The Generation of Antibody-Secreting Plasma Cells

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ABSTRACT

The regulation of antibody production is linked to the generation and maintenance of plasmablasts and plasma cells from their B cell precursors. Plasmablasts are the rapidly produced and short-lived effectors of the early antibody response, while plasma cells are the long-lived mediators of lasting humoral immunity. An extraordinary number of controls, at both the cellular and molecular level, underlie the exquisite regulation of this essential arm of the immune response. Despite this complexity, B cell terminal differentiation can be described as a simple probabilistic process that is governed by a central gene regulatory network and modified by environmental stimuli.

INTRODUCTION

The generation of antibody is one of the most important elements in the immune response, and is the basis for the vast majority of successful vaccination strategies. Antibody is produced from rare populations of terminally differentiated B cells, termed plasmablasts and plasma cells, the formation of which is associated with profound alterations in the morphology, gene expression profile and lifespan of the differentiated antibody-secreting cells (ASCs) compared to their B cell predecessors [1].

The humoral immune system is clearly complex, with many parts. Much research activity has focussed on its different levels – the cellular and anatomical diversity, patterns of transition during activation and differentiation, the molecular control of these processes and the complex microenvironments in vivo that enable signal integration. Through synthesis of insights gained by these broad approaches, some of the important rules that define a canonical B cell response program are being realized. Integrating insights into the molecular, cellular and whole system regulation of antibody is both challenging and vital. It will aid our understanding of the cellular commitment and differentiation processes and the regulatory logic of the system. Ultimately, it will provide tools to better understand and manipulate the humoral immune system to both improve immunity and to thwart autoimmune pathologies. In this Review, we focus on the factors that impinge on the generation, function and maintenance of ASCs, to highlight the progress being made towards an integrated view of the humoral immune response.

B cell terminal differentiation

Mature B cells have three major subsets: follicular, marginal zone (MZ) and B1 B cells (Figure 1) [2, 3]. Follicular B cells are the dominant subset found in the lymphoid follicles of the spleen and lymph nodes. MZ B cells, in contrast, abut the marginal sinus of the spleen, where they are ideally placed to encounter blood-borne pathogens and particulate antigens. B1 B cells reside mostly in the peritoneal and pleural cavities and at mucosal sites, which facilitates their surveillance of tissues that are most susceptible to environmental pathogens [1]. These specialized locations enable MZ and B1 B cells to rapidly respond to T-independent (TI) antigens, such as bacterial components [1]. Follicular B cells, while also capable of responding to TI antigens,

appear more specialized for responding to protein antigens that elicit simultaneous $CD4^+$ helper T cell activation.

The production of ASCs in response to T-dependent (TD) antigens is a two-step process aimed at providing immediate and persistent protection, respectively (Figure 1). In the first 'extrafollicular response', B cells receive an antigen receptor-dependent signal leading to development of B-lymphoblasts that divide (and may undergo immunoglobulin (Ig) class switch recombination (CSR)) and differentiate into short-lived plasmablasts that secrete antibody [4]. The extrafollicular response exhibits little somatic hypermutation (SHM) and therefore the affinity of the resulting antibodies for antigen tends to be moderate and unchanging. Nevertheless, the extrafollicular response is the source of the bulk of the early protective antibody.

In the second phase of a TD response, some of the activated B cells re-enter the B cell follicle and, under the influence of specialised T follicular helper (Tfh) cells, proliferate vigorously to form a germinal center (GC) [5-7]. GCs are sites of extensive proliferation and selection based on antigen receptors that have been modified by SHM. The GC ultimately produces high affinity, long-lived plasma cells that are capable of sustained high-level antibody secretion [6, 8]. The GC reaction also produces memory B cells that maintain a B cell phenotype but appear epigenetically programed to rapidly differentiate into ASCs following re-exposure to antigen [9]. This GC phase of the response appears to have evolved to offer effective protection against a future infection, even though it is running concurrently with the protective and rapid extrafollicular phase.

Many, but not all, aspects of ASC differentiation can be effectively recapitulated *in vitro*, as naïve B cells undergo both CSR and ASC differentiation in response to T-cell derived stimuli (CD40 ligation and cytokines such as IL-4, IL-5 or IL-21) or TI-related signals, such as lipopolysaccharide (LPS) and hyper-methylated CpG DNA [10-13]. The highly reproducible nature of these B cell responses provides a defined system to investigate the biology of ASC differentiation on both a cellular and molecular level (Box 1).

Transcriptional circuitry of B cell terminal differentiation

On a transcriptional level, the transition of an activated B cell to an ASC is, in essence, a lineage-switch, as it requires the co-ordinated regulation of hundreds of gene expression changes and major remodelling of the chromatin landscape. This fundamental change in gene expression results from the silencing of a group of key transcription factors that control B cell identity and the expression of the ASC-specific regulators [14, 15]. The transcription factors that define B cell and ASC identity regulate mutually antagonistic gene expression programs, with the result that the two cellular states, B cells and ASCs, are stable over the long-term [16]. How the B cell program is destabilized, and ultimately silenced, to allow commitment to the ASC fate is discussed below. Failure to complete the B cell to ASC transition, through either the maintenance of the B cell factors or the loss of critical ASC differentiation factors, is a major source of late stage B cell lymphomas (Box 2).

Maintenance of the B cell fate

One group of transcription factors is expressed in mature B cells, where they function to promote the B cell gene expression programs that underlie antigen and T cell sensitivity and to prevent premature ASC differentiation. The best-characterized B cell factors are discussed below.

Pax5. Very few aspects of the B cell transcriptional program can operate independently of Pax5, which binds to many thousands of sites in follicular B cells [17]. Pax5 is expressed throughout B cell development, where it is required for the initial lineage commitment of lymphoid progenitors to the B cell fate [18]. Pax5 continues to reinforce B cell identity throughout the lineage, as conditional inactivation of Pax5 in mature B cells results in the loss of B cell identity and reversion to a progenitor stage [19]. In mature B cells, Pax5 regulates components of the B cell receptor, such as IgH and Igα, other immune receptors such as CD19 and CD21, as well as the transcription factors Irf4, Irf8, Bach2, Aiolos and Spi-B (Figure 2 [20, 21]). Pax5 also represses a suite of genes not normally expressed by B cells, including *Flt3*, *Ccr2* and *Cd28*, which are re-expressed after the down-regulation of *Pax5* in ASCs [11, 22]. While it is assumed that *Pax5* needs to be silenced to allow ASC differentiation, this has not been definitively shown. Enforced *Pax5* expression in B cells causes some repression of ASC potential [23, 24], while deletion of *Pax5* in chicken DT40 cells induces some aspects of ASC differentiation and Ig secretion [25]. Most compellingly, *PAX5* expression is often maintained in human B cell lymphomas, through translocation to the *IGH* locus,

blocking further differentiation [26]. While mouse models that mimic these translocations have been reported, it is not yet known whether ASC differentiation is impaired [27]. An alternative possibility is that it is Pax5 function, and not its expression per se, that is altered to initiate ASC differentiation. Indeed we have shown that a very early event in ASC differentiation is the reactivation of the Pax5-repressed genes [11]. This reactivation occurs in the presence of the normal concentration of Pax5 protein, implying an alteration in Pax5 function, potentially through a post-translational modification or change in co-factor interactions. The precise mechanism of this inactivation of Pax5-mediated gene repression remains to be determined.

Bach2. Bach2 is a transcriptional repressor that is expressed throughout B cell differentiation but, in keeping with its positive regulation by Pax5 [20], Bach2 expression is absent from ASCs. Bach2-deficient mice lack GCs and the expression of *Aicda* (encoding Activation induced cytidine deaminase (AID)), an essential mediator of CSR and SHM [28]. A major target of Bach2 in B cells is *Blimp1* [29, 30] (Figure 2). In the absence of Bach2, mature B cells form relatively normally, but upon activation, Blimp1 is prematurely expressed resulting in *Aicda* repression and greatly enhanced ASC differentiation [31]. Repression of Bach2 was also recently reported to be an important component of the increased propensity of memory B cells to undergo ASC differentiation after re-exposure to antigen [9]. While Bach2 is a crucial component of the genetic network controlling the timing of ASC differentiation, to date there are no global studies of Bach2 DNA binding in B cells and thus there is little understanding of its mechanisms of action beyond *Blimp1* regulation.

Bcl6. Bcl6 is another factor, acting predominantly as a transcriptional repressor, that is highly expressed in GC B cells and is essential for their formation [32]. Bcl6 facilitates both the rapid proliferation of GC B cells and through inhibition of the DNA damage response, their tolerance of the high rates of SHM. Bcl6 binds to the regulatory regions of many thousands of genes in GCs, including those controlling the expression of the cell cycle regulators p21 and p53, and oncogenes including Myc and Bcl2 [33-35]. The repression function of Bcl6 is mediated through direct interaction of its BTB-domain with co-repressors such as NCOR and SMRT [36]. One proposed target of Bcl6-mediated repression is *Blimp1* [15, 37], establishing an antagonism that blocks ASC differentiation in GC B cells (Figure 2). IL-21 [38, 39] and the transcription factor Irf8 [40, 41] are reported to sustain *Bcl6* expression, while Irf4 and Blimp1 are proposed to

repress *Bcl6* (Figure 2) [42, 43]. *Bcl6* downregulation is a normal component of ASC differentiation and *IGH-BCL6* translocations are hallmarks of GC derived lymphomas [32] (Box 2). Interestingly, analyses of a mouse model of the IGH-BCL6 translocation revealed ASC expressing Bcl6 exist in the BM [44]. This suggests that ASC differentiation can occur in the presence of high Bcl6 amounts. Unlike Pax5, Bcl6 appears to play a minimal role in the differentiation of non-GC B cell subsets in vivo, and during in vitro differentiation of ASCs.

Obf1 and Oct2. The octamer binding factor, Oct2 (*Pou2f2*) and its co-activator, Obf1 (*Pou2af1*) were discovered via their binding activity in Ig gene promoters, however *Igh/Igl* gene expression is normal in their absence [45]. In contrast, these factors are required for MZ (both factors) and B1 B cell (Oct2 only) development [46-48]. Obf1 plays a unique role in TD B cell responses, as Obf1-deficient B cells cannot form GC, and when exposed to TD stimuli, prematurely initiate but cannot complete the terminal differentiation pathway and thus fail to generate ASCs [49, 50]. In part, this reflects a defective response to the T cell cytokine IL-4. Oct2 alone is required for the activation of ASC differentiation by IL-5, through direct control of the expression of the IL-5Rα chain [51]. Finally, both Oct2 and Obf1 enable activated B cells to secrete optimal IL-6, an inducer of Tfh cell differentiation [52].

PU.1, Spi-B, Ets1 and Irf8. While it has long been known that the Ets factor PU.1, and the closely related Spi-B, can form complexes on DNA with two members of the interferon regulatory factor (IRF) family of transcription factors (Irf4 and Irf8) [53], the exact function of these complexes in B cells has remained unclear. PU.1 [54] and Irf8 [55] are constitutively expressed throughout B cell differentiation, while Spi-B expression peaks in GC B cells [56], where it is regulated by Obf1 [57]. All three factors are subsequently downregulated in ASCs, with the repression of *Spib* being mediated by Blimp1 (Figure 2) [14]. The B cell specific inactivation of either PU.1 [58, 59] or Irf8 [60] results in only mild alterations in B cell development and function, while the loss of Spi-B impairs GC maintenance [61]. Such relatively mild phenotypes are surprising as PU.1 and Irf8 bind in the regulatory elements of thousands of genes in B cells [62, 63]. Recent evidence suggest that these factors do indeed play an important role in late B cell differentiation that is masked by complex redundancies and dosage sensitivities: PU.1/Irf8 double deficient B cells show a profound hyper-responsiveness to both TD and TI stimuli and generate both increased numbers of ASC and proportions of cells

that have undergone CSR, particularly IgE [41]. On a cellular level, PU.1/Irf8 functions to control the probability of ASC differentiation per cell division in activated B cells, thus providing a molecular mechanism for ASC generation in response to the cellular division history [10]. PU.1/Irf8 maintains the expression of B cell promoting factors such as *Bcl6, Pax5* and *Mef2c*, while concomitantly repressing *Blimp1* [41] (Figure 2).

PU.1 functions in a dose-dependent manner during early hematopoiesis, and in agreement with this enforced expression of PU.1 or SpiB impairs CSR and ASC differentiation [41, 64, 65]. One mechanism by which PU.1 concentration is controlled is through miR155, which binds in the 3' non-translated region of PU.1 [65]. This control appears to be of physiological importance as the specific removal of the mir155 recognition sequence from the *PU.1* gene increased PU.1 concentration 2-fold. This resulted in increased Pax5 expression and decreased TD immune responses in vivo and impaired ASC differentiation in vitro [66]

A third Ets family member, Ets1, also plays a role in B cell terminal differentiation. Ets1-deficient B cells show hyper-ASC differentiation capacity in the presence of CpG [67], a normally inefficient inducer of ASCs. Ets1, like PU.1, functions to promote *Pax5* expression and simultaneously inhibit Blimp1, although in this case the inhibition is proposed to be post-translational [67, 68]. Thus multiple members of the Ets and IRF families are critical for the normal production of ASCs

Drivers of antibody secreting cell differentiation

The generation of ASCs requires the B cell program to be silenced, allowing induction of an ASC transcriptome that is geared towards the production of an enormous amount of Ig, as well as homing to and survival in unique bone marrow niches. This transition is achieved through the action of three transcription factors, Irf4, Blimp1 and Xbp1, whose functions are outlined below.

Irf4. Irf4 sits on both sides of the differentiation fence, being essential both for B cell responses, such as CSR and GC B cell formation, and for ASC differentiation [69-72]. Irf4 binds DNA weakly on its own, but displays strong co-operative binding in the presence of PU.1. Other binding partners include, the BATF component of the AP-1 complex [73-75] and NFAT [76]. The Irf4-BATF complex is likely to be important in activated B cells, as mice lacking either factor have similar defects in CSR and GC

formation [69, 71, 77]. Irf4-Ets complexes are involved in the regulation of the *Igh/Igl* loci [78] and also bind to the B cell specific enhancer of *Pax5* [79]. Irf4 functions in a dose-dependent manner, with low Irf4 promoting GC fate and CSR, through the activation of *Aicda, Obf1* and *Bcl6* [70, 71, 80], while high amounts repress *Bcl6* and activate both *Blimp1* [71] and the Bcl6 relative *Zbtb20* [81], facilitating the ASC fate (Figure 2).

Blimp1/Prdm1. Blimp1 is a transcriptional repressor that in the immune system promotes the terminal differentiation of B and T cells [82]. Within the B cell lineage, Blimp1 is exclusively expressed in ASCs, with cycling plasmablasts being distinguishable from long-lived post-mitotic plasma cells based on their lower expression of Blimp1 in both mice and humans [12, 83, 84] (Figure 3). While Blimp1 expression is known to require Irf4 and the AP-1 factor, c-fos [71, 80, 85, 86] (which is in turn functionally antagonized by Fra1 [87]), the factors controlling the distinct concentrations of Blimp1 in plasmablasts and plasma cells remain unknown. Blimp1 has many proposed functions in ASCs, including repression of the key regulators of the B cell program, including the genes encoding Spi-B, Bcl6, Id3, Myc and Pax5 (Figure 2) [14, 24, 88]. Despite being essential for the formation of mature plasma cells [89], Blimp1 is not required for the initiation of the ASC program, as a pre-plasmablast population can develop in its absence [11]. In these cells, putative targets such as *Pax5* and *Bcl6* are transcriptionally down-regulated independent of functional Blimp1. There is also only limited understanding of the physiological function of Blimp1 in mature plasma cells, with evidence for roles in antigen presentation and responses to cellular stress [90-93]. Thus many questions remain as to how Blimp1 controls ASC fate, in particular its relationship with the other major regulators of the process.

BLIMP1/PRDM1 is expressed in all plasma cell malignancies including multiple myeloma (MM), and appears both essential and normally limiting for MM formation in mouse models [94]. However BLIMP1 is also commonly mutated in the activated B cell (ABC) subset of diffuse large B cell lymphoma (DLBCL) [95] and in MM, [96] suggesting an additional tumour suppressor function (Box 2).

Xbp1. The unfolded protein response (UPR) is induced by endoplasmic reticulum (ER) stress resulting from the accumulation of unfolded proteins. ASCs, due to their enormous rates of Ig synthesis and secretion, are particularly sensitive to ER-stress. The transcription factor Xbp1 is induced by ER stress in many cell types, including ASCs, and is a mediator of the UPR[97]. In B cells, Pax5 is proposed to repress *Xbp1*,

with Pax5 down-modulation during ASC differentiation contributing to *Xbp1* activation [98]. Early work suggested that Xbp1 was essential for both normal serum Ig titres and ASC development [99], however recent studies using B cell specific inactivation of Xbp1 have shown that ASCs form relatively normally in the absence of Xbp1. Instead Xbp1 functions predominantly to promote the *Igh* mRNA processing, Ig secretion and the associated remodelling of the ER that are characteristic of plasma cells [100-102]. Interestingly, activation of the mTOR pathway can partially compensate for Xbp1 loss in ASCs suggesting that metabolic deficiency is a consequence of impaired UPR [103]. Conversely, loss of authophagic activity, which is also thought to counter ER stress, has a negatively impact on ASC homeostasis and Ig production [104]. Similar roles in Ig processing and ER remodelling have also been proposed for the transcriptional elongation factor ELL2 [105-107] and ribonuclease IRE1 [108], suggesting that multiple pathways are operating to facilitate the Ig secretion capacity of ASCs. Due to its high expression in ASCs, the Xbp1 pathway has long been a candidate for the targeted therapies against MM. However a recent study has suggested that XBP-1 may actually function as a tumour suppressor in MM [109] (Box 2).

Cell biology of B cell differentiation

While the generation of antibody in an adaptive immune response appears complex and multifaceted, it can be stripped down to a minimal cellular response model. B cells receive activating signals that elicit a series of programmed cell divisions where each new cycle provides an opportunity for ASC differentiation. Typically only a small proportion of dividing B cells become ASC at each generation. Depending on the strength of initial stimulation and the presence of ongoing stimuli, division will eventually cease with most cells going on to die, although a proportion of long-term surviving memory cells and ASCs can be retained. Variations on this theme result from differences in the nature of the starting cells, in activating signals and in acute versus ongoing stimulation (Box 1). For example, certain B cell subsets, such as MZ and B1 B cells, rapidly generate ASCs without the need for extensive cell division or T cell help [110], while memory B cells require T cells but not extensive cell division. While the bulk of available evidence suggests that the core gene regulatory network is fundamentally conserved in all B cell subsets (Figures 1, 2, and www.immgen.org), it is known that MZ and B1 B cells display higher basal amounts of *Blimp1* and lower

expression of *Pax5* and *Bcl6* compared to follicular B cells, consistent with the more advanced activation state and accelerated differentiation kinetics of these B cell subsets [110, 111].

The GC reaction, while broadly conforming to the canonical pattern, appears to be a special case. Here, the input cells are selected after their initial activation by antigen. The subsequent signals, comprising increased antigen affinity and T cell help (through cytokines such as IL-21 and IL-4 and other ligand-receptor interactions with Tfh cells), function to promote rapid proliferation and to prevent differentiation [5-7, 38, 39, 112]. Exit from the GC results in either the acquisition of a memory phenotype, characterised by the maintenance of the B cell program gene regulators, including Pax5, or the initiation of ASC differentiation through a process that is not entirely resolved, but is known to be dependent on antigen affinity (Figure 1) [113-115]. Memory B cells maintain a lower expression of *Bach2* compared to GC B cells, a finding also consistent with their higher propensity for ASC differentiation [9]. A more complete understanding of the factors that control the different rates of differentiation by B cell subsets is an important goal, and one likely to provide a valuable insight into the complex construction of the humoral immune response.

Impact of cell division

Clonal expansion of selected antigen-specific B cells is necessary for an effective antibody response. Expanding the pool of specific cells provides the cellular substrate for generating the range of antigen-specific effector cell types needed for short and long-term protection. But how is it that these cells are directed in varying and appropriate proportions to numerous cell fates, including ASC that produce antibodies of different isotypes and of varying lifespans?

Remarkably, cell division plays more than a passive role in creating the expanded clone. This was first observed for CSR, where changes in antibody class are intimately linked to cell generation number in mouse and human B cells [116, 117]. CSR frequency per generation can be cytokine independent, as seen for LPS, or driven by cytokines such as IL-4, TGF-β or IFN-γ [118-120]. Furthermore, cytokine concentration strongly influences the link between CSR and division, with low concentrations resulting in more cell divisions before switching occurs [118, 120].

Similarly, ASC differentiation increases in frequency in a division-linked manner under stimulating conditions in vitro [10]. Once generated, ASCs divide, but do not undergo further CSR [14, 31]. Thus, a balance must be achieved for the expanded clone to allocate cells that first undergo CSR and then differentiation into ASCs. Linking both these key B cell fates to cell division enables a range of outcomes to arise as an automatic consequence of clonal expansion itself.

Models of B cell terminal differentiation.

The division-linked changes in B cell fate suggest that some form of 'resetting' of molecular processes takes place upon each division. This question has been investigated by filming cultured B cells continuously over several generations and following four fates – division, death, CSR and ASC development [13]. The behaviour of individual cells was remarkably heterogeneous, but statistical analysis and mathematical modelling showed that it was consistent with internal cellular machinery controlling each of the four fates, but operating independently and in competition within single cells. Despite immense single cell variation, a consistent outcome was observed at the population level. This mechanism of controlling cell fate by competition between internal cellular machinery represents a simple way to allocate cells to multiple outcomes (Box 3) [13, 121].

An alternative mechanism to account for B cell terminal differentiation proposes that GC B lymphocytes asymmetrically divide, resulting in the differential segregation within the cell of key molecules including Bcl6, the IL-21R, and a known polarity protein, atypical protein kinase C (Box 3) [122]. While such asymmetric cell division generates an unequal inheritance of potentially fate-altering molecules in daughter cells, the importance of this observation for GC dynamics and ASC differentiation remains to be shown. One recent study found that while members of the Scribble complex were asymmetrically distributed upon B cell division, deletion of components of this complex in mice had no impact on the antibody response [123]. How initially homogeneous cell types generate so many different cell types over a short period remains one of the compelling mysteries associated with antibody regulation.

The above results illustrate possible mechanisms for fate decisions by dividing B cells. Evidence for an automatic, internal program controlling clone size itself has also been described [124]. Direct imaging of CpG stimulated B cells, which can be followed

individually for several generations in vitro, revealed that cells typically divide 3-6 times under these conditions, then lose the motivation for growth and eventually die. The number of generations each clone undergoes is variable, but the descendants of each founder divide the same number of times - indicating that the total division number (the destiny) is in some way programmed into, and inherited from, this first cell [124]. The type and strength of stimulation influences the size of this generational limit [125]. Thus, we can postulate that a small number of division-linked processes automatically lead to expanded clones with heterogeneous outcomes, and that an internal default on clone size limits the output. A related mechanism was observed for GC using an inducible division-tracking marker [115]. At the height of the response cells undergo a burst of division of duration proportional to the strength of stimulation.

A challenging area for future study will be the integration of the cellular and molecular facets of B cell terminal differentiation in a way that explains the striking single cell heterogeneity. Alteration in extrinsic inputs such as signalling strength (antigen amount and affinity, and concentration of cytokines), as well as intrinsic changes to the concentration or activity of important regulatory intracellular proteins are likely to influence each other and cell division rates in complex ways (Box 1). This complexity and inter-relatedness requires development of quantitative methods to follow multiple simultaneous fate outcomes within single cells and to relate these changes to molecular expression levels. It is, as yet, early days for such an ambitious future understanding. Important research directions that may identify the source of molecular variation that underpins cellular heterogeneity are studies of stochastic variation in transcription factor concentration (or activity) in the differentiation process. For example Irf4 and Bach2 are both known to function as concentration-dependent rheostats for CSR and ASC differentiation [31, 70, 71, 80], while a relatively subtle increase in PU.1 expression maintains Pax5 and impairs ASC differentiation [66]. How these changes in transcription factor abundance arise from epigenetic processes to impact target gene expression and how these changes, in turn, link and alter with progressive cell division and control rich and diverse, but reproducible population outcomes, remain open questions for the field.

Plasmablast to plasma cell transition

As outlined above much research has focused on describing the factors that control the initial commitment to the ASC fate, however less is known about the transition between short-lived, cycling plasmablasts and long-lived, post-mitotic plasma cells (Figure 3). We have previously shown that plasmablasts and plasma cells can be prospectively isolated based on the expression of Blimp1/GFP, with plasmablasts displaying a distinctly lower GFP fluorescence than plasma cells [12]. A similar distinction has been demonstrated in human plasmablasts and plasma cells [84]. Whether the increased Blimp1 concentration is functionally relevant to this transition is unclear, however it is important to note that plasma cells silence the cell cycle program, including the transcription factor Myc, which is a target of Blimp1-repression [88, 126].

It remains an open question whether GC-derived plasma cells pass through a plasmablast-like stage or whether plasma cells are a direct product of GC B cells. Analysis of the circulating Ag-specific cells in the blood of mice shortly after primary immunization shows that the ASCs that presumably constitute the precursor for the long-lived bone marrow plasma cells are Blimp1/GFP-intermediate and thus resemble plasmablasts [127, 128]. Once in the bone marrow, these cells upregulate Blimp1/GFP to the high concentration characteristic of plasma cell. In addition, ASCs in the blood following recall challenge express the proliferation marker Ki-67, consistent with being plasmablasts [129]. Analysis of chemokine and homing molecule sensitivity of plasmablasts showed that only a subset of cells respond to bone marrow trophic factors, suggesting that only these responsive cells are precursors to plasma cells, with the bulk of the cells constituting a distinct, short-lived entity [128]. The factors that mediate the transition to, homing and survival of, fully mature plasma cells is discussed below.

Plasma cell homing and survival

While long-lived plasma cells exist in multiple lymphoid organs in the body and in non-lymphoid organs in disease situations, the bone marrow houses the majority of plasma cells in a healthy individual [113, 130, 131]. The longevity of plasma cells appears to be non-intrinsic, as displacement of the cells from the bone marrow microenvironment results in rapid cell death [132, 133]. Thus key questions are: 1) How do plasma cells, or their plasmablast-like precursor, home to the bone marrow? 2)

What are the niche components that maintain long-lived plasma cells? 3) How are these signals translated into cellular longevity?

Homing

The process of homing and retention in the bone marrow remains poorly understood. Activation of the S1P1 receptor is required for efficient egress of ASCs from secondary lymphoid organs to the blood, while the chemokine CXCL12 and its receptor CXCR4 are important for recruitment and retention of ASCs to the bone marrow [128]. In contrast, inflammatory chemokines such as CXCL9, 10, 11, that signal via CXCR3, promote homing of plasmablasts to sites of inflammation including non-lymphoid organs [134]. Retention in the bone marrow and maturation of ASCs into plasma cells involves engagement of VLA4 [135], CD44 [136], CD28 [22, 137] and CD93 [138] (Figure 4) on the plasma cells as well as the transcription factors, Klf2 [139], Zbtb20 [81, 140] and Aiolos [141].

Niches

Longitudinal studies of the frequency of antigen-specific plasma cells have demonstrated that a finite number of "niches" exist in the bone marrow [8]. The cellular nature of this niche has been the subject of numerous studies and remains contentious, with several distinct cellular lineages proposed as niche components (recently reviewed by [132, 142, 143]). While it is outside the scope of this review to discuss the many competing models of the plasma cell niche, it appears likely in the bone marrow to consist of at least a $CXCL12^{+}VCAM1^{+}$ stromal cell [144] and an APRIL-secreting hematopoietic cell (Figure 4). Recent evidence suggests a key hematopoietic cell type is the eosinophil [145], although it is feasible that multiple distinct blood cells fulfil this function [143]. The essential function of eosinophils is evidenced by the findings that mice lacking eosinophils have decreased numbers and maturation of bone marrow plasma cells, the co-localization of plasma cells with multiple eosinophils in the bone marrow and the secretion of APRIL and IL-6 by these associated eosinophils[145] (Figure 4).

Survival

Several factors have been proposed to mediate plasma cell survival in the bone marrow, including IL-6, TNFα and APRIL [136, 146-148], with genetic evidence suggesting APRIL and its receptor BCMA are the most functionally significant components of the niche [149, 150]. How BCMA expression is regulated in plasma cells is

unclear, although the process is known to be independent of Blimp1 [149]. A key consequence of signalling through the APRIL: BCMA axis is the expression of the anti-apoptotic protein Mcl-1, which is essential for the survival of all ASCs [149]. The very short half-life of Mcl-1 protein suggests a model whereby continuous exposure to niche-derived signals, including APRIL maintains Mcl-1 thereby making the survival of the long-lived plasma cell highly sensitive to its location. Interestingly, the survival promoting activity of APRIL and the cytokine IL-6 require activity of inducible nitric oxide synthase suggesting that nitric oxide is a signalling intermediary in ASC survival [151]. Plasma cells also express the inhibitory Fc receptor, FcγRIIb, whose crosslinking induces plasma cell apoptosis, suggesting a negative regulatory loop where serum antibodies impact on plasma cell homeostasis [152].

Conclusions

The production of antibody by plasma cells is of tremendous importance to human health, providing immunity during initial exposure to a pathogen and mediating the protective effects of vaccination. The ASC differentiation process has also been exploited to provide the highly specific monoclonal antibodies that are used as diagnostics and as therapeutics to treat many diseases. Plasma cells do, however, have a downside, including involvement in some autoimmune diseases and MM. Thus it is critical to understand plasma cell differentiation, particularly as it relates to the selection of high-affinity clones into the long-lived plasma cell pool. As outlined in this review, much has been learnt about this process, but many questions remain, including how long-lived plasma cells are selected and home to their bone marrow niche. Moreover, the transcriptional network that underpins this longevity is comparatively poorly characterized. The advent of high-resolution techniques such as single cell imaging, and the application of powerful high-throughput DNA/RNA sequencing technologies to plasma cells will yield a wealth of new information to permit further manipulation of this cell type for therapeutic benefit.

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Competing interests statement

The authors declare no competing financial interests.

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Figure legends

Figure 1. The cellular stages of late B cell differentiation. The majority of mature B cells are located in the follicles of lymphoid organs (follicular B cells), while other specialized subsets include marginal zone B cells that localize to the region between the red and white pulp in the spleen, and B1 B cells found in the peritoneal and pleural cavities. All mature B cell subsets express Pax5, PU.1, Irf8 and Bach2 while low levels of Irf4 are induced by antigen receptor signalling. B cells activated by antigen are capable of rapid proliferation, Ig class switch recombination and differentiation into short-lived plasmablasts that express high amounts of Irf4, Xbp1 and intermediate (int) amounts of Blimp1 and secreted Ig. Follicular B cells can also upregulate Bcl6 and repress Irf4 during the germinal center (GC) reaction where affinity maturation of the antigen receptor occurs. B cells with high-affinity antigen receptors exit the GC and differentiate into either memory cells, that express a similar transcriptional signature to mature B cells, or long-lived plasma cells that express high amounts of Irf4, Xbp1 and Blimp1 and produce large quantities of Ig. While Blimp1^{high} plasma cells derive from Blimp1^{int} cells, it remains unknown whether plasma cells derive from plasmablasts or directly from an earlier plasma cell committed stage. The contribution of marginal zone and B1 B cells to the long-lived plasma-cell compartment is also poorly characterized.

Figure 2. The gene regulatory network controlling B cell terminal differentiation. Model gene regulatory networks describing the action of key transcription factors in (A) activated B cells and (B) antibody secreting cells. The individual genes are shown in schematic form only. • Indicates functions of the protein encoded by the indicated gene resulting in direct binding in the regulatory regions of the linked target genes, leading to transcriptional activation (↓) or repression (⊥). Linkages are color coded for clarity only. Genes that are expressed are shaded black, while lowly- or non-expressed genes are in gray.

Figure 3. A scheme for plasma cell maturation. Indicative properties of antibody secreting cells from the plasmablast, immature plasma cell and mature plasma cell compartments. Naïve B cells are included as a reference point. While the values used

in the figure are from humans, similar changes occur in mice. For example, the markers CD138, CXCR4, CD19, MHCII, CD20 and CD45 are congruent in both species while CD38 and CD27 are not. More detailed descriptions of changes specific for plasma cell development can be found in references cited in the text. Abbreviations: LO – lymphoid organs; BM – bone marrow; +/- – low level or heterogeneous expression. Plasmablasts are indicated to secrete 'all' Ig Isotypes as they show no constitutive distribution, but depend rather on the stimulus that generated them.

Figure 4. Model of the bone marrow plasma cell survival niche. Plasma cells are recruited to the bone marrow through the expression of the chemokine CXCL12 and cellular adhesion molecules on bone marrow stromal cells. A variety of hematopoietic cell types are then proposed to act as secondary components, secreting the important survival factors APRIL and IL-6. Shown here are eosinophils as one documented APRIL-producing cell type that associates with bone marrow plasma cells. Plasma cells express BCMA, IL-6R, VLA4, CD93, CD28 and CD44, all of which are important for plasma cell homing and survival as well as the transcription factors Blimp1, Xbp1, Irf4 (not shown) and Zbtb20. Signalling through the APRIL: BCMA axis induces large amounts of Mcl-1 the essential anti-apoptotic factor for plasma cells.

BOXES

Box 1 – Sources of Heterogeneity for B cell responses

1. The B cell type – Follicular, marginal zone, B1 and memory B cells all display intrinsically altered propensity to differentiate into ASCs after exposure to the same exogenous stimuli.
2. Antigen – antigen can be presented to a specific B cell in very different physical forms. For the B cell, the antigen provides opportunity for signalling differences that alter the outcome of the response outlined in Figure 1. Classically antigen responses are divided into T-independent (non-protein) and T-dependent (protein), but the response is known to be more complex, being influenced by antigen structure and valency.

3. The co-stimuli – B cells can receive multiple additional activation stimuli that have different consequences and are integrated in unique patterns, presumably reflecting evolution and selection for successful survival strategies. Toll-like receptor ligands such as LPS are associated with T-independent responses and rapid ASC differentiation, while T cell derived signals, CD40L and cytokines such as IL-4 and IL-21, are associated with GC responses, affinity maturation and long-lived plasma cells, while still generating some short-term antibody responses.

Box 2. Malignancies of mature B cells and plasma cells

Late B cell differentiation is a prominent source of human lymphomas, due to the high proliferative rate of germinal center (GC) B cells combined with the expression of AID, the enzyme responsible for initiating class switch recombination and somatic hypermutation, both DNA altering processes. Unsurprisingly, many of the important transcriptional regulators of B cell terminal differentiation are also involved in Diffuse Large B cell Lymphoma (DLBCL) and Multiple Myeloma (MM).

BCL6 expression is deregulated in most DLBCL through a number of mechanisms, while *IGH-BCL6* translocations are characteristic of the activated B cell (ABC) subtype [32]. This translocation both overexpresses BCL6 and prevents its physiological down-regulation in ASCs, thus trapping cells in a GC state. Similar *IGH-PAX5* translocations are found in mature B cell lymphomas [26]. The *BLIMP1*/*PRDM1* gene is also mutated in ~25% ABC-DLBCL, with silencing or loss of the second allele [95]. Similar lymphomas are observed in mouse models of *Bcl6* overexpression or *Blimp1*-deficiency [153-155]. In line the antagonistic function of BLIMP1 and BCL6 in B cell differentiation, the mutations of each factor are mutually exclusive in lymphomas.

Genome sequencing has revealed that *XBP1*, *IRF4* and *BLIMP1* are all mutated in MM [96, 109], which was surprising as all three factors were considered essential for MM survival. While the natures of the IRF4 mutations are untested, IRF4 has been shown to be essential for MM cell survival in a process termed non-oncogene addiction [156]. In contrast, the mutations in *BLIMP1* and *XBP1* are clearly deleterious and suggest that MM do not absolutely require either factor for their survival. While *BLIMP1*

mutations were the most frequent of all plasma cell specific genes [96], it remains to be determined how BLIMP1 functions as a tumor suppressor in both DLBCL and MM.

Box 3. Symmetry and asymmetry, determinism and randomness in plasma cell differentiation

How do plasma cells arise following a B cell response? There is evidence for a number of mechanisms:

1. *Deterministic*. The earliest and most widely held view is that cell fates result from variation in externally delivered signals. Differences in activating signals (such as antigen affinity) as well as cytokines provided by helper T cells can alter the broad features of the antibody response. The rich environments of the germinal center (GC) and the lymphoid organs provide the clear opportunity for orchestrated changes that could easily promote or impede the development of plasma cells.
2. *Programmed automaton*. Under some conditions B cells undertake a programmed response with stimulation leading to a series of divisions, differentiation and the automatic return to quiescence without any further direction [157]. This automated outcome may be a primitive cellular program as it is particularly striking following stimulation by the evolutionary ancient TLR pathway. An autonomous programmed response of limited division burst was also recently noted for GC B cells in vivo [115], and is consistent with early measurement of responses in vivo [158]. A variant of this mechanism includes the possibility of an asymmetric division, setting up two fate lineages early after stimulation. This is a popular theme for allocation of T cell memory and effector cells [159], and has been suggested for B cells based on evidence for asymmetric partitioning of proteins between GC cell daughters [122].
3. *Stochastic competition*. Studies that isolate B cells and measure responses in vitro generate considerable cellular heterogeneity suggesting strong intrinsic mechanisms play a role in fate determination [10]. This result supports a stochastic mechanism for allocating cells to different fates. Direct imaging of B cells undergoing class switch recombination and antibody-secreting cell (ASC) differentiation was consistent with temporal competition for different

fates operating independently within each cell [13] . Variation in times, presumably as the result of differences in molecular construction of each cell, account for all outcomes. Such a mechanism can account for enormous single cell heterogeneity while summing to a robust population outcome [121].

The relative contribution of each process in the many different activation scenarios represents one of the ongoing puzzles of B cell biology. The end result must be consistent with the transcriptional programming of ASC generation described here, and illustrated in Figure 2.

About the authors

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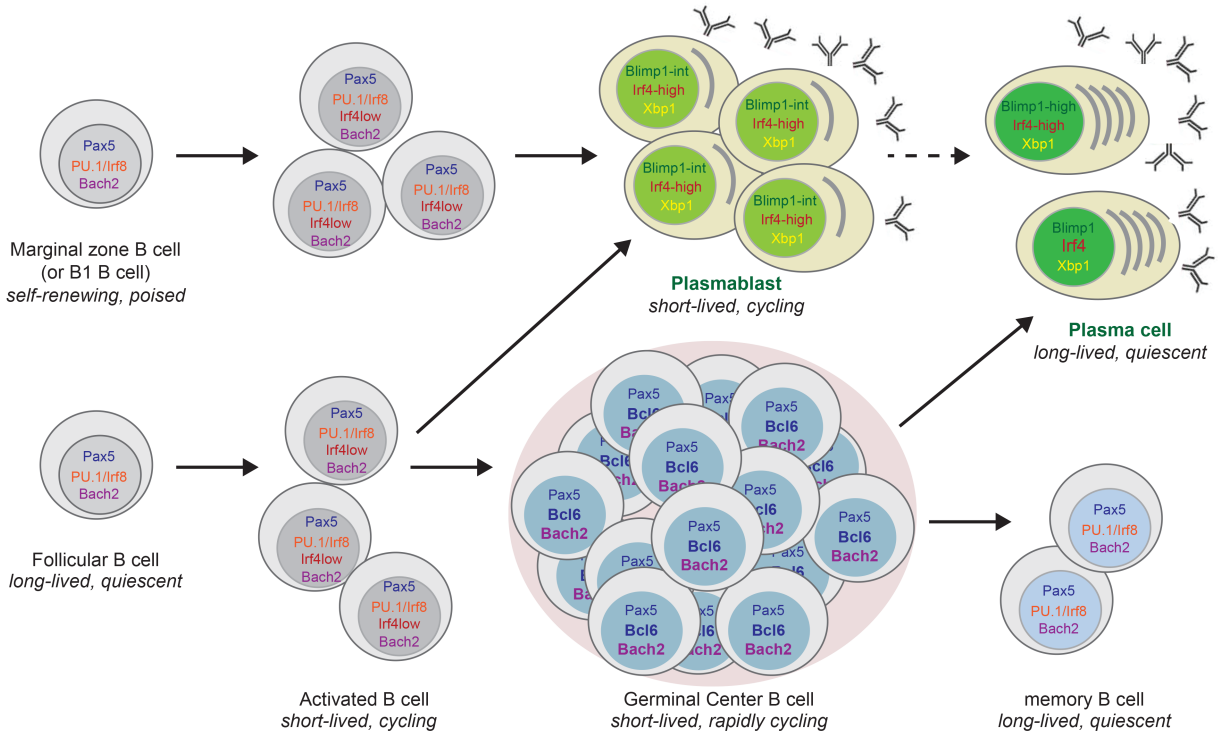
Online Summary

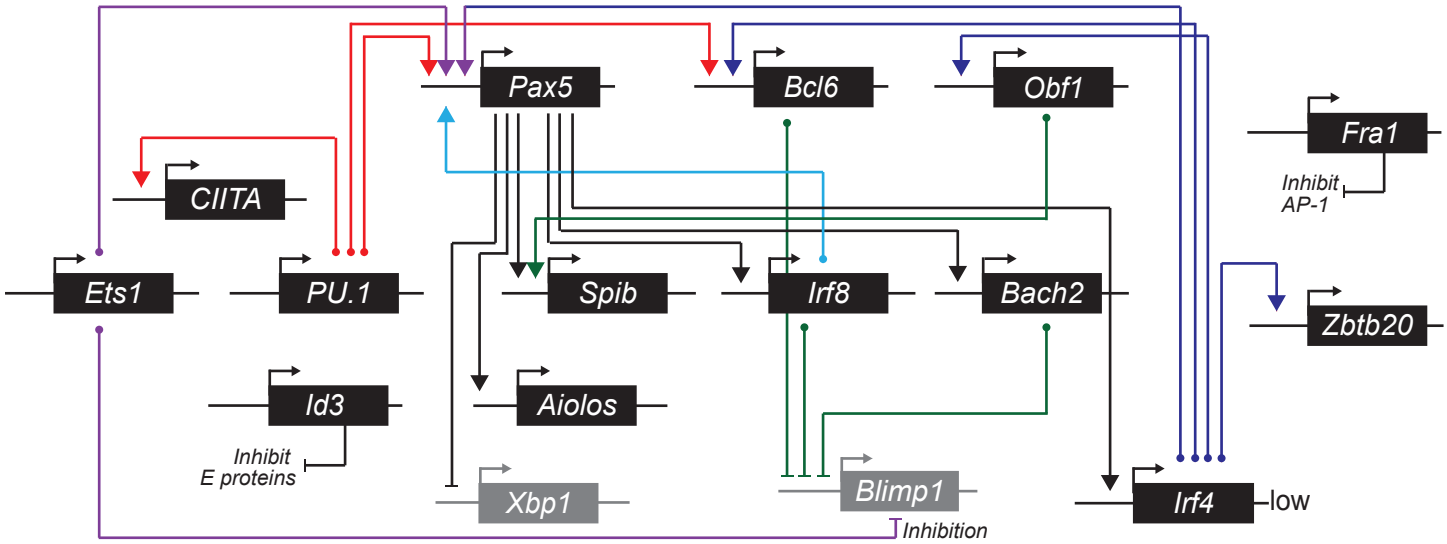
- B cell terminal differentiation generates short-lived plasmablasts and long-lived plasma cells.
- B cells and antibody secreting cells display markedly divergent transcriptomes.
- Differentiation of antibody secreting cells from B cells is controlled by a network of antagonistic transcription factors.
- Despite considerable heterogeneity, a simple probabilistic differentiation process can explain B cell terminal differentiation.
- Transition from short-lived plasmablast to long-lived plasma cell requires homing to the bone marrow niche.
- The plasma cell niche consists of a stromal component and an APRIL-expressing hematopoietic cell.
-

Nutt, Figure 1



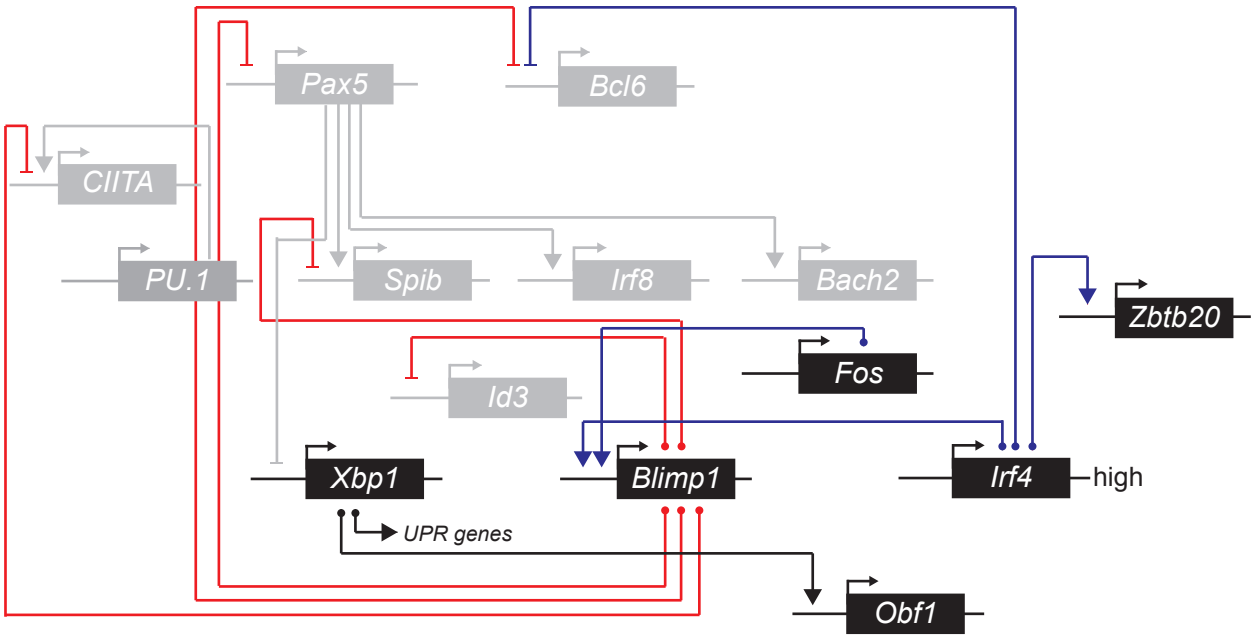
A

Activated B cell network



B

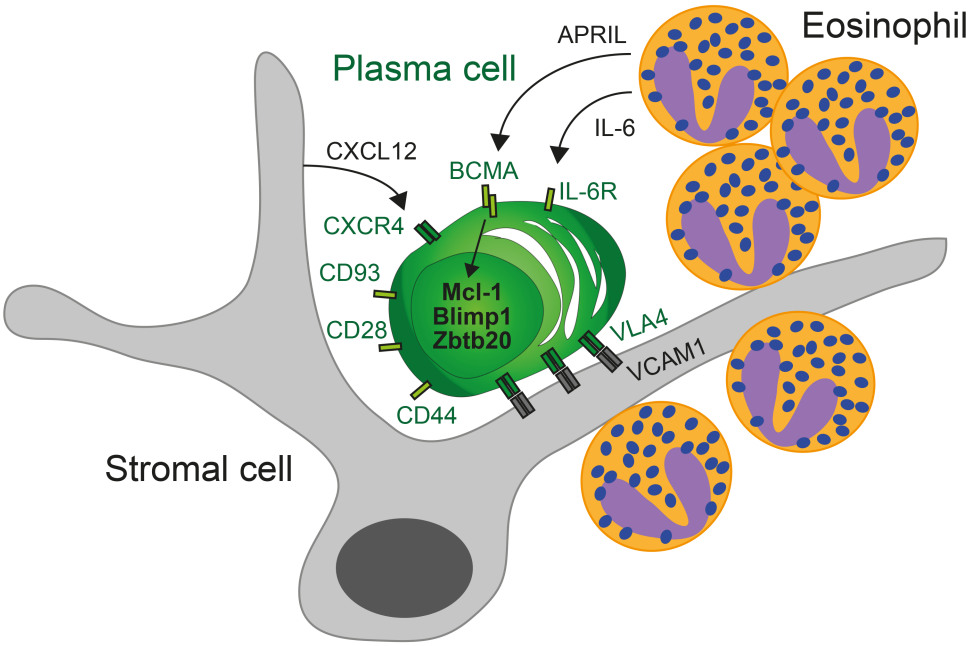
Antibody secreting cell network



Nutt, Figure 2

	Naive B cell	Plasmablast	Immature plasma cells	Mature plasma cells
Lifespan	++	+	+	++++
Proliferation	-	++	-	-
Markers	- +++	+ ++	++ +/-	+++ CD38, CD27 CD138, CXCR4 +/- CD19, CD20 MHCII, CD45
Location	LO	LO, Blood	LO	BM
Isotype	IgM, IgD	all	IgM=IgG>IgA	IgG>>IgA>IgM
Blimp1	-	+	+	++

LO = lymphoid organs



Nutt, Figure 4