Plasma cells: finding new light at the end of B cell development

Kathryn L. Calame

Plasma cells are cellular factories devoted entirely to the manufacture and export of a single product: soluble immunoglobulin (Ig). As the final mediators of a humoral response, plasma cells play a critical role in adaptive immunity. Although intense effort has been devoted to studying the regulation and requirements for early B cell development, little information has been available on plasma cells. However, more recent work—including studies on genetically altered mice and data from microarray analyses—has begun to identify the regulatory cascades that initiate and maintain the plasma cell phenotype. This review will summarize our current understanding of the molecules that regulate commitment to a plasma cell fate and those that mediate plasma cell function.

Plasma cells are interesting for several reasons. On a basic level, they show the general mechanisms involved in the commitment of somatic cells to a terminally differentiated, post-mitotic state. As final mediators of the humoral immune response, they provide insights into the regulation of humoral immunity that may be helpful for vaccine development. They may also aid our understanding of certain human diseases. For example, information about normal plasma cells may help us understand human multiple myeloma, a disease that involves malignant plasma cells. In addition, soluble antibodies or immune complexes are pathological in many autoimmune diseases, including myasthenia gravis, Grave's disease, lupus erythematosus and rheumatoid arthritis. Therefore, knowledge of plasma cell biology may eventually provide a rational basis for designing treatments for these diseases.

Characteristics of plasma cells

Because they are terminally differentiated, end-stage cells, plasma cells do not divide. However, "plasmablasts" in extrafollicular regions or exiting germinal centers (GCs) do undergo cell division just before they become plasma cells[1]. The lifespan of nondividing plasma cells varies from a few days to many months and, as detailed below, this appears to be determined in part by their developmental history and homing capacity as well as by the finite capacity of the spleen to support them[1]. Other characteristics of plasma cells are summarized below (**Fig. 1**).

Upon plasma cell differentiation, there is a marked increase in steady-state amounts of Ig heavy and light chain mRNA and, when required for IgM and IgA secretion, J chain mRNA[2]. Whether the increase in Ig mRNA is due to increased transcription, increased mRNA stability or, as seems likely, both mechanisms, remains controversial[2]. There is also an increase in secreted *versus* membrane forms of heavy chain mRNA, as determined by differential use of poly(A) sites that may involve the availability of one component of the polyadenylation machinery, cleavage-stimulation factor Cst-64[3]. To accommodate translation and secretion of the abundant Ig mRNAs, plasma cells have an increased cytoplasmic to nuclear ratio and prominent amounts of rough endoplasmic reticulum and secretory vacuoles.

Numerous B cell–specific surface proteins are down-regulated upon plasma cell differentiation, including major histocompatibility complex (MHC) class II, B220, CD19, CD21 and CD22. MHC class II, which is required for antigen presentation to T cells, is absent due to lack of class II transactivator (CIITA), a coactivator required for transcription of MHC class II, invariant chain and HLA-DM[4]. The proteoglycan syndecan-1, which recognizes extracellular matrix and growth factors, is induced on antibody-secreting B cells and is often used as a marker of plasma cells[5]. Among integrin family proteins, which mediate cell-matrix and cell-cell adhesion functions, VLA-4 (very late antigen 4) is most predominant on plasma cells. Perhaps most important for differential homing of plasma cells, the chemokine receptors CXCR5 and CCR7 are decreased, which reduces responsiveness to the B and T cell zone chemokines CXCL13, CCL19 and CCL21[6]. In contrast, expression of CXCR4, which recognizes CXCL12 present in splenic red pulp, lymph node medullary cords and in bone marrow, remains high[6]. These changes mediate movement of plasma cells from the follicles to other locations, including the bone marrow.

Several transcription factors required for earlier steps in B cell development are decreased or absent in plasma cells. These include B cell lineage–specific activator (BSAP), encoded by *PAX5*, which is required for commitment to the B cell lineage[7] and for B cell lymphopoeisis in bone marrow and spleen[8,9]. CIITA is also decreased in plasma cells[4]. Other transcription factors expressed at earlier stages in B cell development, such as early B cell factor (EBF), A-Myb and BCL-6 are also decreased in plasma cells. However, a few transcription factors do increase in plasma cells. B lymphocyte–induced maturation protein 1 (Blimp-1) is induced upon plasma cell differentiation[10] and expressed *in vivo* in plasma cells and a subset of GC B cells with a partial plasma cell phenotype[11]. Interferon-regulatory factor 4 (IRF4), which associates with the Ets family protein PU.1[12], is also highly expressed in the Blimp-1$^+$ subset of GC B cells and in plasma cells[13]. X box–binding protein 1 (XBP-1), although ubiquitously expressed, is induced upon activation of B cells and increased in plasma cells[14].

Departments of Microbiology and Biochemistry & Molecular Biophysics, Columbia University College of Physicians and Surgeons, New York, NY 10032, USA. Correspondence should be addressed to K. L. C. (klc1@columbia.edu).

Commitment to a plasma cell fate

Naïve B cell immigrants from the bone marrow mature in a variety of B cell compartments. Outside the bone marrow, spleen is a major site for maturation of conventional B2 cells, whereas B1 cells mature primarily in peritoneal and pleural cavities[15]. In the spleen, most lymphocytes are located in white pulp nodules that are interspersed in the red pulp matrix[16]. In each white pulp nodule, blood is supplied from a central arteriole surrounded by a periarteriolar sheath (PALS). T cells are found primarily in the PALS, whereas most B cells cluster around follicular dendritic cells in follicles adjacent to the PALS. A marginal zone (MZ)—which contains specialized dendritic cells and macrophages, memory and naïve B cells—surrounds the outside of each nodule and acts as an antigen-filtering and scavenging area.

When naïve B cells leave the bone marrow and enter the spleen, a maturation process involving both positive and negative selection ensues and most cells die in the outer PALS. Of the surviving B cells, the majority pass through the T cell zone and enter primary follicles where they continue to mature into naïve follicular B cells ($IgM^{int}IgD^{hi}CD21^{int}CD23^{hi}$)[17]. A smaller fraction are found in the MZ as naïve MZ B cells ($IgM^{hi}IgD^{lo}CD21^{hi}CD23^{lo}$)[18]. Follicular B cells recirculate to bone marrow and lymph nodes but MZ B cells do not recirculate. Antigen, either in soluble form or associated with dendritic cells, activates naïve B cells in various B cell compartments and, ultimately, leads to terminal plasmacytic differentiation. However, plasmacytic differentiation occurs at different times and in different locations during an immune response (**Fig. 2**).

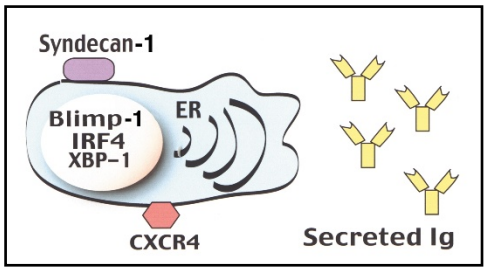

Figure 1. Plasma cell. Two characteristic surface molecules, Syndecan-1 and CXCR4, three key transcription factors, Blimp-1, IRF4 and XBP-1, and increased endoplasmic reticulum (ER) are shown.

MZ and B1 cells respond most rapidly to antigen, giving rise to the first wave of plasma cells and the earliest antibody response to antigen[19]. B1 cells are a self-renewing subset with a restricted repertoire that provides a response to common bacterial antigens and plays a role in autoimmunity. For example, B1 cells provide T cell–independent production of mucosal IgA in response to commensal bacteria[20]. The lineage relationship between B1 and B2 cells remains controversial and will not be reviewed here. However, the size of the B1 *versus* B2 compartments appears to depend on expression and composition of the B cell receptor (BCR)[18,21].

When naïve MZ B cells encounter antigen, they become activated and leave the MZ within 1–3 days. They proliferate and form foci of plasma cells in extrafollicular regions along the periphery of the PALS. Antibodies made by plasma cells that arise from MZ B cells are usually IgM and are often of low affinity[22]. Most of these early plasma cells survive only about 3 days, undergoing apoptosis *in situ*, possibly due to down-regulation of the anti-apoptotic Bcl-2 homolog A1[23]. However, additional factors affect survival in the spleen and a portion of the plasma cells in extrafollicular regions, possibly those associated with dendritic cells, survive longer than 3 days[1].

Because antigen stimulation drives the development of MZ B cells toward early plasma cells, the factors that determine MZ *versus* follicular B cell fate are key to understanding plasma cell development. Although BCR signals are critical for survival of all naïve B cells[24], recent data suggest that migration to the MZ *versus* follicles may be determined by the strength of BCR signals, with lower intensity signals favoring MZ B cells[25]. Supporting this idea, MZ B cells are absent in Aiolos$^{-/-}$ mice, which have enhanced BCR signaling[25,26]. On the other hand, *xid* and Btk$^{-/-}$ mice, which show compromised BCR signaling, have MZ B cells but reduced survival of follicular B cells[27]. However, alternative explanations are possible. For example, decreased MZ B cells in Aiolos$^{-/-}$ mice could result from enhanced negative selection by self-antigen.

Tumor necrosis factor (TNF) family ligands and TNF receptors (TNFRs) play critical roles in determining splenic architecture and B cell development[16]. Lymphotoxins are necessary for the establishment of splenic T cell and B cell zones and TNF is involved in the formation of marginal sinuses and GCs[16]. In addition, two ligands—B lymphocyte stimulator (BLyS) and APRIL (a proliferation-inducing ligand)—and three receptors—TACI (transmembrane activator and CAML interactor) and BCMA (B cell maturation antigen), which recognize both, and BAFF-R (B cell–activating factor receptor), which recognizes BLyS[28]—have important roles in the development of mature follicular and GC B cells and in Ig production[29]. BlyS is required for maturation of follicular B cells and TACI$^{-/-}$ mice have elevated B cells but lack T cell–independent type 2 B cell responses[30]. Further studies on TNF-TNFR proteins are likely to identify additional roles for them in B lymphoiesis and refine our understanding of how they affect humoral immunity and plasmacytic differentiation.

A later response to antigen occurs when follicular B cells are recruited by T cell–dependent antigens into GCs in spleen and lymph nodes. It is also possible, but not yet established, that some MZ or B1 cells may enter follicles and mature in GCs. The GC reaction has been intensively studied[31], but will not be described in detail here. Iterative cycles of proliferation, somatic hypermutation and apoptosis in the GC result in the selection of B cells that make an antibody with high affinity for cognate antigen. In addition, isotype switching occurs during the GC reaction. The GC reaction usually peaks ~10–12 days after immunization, giving rise to two types of B cells: nonsecreting memory B cells that are Ig surface–positive and antibody-secreting plasmablasts. Plasmablasts leave the GC and develop into terminally differentiated plasma cells that secrete high-affinity antibodies. Some of these plasma cells home to the bone marrow where they receive survival signals from stromal cells *via* cell adhesion molecules[32]. Bone marrow plasma cells continue to secrete antibody for many months and are sometimes considered to be a part of the secondary response, although they do not respond to secondary antigenic stimulation[33].

What signals tell a GC B cell to exit the GC? Because somatic mutation, which drives affinity maturation, is a random process, it seems unlikely that a given number of cell divisions determines when a B cell leaves the GC. More likely, signaling *via* high-affinity antibody causes an end to the iterative cycles of mutation and selection, although there is little experimental evidence to support this idea.

What determines memory or plasma cell fate? One study of affinity maturation in antibody-secreting *versus* memory cells suggests that expression of high-affinity antibody may favor plasma cell rather than memory cell development[34]. Another informative approach has been to study B cell differentiation in defined culture systems. These cultures have shown that signals *via* CD40 favor a memory phenotype, whereas in the presence of interleukin 2 (IL-2) and IL-10 and the absence of CD40 ligand (CD40L), terminal plasma cell differentiation occurs[35].

Signals from another TNFR, OX40L, also favor a plasma cell fate[36]. IL-10 also interrupts memory B cell expansion, whereas IL-4 directs B cells to a memory fate[37,38]. Although it has pleiotropic activities, IL-6 is required for differentiation of plasmablasts and antibody secretion[39], in part due to induction of the cyclin-dependent kinase inhibitor p18[40]. IL-6 is of particular interest because it is also required for myeloma cell growth and survival[41]. Finally, differentiation and survival of plasma cells depends on, and may be limited by, specific types of plasmablast-associated dendritic cells[42].

In response to secondary antigenic stimulation, rapid and massive clonal expansion of memory B cells occurs, generating eight- to tenfold more plasma cells than a primary response does[43]. The biology of memory B cells remains somewhat mysterious, although it is known that they are long-lived, even in the absence of antigen[44], and do not secrete antibody[43]. Upon leaving the GC, memory B cells recirculate or home to draining areas of lymph node and spleen. A distinct population of B220$^-$ memory cells has been identified that undergoes affinity maturation in the splenic red pulp, is maintained long-term in spleen and bone marrow and is thought to be a major component of the splenic memory cell compartment[45]. Upon antigen encounter, memory cells are biased to become plasma cells[46]. Although CD40 is important for the development of memory B cells, it does not appear to be required for the development of plasma cells in a secondary response[47]. In addition to formation of plasma cells, some memory cells must be maintained during the secondary response to antigen. Signals influencing the decision between memory maintenance and plasmacytic differentiation are not well understood at present.

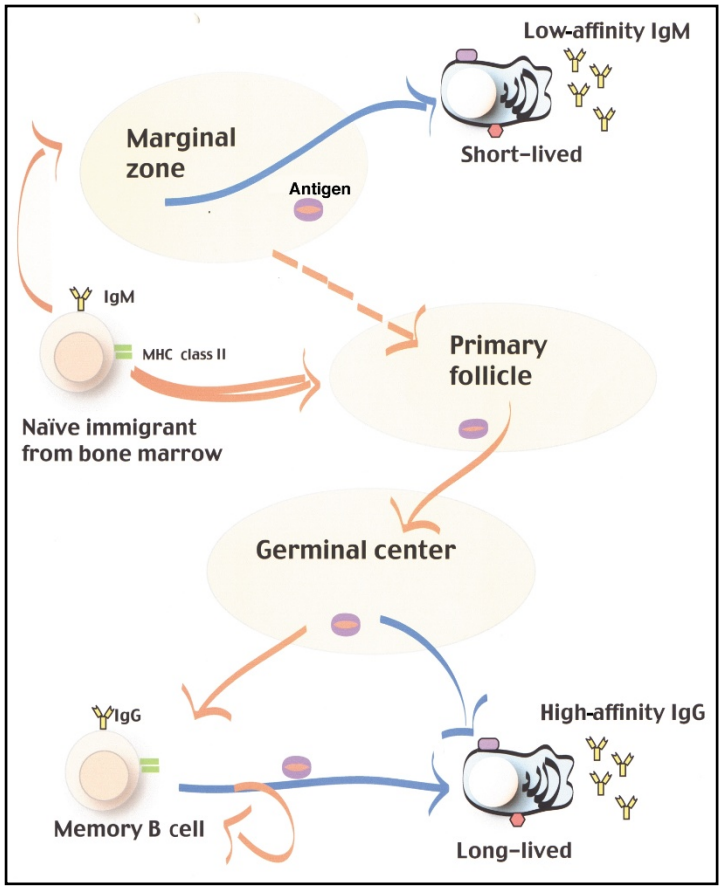

Figure 2. Generation of plasma cells in spleen during a humoral response. Solid orange arrows denote maturation pathways for B cells; dashed orange arrow denotes uncertainty regarding migration of B cells from MZ to follicles. Blue arrows denote three occasions when plasma cells develop: after antigen stimulation of MZ B cells; after the GC reaction; and after secondary antigenic stimulation of memory B cells. This figure is simplified and does not indicate T cells, dendritic cells or the various cytokines that participate in these processes.

Key transcription factors in differentiation

XBP-1 is the only transcription factor that is uniquely required for plasma cell differentiation but is not, apparently, required for any earlier stage of B cell development[14]. XBP-1 was first discovered based on its ability to bind an "X box" sequence in the HLA-DRα promoter[48]. It is a basic-leucine zipper protein that activates transcription. XBP-1 is ubiquitously expressed and mice deficient in XBP-1 die as embryos due to defective hepatocyte growth, which leads to reduced hematopoiesis and anemia[49]. However, an unexpected finding was made when the RAG (recombination-activating gene) complementation system was used to study the effect of XBP-1 deficiency in T and B cells[14]. XBP-1$^{-/-}$ T cells appeared to be normal and the number and distribution of B cells in the bone marrow and spleen of chimeric mice was also normal. However, the chimeric mice had severely reduced concentrations of serum Ig. Upon closer analysis, when XBP-1$^{-/-}$ splenic B cells were activated *in vitro*, the activation markers CD44 and CD69 were induced normally, cells proliferated normally and germ-line transcripts appeared normally. This all suggested that the XBP-1$^{-/-}$ B cells were fully capable of responding to activation. However, the XBP-1$^{-/-}$ splenocytes did not secrete antibody and did not express Syndecan-1 on their surface after activation *in vitro*. In addition, when the chimeric mice were immunized with T cell–independent (TI) or T cell–dependent (TD) antigens or exposed to polyoma virus, secretion of serum Ig of all isotypes was severely reduced.

Although it is expressed ubiquitously, XBP-1 is induced upon activation of splenic B cells and is present at high concentrations in plasma cells[14]. It is repressed by BSAP[50], a regulator that decreases during plasma cell differentiation[9,51]. Therefore, reversal of BSAP repression is likely to account, at least in part, for the induction of XBP-1. In addition, XBP-1 is induced in response to IL-6 signaling in myeloma cells[52] and by activating transcription factor 6 (ATF-6), which is activated in response to endoplasmic stress[53]. These signals may also be important for XBP-1 induction during plasma cell development.

No target genes for XBP-1 have been identified that would explain its critical role in plasma cells. Indeed, the time(s) when XBP-1 is required during the sequence of plasmablast to plasma cell development is not yet clear.

Blimp-1, which has been called a "master regulator of terminal B cell differentiation"[10], was identified in a subtractive screen as a gene that was induced upon IL-2 + IL-5–dependent differentiation of the BCL-1 murine lymphoma to a plasma cell state[10]. A key activity of the protein, first observed in BCL-1 lymphoma cells[10] and subsequently confirmed in primary splenocytes[54,55], is its ability to drive activated or GC-like B cells to become antibody-secreting cells with a plasma cell phenotype. Both human and murine Blimp-1 proteins use five zinc-finger motifs for sequence-specific binding to DNA[56]. Blimp-1 is a transcriptional repressor and a proline-rich region NH_2-terminal to the zinc-finger domains is required for association of the

protein with hGroucho[57] and class I histone deacetylases[58] and for the protein to be able to repress transcription.

Blimp-1 is present in all plasma cells, including those formed in a primary response to either TI or TD antigen, those formed from memory cells in a secondary response and in long-lived plasma cells in human bone marrow[11]. Blimp-1 was not found in memory B cells but is expressed in a subset (5–15%) of GC B cells. Cells in this subset have a partial plasma cell phenotype because they lack expression of BCL-6 and BSAP, but express IRF4 and Sydecan-1, in addition to Blimp-1, which suggests that this subset of GC B cells might be committed to a plasma cell fate. Part of this Blimp-1$^+$ population continues to proliferate, suggesting that mitogenic signals within the GC override Blimp-1–dependent repression of c-*Myc* and other proliferation genes at this stage[11]. Little is known about signals that induce Blimp-1, although signal transducer and activator of transcription 3 (STAT3) may be involved, either directly or indirectly, in activating its expression[59]. Microarray analyses showed that the gene that encodes Blimp-1 (*PRDM1*) is repressed by BCL-6[60] and two BCL-6 response elements have been mapped within the *PRDM1* gene, confirming that *PRDM1*is a direct target of BCL-6 repression (C. Tunyaplin, and K. Calame, unpublished data). Repression of *Prdm1* by BCL-6 was also confirmed in studies that showed ectopic expression of BCL-6 inhibited expression of Blimp-1 and differentiation of primary B cells to antibody-secreting cells[59]. Thus, one function of BCL-6 in GC B cells is to repress *Prdm1,* thus inhibiting Blimp-1–dependent terminal differentiation. Signaling from CD40, which inhibits plasma cell differentiation, also causes down-regulation of Blimp-1 mRNA[61], emphasizing the strong association between Blimp-1 and plasmacytic differentiation.

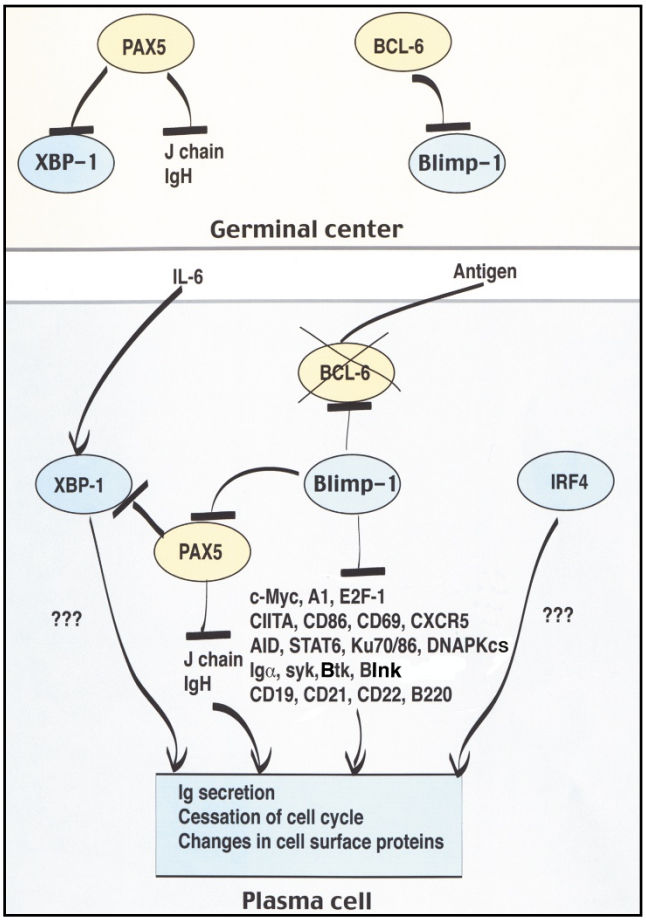

Figure 3. Regulatory cascades in plasma cell development. Transcription factors required for earlier B cells are shown in yellow; those required for plasma cells are shown in blue. Targets that are activated by a particular factor are indicated by arrows; targets that are repressed are indicated by bars. The X indicates degradation of BCL-6 protein after BCR signaling in the GC.

Three gene targets for Blimp-1–dependent repression have been identified; they explain important aspects of the plasma cell phenotype induced by Blimp-1. Blimp-1 represses c-*Myc* transcription[62], which provides an explanation for cessation of the cell cycle in plasma cells, as c-Myc is required for cell proliferation and growth. *MHC2TA* promoter III (the promoter responsible for regulating CIITA in B cells) is also directly repressed by Blimp-1[55]. Repression of CIITA by Blimp-1 explains the down-regulation of MHC class II in plasma cells. Finally, Blimp-1 represses *Pax5,* which encodes BSAP (K.-I. Lin *et al.*, unpublished data). BSAP is required for commitment to the B cell lineage, for B cell development in the bone marrow and for isotype switching in GCs[8]. Down-regulation of BSAP is required for development of antibody-secreting cells[63] (K.-I. Lin *et al.*, unpublished data), probably because it represses XBP-1[50], J chain[64] and Ig heavy chain gene transcription[65].

However, repression of c-*Myc, MHC2TA* and *Pax5* is unlikely to explain the entire program of plasma cell development triggered by Blimp-1[66–68]. With the use of cDNA microarray technology, a more complete understanding of the program of changes in gene expression triggered by expression of Blimp-1 in human B cell lines has been gained (A. Shaffer *et al.*, unpublished data). Changes in mRNA were observed that are consistent with a plasma cell phenotype, including antibody secretion (increases in Ig, J chain and heat-shock protein 70), cessation of cell cycle and increased apoptosis (decreases in c-*Myc,* c-Myc targets and the anti-apoptotic protein A1; increases in the cyclin-dependent kinase inhibitors p18 and p21 and the apoptotic proteins GAD153 and GADD45). We also observed that Blimp-1 represses gene expression programs required for activated B cells and/or GC B cells. Expression of multiple mRNAs encoding proteins, including Syk, Btk, Blnk (B cell linker protein), Igα, CD19, CD20, CD22 and CD45, involved in signaling from the BCR was decreased. Blimp-1 also down-regulated BCL-6, a protein which is required for GC B cells[69,70], and other genes required for T cell–B cells interactions and/or GC functions, such as isotype-switch recombination and somatic hypermutation. These genes included those that encode CD86, AID[71], DNAPKcs, Ku70, Ku86, BSAP, CIITA and STAT6. Thus, Blimp-1 determines plasma cell fate by triggering two programs: inhibition of earlier or GC functions and activation of plasma cell functions. The reciprocal ability of Blimp-1 to repress *BCL6* creates a feedback loop that enforces strict control over the B cell fate decision to become a plasma cell. As long as BCL-6 is expressed in a GC B cell, Blimp-1 expression and plasmacytic differentiation is blocked; if *Prdm1* becomes activated, however, *BCL6* will be repressed, which leads to irreversible plasmacytic differentiation.

Mice that lack IRF4 cannot mount an antibody response and lack plasma cells and cytotoxic T cells[72], which suggests an important role for IRF4 in plasma cell development and/or function. This IRF family protein was identified as a partner for the Ets family protein PU.1[12] and by its homology to other IRF proteins[73]. IRF4 can also associate with the Ets family protein SpiB, the helix-loop-helix protein E47, STAT6[74]

and zinc-finger proteins BCL-6 and Blimp-1[75,76]. Known targets activated by complexes that contain IRF4 include κ and λ light chain enhancers and the human CD23 promoter.

Regulation of IRF4 appears to be complex. It is expressed primarily in lymphoid cells and in B cells IRF4 expression depends on Rel proteins[77]. Also, in B cells its expression pattern parallels that of Blimp-1: it is increased in a plasma cell–like subset of GC cells and in plasma cells[13]. However, IRF4 activity is also post-transcriptionally regulated in several ways. For some sites, binding to DNA is enhanced when IRF4 is associated with another protein, such as PU.1[78]. Its transcriptional activity is negatively regulated by BCL-6 and Blimp-1[75,76].

The predominant NFAT proteins in lymphocytes are NFATc1 and NFATc2[79]. Mice with lymphocytes that are doubly deficient in NFATc1 and NFATc2 have T cells with multiply defective effector functions, B cells that are hyperactivated and increased serum IgG1 and IgE as well as plasma cell expansion[80]. For plasma cells, these results suggest either a B cell–intrinsic mechanism by which NFATc1 and NFATc2 inhibit plasma cell development, or a mechanism by which T cells that lack NFATc1 and NFATc2 cannot appropriately regulate plasma cell development. Given the current understanding that BCL-6 keeps GC B cells from premature plasma cell development by repressing *Prdm1*, it will be interesting to determine whether BCL-6 expression is normal in B cells from mice that lack NFATc1 and NFATc2 expression by their lymphocytes.

Octamer sites are important for transcriptional activity of heavy and light chain promoters and for the 3′ Cα enhancer. Octamer-binding factor 1 (Oct-1), which is ubiquitously expressed, and Oct-2, which has more lymphoid-restricted expression, bind these sites and both associate with B cell–specific octamer cofactor (OCA-B)[81]. Mice with lymphocytes that lack Oct-2 have normal B cell development, but the B cells do not respond to polyclonal mitogens and undergo G1 arrest rather than proliferation, which presumably accounts for the reduced serum Ig in these mice[82]. Mice that lack OCA-B do not make GCs, but plasma cell development is not affected[83]. Although B cells that lack Oct-2 or OCA-B show defective developmental stages before plasma cell differentiation, the role of octamer proteins in Ig promoters and the 3′ Cα enhancer argue that that Oct-1 or Oct-2, along with OCA-B, may be important in plasma cells. The occurrence of plasma cells in the absence of OCA-B is not consistent with this idea, but there have been suggestions of additional, currently unidentified, octamer cofactors[84] that may be important in plasma cells.

Unifying concepts

Because several key transcriptional regulators involved in plasma cell development have been identified, it is important to speculate on how their functions may be related. Although there is no direct evidence, it seems logical that a single developmental sequence may exist in which these regulators cooperate to bring about plasmacytic differentiation. We suggest the following working model to integrate the regulation and action of three major transcription factors: XBP-1, Blimp-1 and IRF4 (**Fig. 3**).

Because these regulators appear to be required for TI and TD responses, appropriate signals by antigen *via* BCR probably induce XBP-1, Blimp-1 and IRF4 in naïve MZ or follicular B cells. This would allow MZ B cells to develop rapidly into plasma cells. However, terminal differentiation is inhibited as follicular B cells enter the GC. This is due, at least in part, to repression of Blimp-1 by BCL-6[59,60]. It may be that *XBP1* and/or *IRF4* are also repressed by BCL-6. In addition, BSAP, which is expressed and required in GC B cells, represses *XBP1*[50] and lack of XBP-1 also blocks plasma cell development[14].

BCL-6– and/or BSAP-dependent repression is probably relieved by some appropriate combination of signals that occurs when the GC reaction is complete. This signal may involve BCR-dependent activation *via* surface Ig with a high affinity for antigen. Signals from BCR are known to cause mitogen-activated protein kinase–dependent phosphorylation and subsequent degradation of BCL-6[85]. This would be expected to cause derepression of Blimp-1, thus initiating plasma cell development. Appropriate combinations of cytokines are probably also important. IL-6 induces XBP-1[52] and other cytokines and TNFR signals are also likely to play a role in inducing XBP-1, IRF4 and/or Blimp-1. Blimp-1 would then repress the genes that encode BCL-6, AID, CIITA, BSAP and other genes required for GC functions, thus inhibiting GC reactions and ensuring irreversible plasma cell development. Repression of *PAX5* by Blimp-1 (K.-I. Lin, C. Angelin-Duclos and K. Calame, unpublished data) is probably critical to drive plasma cell development because it relieves BSAP-dependent repression of *XBP1*[50], which is required for plasma cell function[14]. It also relieves BSAP-dependent repression of IgH and IgJ chain[64] transcription, which leads to IgM secretion. Repression of c-*Myc* by Blimp-1 is important for cessation of cell cycle[67] in plasma cells and repression of other targets, including Bcl-2 family member *A1* and *MHC2TA*, also contributes to the plasma cell phenotype.

Many interesting issues remain unanswered at this point: some may yield answers rapidly, whereas others may be more challenging. It should be relatively easy to determine whether certain features of this model are correct and to expand some aspects of it. For example, do XBP-1, Blimp-1 and IRF4 function in a single pathway or parallel pathways? What causes induction of IRF4, Blimp-1 and XBP-1 in MZ B cells after antigen stimulation? Does BCL-6 repress *IRF4* or *XBP1* as well as *Prdm1* in GC B cells? What are the relevant targets of XBP-1 and IRF4 in plasmacytic differentiation? It will also be interesting, but more challenging, to identify individual signals and particular signal strengths or combinations of signals that determine developmental fate decisions. These should include developmental fate choices between B1 and B2 cells, MZ and follicular B cells, plasma cells and memory cells after the GC reaction and plasma cells and maintenance of memory cells upon secondary stimulation of memory cells by antigen.

Acknowledgments

I thank members of my laboratory and many other colleagues for helpful discussions. Supported by grant numbers GM29361 and AI43576.

1. Sze, D. M., Toellner, K. M., Garcia de Vinuesa, C., Taylor, D. R. & MacLennan, I. C. Intrinsic constraint on plasmablast growth and extrinsic limits of plasma cell survival. *J. Exp. Med.* **192**, 813–821 (2000).
2. Chen-Bettecken, U., Wecker, E. & Schimpl, A. Transcriptional control of μ- and κ-gene expression in resting and bacterial lipopolysaccharide-activated normal B cells. *Immunobiology* **174**, 162–176 (1987).
3. Takagaki, Y. & Manley, J. L. Levels of polyadenylation factor CstF-64 control IgM heavy chain mRNA accumulation and other events associated with B cell differentiation. *Mol. Cell.* **2**, 761–771 (1998).
4. Silacci, P., Mottet, A., Steimle, V., Reith, W. & Mach, B. Developmental extinction of major histocompatibility complex class II gene expression in plasmocytes is mediated by silencing of the transactivator gene CIITA. *J. Exp. Med.* **180**, 1329–1336 (1994).
5. Sanderson, R. D., Lalor, P. & Bernfield, M. B lymphocytes express and lose syndecan at specific stages of differentiation. *Cell. Reg.* **1**, 27–35 (1989).
6. Hargreaves, D. C. *et al.* A coordinated change in chemokine responsiveness guides plasma cell movements. *J. Exp. Med.* **194**, 45–56 (2001).
7. Nutt, S. L., Heavey, B., Rolink, A. G. & Busslinger, M. Commitment to the B-lymphoid lineage depends on the transcription factor Pax5. *Nature* **401**, 556–562 (1999).
8. Nutt, S. L., Eberhard, D., Horcher, M., Rolink, A. G. & Busslinger, M. Pax5 determines the identity of B cells from the beginning to the end of B-lymphopoiesis. *Int. Rev. Immunol.* **20**, 65–82 (2001).
9. Barberis, A., Widenhorn, K., Vitelli, L. & Busslinger, M. A novel B-cell lineage-specific transcription factor present at early but not late stages of differentiation. *Genes Dev.* **4**, 849–859 (1990).
10. Turner, C. A., Jr., Mack, D. H. & Davis, M. M. Blimp-1, a novel zinc finger-containing protein that can drive the maturation of B lymphocytes into immunoglobulin-secreting cells. *Cell* **77**, 297–306 (1994).
11. Angelin-Duclos, C., Cattoretti, G., Lin, K. I. & Calame, K. Commitment of B lymphocytes to a plasma cell fate is associated with blimp-1 expression *in vivo*. *J. Immunol.* **165**, 5462–5471 (2000).

12. Eisenbeis, C. F., Singh, H. & Storb, U. Pip, a novel IRF family member, is a lymphoid-specific, PU. 1-dependent transcriptional activator. *Genes Dev.* **9**, 1377–1387 (1995).
13. Falini, B. *et al.* A monoclonal antibody (MUM1p) detects expression of the MUM1/IRF4 protein in a subset of germinal center B cells, plasma cells, and activated T cells. *Blood* **95**, 2084–2092 (2000).
14. Reimold, A. M. *et al.* Plasma cell differentiation requires the transcription factor XBP-1. *Nature* **412**, 300–307 (2001).
15. Macpherson, A. J. *et al.* IgA production without μ or δ chain expression in developing B cells. *Nature Immunol.* **2**, 625–631 (2001).
16. Fu, Y. X. & Chaplin, D. D. Development and maturation of secondary lymphoid tissues. *Annu. Rev. Immunol.* **17**, 399–433 (1999).
17. Loder, F. *et al.* B cell development in the spleen takes place in discrete steps and is determined by the quality of B cell receptor-derived signals. *J. Exp. Med.* **190**, 75–89 (1999).
18. Martin, F. & Kearney, J. F. B-cell subsets and the mature preimmune repertoire. Marginal zone and B1 B cells as part of a "natural immune memory". *Immunol. Rev.* **175**, 70–79 (2000).
19. Oliver, A. M., Martin, F. & Kearney, J. F. $IgM^{high}CD21^{high}$ lymphocytes enriched in the splenic marginal zone generate effector cells more rapidly than the bulk of follicular B cells. *J. Immunol.* **162**, 7198–7207 (1999).
20. Macpherson, A. J. *et al.* A primitive T cell-independent mechanism of intestinal mucosal IgA responses to commensal bacteria. *Science* **288**, 2222–2226 (2000).
21. Fagarasan, S., Watanabe, N. & Honjo, T. Generation, expansion, migration and activation of mouse B1 cells. *Immunol. Rev.* **176**, 205–215 (2000).
22. Dal Porto, J. M., Haberman, A. M., Shlomchik, M. J. & Kelsoe, G. Antigen drives very low affinity B cells to become plasmacytes and enter germinal centers. *J. Immunol.* **161**, 5373–5381 (1998).
23. Tomayko, M. M. & Cancro, M. P. Long-lived B cells are distinguished by elevated expression of A1. *J. Immunol.* **160**, 107–111 (1998).
24. Lam, K. P., Kuhn, R. & Rajewsky, K. *In vivo* ablation of surface immunoglobulin on mature B cells by inducible gene targeting results in rapid cell death. *Cell* **90**, 1073–1083 (1997).
25. Cariappa, A. *et al.* The follicular *versus* marginal zone B lymphocyte cell fate decision is regulated by Aiolos, Btk, and CD21. *Immunity* **14**, 603–615 (2001).
26. Wang, J. H. *et al.* Aiolos regulates B cell activation and maturation to effector state. *Immunity* **9**, 543–553 (1998).
27. Khan, W. N. *et al.* Defective B cell development and function in Btk-deficient mice. *Immunity* **3**, 283–299 (1995).
28. Thompson, J. S. *et al.* Baff-R, a newly identified TNF receptor that specifically interacts with BAFF. *Science* **293**, 2108–2111 (2001).
29. Marsters, S. A. *et al.* Interaction of the TNF homologues BLyS and APRIL with the TNF receptor homologues BCMA and TACI. *Curr. Biol.* **10**, 785–788 (2000).
30. Do, R. & Chen-Kiang, S. Mechanism of BLyS action in B cell immunity. *Cytok. Growth Factor Rev.* **199**, 1–8 (2001).
31. McHeyzer-Williams, L. J., Driver, D. J. & McHeyzer-Williams, M. G. Germinal center reation. *Curr. Opin. Hematol.* **8**, 52–59 (2001).
32. Merville, P. *et. al.* Bcl-2$^+$ tonsillar plaoma cells are rescued from apoptosis by bone marrow fibroblasts. *J. Exp. Med.* **183**, 227–236 (1996).
33. Slifka, M. K., Antia, R., Whitmire, J. K. & Ahmed, R. Humoral immunity due to long-lived plasma cells. *Immunity* **8**, 363–372 (1998).
34. Smith, K. G., Light, A., Nossal, G. J. & Tarlinton, D. M. The extent of affinity maturation differs between the memory and antibody-forming cell compartments in the primary immune response. *EMBO J.* **16**, 2996–3006 (1997).
35. Arpin, C. *et al.* Generation of memory B cells and plasma cells *in vitro*. *Science* **268**, 720–722 (1995).
36. Stuber, E. & Strober, W. The T cell-B cell interaction *via* OX40-OX40L is necessary for the T cell–dependent humoral immune response. *J. Exp. Med.* **183**, 979–989 (1996).
37. Choe, J. & Choi, Y. S. IL-10 interrupts memory B cell expansion in the germinal center by inducing differentiation into plasma cells. *Eur. J. Immunol.* **28**, 508–515 (1998).
38. Zhang, X. *et al.* The distinct roles of T cell-derived cytokines and a novel follicular dendritic cell-signaling molecule 8d6 in germinal center-B cell differentiation. *J. Immunol.* **167**, 49–56 (2001).
39. Kawano, M. M., Mihara, K., Huang, N., Tsujimoto, T. & Kuramoto, A. Differentiation of early plasma cells on bone marrow stromal cells requires interleukin-6 for escaping from apoptosis. *Blood* **85**, 487–494 (1995).
40. Morse, L., Chen, D., Franklin, D., Xiong, Y. & Chen-Kiang, S. Induction of cell cycle arrest and B cell terminal differentiation by CDK inhibitor p18(INK4c) and IL-6. *Immunity* **6**, 47–56 (1997).
41. Gado, K., Domjan, G., Hegyesi, H. & Falus, A. Role of interleukin-6 in the pathogenesis of multiple myeloma. *Cell. Biol. Int.* **24**, 195–209 (2000).
42. Garcia De Vinuesa, C. *et al.* Dendritic cells associated with plasmablast survival. *Eur. J. Immunol.* **29**, 3712–3721 (1999).
43. McHeyzer-Williams, M. G. & Ahmed, R. B cell memory and the long-lived plasma cell. *Curr. Opin. Immunol.* **11**, 172–179 (1999).
44. Maruyama, M., Lam, K. P. & Rajewsky, K. Memory B-cell persistence is independent of persisting immunizing antigen. *Nature* **407**, 636–642 (2000).
45. Driver, D., McHeyzer-Williuams, L., C., M., Stetson, D. & McHeyzer-Williams, M. Development and maintenance of a B220-memory B cell compartment. *J. Immunol.* **167**, 1393–1405 (2001).
46. Arpin, C., Banchereau, J. & Liu, Y. J. Memory B cells are biased towards terminal differentiation: a strategy that may prevent repertoire freezing. *J. Exp. Med.* **186**, 931–940 (1997).
47. Silvy, A., Lagresle, C., Bella, C. & Defrance, T. The differentiation of human memory B cells into specific antibody- secreting cells is CD40 independent. *Eur. J. Immunol.* **26**, 517–524 (1996).
48. Liou, H. C. *et al.* A new member of the leucine zipper class of proteins that binds to the HLA DR α promoter. *Science* **247**, 1581–1584 (1990).
49. Reimold, A. M. *et al.* An essential role in liver development for transcription factor XBP-1. *Genes Dev.* **14**, 152–157 (2000).
50. Reimold, A. M. *et al.* Transcription factor B cell lineage-specific activator protein regulates the gene for human X-box binding protein 1. *J. Exp. Med.* **183**, 393–401 (1996).
51. Wallin, J. J., Gackstetter, E. R. & Koshland, M. E. Dependence of BSAP repressor and activator functions on BSAP concentration. *Science* **279**, 1961–1964 (1998).
52. Wen, X. *et al.* Identification of c-myc promoter-binding protein and X-box binding protein 1 as interleukin-6 target genes in human multiple myeloma cells. *Int. J. Oncol.* **15**, 173–178 (1999).
53. Yoshida, H. *et al.* ATF6 activated by proteolysis binds in the presence of NF-Y (CBF) directly to the *cis*-acting element responsible for the mammalian unfolded protein response. *Mol. Cell. Biol.* **20**, 6755–6767 (2000).
54. Schliephake, D. E. & Schimpl, A. Blimp-1 overcomes the block in IgM secretion in lipopolysaccharide/anti- μ F(ab′)2-co-stimulated B lymphocytes. *Eur. J. Immunol.* **26**, 268–271 (1996).
55. Piskurich, J. F. *et al.* BLIMP-I mediates extinction of major histocompatibility class II transactivator expression in plasma cells. *Nature Immunol.* **1**, 526–532 (2000).
56. Keller, A. D. & Maniatis, T. Only two of the five zinc fingers of the eukaryotic transcriptional repressor PRDI-BF1 are required for sequence-specific DNA binding. *Mol. Cell. Biol.* **12**, 1940–1949 (1992).
57. Ren, B., Chee, K. J., Kim, T. H. & Maniatis, T. PRDI-BF1/Blimp-1 repression is mediated by corepressors of the Groucho family of proteins. *Genes Dev.* **13**, 125–137 (1999).
58. Yu, J., Angelin-Duclos, C., Greenwood, J., Liao, J. & Calame, K. Transcriptional repression by blimp-1 (PRDI-BF1) involves recruitment of histone. *Mol. Cell. Biol.* **20**, 2592–2603 (2000).
59. Reljic, R., Wagner, S. D., Peakman, L. J. & Fearon, D. T. Suppression of signal transducer and activator of transcription 3-dependent B lymphocyte terminal differentiation by BCL-6. *J. Exp. Med.* **192**, 1841–1848 (2000).
60. Shaffer, A. L. *et al.* BCL-6 represses genes that function in lymphocyte differentiation, inflammation, and cell cycle control. *Immunity* **13**, 199–212 (2000).
61. Randall, T. D. *et al.* Arrest of B lymphocyte terminal differentiation by CD40 signaling: mechanism for lack of antibody-secreting cells in germinal centers. *Immunity* **8**, 733–742 (1998).
62. Lin, Y., Wong, K. & Calame, K. Repression of c-myc transcription by Blimp-1, an inducer of terminal B cell differentiation. *Science* **276**, 596–599 (1997).
63. Usui, T. *et al.* Overexpression of B cell-specific activator protein (BSAP/Pax-5) in a late B cell is sufficient to suppress differentiation to an Ig high producer cell with plasma cell phenotype. *J. Immunol.* **158**, 3197–3204 (1997).
64. Rinkenberger, J. L., Wallin, J. J., Johnson, K. W. & Koshland, M. E. An interleukin-2 signal relieves BSAP (Pax5)-mediated repression of the immunoglobulin J chain gene. *Immunity* **5**, 377–386 (1996).
65. Singh, M. & Birshtein, B. K. NF-HB (BSAP) is a repressor of the murine immunoglobulin heavy-chain 3′ α enhancer at early stages of B-cell differentiation. *Mol. Cell. Biol.* **13**, 3611–3622 (1993).
66. Waldburger, J. M. *et al.* Lessons from the bare lymphocyte syndrome: molecular mechanisms regulating MHC class II expression. *Immunol. Rev.* **178**, 148–165 (2000).
67. Lin, K. I., Lin, Y. & Calame, K. Repression of c-myc is necessary but not sufficient for terminal differentiation of B lymphocytes *in vitro*. *Mol. Cell. Biol.* **20**, 8684–8695 (2000).
68. Horcher, M., Souabni, A. & Busslinger, M. Pax5/bsap maintains the identity of B cells in late b lymphopoiesis. *Immunity* **14**, 779–790 (2001).
69. Dent, A. L., Shaffer, A. L., Yu, X., Allman, D. & Staudt, L. M. Control of inflammation, cytokine expression, and germinal center formation by BCL-6. *Science* **276**, 589–592 (1997).
70. Ye, B. H. *et al.* The BCL-6 proto-oncogene controls germinal-centre formation and Th2- type inflammation. *Nature Genet.* **16**, 161–170 (1997).
71. Muramatsu, M. *et al.* Class switch recombination and hypermutation require activation-induced cytidine deaminase (AID), a potential RNA editing enzyme. *Cell* **102**, 553–563 (2000).
72. Mittrucker, H. W. *et al.* Requirement for the transcription factor LSIRF/IRF4 for mature B and T lymphocyte function. *Science* **275**, 540–543 (1997).
73. Matsuyama, T. *et al.* Molecular cloning of LSIRF, a lymphoid-specific member of the interferon regulatory factor family that binds the interferon-stimulated response element (ISRE). *Nucleic Acids Res.* **23**, 2127–2136 (1995).
74. Mamane, Y. *et al.* Interferon regulatory factors: the next generation. *Gene* **237**, 1–14 (1999).
75. Gupta, S., Jiang, M., Anthony, A. & Pernis, A. B. Lineage-specific modulation of interleukin 4 signaling by interferon regulatory factor 4. *J. Exp. Med.* **190**, 1837–1848 (1999).
76. Gupta, S., Anthony, A. & Pernis, A. B. Stage-specific modulation of IFN-regulatory factor 4 function by kruppel-type zinc finger proteins. *J. Immunol.* **166**, 6104–6111 (2001).
77. Grumont, R. J. & Gerondakis, S. Rel induces interferon regulatory factor 4 (IRF-4) expression in lymphocytes. Modulation of interferon-regulated gene expression by rel/nuclear factor κB. *J. Exp. Med.* **191**, 1281–1292 (2000).
78. Brass, A. L., Kehrli, E., Eisenbeis, C. F., Storb, U. & Singh, H. Pip, a lymphoid-restricted IRF, contains a regulatory domain that is important for autoinhibition and ternary complex formation with the Ets factor PU. 1. *Genes Dev.* **10**, 2335–2347 (1996).
79. Rao, A., Luo, C. & Hogan, P. G. Transcription factors of the NFAT family: regulation and function. *Annu. Rev. Immunol.* **15**, 707–747 (1997).
80. Peng, S. L., Gerth, A. J., Ranger, A. M. & Glimcher, L. H. NFATc1 and NFATc2 together control both T and B cell activation and differentiation. *Immunity* **14**, 13–20 (2001).
81. Matthias, P. Lymphoid-specific transcription mediated by the conserved octamer site: who is doing what? *Semin. Immunol.* **10**, 155–163 (1998).
82. Corcoran, L. M. & Karvelas, M. Oct-2 is required early in T cell-independent B cell activation for G1 progression and for proliferation. *Immunity* **1**, 635–645 (1994).
83. Qin, X. F., Reichlin, A., Luo, Y., Roeder, R. G. & Nussenzweig, M. C. OCA-B integrates B cell antigen receptor-, CD40L- and IL 4-mediated signals for the germinal center pathway of B cell development. *EMBO J.* **17**, 5066–5075 (1998).
84. Schubart, K. *et al.* B cell development and immunoglobulin gene transcription in the absence of Oct-2 and OBF-1. *Nature Immunol.* **2**, 69–74 (2001).
85. Niu, H., Ye, B. H. & Dalla-Favera, R. Antigen receptor signaling induces MAP kinase-mediated phosphorylation and degradation of the BCL-6 transcription factor. *Genes Dev.* **12**, 1953–1961 (1998).