transcriptional pathway.

B-cell destiny controlled by BCR affinity

Upon challenge with a TD antigen, responding oligoclonal B cells will give rise to short-lived extrafollicular PCs as well as GC B cells, wherein the molecular machinery for affinity maturation, isotype-switching, and production of B_{mem} cells and long-lived PCs is orchestrated. How each of these B-cell fates develops *in vivo* remains unclear. Because GC responses originate from several oligoclonal B cells, the role of their BCR affinity for the initiating antigen in GC recruitment has been intensely studied. In sum, these studies show that low-affinity B cells can form GCs and that there does not appear to be an affinity threshold for entry into the GC [7–9]. Furthermore, B cells that exit the GC show varying affinities for antigen and are subject to selection [10–13]. Those that demonstrate enhanced affinity relative to their lower affinity counterparts will terminally differentiate to B_{mem} cells and PCs [14,15,16**]. Clones that have high affinity for antigen are actively recruited into the PC pool throughout the duration of the GC, whereas B_{mem} cells exhibit moderate to high affinities to immunizing antigen and appear in the secondary lymphoid organs during the dissolution of the GC, where they reside for the lifetime of the individual [17,18]. These studies have provided a strong basis for affinity-independent cell entry into GCs, wherein clonal competition occurs among higher affinity clones for selection of post-GC B cells. However, it is unknown whether innate heterogeneity of BCR affinity within the oligoclonal B cell population that gives rise to a GC serves a specific biological purpose in humoral immunity through controlling the emergence of short- and/or long-lived PCs and B_{mem} cells.

To evaluate whether the intrinsic BCR affinity controls the fate of naïve B cells, we analyzed the *in vivo* differentiation capacity of heterogeneous B-cell populations from immunoglobulin transgenic (Tg) mice that recognize the same antigen yet vary stepwise in affinity. B cells from these mice share a rearranged V_H chain that confers recognition to the hapten (4-hydroxy-3-nitrophenyl)acetyl (NP), and proliferate, differentiate, isotype-switch and produce Ig in response to NP-conjugated TD antigen [19,20]. Upon adoptive transfer and challenge, low-affinity NP-reactive Tg B cells form GCs, undergo somatic hypermutation, and generate B_{mem} cells and long-lived bone marrow PCs [21**]. Adoptively transferred Tg B cells that have medium affinity exhibit a hastened GC response, have a dramatically reduced capacity to differentiate into B_{mem} cells, and are skewed to differentiate into long-lived PCs. Finally, B cells that display high-affinity NP-reactive BCRs are skewed away from seeding long-lived humoral protection and instead rapidly differentiate into short-lived extrafollicular PCs upon

antigen encounter and fail to enter the GC [16^{••},21^{••}]. Similar findings have been reported in systems using B cells of other antigen specificities [16^{••}].

The physiological impact of the control of humoral fate by germline BCR affinity is one of rapid protection followed by sustained and robust protection, as the body is provided with protective antibody throughout the course of an adaptive immune response. The initial proliferative burst of germline-encoded extrafollicular PCs generated from high-affinity NF B cells supplies the body with a rapid low-affinity immediate defense as the GC gears up to generate the more sophisticated antigen-selected and somatically hypermutated high-affinity PCs and B_{mem} cells. NF B cells that have moderate BCR affinity are skewed to supply the long-lived PC pool; however, these PCs have a finite lifespan and need to be continually generated to avoid protective serum antibody levels being diminished over time [22]. It is hypothesized that the long-lived PC pool is maintained by continuous turnover of B_{mem} cells into PCs, with B_{mem} cells also able to rapidly proliferate into PCs upon secondary antigen encounter [23]. As B_{mem} cells are generated from NF B cells that initially exhibit a low affinity for antigen, the requirements for low-, moderate- and high-affinity NF B cells in generating long-lived humoral immunity is evident because together they seed the entire effector arm of adaptive humoral immunity.

In addition to BCR affinity for antigen, other intrinsic factors control B-cell fate; examples of this include complement receptors and CD40, which also influence B-cell differentiation. Experiments in which BCR signaling is impaired through the elimination of the complement receptors CD21/CD35 in mice responding to a TD antigen showed defective PC generation with normal B_{mem} cell development [6^{••}]. The absence of CD21/CD35 prevents the BCR signal strength from reaching the threshold required for the initiation of the PC transcriptional pathway, and thus mimics the situation of a GC B cell exhibiting a moderate-affinity BCR–antigen interaction. Consequently, a high-affinity BCR–antigen interaction is never achieved in the absence of CD21/CD35, and allows B_{mem} cell generation but impairs PC generation [6^{••}]. Enhanced signaling through CD40 can also attenuate long-lived immunity by inducing antigen-specific B cells to differentiate to short-lived PCs at the expense of GC formation and induction of B-cell memory. We have shown that a CD40 agonist coupled with a TD antigen ablates long-lived humoral immunity in favor of short-lived extrafollicular PCs, with this situation partially mimicked *in vivo* by enhancing T-cell help [19]. Other factors that impact the B cell include the interactions of BAFF (B-cell activating factor belonging to the TNF family) and APRIL (a proliferation inducing ligand) with their B-cell expressed receptors, Toll-like receptor ligation, cytokines and downstream signaling mediators of the BCR such as Lyn tyrosine kinase, which can attenuate or exacerbate total signal strength to the GC B cell and thus influence terminal B-cell differentiation [24–27].

Taken together, these data indicate that the intrinsic BCR affinity and, moreover, total signal strength (from complement receptors, CD40 and probably others) to the naïve B cell is a determinant that controls the B-cell differentiation pathway and commitment to short- or long-lived effector cell fates. We put forth the hypothesis that, in addition to the classical competition pathway, a non-competitive pathway exists that is determined by germline encoded BCR affinity to provide the host with a spectrum of antibody protection (Figure 1). In this regard, the differentiation potential of an antigen-specific B cell is partially determined when it first encounters antigen, before competition occurs. Thus, within the BCR affinity continuum that permits entrance into B-cell follicles and GC responses, a checkpoint exists that governs which long-lived effector B cells are created, independent from antigen-driven selection. Whereas B cells that have intrinsic high affinity might have a competitive advantage, their contribution to the longevity and specificity of the humoral immune response is dampened owing to limited somatic hypermutation and B_{mem} cell production, and they function in a manner similar to the behavior of B cells that have engaged T cell independent antigen as a first line of antibody

defense (Figure 1c). As the immune response matures, additional layers of antibody competency and longevity are generated by the GC reaction from clones of variably lower affinities (Figure 1a and 1b). Thus, having an antigen-specific response from oligoclonal B cells that have disparate affinities provides the host with holistic antibody protection.

Transcriptional development of long-lived plasma cells

After antigen encounter and proliferation, B cells that have sufficiently generated a high-affinity BCR within the GC undergo a transcriptional program in which an overhaul of B-cell character occurs and a PC is generated. The transcription factors that drive PC character have been identified, and the dynamic interplay between GC transcription factors and PC transcription factors has begun to be unraveled.

Within a GC B cell, the transcription factor paired box gene 5 (Pax5) maintains B-cell identity by sustaining surface expression of immunoglobulin, maintaining the machinery required for BCR signalling and suppressing the PC transcriptional pathway [28,29]. Pax5 induces the expression of the enzyme activation-induced cytidine deaminase (AID), which controls isotype switching and somatic hypermutation within the GC [30]. B cell lymphoma 6 (BCL-6), a transcriptional repressor whose expression drives GC B cells and whose induction has been postulated to be maintained by Pax5, additionally inhibits PC differentiation by directly suppressing B lymphocyte-induced maturation protein-1 (Bim-1) expression and enhancing proliferation of the GC B cell [28,31,32]. The transcriptional program that drives PC character is mediated by Blimp-1, a zinc-finger transcriptional repressor that is necessary and sufficient for PC differentiation and is required for PC maintenance [33–35]. As a GC B cell differentiates into a proliferating plasmablast within the spleen and subsequently to quiescent long-lived PCs in the bone-marrow, Blimp-1 expression is increased. Concomitant with heightened Blimp-1 expression, immunoglobulin secretion is enhanced and is accompanied by increases in interferon regulatory factor-4 (IRF-4) expression whereas the expression of Pax5 and the surface markers B220 and CD19 is suppressed [36]. By antagonizing the expression of Pax5, Blimp-1 releases the PC transcriptional program repressed by Pax5 and allows expression of the spliced form of the transcription factor X-box binding protein-1 (XBP-1) [37]. XBP1 is required for PC development as it enables the cell to secrete copious amounts of immunoglobulin through mediation of the protein secretory apparatus [38,39]. It is within the light zone of GC structures that Blimp-1 is initially expressed — the anatomical site at which GC B cells contact antigen and receive help by a developmentally distinct subset of CD4⁺ T cells demarcated by Ly6c expression. It is here that the fate of GC B cell differentiation into PCs is determined by down-regulation of Bcl6 and expression of IRF-4 and Blimp-1 [40, 41,42]. If the combined signal strength through the BCR and CD40 reaches the proper threshold, a productive terminally differentiated PC is generated.

Graded levels of IRF-4 appear to dictate cellular fate within post-GC B cells: moderate levels of IRF-4 expression *in vitro* direct AID expression and accompanying isotype switching whereas high levels of IRF-4 expression such as those seen in PCs direct expression of Blimp-1 [43,44]. Whether graded expression of IRF-4 directs AID versus Blimp-1 expression *in vivo* has not been reported to date, but it is tempting to speculate that a strong BCR signal in a GC B cell imposed through a high-affinity BCR–antigen interaction leads to an elevation in IRF-4 expression that sends the GC B cell down the PC developmental pathway through induction of Blimp-1, with IRF-4 thus acting as a molecular ‘switch’ of B-cell differentiation (Figure 2). In turn, Blimp-1 further enhances IRF-4 expression in a positive autoregulatory feedback loop [36,45]. It is likely that IRF-4 initiates PC character through the direct induction of Blimp-1, because IRF-4 interacts with a conserved region of the *Prdm1* gene that encodes Blimp-1 rather than inducing the PC transcriptional pathway through direct suppression of Bcl-6 and Pax5; this latter function is instead performed directly by Blimp-1 [43]. It appears

that IRF-4 plays no role in the maintenance of B_{mem} cells, as its deletion after class switch recombination within the GC has no impact on the size of the memory pool [40]. In sum, the transcriptional regulators that drive the GC and PC are rapidly being revealed. These transcriptional regulators might be directly regulated by BCR signal strength and are probably perturbed by other extrinsic signals.

Conclusions

Our capacity to prevent re-infection and the success of vaccines in eradicating some of civilizations worst plagues is a result of immune system diversity; this allows us to produce both short- and long-lived immune responses as well as immunological memory. The newly unraveled contribution of the ‘breadth’ of BCR affinity in controlling the destination of NF B cells to the effector and memory phase of humoral immunity provides further insights to the advantage of generating a diverse antibody repertoire. Although BCR diversity provides protection against an unlimited spectrum of pathogens that contain distinct antigens, the spectrum of BCR affinity amongst oligoclonal B cells specific to a single antigen also controls the fate of B cells. Finally, we propose that the combined signal strength imposed on the GC B cell and which is predominated by BCR signaling can drive it towards a PC character through an IRF-4 threshold ‘trigger’.

So where do we go from here? The hypothesis that IRF-4 is an upstream inducer of Blimp-1 is still contentious, and whether graded BCR signal strength induces IRF-4 directly is hypothetical at this stage and has not yet been shown directly in a controlled *in vitro* experiment [40,43]. A gap in our understanding of the transcriptional regulation of the mature B-cell subsets appears when asking which transcriptional regulators drive B_{mem} cells. Transcriptional profiling of B_{mem} cells has linked their genome-wide expression pattern to those of hematopoietic stem cells and has allowed a glimpse into the genes that sustain this cell type [46]. The role of Pax5 in B_{mem} cells is still unknown, as are the extrinsic signals that sustain B_{mem} cell survival. Whether development of B_{mem} cells occurs as a ‘default’ differentiation pathway in GCs wherein IRF-4 expression is not sufficient to drive a PC transcriptional pathway is uncertain. All of these questions remain to be explored.

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Figure 1.

Proposed model of how BCR affinity controls long-lived humoral immune responses. During an oligoclonal B-cell response, antigen-specific B cells ranging in affinity are activated and undergo proliferation. **(a)** From this oligoclonal response, B cells with initially low affinity towards antigen will form germinal centers, undergo somatic hypermutation, and differentiate to long-lived memory B (B_{mem}) cells, plasma cell precursors (PC_{pre}), and bone marrow plasma cells (PCs), each of which has improved antibody affinity. **(b)** Oligoclonal responding B cells that have moderate affinity for antigen will also form germinal centers yet show a skewing in terminal differentiation whereby they can become long-lived PC_{pre} and PCs, but do not commit to B_{mem}. **(c)** When the innate BCR affinity on a naïve B cell exceeds a certain threshold for antigen, its differentiation fate is to become a short-lived PC. Shuttling intrinsically high-affinity B cells towards an extra-follicular pathway provides rapid local antibody production with the best possible protection early during an immune response while preventing the development of B-cell memory.

Figure 2.

The transcriptional development of plasma cells is mediated by the total signal strength on the B cell. We propose that the sum signal strength induces high IRF-4 expression in a subset of light-zone centrocytes exiting the germinal center (GC). These signals are composed of BCR signal strength and controlled by BCR affinity to antigen as well as complement receptor and CD40 engagement. Low IRF-4 expression induces AID and thus may play a role in GC B cells, although this link has not yet been made *in vivo*. (a) Within GC B cells, AID expression is induced by Pax5, which is also responsible for enhancing Bcl-6 expression. To maintain GC B-cell character, Pax5 suppresses XBP-1 whereas Bcl-6 represses Blimp-1, prohibiting differentiation into PCs. (b) High expression of IRF-4 in light-zone centrocytes would lead to the induction of Blimp-1, which represses Pax5 and Bcl-6 and allows for the expression of XBP-1 and terminal differentiation into a plasma cell (PC) secreting immunoglobulin (c). The second major cellular fate of GC B cells is the generation of B_{mem} (d). IRF-4 does not appear to be required for this fate and the transcriptional pathway driving and sustaining B_{mem} remains unknown. The B_{mem} pool supplies the body with a burst of PCs upon secondary antigen encounter and continually replenishes the PC pool through polyclonal stimulation such as TLR ligation and bystander T-cell help. During the transition from a B_{mem} to a PC, IRF-4 again induces the PC transcriptional pathway through Blimp-1.