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 effector cells of the early antibody response, whereas plasma cells are the long-lived mediators of lasting humoral immunity. An extraordinary number of control mechanisms, at both the cellular and molecular levels, underlie the regulation of this essential arm of the immune response. Despite this complexity, the terminal differentiation of B cells can be described as a simple probabilistic process that is governed by a central gene-regulatory network and modified by environmental stimuli.

Plasmablasts
Dividing antibody-secreting cells of the B cell lineage that have migratory potential. These cells can further mature into plasma cells, which do not divide.

Plasma cells
Terminally differentiated quiescent B cells that develop from plasmablasts and are characterized by their capacity to secrete large amounts of antibodies.

Antibody-secreting cells
(ASCs). Refers to both proliferating plasmablasts and non-proliferating plasma cells. The term is used when both cell types might be present.

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

The humoral immune system is clearly complex and has many components. Research activity has focused on the different levels of the humoral immune system: the cellular and anatomical diversity, the patterns of transition during activation and differentiation, the molecular control of these processes and the complex microenvironments *in vivo* that enable signal integration. Insights gained by these broad approaches have enabled the realization of some of the important rules that define a canonical B cell response programme. Integrating such 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 that enable a better understanding and a greater manipulation of the humoral immune system both to improve immunity and to thwart autoimmune pathologies. In this Review, we focus on the factors that influence the generation, function and maintenance of ASCs to highlight the progress being made towards achieving an integrated view of the humoral immune response.

B cell terminal differentiation

Mature B cells have three major subsets: follicular B cells, marginal zone (MZ) B cells and B1 cells[2,3] (FIG. 1). Follicular B cells are the dominant subset, and they are located in the lymphoid follicles of the spleen and lymph nodes. MZ B cells, by contrast, abut the marginal sinus of the spleen, where they are ideally placed to encounter blood-borne pathogens and particulate antigens. B1 cells are located mostly in the peritoneal and pleural cavities and at mucosal sites, which facilitates their surveillance of the tissues that are the most susceptible to environmental pathogens[1]. These specialized locations enable B1 cells and MZ B cells to rapidly respond to T cell-independent antigens (TI antigens), such as bacterial components[1]. Follicular B cells, although also capable of responding to TI antigens, seem to be more specialized for responding to protein antigens that elicit simultaneous $CD4^+$ T helper cell activation.

The production of ASCs in response to T cell-dependent (TD) antigens is a two-step process, with the first and second steps providing immediate and persistent protection, respectively (FIG. 1). In the first step, known as the 'extrafollicular response', B cells receive an antigen receptor-dependent signal, which leads to the development of B lymphoblasts that divide (and may undergo immunoglobulin 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 majority of the early protective antibodies that are produced.

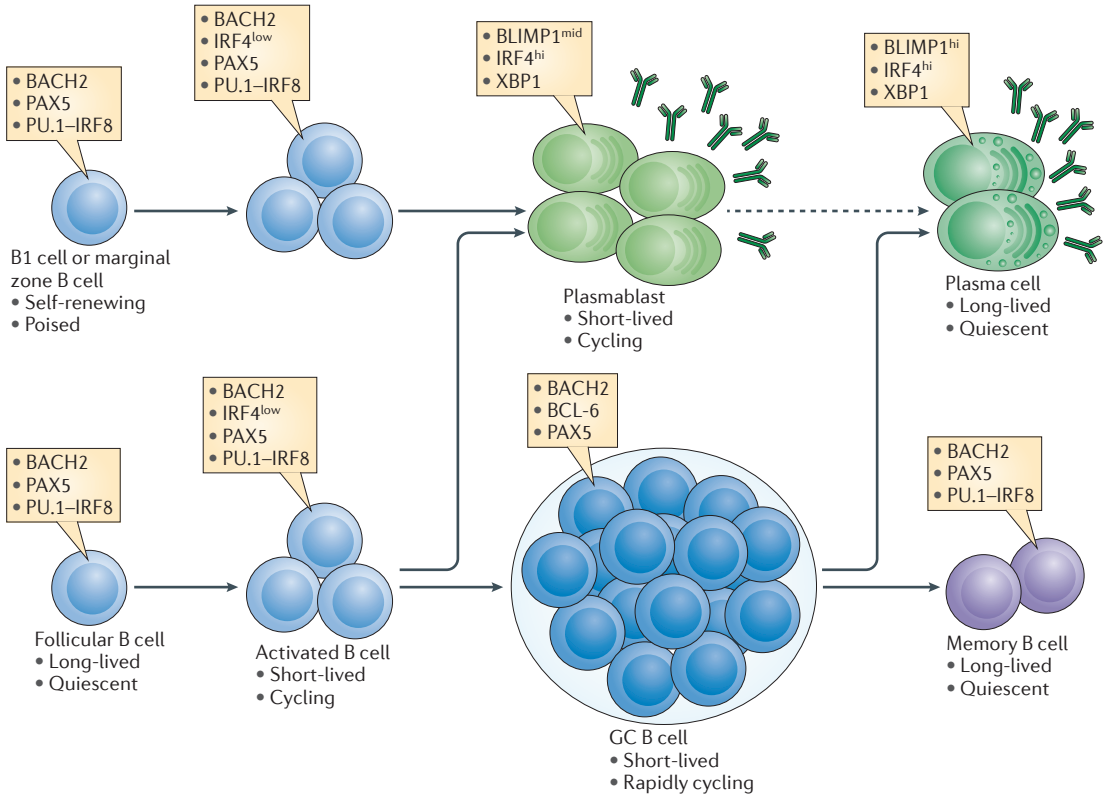

Figure 1 | **The cellular stages of late B cell differentiation.** The majority of mature B cells are located in the follicles of lymphoid organs and are known as follicular B cells. Other specialized B cell subsets include marginal zone B cells, which localize to the region between the red and white pulp in the spleen, and B1 cells, which are found in the peritoneal and pleural cavities. All mature B cell subsets express the transcription factors paired box protein 5 (PAX5), PU.1, interferon-regulatory factor 8 (IRF8), and BTB and CNC homologue 2 (BACH2), whereas low levels of IRF4 are induced by antigen receptor signalling. B cells activated by antigen (also termed B lymphoblasts) are capable of rapid proliferation, immunoglobulin class-switch recombination, and differentiation into short-lived plasmablasts that express high levels of IRF4 and X-box-binding protein 1 (XBP1), and intermediate (mid) levels of B lymphocyte-induced maturation protein 1 (BLIMP1) and secreted antibody. Follicular B cells can also upregulate B cell lymphoma 6 (BCL-6) and repress IRF4 expression during the germinal centre (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 B cells, which express a similar transcriptional signature to mature B cells, or long-lived plasma cells, which express high levels of BLIMP1, IRF4 and XBP1, and produce large quantities of antibody. Although BLIMP1^{hi} plasma cells derive from BLIMP1^{mid} cells, it remains unknown whether plasma cells are derived from plasmablasts (indicated by the dashed arrow) or from an earlier plasma cell-committed stage. The contribution of marginal zone B cells and B1 cells to the long-lived plasma cell compartment is also poorly characterized.

T cell-independent antigens (TI antigens). Antigens that directly activate B cells, without the need for cognate T cell help.

Class-switch recombination (CSR). The process by which proliferating B cells rearrange their DNA to switch from expressing IgM (or another class of immunoglobulin) to expressing a different immunoglobulin heavy chain constant region, thereby producing antibody with different effector functions.

Somatic hypermutation (SHM). A unique mutation mechanism that is targeted to the variable regions of rearranged immunoglobulin gene segments. Along with selection for B cells that produce high-affinity antibody, SHM leads to the affinity maturation of B cells in germinal centres.

Germinal centre (GC). A highly specialized and dynamic microenvironment that gives rise to secondary B cell follicles during an immune response. It is the main site of B cell maturation, which leads to the generation of memory B cells and plasma cells that produce high-affinity antibody.

In the second step of a TD response, some of the activated B cells re-enter the B cell follicle and, under the influence of specialized T follicular helper (T_{FH}) cells, proliferate vigorously to form a germinal centre (GC)[5–7]. GCs are sites of extensive proliferation and selection that is based on antigen receptors that have been modified by SHM. The GC ultimately produces high-affinity, long-lived plasma cells that are capable of sustaining a high level of antibody secretion[6,8]. The GC also produces memory B cells that maintain a B cell phenotype but that seem to be epigenetically programmed to rapidly differentiate into ASCs following re-exposure to antigen[9]. This GC phase of the response seems to have evolved to provide effective protection against a future infection, even though it occurs in parallel with the protective and rapid extrafollicular phase.

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

Transcriptional circuitry of B cell differentiation

At the transcriptional level, the transition of an activated B cell into an ASC is essentially a lineage switch, as it requires the coordinated regulation of hundreds of gene

Box 1 | **Sources of heterogeneity in B cell responses**

Type of B cell
Follicular, marginal zone, B1 and memory B cells all display an intrinsically altered propensity to differentiate into antibody-secreting cells (ASCs) after exposure to the same exogenous stimuli.

Antigen
Antigen can be presented to a specific B cell in very different physical forms and this provides an opportunity for differences in signalling to arise that alter the outcome of the B cell response (FIG. 1). Classically, antigen responses are divided into those that are T cell-independent (non-protein) and those that are T cell-dependent (protein); however, the influence of antigen structure and valency increases the complexity of these responses.

Co-stimuli
B cells can receive multiple additional activation stimuli (co-stimuli) that have different consequences and are integrated in unique patterns, presumably reflecting the evolution of and selection for successful survival strategies. Toll-like receptor ligands such as lipopolysaccharide are associated with T cell-independent responses and rapid ASC differentiation, whereas T cell-derived signals (such as CD40 ligand, and cytokines such as interleukin-4 (IL-4) and IL-21) are associated with germinal centre responses, affinity maturation and long-lived plasma cells, while still generating some short-term antibody responses.

expression changes along with major chromatin remodelling. These fundamental changes in gene expression result from the silencing of a group of key transcription factors that control B cell identity and the expression of ASC-specific regulators[14,15]. The transcription factors that define B cell and ASC identity regulate mutually antagonistic gene expression programmes, resulting in two cellular states — B cells and ASCs — that are stable in the long-term[16]. How the B cell gene expression programme is destabilized, and ultimately silenced, to allow cells to commit to the ASC fate is discussed below. Failure to complete the B cell to ASC transition, through either the maintenance of factors that specify B cell identity or the loss of crucial ASC differentiation factors, is a major cause 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, in which they promote the B cell gene expression programmes that underlie antigen and T cell sensitivity, and that prevent premature ASC differentiation. The best-characterized B cell factors are discussed below.

PAX5. Very few aspects of the B cell transcriptional programme can operate independently of the transcription factor paired box protein 5 (PAX5), which binds to many thousands of sites in the DNA of follicular B cells[17]. PAX5 is expressed throughout B cell development, during which 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 lineage development, as the 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 the immunoglobulin heavy chain (IgH) and the signal transduction chain Igα (also known as CD79A), other immune receptors such as CD19 and CD21, and also the transcription factors interferon-regulatory factor 4 (IRF4), IRF8, BACH2 (BTB and CNC homologue 2), Aiolos (also known as IKZF3) and SPIB[20,21] (FIG. 2). PAX5 also represses the expression of a set of genes that are not normally expressed by B cells, including FMS-related tyrosine kinase 3 (*Flt3*), CC-chemokine receptor 2 (*Ccr2*) and *Cd28*, which are re-expressed after the downregulation of *Pax5* in ASCs[11,22].

Although 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 development[23,24], whereas deletion of *Pax5* in chicken DT40 cells induces some aspects of ASC differentiation and immunoglobulin secretion[25]. Most compellingly, *PAX5* expression is often maintained in human B cell lymphomas through its translocation to the *IGH* locus, which blocks further B cell differentiation[26].

Box 2 | **Malignancies of mature B cells and plasma cells**

Late B cell differentiation is a prominent source of human lymphomas owing to the high proliferative rate of germinal centre (GC) B cells combined with the expression of activation-induced cytidine deaminase (AID), the enzyme responsible for initiating class-switch recombination and somatic hypermutation (both of which are 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.

B cell lymphoma 6 (*BCL6*) expression is dysregulated in most DLBCL through a number of mechanisms, whereas translocations of *BCL6* to the *IGH* locus are characteristic of the activated B cell (ABC) subtype of DLBCL[32]. This translocation results in the overexpression of *BCL6* and prevents its physiological downregulation in antibody-secreting cells, thus trapping the cells in a GC state. Similar translocations of *PAX5* to the *IGH* locus are found in mature B cell lymphomas[26]. The B lymphocyte-induced maturation protein 1 (*BLIMP1*) gene is also mutated in ~25% ABC-DLBCL cases, 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 agreement with the antagonistic function of BLIMP1 and BCL-6 in B cell differentiation, the mutations of each factor are mutually exclusive in lymphomas.

Genome sequencing has revealed that X-box-binding protein 1 (*XBP1*), interferon-regulatory factor 4 (*IRF4*) and *BLIMP1* are all mutated in multiple myeloma[96,109], which was surprising as all three factors were considered to be essential for the survival of multiple myeloma tumour cells. Although the nature of the *IRF4* mutations remains untested, IRF4 has been shown to be essential for multiple myeloma tumour cell survival in a process termed 'non-oncogene addiction' (REF. 156). By contrast, the mutations in *BLIMP1* and *XBP1* are clearly deleterious and suggest that multiple myeloma tumour cells do not absolutely require either factor for their survival. Although *BLIMP1* mutations were the most frequent of all plasma cell-specific gene mutations[96], the mechanisms by which BLIMP1 functions as a tumour suppressor in both DLBCL and multiple myeloma remain to be determined.

Although mouse models that mimic these translocations have been reported, it is not yet known whether these translocations impair ASC differentiation[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 the reactivation of the PAX5-repressed genes is a very early event in ASC differentiation[11]. This reactivation occurs in the presence of the normal concentration of PAX5 protein, which implies that there is an alteration in PAX5 function, potentially through a post-translational modification or a change in cofactor interactions. The precise mechanism of this inactivation of PAX5-mediated gene repression remains to be determined.

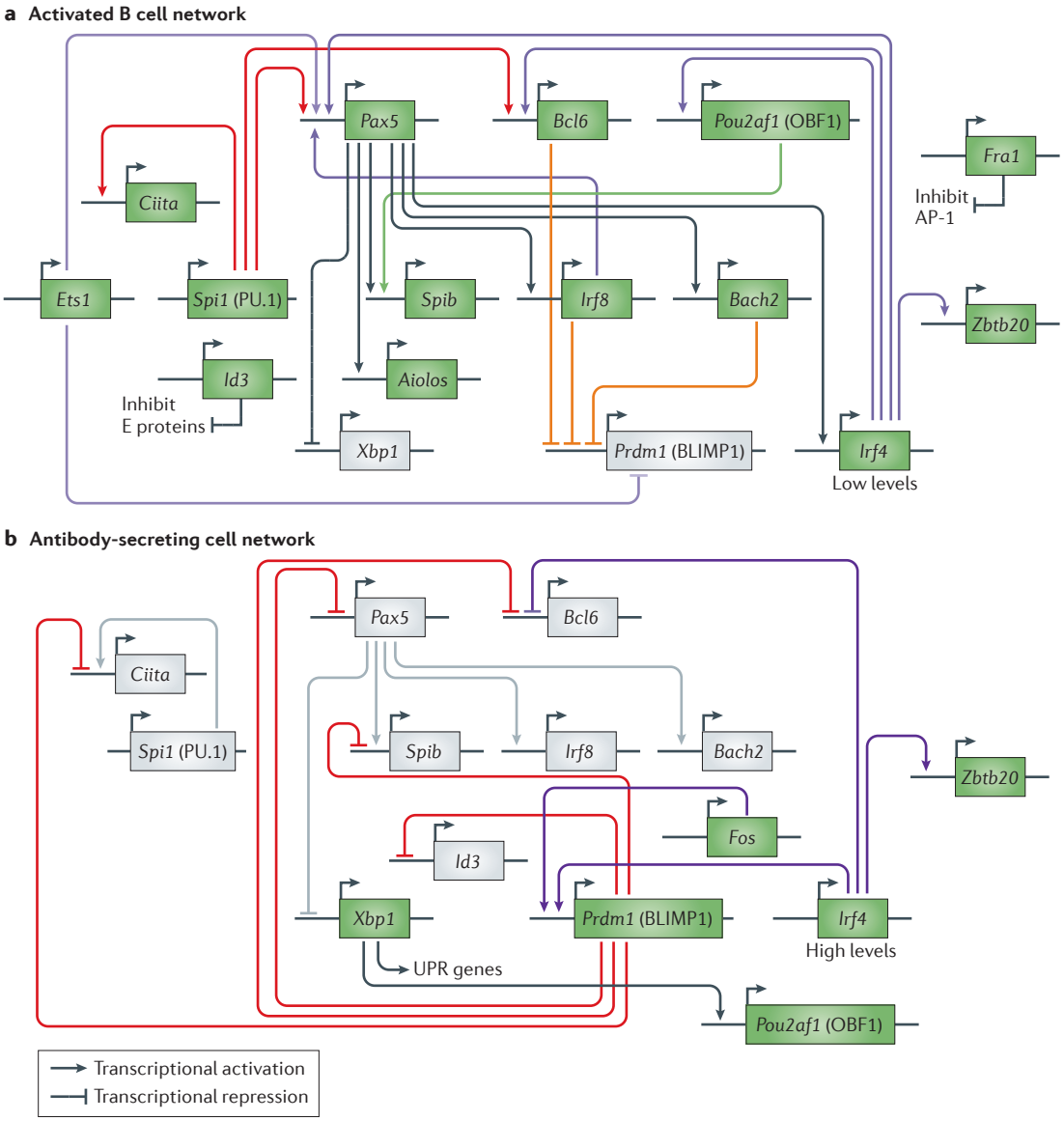

Figure 2 | **The gene-regulatory network controlling B cell terminal differentiation.** This figure depicts model gene-regulatory networks and shows the action of key transcription factors in activated B cells (part **a**) and antibody-secreting cells (that is, plasmablasts and plasma cells; part **b**). The individual genes are shown in schematic form only. The lines indicate the direct binding of the protein encoded by the indicated gene to the regulatory regions of the linked target genes, which leads to transcriptional activation or repression. When the protein name is different to the gene name, the protein name is included in parentheses. Linkages are colour-coded for clarity only. Genes that are expressed are shaded green, whereas genes that are expressed at low levels or not at all are shaded grey. AP-1, activator protein 1; *Bach2*, BTB and CNC homologue 2; *Bcl6*, B cell lymphoma 6; BLIMP1, B lymphocyte-induced maturation protein 1; *Ciita*, MHC class II transactivator; *Fra1*, FOS-related antigen 1; *Id3*, DNA-binding protein inhibitor ID3; *Irf*, interferon-regulatory factor; *Pax5*, paired box protein 5; *Pou2af1*, POU domain class 2 associating factor 1; *Prdm1*, PR domain zinc finger protein 1; UPR, unfolded protein response; *Xbp1*, X-box-binding protein 1; *Zbtb20*, zinc finger and BTB domain-containing protein 20.

BACH2. BACH2 is a transcriptional repressor that is expressed throughout B cell differentiation but, in keeping with its positive regulation by PAX5 (REF. 20), BACH2 expression is absent from ASCs. BACH2-deficient mice lack GCs and the expression of *Aicda* (which encodes the essential mediator of CSR and SHM, activation-induced cytidine deaminase (AID))[28]. A major target of BACH2 in B cells is the gene encoding B lymphocyte-induced maturation protein 1 (*Blimp1*; also known as *Prdm1*)[29,30] (FIG. 2). In the absence of BACH2, the formation of mature B cells is relatively normal 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]. BACH2 is a crucial component of the genetic network that controls the timing of ASC differentiation; however, to date, there are no studies of global DNA binding by BACH2 in B cells, and thus there is little understanding of the mechanisms of its action beyond *Blimp1* regulation.

BCL-6. B cell lymphoma 6 (BCL-6), which predominantly functions as a transcriptional repressor, is another factor that is highly expressed in GC B cells and is essential for their formation[32]. BCL-6 facilitates the rapid proliferation of GC B cells and, through the inhibition of the DNA damage response, facilitates their tolerance of the high rates of SHM. BCL-6 binds to the regulatory regions of many thousands of genes in GC B cells, including those that control the expression of the cell cycle regulators p21 and p53, and of oncogenes, including *Myc* and *Bcl2* (REFS 33–35). The repressive function of BCL-6 is mediated through the direct interaction of its BTB-domain with co-repressors such as nuclear receptor co-repressor 1 (NCOR1) and NCOR2 (also known as SMRT)[36].

One proposed target of BCL-6-mediated repression is *Blimp1* (REFS 15,37), and this repression establishes an antagonism that blocks ASC differentiation in GC B cells (FIG. 2). IL-21 (REFS 38,39) and the transcription factor IRF8 (REFS 40,41) are reported to sustain *Bcl6* expression, whereas IRF4 and BLIMP1 are proposed to repress *Bcl6* (REFS 42,43) (FIG. 2). Downregulation of *Bcl6* expression 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 that ASCs expressing BCL-6 exist in the bone marrow[44]. This suggests that ASC differentiation can occur in the presence of high levels of BCL-6. Unlike PAX5, BCL-6 seems to have 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 (encoded by *Pou2f2*) and its co-activator OBF1 (encoded by *Pou2af1*) were discovered via their binding activity in immunoglobulin gene promoters; however, gene expression of IgH and immunoglobulin light chain (IgL) is normal in their absence[45]. By contrast, these factors are required for the development of MZ B cells (both factors) and B1 cells (OCT2 only)[46–48].

OBF1 has a unique role in TD B cell responses as OBF1-deficient B cells cannot form GCs and when exposed to TD stimuli, they 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-5 receptor α-chain[51]. Finally, both OCT2 and OBF1 enable activated B cells to secrete optimal levels of IL-6, an inducer of T_{FH} cell differentiation[52].

PU.1, SPIB, ETS1 and IRF8. It has long been known that the ETS family transcription factor PU.1 (also known as SPI1), and the closely related factor SPIB, can form complexes on DNA with IRF4 and IRF8 (REF. 53), although the exact function of these complexes in B cells has remained unclear. PU.1 (REF. 54) and IRF8 (REF. 55) are constitutively expressed throughout B cell differentiation, whereas SPIB expression peaks in GC B cells[56], where it is regulated by OBF1 (REF. 57). The expression of all three factors (PU.1, IRF8 and SPIB) is subsequently downregulated in ASCs, with the repression of *Spib* being mediated by BLIMP1 (REF. 14) (FIG. 2).

The B cell-specific inactivation of either PU.1 (REFS 58,59) or IRF8 (REF. 60) results in only mild alterations in B cell development and function, whereas the loss of SPIB impairs GC maintenance[61]. Such relatively mild phenotypes are surprising, as PU.1 and IRF8 bind the regulatory elements of thousands of genes in B cells[62,63]. Recent evidence suggest that these factors do indeed play an important part in late B cell differentiation that is masked by complex redundancies and dosage sensitivities; B cells lacking both PU.1 and IRF8 show a marked hyperresponsiveness to both TD and TI stimuli, and generate both increased numbers of ASCs and proportions of cells that have undergone CSR, particularly switching to IgE production[41]. On a cellular level, a PU.1–IRF8 complex 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]. Furthermore, PU.1–IRF8 maintains the expression of B cell promoting factors such as *Bcl6, Pax5* and myocyte-specific enhancer factor 2C (*Mef2c*), while concomitantly repressing *Blimp1* (REF. 41) (FIG. 2).

PU.1 functions in a dose-dependent manner during early haematopoiesis 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 the microRNA miR-155, which binds to the 3′ untranslated region of PU.1 (REF. 65). This control seems to be of physiological importance as the specific removal of the miR-155 recognition sequence from the gene encoding PU.1 (*Spi1*) increased PU.1 concentration twofold. 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 has a role in B cell terminal differentiation. ETS1-deficient B cells show a greatly increased capacity to differentiate into ASCs in the presence of CpG oligodeoxynucleotides[67], which

MicroRNA
A single-stranded RNA molecule of approximately 21–23 nucleotides in length that regulates the expression of genes by binding to the 3′ untranslated regions of specific mRNAs.

Multiple myeloma
A cancer of plasma cells that is characterized by an increased frequency of malignant cells in the bone marrow and high levels of monoclonal immunoglobulin in the serum.

is normally an inefficient inducer of ASCs. Similar to PU.1, ETS1 functions to promote *Pax5* expression and to 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 crucial for the normal production of ASCs.

Drivers of ASC differentiation. The generation of ASCs requires the B cell transcriptional programme to be silenced, allowing induction of an ASC transcriptome that is geared towards the production of an enormous amount of immunoglobulin, as well as homing to, and surviving in, unique bone marrow niches. This transition is achieved through the action of three transcription factors — IRF4, BLIMP1 and X-box-binding protein 1 (XBP1) — the functions of which 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 to DNA weakly on its own, but displays strong cooperative binding in the presence of PU.1. Other binding partners include the basic leucine zipper transcriptional factor ATF-like (BATF) component of the activator protein 1 (AP-1) complex[73–75] and nuclear factor of activated T cells (NFAT)[76]. The IRF4–BATF complex probably has an important role 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* and *Igl* loci[78], and also bind to the B cell-specific enhancer of *Pax5* (REF. 79). IRF4 functions in a dose-dependent manner, with low IRF4 levels promoting GC fate and CSR through the activation of *Aicda*, *Pou2af1* and *Bcl6* (REFS 70,71,80), whereas high amounts of IRF4 repress *Bcl6* and activate both *Blimp1* (REF. 71) and the BCL-6-related transcription factor zinc finger and BTB domain-containing protein 20 (*Zbtb20*)[81], facilitating the ASC fate (FIG. 2).

BLIMP1. BLIMP1 is a transcriptional repressor that, in the immune system, promotes the terminal differentiation of B cells and T cells[82]. Within the B cell lineage, BLIMP1 is exclusively expressed in ASCs, with cycling plasmablasts being distinguishable from long-lived postmitotic plasma cells based on their lower expression of BLIMP1 in both mice and humans[12,83,84] (TABLE 1). Although BLIMP1 expression is known to require IRF4 and the AP-1 factor FOS[71,80,85,86] (which is in turn functionally antagonized by FOS-related antigen 1 (REF. 87)), the factors that control 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 programme, including the genes that encode SPIB, BCL-6, ID3, MYC and PAX5 (REFS 14,24,88) (FIG. 2). Despite being essential for the formation of mature plasma cells[89], BLIMP1 is not required for the initiation of the ASC programme, as a pre-plasmablast population can develop in its absence[11]. In these cells, putative targets such as *Pax5* and *Bcl6* are transcriptionally downregulated independently of functional BLIMP1. There is also only a limited understanding of the physiological function of BLIMP1 in mature plasma cells, with evidence suggesting that it has roles in antigen presentation and in 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 is expressed in all plasma cell malignancies, including multiple myeloma, and it seems to be essential for disease progression in mouse models[94]. However, BLIMP1 is also commonly mutated in the activated B cell (ABC) subset of diffuse large B cell lymphoma (ABC-DLBCL)[95] and in multiple myeloma[96], which suggests that this transcription factor has an additional tumour-suppressive function (BOX 2).

Table 1 | **A summary of plasma cell maturation**

	Naive B cell	Plasmablast	Immature plasma cell	Mature plasma cell
Lifespan	++	+	+	++++
Proliferation	–	++	–	–
CD27*, CD38*, CD138 and CXCR4 expression	–	+	++	+++
CD19, CD20, CD45 and MHC class II expression	+++	++	+/–	+/–
Location	Lymphoid organs	Lymphoid organs and blood	Lymphoid organs	Bone marrow
Isotype	IgM and IgD	All‡	IgM=IgG>IgA	IgG>>IgA>IgM
BLIMP1 expression	–	+	+	++

*Values used in the table are from humans; similar changes occur in mice with the exception of CD27 and CD38. ‡Plasmablasts are indicated to secrete 'all' immunoglobulin isotypes as they show no constitutive distribution, but instead depend on the stimulus that generated them. +/–, low level or heterogeneous expression. BLIMP1, B lymphocyte-induced maturation protein 1; CXCR4, CXC-chemokine receptor 4.

XBP1. The unfolded protein response (UPR) is induced by endoplasmic reticulum (ER) stress that results from the accumulation of unfolded proteins. Owing to their enormous rates of immunoglobulin synthesis and secretion, ASCs 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 the downregulation of PAX5 expression during ASC differentiation contributing to *Xbp1* activation[98]. Early work suggested that XBP1 was essential for both normal serum immunoglobulin levels 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 (REFS 100–102). Instead, XBP1 predominantly functions to promote the *Igh* mRNA processing, immunoglobulin secretion and associated remodelling of the ER that is characteristic of plasma cells[100–102]. Interestingly, activation of the mammalian target of rapamycin (mTOR) pathway can partially compensate for the loss of XBP1 in ASCs, suggesting that metabolic deficiency is a consequence of an impaired UPR[103]. Conversely, loss of autophagic activity, which is also thought to counter ER stress, has a negative impact on ASC homeostasis and immunoglobulin production[104].

Similar roles in immunoglobulin processing and ER remodelling have also been proposed for the transcriptional elongation factor ELL2 (REFS 105–107), as well as for the ribonuclease inositol-requiring enzyme 1 (IRE1)[108], suggesting that multiple UPR pathways are operating to facilitate the immunoglobulin secretion capacity of ASCs. Owing to its high expression in ASCs, the XBP1 pathway has long been a candidate for targeted therapies against multiple myeloma. However, a recent study has suggested that XBP1 may actually function as a tumour suppressor in multiple myeloma[109] (BOX 2).

Cell biology of B cell differentiation

Although the generation of antibody in an adaptive immune response seems complex and multifaceted, it can be stripped down to a minimalistic cellular response model. B cells receive activating signals that elicit a series of programmed cell divisions in which each new cycle provides an opportunity for ASC differentiation. Typically, only a small proportion of dividing B cells become ASCs at each generation. Depending on the strength of the initial stimulation and the presence of ongoing stimuli, division eventually ceases, 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 the differences in the nature of the starting cells, the activating signals and the stimulus (which can be acute or chronic) (BOX 1). For example, certain B cell subsets — such as B1 cells and MZ B cells — rapidly generate ASCs without the need for extensive cell division or T cell help[110], whereas memory B cells require T cells but not extensive cell division. While the bulk of the available evidence suggests that the core gene-regulatory network is fundamentally conserved in all B cell subsets (FIGS 1,2) (also see Immunological Genome Project), it is known that B1 cells and MZ B cells display a higher basal induction of *Blimp1* expression and lower levels of *Pax5* and *Bcl6* expression than follicular B cells, which is consistent with the more advanced activation state and the accelerated differentiation kinetics of these B cell subsets[110,111].

The GC reaction, although broadly conforming to the canonical pattern of B cell differentiation, seems to be somewhat unique. 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-4 and IL-21, and other ligand–receptor interactions with T_{FH} cells), promote the rapid proliferation and prevent the differentiation of B cells[5–7,38,39,112]. Exit from the GC results in either the acquisition of a memory phenotype, which is characterized by the maintenance of the B cell programme gene regulators (including PAX5) or by the initiation of ASC differentiation through a process that is not entirely resolved but is known to be dependent on antigen affinity[113–115] (FIG. 1). Memory B cells maintain a lower level of *Bach2* expression than GC B cells, a finding that is also consistent with their higher propensity for ASC differentiation[9]. A more complete understanding of the factors that control the different rates of differentiation of B cell subsets is an important goal, and one that is likely to provide valuable insight into the complex construction of the humoral immune response.

The effect of cell division. The clonal expansion of selected antigen-specific B cells is necessary for an effective antibody response. Expanding the pool of specific cells provides the cellular substrates that are needed to generate the range of antigen-specific effector cell types that are required for short-term and long-term immune protection; but how is it that these cells are directed in varying and appropriate proportions to numerous cell fates, including to ASCs that produce antibodies of different isotypes and of varying lifespans?

Remarkably, cell division plays more than a passive part in creating the expanded B cell clones. This was first observed for CSR in which changes in antibody class are intimately linked to the generation number of the B cells in mice and humans[116,117]. CSR frequency per generation can be cytokine independent, as seen for LPS, or can be driven by cytokines such as IL-4, transforming growth factor-β (TGFβ) or interferon-γ (IFNγ)[118–120]. Furthermore, cytokine concentrations strongly influence the link between CSR and cell division, with low concentrations resulting in more cell divisions before CSR 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 of these key B cell fates to cell division enables a range of outcomes to arise as an automatic consequence of clonal expansion itself.

Mammalian target of rapamycin
(mTOR). A conserved serine/threonine protein kinase that regulates cell growth and metabolism, as well as cytokine and growth factor expression, in response to environmental cues. mTOR receives stimulatory signals from RAS and phosphoinositide 3-kinase downstream of growth factors and nutrients, such as amino acids, glucose and oxygen.

Gene-regulatory network
Diagrams that are used to visualize the relationship between large numbers of genes and their regulators that are involved in distinct biological processes.

Models of B cell terminal differentiation. The division-linked changes in the fate of a B cell suggest that some form of 'resetting' of molecular processes takes place upon each division. This question has been investigated by the filming of cultured B cells continuously over several generations and following four cellular fates — division, death, CSR development and ASC development[13]. The behaviour of the individual cells was remarkably heterogeneous, but statistical analysis and mathematical modelling showed that it was consistent with the internal cellular machinery that controls each of the four fates, which were acting independently and competitively within single cells. Despite the 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 is a simple way to allocate cells to multiple outcomes[13,121] (BOX 3).

An alternative mechanism to account for B cell terminal differentiation proposes that GC B cells divide asymmetrically, resulting in the differential segregation of key molecules within the cell, including BCL-6, the IL-21 receptor and a known polarity protein, atypical protein kinase C[122] (BOX 3). Although 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 although members of the Scribble polarity 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 of time remains one of the compelling mysteries that is associated with antibody regulation.

The results described above illustrate possible mechanisms for the determination of the fate of dividing B cells. Evidence for an automatic, internal programme that controls 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 the cells typically divide 3–6 times under these conditions, then lose the capacity for growth and eventually die. The number of cell divisions each clone undergoes is variable, but the descendants of each founder cell divide the same number of times, which indicates 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 the 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 B cells in the GC reaction using an inducible division-tracking marker[115]. At the height of the response, cells undergo a burst of division for a duration that is 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. Alterations in extrinsic inputs such as signalling strength (antigen amount and affinity and the concentration of cytokines), as well as intrinsic changes to the concentration or activity of important intracellular regulatory proteins, are likely to influence each other and cell division rates in complex ways (BOX 1). This complexity and inter-relatedness requires the 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. An important research direction that may identify the source of the molecular variations that underpin cellular heterogeneity is the study of stochastic variations in transcription factor concentrations (or activities) 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], whereas a relatively subtle increase in PU.1 expression maintains PAX5 expression and impairs ASC differentiation[66]. How these changes in transcription factor abundance arise from epigenetic processes to affect 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.

Box 3 | **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.

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 T helper cells, can alter the broad features of the antibody response. The rich environments of the germinal centre (GC) and the lymphoid organs provide the clear opportunity for coordinated changes that could easily promote or impede the development of plasma cells.

Programmed automaton

Under some conditions, B cells undertake a programmed response with their 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 programme as it is particularly striking following stimulation by the evolutionary ancient Toll-like receptor pathway. An autonomous programmed response of limited division burst was also recently noted for GC B cells *in vivo*[115], and it is consistent with the 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 the development of memory and effector T cells[159], and it has been suggested for B cells based on evidence for asymmetric partitioning of proteins between daughters of GC B cells[122].

Stochastic competition

Studies that isolate B cells and measure responses *in vitro* generate considerable cellular heterogeneity, suggesting that strong intrinsic mechanisms have a role in fate determination[10]. This 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 a result of the differences in the molecular constructions 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 (see FIG. 2).

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, postmitotic plasma cells (TABLE 1). We have previously shown that plasmablasts and plasma cells can be prospectively isolated based on the expression of BLIMP1, with plasmablasts displaying a distinctly lower level of BLIMP1 expression than plasma cells[12]. A similar distinction has been demonstrated between human plasmablasts and plasma cells[84]. Whether the increased BLIMP1 expression is functionally relevant to this transition is unclear; however, it is important to note that plasma cells silence the cell cycle programme, including the expression of the transcription factor MYC, which is a target of BLIMP1-mediated 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 antigen-specific cells in the blood of mice shortly after primary immunization shows that the ASCs that presumably constitute the precursors for the long-lived bone marrow plasma cells express intermediate levels of BLIMP1, and thus resemble plasmablasts[127,128]. Once in the bone marrow, these cells upregulate BLIMP1 expression to the high levels that are characteristic of plasma cells. In addition, ASCs in the blood following recall challenge express the proliferation marker Ki67, which is 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 tropic factors, suggesting that only these responsive cells are the precursors of plasma cells, with the bulk of the cells constituting a distinct, short-lived entity[128].

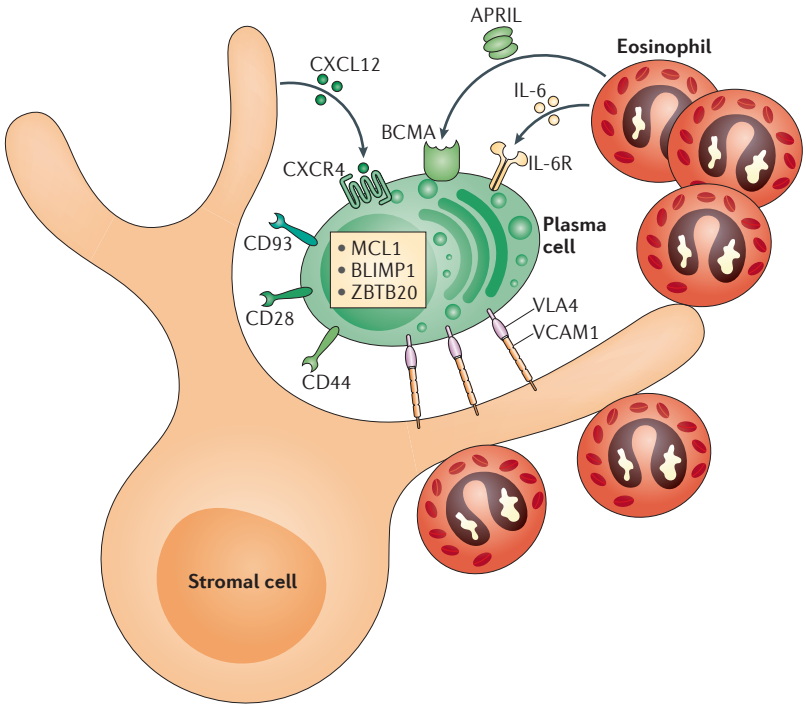

Figure 3 | **Model of the bone marrow plasma cell survival niche.** Plasma cells are recruited to the bone marrow through the expression of CXC-chemokine ligand 12 (CXCL12) and cellular adhesion molecules, such vascular cell adhesion molecule 1 (VCAM1) on bone marrow stromal cells. A variety of haematopoietic cell types are then proposed to act as secondary components of the plasma cell niche, secreting the important plasma cell survival factors APRIL (a proliferation-inducing ligand) and interleukin-6 (IL-6). Shown here are eosinophils as one documented APRIL-producing cell type that associates with bone marrow plasma cells. Plasma cells express B cell maturation antigen (BCMA), IL-6 receptor (IL-6R), very late antigen 4 (VLA4), CD28, CD44 and CD93, all of which are important for plasma cell homing and survival. Plasma cells also express the transcription factors B lymphocyte-induced maturation protein 1 (BLIMP1), X-box-binding protein 1 (XBP1; not shown), interferon-regulatory factor 4 (IRF4; not shown) and zinc finger and BTB domain-containing protein 20 (ZBTB20). Signalling through the APRIL–BCMA axis induces the expression of high levels of myeloid cell leukaemia 1 (MCL1), which is the essential anti-apoptotic factor for plasma cells. CXCR4, CXC-chemokine receptor 4.

Plasma cell homing and survival

Although long-lived plasma cells exist in multiple lymphoid organs in the body and in non-lymphoid organs in disease states, the bone marrow houses the majority of plasma cells in healthy individuals[113,130,131]. The longevity of plasma cells seems to be non-intrinsic, as displacement of these cells from the bone marrow microenvironment results in rapid cell death[132,133]. Thus, the key questions are: first, how do plasma cells, or their plasmablast-like precursors, home to the bone marrow? Second, what are the niche components that maintain long-lived plasma cells? Third, how are these signals translated into cellular longevity?

Homing. The process of homing to and retention in the bone marrow remains poorly understood. Activation of sphingosine-1-phosphate receptor 1 (S1PR1) is required for the efficient egress of ASCs from secondary lymphoid organs into the blood, whereas the chemokine CXC-chemokine ligand 12 (CXCL12) and its receptor CXC-chemokine receptor 4 (CXCR4) are important for the recruitment of ASCs to the bone marrow and their retention at this site[128]. By contrast, inflammatory chemokines, such as CXCL9, CXCL10 and CXCL11 — which signal through CXCR3 — promote the homing of plasmablasts to sites of inflammation, including non-lymphoid organs[134]. ASC retention in the bone marrow and maturation into plasma cells involves the engagement of very late antigen 4 (VLA4)[135], CD44 (REF. 136), CD28 (REFS 22,137) and CD93 (REF. 138) (FIG. 3) on the plasma cells, as well as the transcription factors Kruppel-like factor 2 (KLF2)[139], ZBTB20 (REFS 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 these niches has been the subject of numerous studies and remains contentious, with several distinct cellular lineages proposed as niche components (recently reviewed in REFS 132,142,143). While it is outside the scope of this Review to discuss the many competing models of the plasma cell niche, it seems likely in the bone marrow to consist of at least a $CXCL12^{+}VCAM1^{+}$ stromal cell[144] and a haematopoietic

cell that secretes the B cell survival factor a proliferation-inducing ligand (APRIL; also known as TNFSF13) (FIG. 3). Recent evidence suggests a key haematopoietic cell type is the eosinophil[145], although it is feasible that several distinct types of blood cell fulfil this role[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, that plasma cells colocalize with multiple eosinophils in the bone marrow, and that the associated eosinophils secrete APRIL and IL-6 (REF. 145) (FIG. 3).

Survival. Several factors have been proposed to mediate plasma cell survival in the bone marrow, including IL-6, tumour necrosis factor (TNF) and APRIL[136,146–148], with genetic evidence suggesting that APRIL and its receptor, B cell maturation antigen (BCMA; also known as TNFRSF17), are the most functionally important 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 (REF. 149). A key consequence of signalling through the APRIL–BCMA axis is the expression of the anti-apoptotic protein myeloid cell leukaemia 1 (MCL1), which is essential for the survival of all ASCs[149]. The very short half-life of MCL1 suggests a model in which continuous exposure to niche-derived signals, including APRIL, maintains MCL1 levels, thereby making the survival of the long-lived plasma cell highly sensitive to its location. Interestingly, the survival-promoting activities of both APRIL and the cytokine IL-6 require the activity of inducible nitric oxide synthase, which suggests that nitric oxide is a signalling intermediary in ASC survival[151]. Plasma cells also express the inhibitory Fc receptor FcγIIB, the crosslinking of which induces plasma cell apoptosis, suggesting that a negative regulatory loop exists in which serum antibodies affect 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 diagnostic tools and as therapeutics to treat many diseases. Plasma cells do, however, have a downside, which includes an involvement in some autoimmune diseases and in multiple myeloma. Thus, it is crucial 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 relatively poorly characterized. The advent of high-resolution techniques — such as single-cell imaging and the application of powerful high-throughput DNA and RNA sequencing technologies — to plasma cells will yield new information to permit further manipulations of this cell type for therapeutic benefit.

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