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Review Article

Gene interaction network regulates plasma cell differentiation

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Abstract

Effective humoral immunity depends on B cells, plasma cells and follicular helper T cells (TFH) and secreted high-affinity antibodies. The differentiation of mature B cell into plasma cells is ultimately hardwired in a regulatory network of transcription factors. This circuitry is responding to extracellular stimuli, which leads to production of higher affinity antibodies after germinal center (GC) reaction. The understanding of the transcriptional regulation of GCs and the initiation of plasma cell differentiation is becoming increasingly clear. It is evident that transcriptional repressor Blimp-1 can drive the plasma cell differentiation, but the initiation of plasma cell differentiation in GCs is likely coupled to the loss of B cell characteristics maintained by transcription factors Pax5 and Bcl6.

Introduction

Upon activation with appropriate stimuli, most notably the antigen recognized by the B cell antigen receptor (BCR), the resting naive B cells start to proliferate. A subset of these cells starts to secrete antibody and are referred to as plasmablasts. These cells may undergo terminal differentiation in tissues, where they continue antibody secretion and stop the proliferation, and are defined as plasma cells. Plasma cells represent the final differentiation stage of the B cell lineage and are the professional antibody secreting cells constituting a major branch of humoral immunity. They are highly specialized to secrete vast amounts of soluble immunoglobulin (Ig) that can facilitate engulfment of bacteria by opsonisation, neutralize invading pathogens and activate complement via classical pathway.

It has become clear that plasma cells are not all alike. Plasma cells differ in their lifespan, differentiation route, the nature of the produced Ig and their anatomical location [1]. The exact pathways that result in different types of plasma cells are not fully understood, but are suggested to depend on which B cell subset the plasma cells are derived from and which type of signals are needed to stimulate their differentiation [1, 2]. The B1 cells, marginal zone B cells and follicular B cells can all give rise to plasma cells when activated. The differentiation of these cells is a complex process and involves integration of extracellular stimuli to the highly interacting network of transcription factors.

The differentiation of B2 cells into antibody-secreting plasma cells can occur via two prominent routes. The cells either differentiate along extrafollicular pathway, creating short-lived plasma cells that produce low-affinity antibodies or proceed to the follicular pathway to generate GCs that support the maturation of antibody affinity and Ig class switching and long-lived plasma cells (Figure 1). The type of antigen, the cellular niche and the affinity of B cell antigen receptor towards an antigen determine which differentiation route is chosen with higher affinity antigen recognition giving rise to extrafollicular pathway and B cells with lower affinity start to form GCs [3]. Type II antigens, that usually contain repeating antigen determinants on a large polysaccharide backbone, can initiate the extrafollicular pathway. The plasma cells from the extrafollicular pathway are sustained in regions such as splenic extrafollicular foci and lymph node medullary chords where $CD11c^{high}$ dendritic cells provide a proliferation-induced ligand (APRIL) and B cell activating factor (BAFF) [4]. Depending on the subtype, these plasma cells have a half life ranging from hours to days and usually secrete IgM class antibody and to a lower extent other Ig classes. The follicular pathway is related to GCs, a specialized structure to

support affinity maturation and class switching of Ig. This follicular pathway is known to produce long-lived high-affinity plasma cells that find their survival niches in the bone marrow where they can survive for longer periods [5].

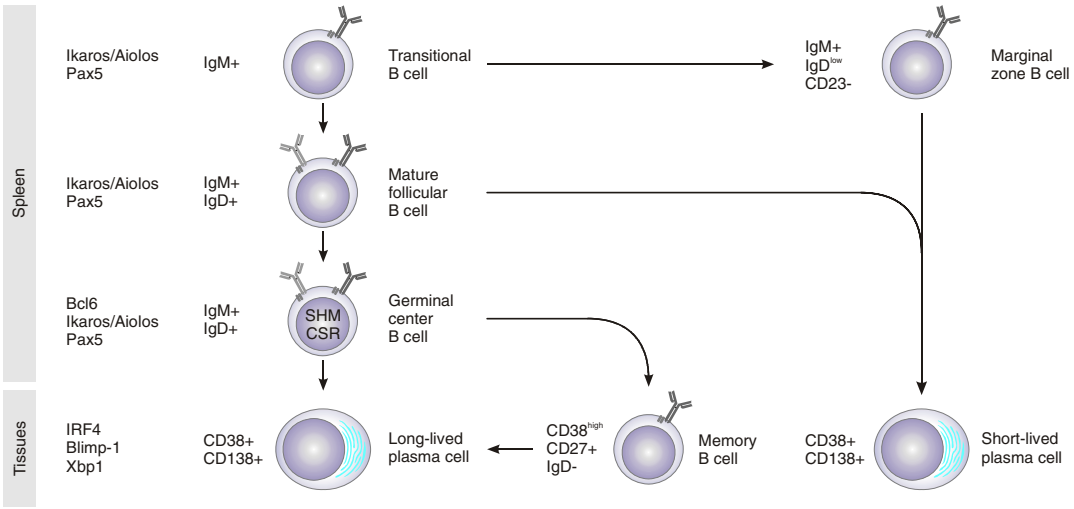

Figure 1. Pathways for generating functional plasma cell compartment. Developing B cells require Pax5, Ikaros, and Aiolos for generating a competent B cell compartment by regulating B cell antigen receptor signaling [6-12]. Aiolos contributes to the decision how transitional B cells give rise to mature B cells that reside in follicles or marginal zones [13]. Upon antigen-specific activation, follicular B cells either differentiate into plasma cells via extrafollicular pathway or form GCs by upregulation of Bcl6. GC B cells can give rise to memory B cells or long lived plasma cell pool in the bone marrow. Blimp-1, IRF4 and Xbp1 are universally required for antibody secreting plasma cell differentiation and Aiolos contributes to high affinity B cell memory [14].

Plasma cell transcription factors drive the terminal differentiation

The response to extracellular stimuli and the ability to undergo differentiation are ultimately dictated by transcription factors. The differentiation of B cells into plasma cells involves an substantial change of the gene expression program, including the repression of B cell transcription factors and other B cell properties [15] as well as induction of plasma cell transcription factors responsible for properties such as active Ig secretion and cessation of cell cycle.

The transcription factors that are generally required for the plasma cell differentiation are B-lymphocyte induced maturation protein 1 (Blimp-1), interferon regulatory factor 4 (IRF4) and X-box binding protein 1 (Xbp1) [16-18]. Short-lived plasmablasts express intermediate level of Blimp-1 whereas long-lived plasma cells express high amounts of Blimp-1 [19, 20]. Blimp-1 is universally required for the formation of competent plasma cells. Blimp-1-deficient mice fail to generate antibody secreting cells [18, 20, 21] and ectopic expression of Blimp-1 is sufficient to induce antibody secreting cell differentiation [22]. Blimp-1 can efficiently shut down the B cell gene expression program and promote the exit from the cell cycle by repressing mature B cell associated transcription factor genes such as Pax5, CIITA, SpiB, c-Myc and genes important for GC formation including Bcl6 and AID [15, 23-25]. However, Blimp-1 is not only needed to drive the plasmacytic properties but is also required for the maintenance of long-lived plasma cells [26]. These findings led to the conclusion that Blimp-1 is a master regulator of the initiation of plasma cell differentiation. This concept, however, is challenged by a parallel mouse model,

where Blimp-1 gene is engineered to harbor a green fluorescent protein reporter gene [20]. This model was used to discover a subset of cells called pre-plasmablast that have downregulated the expression of a central B cell transcription factor Pax5 but not yet induced the expression of Blimp-1 [27]. This finding fits with other models, where deletion of Pax5 gene in DT40 B cell line induced spontaneous plasma cell differentiation [8, 9] and inactivation of Pax5 in mature mouse B cells induces Blimp-1 expression [28]. Collectively these findings suggest that Blimp-1 drives the differentiation of plasma cells but the initiation of plasma cell differentiation precedes the induction of Blimp-1, and is caused by downregulation of B cell properties.

IRF4 has a two-phase expression pattern during the B cell development. While it is expressed in immature B cells in the bone marrow, it is lost in proliferating GC centroblasts [29, 30]. However, its expression starts to gradually increase again in some centrocytes and plasmablasts and reaches its highest level in plasma cells [30, 31]. In addition to Blimp-1, IRF4 is generally required for plasma cell differentiation. IRF4 deficient mice lack plasma cells, their serum Ig levels are low and their B cells cannot form plasma cells *in vitro* [16, 32, 33]. IRF4 seems to act upstream of Blimp-1, as IRF4 can bind to Blimp-1 gene and B cells cannot express Blimp-1 in the absence of IRF4 [33].

Xbp1 is also necessary for effective plasma cell formation [17], but it cannot initiate the process in the absence of Blimp-1 [18]. Xbp1 is required for secretion of antibody in plasma cells [34]. Within the B cells the expression of Xbp1 is suppressed by Pax5 [35] and its overexpression in B cells expands the protein secretory apparatus [34]. Xbp1 acts downstream of IRF4 and Blimp-1 [18, 32]. In the response to ER stress the Xbp1 transcription is initiated by activating transcription factor 6 (ATF6) and the differential splicing of the transcript by IRE1 renders the Xbp1 in its active form [36, 37], that seems to regulate chaperones involved in handling the load of the increased Ig synthesis [38].

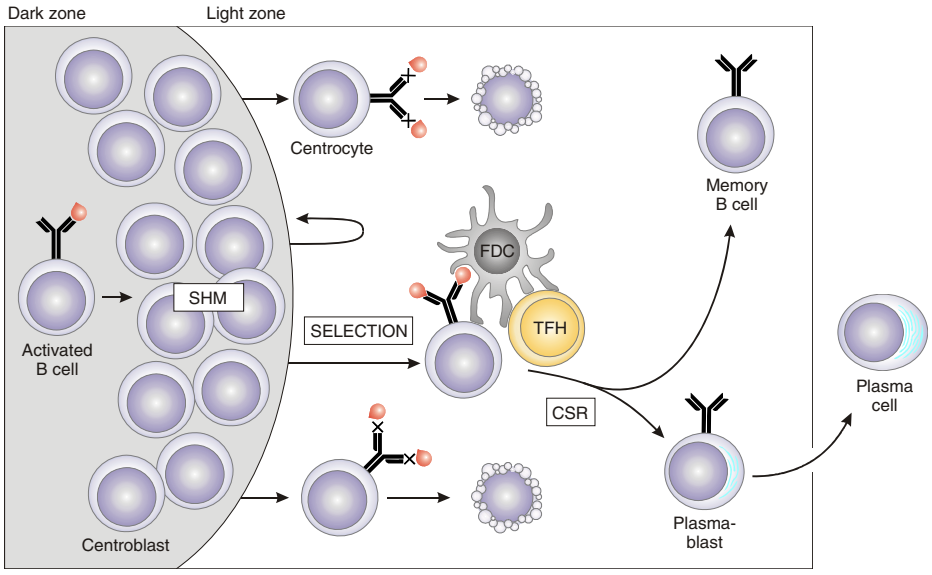

Figure 2. Long-lived plasma cells arise from GCs. Antigen-activated follicular B cell can differentiate into centroblast that proliferate and induce point mutation into Ig genes by somatic hypermutation (SHM). Centroblasts migrate into light zone, where they are probed for increased Ig affinity and are called centrocytes. Autoreactive clones or clones with weak affinity die of apoptosis or undergo further rounds of SHM in the dark zone. The clones with increased

affinity receive help from TFH cells or follicular dendritic cells (FDC) to undergo class switch recombination (CSR) and start differentiation into plasma cell or memory B cell fate. The high affinity and class-switched plasma cells survive for long periods in survival niches such as in bone marrow.

Bcl6 supports GC stage before the induction of terminal differentiation

In the follicular pathway, the B cells can differentiate into GC B cells: centroblasts and centrocytes. GCs are specialized structures forming in the B cell follicles that provide an environment for antibody affinity maturation, class switching and induction of plasmacytic differentiation. The affinity maturation produces the high affinity B cell clones by cycles of cell proliferation, SHM of Ig gene variable regions and selection gaining increased Ig affinity [39] (Figure 2). The key transcriptional regulator of GC formation and function is Bcl6 (encoded by the B cell lymphoma 6 gene). Bcl6-deficient mice lack GCs and affinity matured B cells [40, 41]. On the other hand, constitutive expression of Bcl6 in B cells *in vivo* results in increased size of GCs [42]. Within the B cell lineage Bcl6 mRNA is observed already in pre B cells, mature B cells and GC B cells but not in plasma cells [43-45]. The expression of Bcl6 protein is highly increased in GC B cells [44] with higher expression in centroblasts than in centrocytes [46]. The expression of Bcl6 in GCs is maintained for example by interleukin-21 (IL-21) [47-49] that is secreted by many cell types, particularly by TFHs [50, 51]. IL-21 receptor signals via STAT3 and STAT5, that promote Bcl6 expression [48, 52, 53]. Analyses of Bcl6 target genes have revealed that Bcl6 maintains the centroblast gene expression signature that includes repression of genes involved in the detection and response to DNA damage (such as p53, ATR and CHEK1) to allow physiological genomic instability associated with SHM and CSR while promoting cell cycle by repressing genes such as CCND2, CDKN1A, CDKN1B [39, 54, 55].

Activation-induced cytidine deaminase is absolutely needed for both SHM and CSR [56-58]. Pax5 controls the expression of AID, since the AID gene has a binding site for Pax5 that is needed for its expression [59], and the expression of AID in DT40 B cell line depends on Pax5 expression [8]. Interestingly, re-expression of Pax5 in Bcl6-deficient DT40 cells that also undergo spontaneous plasma cell differentiation, cannot support the expression of AID (Alinikula et al., submitted), showing that also Bcl6 is necessary to sustain SHM and CSR via regulation of AID. Bcl6 knockout mice are capable of producing plasma cells, but not efficiently the long-lived population, supporting the role of Bcl6 in promoting GC B cell functions [41, 60, 61]. Thus Pax5 and Bcl6 co-operate to maintain the GC phenotype before the induction of plasma cell differentiation.

The promotion of GC features is elegantly coupled to repression of further differentiation by the action of Bcl6. Bcl6 can efficiently repress the expression of Blimp-1 and subsequent plasma cell differentiation ([8, 54, 61, 62], Alinikula et al., submitted). The repression can occur directly by interfering with the function of Blimp-1-inducing STAT3 [62] and independently by binding to Blimp-1 intronic sequences [61, 63]. Additionally, Bcl6 may repress Blimp-1 via regulating the other repressors of Blimp-1, such as Bach2 (Alinikula et al., submitted). Thus the function of Bcl6 is to prevent the premature differentiation of plasma cells to allow effective Ig SHM and CSR during the GC response (Figure 3).

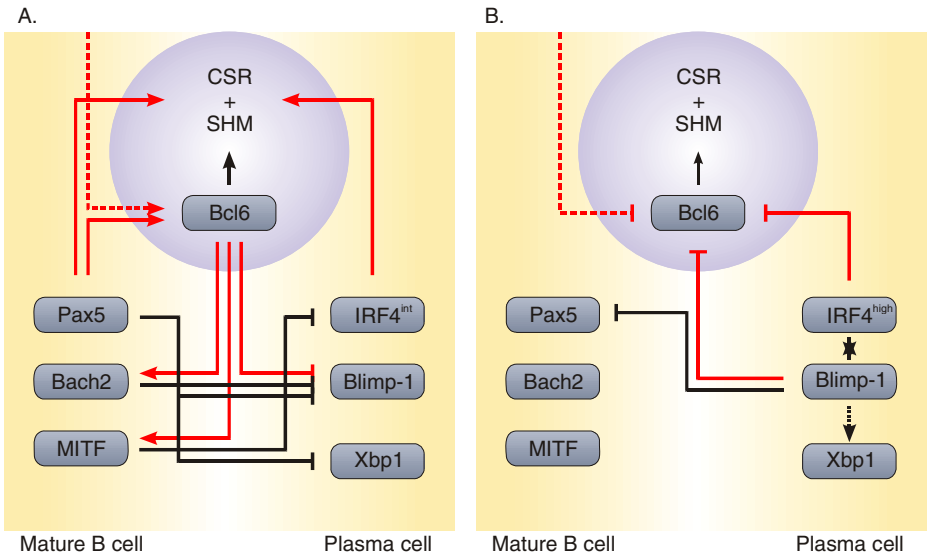

Figure 3. Transcription factor network guiding plasma cell differentiation. *A.* In the mature B cells the B cell transcription factors maintain the repressed state of plasma cell transcription factors (black arrows). In the GCs Bcl6 expression is induced by extracellular signals (dotted line). Pax5 maintains Bcl6 expression and both Bcl6 and Pax5 promote the expression of AID to allow SHM. Intermediate expression of IRF4 facilitates also CSR. Bcl6 is also critically required to repress Blimp-1 directly and via Bach2 expression. *B.* Degradation of Bcl6 protein by high-affinity BCR signals allows the Blimp-1 to be activated by increased IRF4 expression among other mechanisms. IRF4 together with Blimp-1 represses Bcl6 and Pax5 to allow full plasma cell differentiation including Xbp1 expression. The regulation in the universal plasma cell differentiation is depicted with black indicators, whereas GC-specific regulation is depicted with red indicators.

Initiation of post-GC plasma cell differentiation

In addition to inducing the activators of plasma cell differentiation, the repressors of plasma cell differentiation Pax5, MITF, Bach2 and Bcl6 [8, 28, 61, 62, 64, 65], need to be suppressed (Figure 3). The downregulation of Pax5, a central factor for the commitment and maintenance of B cell phenotype [66], is crucial for the plasma cell differentiation [9]. Pax5 expression can efficiently prevent the differentiation of antibody secreting plasma cells and the expression of Blimp-1 [9, 28, 67-69]. Pax5 also represses the expression of several genes associated with immunglobulin secretion such as Ig J-chain [70-72] and Xbp1 [8, 9, 35], and inhibits high level transcription of Ig loci [73]. Inactivation of Pax5 gene in DT40 B cells induces plasma cell transcription program and Ig secretion [8]. Conditional inactivation of Pax5 in mature B cells induces also a similar phenotype [28].

The downregulation of Pax5 is one of the initiating mechanisms of plasma cell differentiation in GCs. The evidence for this comes from the experiments where Blimp-1 gene was engineered to harbor a GFP reporter gene [20]. This model was used to discover a population of GC cells called pre-plasmablasts that have downregulated the expression of Pax5 but not yet induced the expression of Blimp-1 [27] suggesting that B cell properties are not lost only after the induction of Blimp-1 but rather precede the Blimp-1 expression. Pax5 can also directly repress the Blimp-1 expression [67]. In line with these results, inactivation of Pax5 in DT40 cells leads to spontaneous differentiation to plasma cells [8]. The mechanism for physiological suppression of Pax5 expression in GCs is however currently unknown.

The Pax5 deficient DT40 cells have, however, also lost their Bcl6 expression [8] warranting the possibility that Pax5 deletion induces plasma cell differentiation via upregulation of Blimp-1 after losing Bcl6-mediated Blimp-1 repression. Indeed, Bcl6 expression in Pax5 deficient cells can repress Blimp-1 [8], but not vice versa: enforced Pax5 expression in Bcl6-deficient cells cannot repress Blimp-1 (Alinikula et al. submitted), suggesting that Pax5 cannot repress Blimp-1 alone, but also the downregulation of Bcl6 is definitely needed. Therefore, the expression of Bcl6 in GC B cells may help Pax5 to maintain the repressed state of Blimp-1 to allow sufficient rounds of SHM to yield the formation of higher affinity antibody producing plasma cells. The finding that BCR signal can induce the phosphorylation and rapid degradation of Bcl6 protein in ubiquitin-proteasome pathway [74], provides a fascinating physiological mechanism how the suppression of Blimp-1 may be relieved. In this model, once the affinity of BCR reaches a certain level, the BCR would signal the cell to lose Bcl6 expression and to initiate the plasma cell program then driven by Blimp-1. The Blimp-1-mediated repression of Bcl6 and Pax5 gene expression [15, 25, 75] can later lock the terminal differentiation into plasma cell fate.

The CD40-induced increased expression of IRF4 is known to downregulate Bcl6 expression [76]. This event may serve as another mechanism by which downregulation of Bcl6 is achieved in GCs to allow Blimp-1 expression and full plasma cell differentiation. This mechanism may also mark the end of centroblast stage and induce class switching of high-affinity B cells [32, 33]. Unstimulated B cells express IRF4 at low levels, but T-dependent and T-independent activation induces IRF4 expression first to intermediate levels that can support the expression of AID and then to higher levels able to induce Blimp-1 expression [33] (Figure 3).

In addition to Pax5 and Bcl6, another repressor expressed in GCs, but not in plasma cells, is Bach2 [77]. Bach2 deficient mice have relatively normal B cell development but produce only low affinity IgM secreting plasma cells [78]. However, Bach2-deficent mice produce less isotype-switched antibodies and have dramatically less mutations in IgM V regions showing that Bach2 promotes efficient SHM and CSR [78]. Like Pax5 and Bcl6, also Bach2 can repress Blimp-1 expression and prevent plasma cell differentiation [63, 65, 79] and Bach2 may prevent full activation of Ig heavy chain locus [80]. It seems that, similarly to Bcl6, Bach2 can delay the differentiation of plasma cells to allow a developmental window for Ig class switching [79]. Therefore losing the Bach2 expression in GCs represents another mechanism by which plasma cell differentiation is initiated. Interestingly, Bcl6 contributes to repression of Blimp-1 together with Bach2 [63] and by regulating Bach2 expression directly (Alinikula et al, submitted).

Feedback regulation of plasma cell differentiation in GCs

Recently Bcl6 has also been shown to critically contribute to the development of TFH cells, a subset of helper T cells that is specialized to provide antigen-specific B cell help in splenic and lymph node GCs [81-83]. Mice with T cells lacking Bcl6 expression are incapable of forming GCs [81-83]. IL-21 is also reported to be required for TFH cell differentiation [81, 84, 85] as IL-21 upregulates Bcl6 expression in naïve helper T cells [81-83]. IL-21 receptor expression in TFH cells and GC B cells is required for the maintenance of GCs [47] and its expression on B cells is needed for maximal Bcl6 expression in GC B cells [47]. IL-21-signaling activates STAT3 that can bind to Bcl6 promoter and activate its expression [86]. Furthermore, Bcl6 and Blimp-1 appear to conform a mutually repressive loop to regulate both GC B cell and TFH cell development [87].

Interestingly, class-switched plasma cells are able to suppress the function of TFH cells. In contrast to previous assumptions, plasma cells seem to retain the possibility to present antigens to T cells [88]. They are capable of decreasing IL-21 and Bcl6 expression in antigen-specific TFH cells [88], which can potentially reduce the capacity of T cells to help follicular B cells. As the T cell help seems to be the limiting factor for high affinity B cell selection in GCs [89], the loss of TFH function can therefore serve as a novel way to prevent further GC reaction when the sufficient high affinity plasma cells are already formed.

The similar function of Bcl6 and Blimp-1 in both TFH and GC B cells represent an interesting regulatory loop that controls the T cell dependent plasma cell formation. The antagonistic function of Bcl6 and Blimp-1 in directing the differentiated vs. undifferentiated developmental stage during the GC-derived plasma cell differentiation represents a genetic switch that can be functional even in different cell types to regulate a common function.

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