Regulation and Functions of Blimp-1 in T and B Lymphocytes

Gislâine Martins and Kathryn Calame

Department of Microbiology, Columbia University College of Physicians and Surgeons, New York, New York 10032; email: gm2141@columbia.edu, klc1@columbia.edu

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transcription, repressor, terminal differentiation, cytokines

Abstract

B lymphocyte–induced maturation protein-1 (Blimp-1), discovered 16 years ago as a transcriptional repressor of the *IFNβ* promoter, plays fundamentally important roles in many cell lineages and in early development. This review focuses on Blimp-1 in lymphocytes. In the B cell lineage, Blimp-1 is required for development of immunoglobulin-secreting cells and for maintenance of long-lived plasma cells (LLPCs). Direct targets of Blimp-1 and the transcriptional cascades Blimp-1 initiates to trigger plasmacytic differentiation are described. Blimp-1 also affects the homeostasis and function of $CD4^+$, $CD8^+$, and regulatory $CD4^+$ T cells, and Blimp-1 levels are highest in antigen-experienced T cells. Blimp-1 attenuates T cell proliferation and survival and modulates differentiation. Roles for Blimp-1 in Th1/Th2 specification, regulatory T cell function, and CD8 differentiation and function are under investigation. Signals that induce Blimp-1 in B cells include Toll-like receptor ligands and cytokines; in T cells, T cell receptors and cytokines induce Blimp-1. In spite of some commonalities, different targets and regulators of Blimp-1 in B and T cells suggest intriguing evolutionary divergence of this regulatory machinery.

INTRODUCTION

Zinc finger: the structural domain of a protein, composed of ~30 amino acids binding a zinc ion, that mediates sequence-specific binding to DNA

Although transcription factors containing zinc finger DNA-binding domains constitute the largest family of transcription factors (1), a single zinc finger–containing transcriptional repressor, B lymphocyte–induced maturation protein-1 (Blimp-1, also called PRDIBF1) plays critical and nonredundant roles in both B and T lymphocytes. In addition, Blimp-1 plays important roles in certain nonlymphoid lineages in adults and in various aspects of embryonic development in many organisms. In this article, we review the role of Blimp-1 in B and T lymphocytes in detail, with an emphasis on recent findings. Other reviews including this topic have appeared recently (2–7).

BLIMP-1 BASICS

Although there is nothing unusual about its structure, an unusual role for Blimp-1 in B cells was indicated in early work.

Discovery

Blimp-1 cDNA was first cloned by Maniatis and colleagues (8), who used expression cloning to identify a human cDNA encoding a zinc finger–containing protein that bound to the positive regulatory domain I (PRDI) of the human *IFNβ* promoter. They named the protein PRDIBF1 (positive regulatory domain I-binding factor 1) and, in addition to verifying its binding specificity, demonstrated that the protein was a transcriptional repressor, which was induced upon virus infection of the human osteosarcoma line U20S. Three years later, Davis and colleagues (9) isolated a murine cDNA from a subtractive screen of BCL1 lymphoma cells compared before and after induction of differentiation to Ig-secreting plasma cells by treatment with IL-2+IL-5. Because the message was induced following cytokine-dependent differentiation of the cells, they called it B lymphocyte-induced maturation protein-1, or Blimp-1 (9). Although they did not show directly that the zinc finger protein encoded by their cDNA was a transcription factor or define a binding site, they made the critical observation that ectopic expression of the protein was sufficient to drive the BCL1 cells to mature into Ig-secreting plasmacytoid cells. Later that year, Huang (10) brought these two lines of study together by showing that Blimp-1 was the murine homolog of PRDIBF1, although the mouse protein had a slightly different N terminus from the human, containing 67 additional amino acids (10). Indeed, the human and mouse proteins are highly homologous and are interchangeable in functional assays. (For simplicity, we use the name Blimp-1 for both the human and mouse proteins in this review.) Huang's recognition that PRDIBF1 and Blimp-1 were homologs identified this protein as a transcriptional repressor, with a defined binding specificity, that was capable of driving BCL1 lymphoma cells to differentiate into Ig-secreting plasma cells.

Subsequently the gene structure of *prdm1* (11), encoding Blimp-1, as well as its location on human chromosome 6q21, and the syntenic region of mouse chromosome 10 (12) were defined. The mouse gene extends over ~23 kb and contains 8 exons. Exons 6, 7, and 8 encode the zinc finger domains (**Figure 1*a*,*b***).

Protein Domains and Biochemical Mechanism of Action

Murine Blimp-1 contains 856 amino acids and is predicted to be a 95,835-Da protein. Human Blimp-1 has 789 amino acids and a predicted molecular weight of 87,990 Da. The five C_2H_2 zinc finger motifs in the C terminus of Blimp-1 were clearly implicated as the DNA-binding domain; however, further study showed that only the first two finger motifs are necessary for recognition of the PRDI region in the *IFNβ* promoter (13). The consensus-binding site for Blimp-1 was determined and, consistent with the Maniatis papers (8, 13), was very similar to that of IFN regulatory factor (IRF)1 and IRF2 (14). In fact, Blimp-1 and IRF1/2 compete for binding to the site in the *IFNβ* promoter (14).

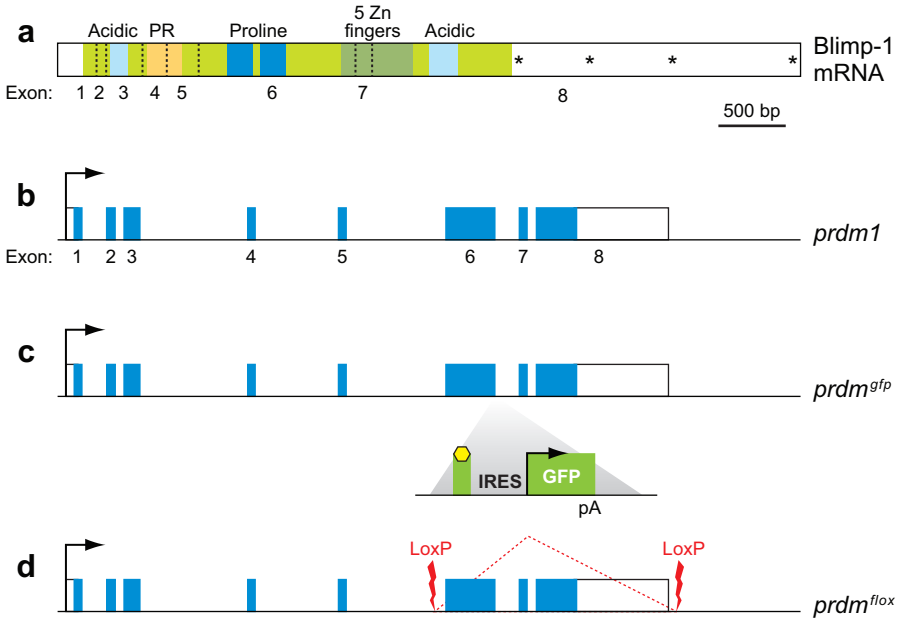

Figure 1

Blimp-1 mRNA and the *prdm1* gene. (*a*) Murine Blimp-1 mRNA showing which portions are encoded by each exon. 5′ and 3′ noncoding regions are white. Coding regions are colored, with acidic regions light blue, PR region orange, proline-rich region dark blue, and zinc fingers dark green (11). (*b–d*) Murine *prdm1* gene with exons shown as raised boxes. Coding regions are blue, and noncoding are white. The approximate location of clustered transcription start sites is indicated by an arrow. The gene covers about 23 kb; in the figure, the scale for introns is 2 times less than the scale for exons. (*c*) Alterations in *prdm1* to make the *prdm1*^{gfp} knockin allele. A region containing a splice acceptor site followed by stop codons in all three frames (*yellow hexagon*), an internal ribosome entry site (IRES), and cDNA encoding e-GFP (green fluorescent protein) followed by a SV40 polyadenylation site (pA) was inserted into intron 6 (24). (*d*) Structure of the *prdm1*^{flox} allele. LoxP sites (*red bolts*) were inserted in intron 5 and 3′ to exon 8. Upon Cre-dependent deletion, exons 6–8, encoding the zinc finger domains, are deleted (34).

Other domains of the protein (**Figure 1*a***) include a proline-rich region N-terminal to the zinc fingers and a PR domain conserved between Blimp-1 and the Rb-binding protein RIZ1 (encoded by *prdm2*) (15, 16). The proline-rich region along with the zinc fingers is required for transcriptional repression and has been shown to mediate association of Blimp-1 with transcriptional corepressor hGroucho (17) and histone deacetylases 1 and 2 (18). Deacetylation of histone lysine residues is associated with a repressive chromatin structure.

The PR domain was named for the first two proteins where it was discovered, PRDI-BF1 and RIZ. PR domains in Blimp-1 and RIZ have similarity to SET domains found in histone methyl transferases (HMT) (19), and the PR domain of RIZ1 does have HMT activity (20). Although the PR domain of Blimp-1 does not have demonstrable HMT activity, Blimp-1 recruits the G9a HMT to the *IFNβ* promoter (21). G9a methylates lysine 9 on histone 3, a repressive histone modification. H3K9 methylation occurs in the IFN-β promoter upon ectopic expression of Blimp-1 (21). Finally, in primordial germ cells, Blimp-1 complexes with prmt5, an arginine HMT that catalyzes symmetrical dimethylation of arginine 3 on H2A and H4 (22). Thus, Blimp-1 appears to repress transcription by recruiting proteins or corepressor complexes that modify histones (by deacetylation, H3K9 methylation, and

arginine methylation) to create a more closed or repressive chromatin structure. However, further work is necessary to learn exactly how chromatin is modified at specific Blimp-1 target genes when Blimp-1 represses them and to learn if chromatin modification is the only mechanism by which Blimp-1 represses transcription.

IHC: immunohistochemistry

GC: germinal center

Cre and "floxed" alleles: bacteriophage P1's Cre recombinase excises DNA between two similarly oriented 34 bp loxP recombination sites, leaving a single loxP site. DNA flanked by loxP sites is said to be "floxed" and is a target for Cre-dependent deletion

BLIMP-1 IN B CELL BIOLOGY

Based on the demonstration by Davis and colleagues (9) that Blimp-1 was sufficient to drive plasmacytic differentiation, much work has focused on the role of Blimp-1 in the B cell lineage. This work provides a paradigm for exploring its roles in other cell lineages.

Expression Pattern of Blimp-1

Blimp-1 expression in the B cell lineage has been studied by immunohistochemistry (IHC) in mice and humans and using a green fluorescent protein (GFP) reporter gene knocked into one allele of the *prdm1* locus in mice (**Figure 1*c***). In mice, both IHC (23) and GFP (24) studies show that some plasmablasts and all plasma cells express Blimp-1. GFP analyses provide evidence that increasing levels of Blimp-1 correspond to stages of plasma cell differentiation from plasmablasts to long-lived plasma cells (LLPCs) in the bone marrow (24). Consistent with this idea, plasma cells resulting from a secondary response had higher levels of Blimp-1 compared with those formed in a primary response (25). No Blimp-1 expression has been observed in memory B cells in mouse (26) or human (27).

Although peritoneal B-1 cells express low levels of Blimp-1 mRNA (28) and protein, indicated by the GFP reporter (29), Blimp-1 mRNA is induced by lipopolysaccharide (LPS) upon Toll-like receptor (TLR) 4 signaling in B-1 cells (30), and the kinetics of induction, measured using the GFP reporter, are more rapid than that for B2 cells (29).

Although IHC is not quantitative, it is probably more sensitive than the GFP reporter. Furthermore, half-lives of Blimp-1 and GFP mRNA and protein are probably not exactly the same, introducing some doubt into using GFP as an absolute indicator of endogenous Blimp-1 expression. IHC for endogenous Blimp-1 has revealed that a small fraction (~5%–15%) of cells in germinal centers (GCs) also express Blimp-1. (Blimp-1 has not been detected in B cells at earlier stages.) Blimp-1$^+$ cells in GCs do not express Bcl-6 but do contain cytoplasmic Ig and probably represent centrocytes that are fated to leave the GC as plasmablasts (23). Blimp-1 appears in these GC cells before Syndecan-1 (CD138) (31).

Blimp-1 expression patterns in humans are similar to those in mice (32, 33). The GC B cells that express Blimp-1 are Pax5$^+$Bcl-6$^-$; outside the GC, human plasma cells do not express Pax5 (32, 33). In an in vitro system in which human centrocytes were induced to become Ig-secreting plasma cells, high levels of Blimp-1 mRNA were not achieved until levels of Bcl-6, Pax5, and Bach2 had fallen significantly (27).

Blimp-1 Is Required for Ig Secretion

Mice with a conditional deletion of *prdm1* in the B lineage were created by crossing mice with "floxed" *prdm1* alleles to CD19Cre transgenic mice (34). In these mice, exons 6–8, which encode all the zinc finger domains, are deleted in mature B cells in the presence of Cre recombinase (**Figure 1*d***). Consistent with Blimp-1's expression pattern, these mice revealed that Blimp-1 is required for plasma cell formation and for normal Ig secretion in response to both T-independent (TI) and T-dependent (TD) antigens. In the B cell conditional knockout (CKO) mice, peripheral B cell subsets were normal, and GCs formed in response to the TD antigen. Class switch recombination (CSR) occurred normally, as evidenced by the appearance of switch circles (M. Shapiro-Shelef & K. Calame, unpublished),

but secretion of all isotypes was severely reduced. In addition, GCs were enlarged in CKO mice, suggesting a developmental block at the late/post-GC stage. Subsequent studies on the CD19Cre CKO mice revealed that, although B1 B cells were present in normal numbers and were capable of self-renewal, they failed to secrete Ig normally (30). Thus, Blimp-1 is required for normal Ig secretion in all B cell subsets.

A recent report (110) has investigated in more detail the low Ig secretion observed in mice lacking Blimp-1 in the B cell lineage (34). Using reconstitution of $Rag^{-/-}$ mice with fetal liver cells from $prdm1^{gfp/gpf}$ mice, these researchers showed that there is an early phase in plasmacytic differentiation, which they call a "preplasmablast," during which Pax5 activity is inhibited and genes repressed by Pax5 and other plasma cell genes, but not Blimp-1, are expressed. These cells secrete low amounts of Ig and provide evidence for an early phase of plasmacytic differentiation that is independent of Blimp-1 but requires inhibition of Pax5. Thus, these authors conclude that, although Blimp-1 is required for full plasmacytic differentiation and normal levels of Ig secretion, B cells can enter an initial phase of plasmacytic differentiation without Blimp-1. One caveat regarding this work, however, is the question of whether the $prdm1^{gfp}$ allele used in this study is a true null allele or a hypomorphic allele, owing to the potential, via differential splicing, to form Blimp-1 mRNA (**Figure 1*c***). Embryos homozygous for the $prdm1^{gfp}$ allele live until at least E13 or E14, allowing transfer of fetal liver cells for reconstitution studies (35). In contrast, embryos homozygous for either of two different deletion alleles, including one generated from the $prdm1^{flox}$ allele (**Figure 1*d***), die significantly earlier at E10.5 (36).

Blimp-1 is not required for formation of memory B cells because in the CKO mice there is an exaggerated GC response upon a secondary challenge even though these cells are unable to differentiate into plasma cells (34). This is consistent with the finding that Blimp-1 mRNA is not present in human memory B cells formed in in vitro cultures (27) or in ex vivo purified human memory cells (26). Preplasma memory cells in the bone marrow, however, were dramatically reduced in the CKO mice, suggesting a requirement for Blimp-1 to form this interesting but controversial (26, 37, 38) subset.

Using an inducible gene deletion system, investigators showed that LLPCs (see the Long-Lived Plasma Cells sidebar) in the bone marrow, formed in the presence of Blimp-1, require continued expression of Blimp-1 for their maintenance (39). LLPCs in the bone marrow provide a second form of humoral memory by providing continuous immunity to pathogens that have been previously encountered. They survive in the bone marrow without proliferation or antigen stimulation for long periods of time (40). The continued requirement for Blimp-1 shows that the repressive changes in chromatin that Blimp-1 facilitates must be labile on at least an important subset of its target genes, requiring continued activity of Blimp-1 to maintain repression. These studies showed that when *prdm1* was deleted, $CD138^{+}$, Ig-secreting cells, resident in the bone marrow disappeared; however, whether they died or dedifferentiated was not established and requires further study. Thus, Blimp-1 is uniquely required for formation and maintenance of all Ig-secreting B cells, and interfering with its activity might be a way to target LLPC in autoimmunity or other pathological conditions.

Plasmacytic differentiation: terminal differentiation of an activated B cell to become an Ig-secreting plasma cell

Targets of Blimp-1

Not only is Blimp-1 necessary for plasma cell formation and function, it is also sufficient. The striking ability of ectopically expressed Blimp-1 to drive plasmacytic development, originally demonstrated in 1994 by Davis and colleagues (9), was subsequently extended beyond BCL1 cells to normal splenic B cells (47, 48). Importantly, however, when Blimp-1 expression is forced in B cells at earlier developmental stages, it causes cell death (49).

LONG-LIVED PLASMA CELLS

In a primary response, most plasma cells formed after a T cell–dependent GC reaction secrete antibodies with high affinity and switched isotypes. Because of changes in chemokine receptors such as CXCR5, they exit the follicles, and some migrate to niches in the bone marrow. In the bone marrow niches, they receive survival signals that include IL-6, made by bone marrow stromal cells (41), and TNF family members BAFF (B cell-activating factor) or APRIL (a proliferation-inducing ligand) that signal through BCMA (B cell maturation antigen) (42). LLPCs have been shown to survive for months to years, sometimes for the lifetime of the organism (43) in the absence of an antigen (44) or cell division (45). Interestingly, when an organism mounts a new primary response, some previously formed LLPCs, resident in the bone marrow, are mobilized to leave the bone marrow survival niches, presumably providing space for newly formed plasma cells and ensuring a dynamic repertoire of LLPCs, reflecting the immunological experience of the organism (46). Because they provide constant immunological vigilance in the form of secreted antibodies, LLPCs are considered one form of humoral immunity. For more details on LLPCs, see a recent review (40).

Ig-secreting plasma cells differ significantly, both morphologically and functionally, from activated B cells; they express neither BCR nor MHC class II on their surface, and they are postmitotic (6). The ability of a single transcription factor to trigger such a complex and dramatic developmental decision is unusual and was a driving force behind efforts to identify targets of Blimp-1-dependent repression in B cells.

Gene expression analyses comparing Burkitt lymphoma lines with or without forced expression of Blimp-1 revealed more than 250 genes whose expression was altered by Blimp-1 (50). Obviously such experiments reveal both direct and indirect Blimp-1 targets (**Figure 2**). Three main programs of gene expression were altered by Blimp-1. (*a*) A proliferative program including *cMYC*, *E2F1*, and other genes required for entry into cycle and cell division was repressed. *Myc* had previously been shown to be a direct target of Blimp-1-dependent repression (51, 52). (*b*) A program involved in Ig secretion, including *J chain*, *X-box binding protein-1 (XBP-1)*, as well as Ig heavy and Ig light chain genes, was induced. (*c*) An extensive program of gene expression characteristic of activated or GC B cells was repressed, including genes encoding the critical transcription factors Pax5 and Bcl-6. *Pax5* was previously shown to be a direct target of Blimp-1 (53), and recent studies show that *bcl6* is also directly repressed by Blimp-1 (L. Cimmino & K. Calame, unpublished). The "B cell program" repressed by Blimp-1 includes repression of genes involved in response to signals from the BCR; genes, notably *AICDA*, involved in CSR and somatic hypermutation (SHM); genes encoding costimulatory molecules for T cells; and genes for chemokine receptors (**Figure 2**). Direct targets among this group also include class II transactivator (*CIITA*) via promoter III, required for MHC class II expression in B cells (48, 54), *ID3* and *SPIB* (50) (**Figure 3**, **Table 1**).

Another gene expression study identified genes regulated by Blimp-1 in BCL1 and M12 cells (55). This study confirmed much of the earlier work but also identified other interesting genes regulated by Blimp-1 in B cells, including induction of *irf4* and repression of *taci*. Using mutant forms of Blimp-1, investigators also identified genes whose regulation depended on the PR domain of Blimp-1, including *ell2* (55).

XBP-1 is a transcriptional activator that is required for plasma cell development and function (56). When the gene expression profiles of splenic B cells lacking Blimp-1 and XBP-1 were compared (57), it was clear that Blimp-1 expression is necessary for expression of XBP-1 and that XBP-1 is the proximate activator of multiple genes necessary for expanded endoplasmic reticulum and protein secretion as well as of genes causing increased cell size, lysosome content, mitochondrial mass and function, ribosome numbers, and total protein synthesis. In the

XBP-1: X-box binding protein-1

Gene expression profile: the unique, global pattern of genes expressed as steady-state mRNA in a given cell type

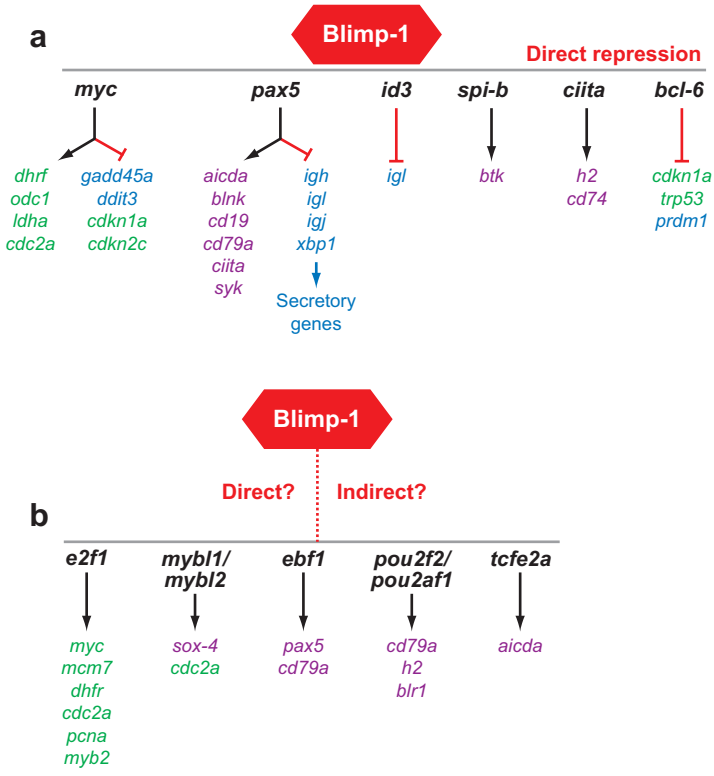

Figure 2

Transcription factors that are targets of Blimp-1 in B cells. Panel (*a*) indicates genes shown to be directly repressed by Blimp-1, and panel (*b*) indicates genes Blimp-1 represses by a currently unknown mechanism. In both cases, target genes that are known to be regulated by the indicated transcription factors and that had their expression altered by Blimp-1 (50) are shown. Green indicates genes involved in cell proliferation, purple, genes involved in B cell phenotype and function, and blue, genes involved in Ig secretion. All genes are given murine designations for clarity and consistency.

absence of Blimp-1, neither XBP-1 nor its target genes are expressed normally (57). The mechanism responsible for Blimp-1-dependent induction of XBP-1 may be via repression of *pax5* since Pax5 has been reported to repress *xbp1* (59). However, a detailed gene expression study in B cells lacking Pax5 did not show that XBP-1 was elevated (60), suggesting another, currently unknown, mechanism may be responsible for Blimp-1-dependent expression of XBP-1. In chicken DT40 cells lacking Pax5, both Blimp-1 and XBP-1 were elevated (61), providing evidence that Pax5 does repress *xbp1* in this setting. Thus, although it is established that Blimp-1 is necessary for XBP-1 induction in B cells, the exact mechanism by which Blimp-1 regulates XBP-1 remains uncertain.

Regulation of Blimp-1 and Plasmacytic Differentiation

Plasmacytic differentiation must be strictly regulated. Failure to mount a humoral response in a timely fashion would clearly jeopardize the organism. However, if plasmacytic differentiation occurred too soon during a TD response, before germinal center reactions were completed, affinity maturation and class switching would be compromised, and the strength and quality of both primary and secondary responses would be

Germinal center (GC) reaction: In response to T cell–dependent antigens and T cell help, B cells form GCs, where they undergo rapid proliferation, affinity maturation, and CSR. Memory cells and plasmablasts result from the GC reaction

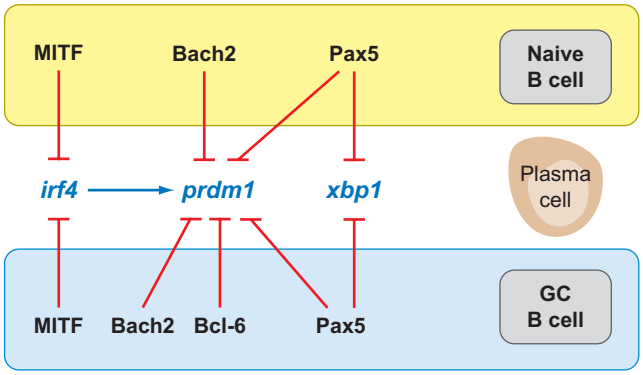

Figure 3

Inhibition of genes required for plasma cell differentiation in earlier naive follicular B cells and by germinal center (GC) B cells. Red bars indicate transcriptional repression, and the blue arrow indicates induction. Factors in naive B cells are shown in the yellow box; factors in GC B cells are shown in the blue box (59–61, 90, 95–97, 100, 103, 106).

diminished. In addition, peripheral tolerance requires B cells to selectively respond to signals from self-antigens by dying or becoming anergic rather than undergoing plasmacytic differentiation. Finally, it is important that memory B cells be poised to differentiate to plasma cells rapidly in response to secondary antigenic challenge but also be regulated so that spontaneous differentiation does not occur in the absence of a stimulus. Expression of Blimp-1 sets plasmacytic differentiation into effect in what is normally an irreversible process. Thus, understanding the regulation of Blimp-1 expression is critical for understanding how appropriate primary humoral responses, peripheral B cell tolerance, and B cell memory are regulated. The data detailed below support a general model in which (*a*) the B cell developmental stage and (*b*) the nature, strength, and duration of signals that B cells receive from antigen, TLRs, and cytokines combine to determine Blimp-1 expression and plasmacytic development.

In B cells, Blimp-1 expression is regulated primarily at the level of transcription initiation (11). Multiple, clustered transcription initiation sites have been mapped in the mouse gene that give rise to a full-length protein (11). An alternative transcription start site, located 5′ of exon 4 in the human gene,

Table 1 Direct targets of Blimp-1 repression in lymphocytes

Gene	Transcriptional regulator?	B cell target	T cell target	References
bcl6	Yes, repressor	Yes	Yes	L. Cimmino & K. Calame, unpublished
myc	Yes, activator and repressor	Yes	No	51; G. Martins, L. Cimmino & K. Calame, unpublished
ciita (promoter III)	Yes, coactivator	Yes	Not expressed	48, 54
fos	Yes, activator	n.d.	Yes	162a; E. Magnusdottis, G. Martins & K. Calame, unpublished
Id3	Yes, inhibitor of E proteins	Yes	n.d.	50
ifng	No	Not expressed	Yes	L. Cimmino & K. Calame, unpublished
il2	No	n.d.	Yes	62; G. Martins & K. Calame, unpublished
pax5	Yes, activator and repressor	Yes	Not expressed	53
spib	Yes, activator	Yes	n.d.	50
tbet	Yes, activator	n.d.	Yes	L. Cimmino & K. Calame, unpublished

n.d., not determined.

gives rise to a shorter protein known as the beta form, which lacks 101 N-terminal amino acids, contains only a partial PR domain, and has reduced transcription repression activity (62). This form is usually present in submolar amounts relative to the full-length form. Blimp-1 mRNA and protein are labile, with half-lives of less than 2 h (C. Tunyaplin & K. Calame, unpublished). Although phosphorylation of the protein has been observed (J. Noronha & K. Calame, unpublished), there is no evidence that levels of phosphorylation are regulated, and the effect of phosphorylation on function has not been assessed. IHC shows Blimp-1 is localized entirely in the nucleus in mouse (23) and human (32) B cells.

Activators of *prdm1* transcription. Pattern-recognition receptors (63), including TLRs, RIG-I-like receptors, and possibly NOD-like receptors, induce Blimp-1 in many settings. This was first demonstrated by induction of Blimp-1 by Sendai virus (double-stranded RNA) infection of U20S cells (8). In B cells treated in vitro, LPS, which activates TLR4, is a strong inducer of Blimp-1 mRNA for murine splenic B cells and for B-1 cells from the peritoneal cavity (30, 47). CpG, which activates TLR9, induces Blimp-1 in human tonsillar B cell cultures (T. Kuo & K. Calame, unpublished). Reishi polysaccharides, which activate TLR4/TLR2, also induce Blimp-1, apparently using different signaling pathways in human and murine B cells (64).

These data are consistent with an analysis of TLR4$^{-/-}$ and MyD88$^{-/-}$ mice (65). [MyD88 is an obligate signal transducer for all TLRs except TLR3 and TLR4, which have both MyD88-dependent and MyD88-independent pathways (63, 66).] B cells from these mice do not secrete IgM or IgG antibodies normally. Further analysis showed that for a TI-2 response to flagellin, which activates TLR5, MyD88$^{-/-}$ B cells had a defective IgM and IgG1, but not IgG3, response. In a TD GC reaction, both TLR4$^{-/-}$ and MyD88$^{-/-}$ B cells were defective in formation of GC B cells. GC B cells lacking MyD88 had decreased Blimp-1 mRNA and increased Bcl-6 mRNA. These data are consistent with the idea that TLR signals induce Blimp-1 in both TI and TD responses. However, a more recent paper (67) calls into question the role of TLR signals for B cell responses as it showed that mice lacking all TLR-dependent signaling mounted normal responses to TD antigens delivered in various adjuvants. Thus, the role of TLRs in B cell responses is unclear at present, although their ability to induce Blimp-1 is unquestioned.

Pattern recognition receptors activate NF-κB (63), and NF-κB appears to be a direct activator of *prdm1* transcription (**Table 3**), as evidenced by the failure of p65$^{-/-}$p50$^{-/-}$ 3T3 cells to induce Blimp-1 in response to Sendai virus infection, failure of splenic B cells to induce Blimp-1 in response to LPS in the presence of NF-κB inhibitors, and the binding of p65 in vivo to multiple κB sites in the region 5′ to transcription initiation on *prdm1* (T. Kuo, E. Magnusdottir & K. Calame, unpublished). TLR or RIG-I activation of Blimp-1 mRNA is sufficient to induce plasmacytic differentiation in activated B cells. However, activated NF-κB is apparently not sufficient to induce Blimp-1 in all stages of B cell development because it plays important roles earlier in B cell development (68), yet Blimp-1 is not expressed in these earlier B cells. Furthermore, BCR, CD40 ligation, and B cell–activating factor (BAFF)-dependent signaling also activate NF-κB but do not induce Blimp-1 mRNA (69). In fact, BCR, CD40, and IL-4 signals block LPS-dependent induction of Blimp-1 in murine splenic B cells (69), although in human B cells BCR and CD40 activation enhance IL-21's ability to induce plasmacytic differentiation and Blimp-1 expression (70). There has, however, been a report that a stress response in macrophage and B cell lines induces Blimp-1 in a way that depends on NF-κB (71). Other transcription factors, including NF-IL6 and IRF1, IRF3, and IRF8 are also induced by TLR signaling (72) and may also induce Blimp-1, especially

BAFF: B cell activating factor

Table 2 Signals that regulate Blimp-1 expression

Function	Signal	B cells	References	T cells	References
Cytokine					
	IL-2	Yes (BCL1 line)	9	Yes	74, 76
	IL-4	n.d.	—	Yes	132
	IL-5	Yes	9	n.d.	—
	IL-6	Yes (lymphoma lines, bone marrow)	K.L. Lin & K. Calame, unpublished	n.d.	—
	IL-10	Yes	27	n.d.	—
	IL-21	Yes	77	n.d.	—
Clonotypic receptor	B/TCR	Indirect via Bcl-6 or others	78	Yes	76, 35
TLR ligand					
	LPS	Yes	47	n.d.	—
	CpG	Yes	29	n.d.	—

n.d., not determined.

because IRF8 induces Blimp-1 in myeloid cells (73).

Several cytokines, including IL-2, IL-5, IL-6, IL-10, and IL-21, induce Blimp-1 mRNA, as first noted by Davis and colleagues (9) who showed that Blimp-1 mRNA was induced by IL-2+IL-5 in BCL1 cells (**Table 2**). Indeed, in T cells as well as B cells, IL-2 is a strong inducer of Blimp-1 mRNA (74).

IL-6 induces plasmacytic differentiation of certain human B cell lines (75), and this is accompanied by induction of Blimp-1 mRNA (K.-I. Lin & K. Calame, unpublished). Although there is little evidence that IL-6 is important for driving plasmacytic differentiation during a primary response in vivo, IL-6, secreted by stromal cells, is critical for maintenance of LLPCs in the bone marrow (41). One of its important functions in this setting may be to induce Blimp-1, which is continuously required for the maintenance of these plasma cells (39), although this has not been directly demonstrated.

Plasmacytic differentiation of human B cells can be induced by IL-10 following activation by BCR and CD40 signals (79–81). Indeed, if IL-10 is added to human memory cell cultures, rapid plasmacytic differentiation ensues (82) and is accompanied by induction of Blimp-1 mRNA (27).

IL-21 is necessary for normal Ig secretion (83, 84) and induces differentiation of B cells to plasma cells in mice (77). Interestingly, IL-21 induces both Blimp-1 and Bcl-6 mRNA (77). Similarly, when human B cells are stimulated via BCR and CD40, IL-21 is a strong inducer of both plasmacytic differentiation and Blimp-1 mRNA, and the effect of IL-21 is enhanced in combination with IL-2 (70).

IL-2, IL-6, IL-10, and IL-21 all activate signal transducer and activator of transcription 3 (STAT3), strongly implicating STAT3 as a direct activator of *prdm1* transcription. Although this has not been definitely demonstrated, forced expression of a dominant-negative form of STAT3 inhibited Blimp-1 mRNA induction in BCL1 cells (85), providing evidence that STAT3 does activate *prdm1* transcription. In addition, and consistent with early observations in BCL1 cells, IL-5, which primarily activates STAT5 (86), induces Blimp-1 mRNA and, like IL-21, also induces genes involved in GC B cells such as *bcl6* and *aicda* (87). IL-2 also activates STAT5 (88), and STAT5 may be important in the IL-2-dependent activation of Blimp-1. However, one study (89) has shown that activated

STAT: signal transducer and activator of transcription

Table 3 Transcription factors that regulate *prdm1* transcription[a]

Transcription factor	Activity	B cell	References	T cell	References
AP-1 (fos-Jun)	Activator	Yes	90	(Yes)	—
Bach2	Repressor	Yes	95	Not expressed	96
Bcl-6	Repressor	Yes	97	Yes	L. Cimmino & K. Calame, unpublished
Blimp-1	Autorepressor	n.d.	—	Yes	L. Cimmino & K. Calame, unpublished; 162a
IRF4	Activator	Yes	93	n.d.	—
Foxp3	Activator	Not expressed	—	Yes	98
NFAT	Activator	Yes	99	(Yes)	—
NF-κB	Activator	Yes	T. Kuo and K. Calame, unpublished	(Yes)	—
Pax5	Repressor	Yes	102a	(No)	—
STAT3	Activator	Yes	85	(Yes)	—
STAT5	Activator	n.d.	—	Yes	D. Gong & T. Malek, personal communication

[a]Parentheses indicate activities implied but not experimentally demonstrated.
n.d., not determined.

STAT5 blocks plasmacytic differentiation, so the role of STAT5 in Blimp-1 regulation in B cells is currently unclear. In addition to Jak/STAT pathways, these cytokines also activate other signaling pathways, including the Ras-Raf-ERK, the phosphatidylinositol 3-kinase (PI3K), the JNK/SAPK, and the p38 signaling pathways, and these may play a role in induction of Blimp-1 as well, although most of these possibilities remain unexplored.

Two other transcriptional regulators, IRF4 and AP-1, bind *prdm1* and directly activate its transcription (**Table 3**). AP-1 was first implicated as an activator of the human gene when it was found that Bcl-6 repressed Blimp-1 transcription by associating with c-Jun and inhibiting the ability of AP-1 to activate *PRDM1* (90). Evidence that AP-1 also induces murine *prdm1* transcription comes from studies using c-Fos transgenic mice. Splenic B cells from these mice undergo more significant plasmacytic differentiation in response to CD40 and IL-4 than do controls. Further studies on these mice also demonstrated binding of AP-1 to the *prdm1* gene by chromatin immunoprecipitation (ChIP) (91). Because Blimp-1 can be induced in mice lacking c-Fos, it seems that, although AP-1 activates Blimp-1 transcription, AP-1 is not essential (91).

IRF4 is necessary for plasma cell formation (92) and more recently has also been shown as a requirement for CSR (93, 94). Mice lacking IRF4 in their B cells have been analyzed to determine if IRF4 might be upstream of Blimp-1 in a regulatory cascade. Two groups obtained different answers to this question. Klein et al. (94) created mice with a conditional deletion of *irf4* in GC B cells. Upon LPS stimulation of splenic B cells in vitro, plasmacytic differentiation was blocked, but Blimp-1 mRNA was similarly induced in both wild-type and CKO, providing evidence that IRF4 does not act upstream of Blimp-1 and is not required for Blimp-1 induction. However, when Sciammas et al. (93) studied B cells from the original knockout mice (92), they also observed a failure in plasmacytic development after LPS stimulation, but in their studies, Blimp-1 mRNA failed to be induced. They went on to perform ChIP studies, which

ChIP: chromatin immunoprecipitation

identified a site between exon 5 and 6 of *prdm1* that was occupied by IRF4 in vivo in stimulated, but not in naive, B cells. They concluded that IRF4 is a direct transcriptional activator of *prdm1*. Although the discrepancy between the two studies remains unexplained, it may be that minor differences in activation conditions or the developmental stage of the cells studied altered the availability of IRF4 partners or other regulators of *prdm1* in the two studies, accounting for different results. The ChIP studies (93), however, strongly suggest that IRF4 does indeed induce *prdm1* transcription in at least some conditions.

Repressors of *prdm1* transcription. It is becoming increasingly clear that repression of Blimp-1 is important for its regulated expression in B cells. Bcl-6 was the first repressor of Blimp-1 to be identified, based on microarray analyses of Burkitt cell lines expressing Bcl-6 or a dominant-negative Bcl-6 (97). Forced expression of Bcl-6 also inhibited plasmacytic differentiation and Blimp-1 expression in murine splenic B cells following activation (85). Bcl-6 represses *prdm1* transcription by interfering with the AP-1 activator (90) and by binding directly to a conserved site in exon 5 of the murine gene (100). MTA3, a cell type-specific subunit of the corepressor complex Mi-2/NuRD, acts as a corepressor with Bcl-6, and when MTA3 and Bcl-6 expression is forced in myeloma cell lines, Ig secretion and Blimp-1 mRNA expression are inhibited, essentially causing dedifferentiation of these transformed plasma cells (101).

Signals that lead to strong activation of PI3K in B cells favor plasmacytic differentiation and inhibit CSR (102). Part of this effect appears to depend on Blimp-1. The mechanism involves PI3K-dependent activation of Akt, which inhibits FoxO-dependent activation of Bcl-6, thus decreasing Bcl-6 and allowing derepression of *prdm1* when PI3K is strongly activated (102).

Loss of Pax5 in mature murine B cells leads to increased expression of Blimp-1 (60), and similar results are observed in chicken DT40 cells (61). However, whether this effect is due to direct repression of *prdm1* by Pax5 or to Pax5-dependent induction of Bcl-6, which then represses *prdm1*, or both was not clear in those studies. In mice, loss of Pax5 did not alter Bcl-6 mRNA levels, but in DT40 cells, Bcl-6 was diminished in the absence of Pax5, and induction of Blimp-1 in the absence of Pax5 could be blocked by Bcl-6 (61). A very recent report shows that Pax5 binds to *prdm1* and directly represses it (102a).

Another repressor of *prdm1* transcription is Bach2, a repressor that interacts with small Maf proteins (95). Bach2 is specifically expressed in B cells and required for normal CSR and SHM. Bach2$^{-/-}$ mice have a hyper-IgM syndrome and spontaneous plasmacytic differentiation of IgM-secreting cells (95). LPS treatment of Bach2$^{-/-}$ splenic B cells led to abnormally elevated Blimp-1 and XBP-1 mRNA and to repression of AID mRNA (95). Subsequently, a binding site for the Bach2-MalK heterodimer in the *prdm1* promoter region was identified, and binding of MalK in vivo was demonstrated by ChIP (103), providing additional evidence that Bach2 directly represses *prdm1*.

Repression of plasmacytic differentiation and Blimp-1 expression. There are several mechanisms that prevent premature plasmacytic differentiation by repressing transcription factors required by plasma cells, and most of these mechanisms involve Blimp-1 (**Figure 3**). Of the three *prdm1* repressors discussed above, Bach2 and Pax5 are expressed in preB and naive B cells (96), as well as in GC B cells. Additionally, Pax5 has been reported to repress *xbp1* (59, 61), although there are data to the contrary (60). Finally, microphthalmia-associated transcription factor MITF, which is most abundant in naive B cells, represses IRF4 mRNA by an undetermined mechanism. B cells lacking MITF have spontaneous plasmacytic differentiation (106), providing direct evidence that MITF is important for normal control of terminal differentiation.

Because IRF4 is a direct activator of *prdm1* (93), its repression indirectly represses *prdm1*.

Expression of Bcl-6 protein is highly restricted to GC B cells (33, 104, 105). Thus, Bcl-6 appears to add a second layer of *prdm1* repression specifically at the GC stage. Thus, Bach2, MITF, and Pax5 repress plasmacytic differentiation in naive B cells and at developmental stages before and during the GC reaction; in the GC, Bcl-6 is present to further ensure that plasmacytic differentiation is inhibited. Interestingly, when human memory B cells develop in vitro from tonsillar centrocytes, Bach2, Pax5, and Bcl-6 mRNA levels decrease significantly, suggesting that one reason memory cells can differentiate into plasma cells quickly is because they lack these repressors (27).

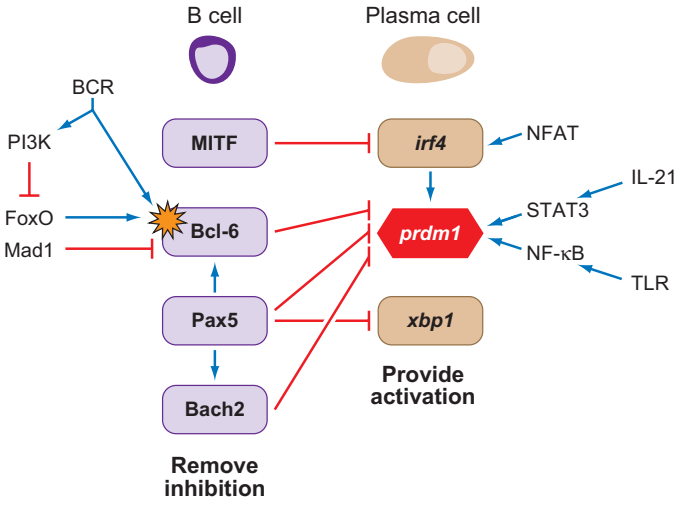

Figure 4

Two signals necessary for plasma cell differentiation. Arrows and bars indicate positive or negative regulation, and the orange star indicates protein degradation (27, 29, 30, 47, 78, 93, 99, 100, 108–112).

Induction and establishment of plasmacytic differentiation. Current understanding of Blimp-1 regulators, as detailed above, shows that Blimp-1 expression and the plasmacytic differentiation that ensues require a combination of two kinds of events: (*a*) removal of inhibitors Bcl-6, Pax5, MITF, and Bach2 and (*b*) induction of activators including IRF4, AP-1, NF-κB, and STAT3 (**Figure 4**). Interestingly, all three general signals necessary for Ig secretion—antigen, TLR ligands, and cytokines—provide one or more of these signals. The requirement both to remove inhibitors and to supply activators may help explain why LPS is such a potent immunogen because TLR4 activates both MyD88-dependent and -independent pathways (107). The requirement may also explain why activated NF-κB present in B cells at earlier developmental stages or BCR signals alone are unable to induce Blimp-1 mRNA.

How are the inhibitors removed? This is a critical question that relates directly to the question of how exit of plasmablasts from the GC is regulated. Unfortunately, information is far from complete, but dissecting the regulation of Blimp-1 in GC and post-GC B cells provides one way to approach the question. Recent studies establish that high-affinity BCRs direct GC B cells to a plasmacytic fate (108, 109), so models must incorporate BCR signals. Studies using in vitro cultures of human tonsillar centrocytes that develop into plasma cells in response to IL-10 (27) show that levels of Bcl-6, Pax5, and Bach2 mRNA drop significantly before large increases in Blimp-1 mRNA occur. There is little information available concerning the mechanisms responsible for loss of Pax5 and Bach2 expression, although decreased Pax5 was observed in preplasmablasts prior to Blimp-1 induction (110).

Several mechanisms remove Bcl-6 from GC B cells. BCR-dependent activation of mitogen-activated protein kinase (MAPK) leads to phosphorylation of Bcl-6, which targets Bcl-6 for ubiquitin-dependent degradation (78). Because Bcl-6 is abundant in GC B cells, this mechanism may only be operative in response to strong BCR signals or in combination with other signals, and it may initiate exit of B cells from the GC, as a decrease in Bcl-6 activity is required for both post-GC plasma cell and memory B cell differentiation

(27). Acetylation inactivates the ability of Bcl-6 to repress transcription, and Bcl-6 is acetylated in GC B cells, although there is no knowledge of if or how acetylation of Bcl-6 may be regulated in GC B cells (111). The E box factor Mad1 is elevated in plasma cells and directly represses Bcl-6 (112). Finally, because FoxO3A activates Bcl-6 (113) and Akt inactivates FoxO transcription factors (114), strong BCR signals, activating PI3K and Akt and inactivating FoxO, lead to a reduction in Bcl-6 and an increase in Blimp-1 in B cells (102). Interestingly, Bcl-6 is not abundant in marginal zone B cells or B1 B cells, and neither Bach2, Pax5, nor Bcl-6 mRNA are found in human memory B cells formed in vitro (27). Thus, in these settings, induction of Blimp-1 can occur more rapidly when appropriate activators are induced.

Mice lacking OBF-1 have impaired plasmacytic differentiation in response to certain kinds of stimulation (115). Without OBF-1, *bcl6* and *pax5* are not repressed, and *prdm1* is not induced when B cells are activated in vitro with CD40L and IL-4. However, stimulation by LPS is normal in the absence of OBF-1 (115). The mechanism requiring OBF-1 for induction of Blimp-1 is not clear, although ChIP assays provided no evidence for direct binding of octamer-OBF-1 complexes to *prdm1*. OBF-1 may indeed be needed to remove one or more repressors such as Bcl-6 in response to certain signals.

Many mechanisms are likely to induce or activate activators of *prdm1*. TLR signals, activating NF-κB, induce Blimp-1 in GC B cells (27) and B-1 B cells (29, 30) and probably in naive and marginal zone B cells present in splenic B cell preparations following treatment with LPS (47). Vav signaling is required downstream of TLR signals to induce Blimp-1 mRNA and to form Ig-secreting plasma cells (116). Mature marginal zone B cells, stimulated with TLR ligands, proliferate and express IRF4 but do not express Blimp-1 when they lack Vav proteins, demonstrating that IRF4 alone is not sufficient to induce Blimp-1. NFAT (nuclear factor of activated T cells) factors are important in B cells, and calcineurin/NFAT-dependent induction of IRF4 is important for post-GC plasmacytic development (99). Because IRF4 is probably a direct activator of *prdm1* (93), it follows that NFAT signaling indirectly induces Blimp-1 mRNA in post-GC plasma cells.

The role of BCR in regulating *prdm1* is interesting and, given rather fragmentary data, certainly deserves further study. There is no evidence that BCR signals alone are sufficient to induce *prdm1*, and in combination with TLR signals, BCR signals actually block plasma cell formation in vitro (117) and Blimp-1 expression (47). However, NFAT, an indirect activator of *prdm1* (99), and AP-1, a direct activator (90), are activated by BCR signals (118). Furthermore, in anergic B cells that do not induce Blimp-1 or develop into plasma cells, NFAT signaling downstream of BCR is uncoupled, whereas extracellular signal regulated kinase (ERK) signaling downstream of BCR is maintained (119). BCR signaling via Ras and MAPK kinase (MEK) to ERK integrates BCR and cytokine signals. ERK activation, via continuous BCR signaling, inhibits Blimp-1 induction, and IL-2 and IL-5 inactivate ERK via induction of the DUSP 5 phosphatase (120). The mechanism for ERK-dependent inhibition of Blimp-1 was not identified, but it might involve Bcl-6 or its cofactors. It is also interesting that in mice lacking Bruton's tyrosine kinase, TI-2 activation of B cells initially induces Blimp-1, but this is not sustained, and the cells do not progress to become plasmablasts (121).

Once Blimp-1 is expressed, it is sufficient to cause plasmacytic differentiation. Blimp-1 also specifically represses genes encoding two critical transcription factors required for GC B cells—*pax5* (53) and *bcl6* (90, 100). Thus, the plasma cell program is enforced, and earlier stages of B cell development are inhibited by Blimp-1.

Blimp-1 in B Cell Malignancies

Recent studies provide evidence that abnormal regulation or abnormal activity of Blimp-1 may be one causal event in some B cell malignancies. If Blimp-1 acts as a tumor suppressor, lack of its activity could be important in some forms of lymphoma. Alternatively, its ability to enforce a plasma cell phenotype may be important in plasma cell tumors.

Diffuse large B cell lymphoma. When an early study identified *myc* as a direct target of Blimp-1-dependent transcriptional repression (51), it was logical to speculate that Blimp-1 might function as a tumor suppressor, especially given the prominent role of dysregulated c-Myc expression in B cell tumors (122). Interestingly, however, aged mice with conditional deletion of *prdm1* in the B cell lineage do not develop B cell tumors spontaneously (M. Shapiro-Shelef & K. Calame, unpublished), indicating that loss of Blimp-1 alone is not sufficient for B lymphomagenesis. Nevertheless, there is growing evidence that deletion or mutation of *PRDM1* is frequently found in a subset of diffuse large B cell lymphoma (DLBCL), providing evidence that it may indeed function as a tumor suppressor (123–125). Similar *PRDM1* mutations were not found in B or T or myeloid leukemias or in the 467 common carcinomas examined (126). In the most thorough study on DLBCL, *PRDM1* was inactivated by structural alterations in 24% (8 out of 34) activated B cell–like diffuse large cell lymphomas but not in GC B cell–like (n = 0/37) or unclassified (n = 0/21) DLBCLs (125). However, a subset of DLBCL, which expressed Blimp-1, lacked detectable plasmablastic or immunoblastic changes and displayed more aggressive behavior, with a shorter failure-free survival (127). Thus, further studies are warranted to determine how expression and lack of expression of Blimp-1 affect the formation and properties of different subsets of DLBCL.

Murine plasmacytoma and human multiple myeloma. Malignant plasma cells in both mice (plasmacytoma) and humans (multiple myeloma) express abundant levels of Blimp-1, which likely reflects their differentiated state as plasma cells and the fact that they usually secrete Ig. Blimp-1 is also present in a subset of DLBCL but not in marginal zone lymphomas or chronic lymphocytic leukemia (32), and abortive plasmacytic differentiation in some Hodgkin and Reed Sternberg cells is indicated by the presence of Blimp-1 (128). Apparently, the ability of Blimp-1 to repress *myc* is overcome in these tumors by oncogenic changes, such as chromosomal translocation, that activate and dysregulate *myc* transcription or the dominant activity of other oncoproteins. [However, Blimp-1 is apparently able to repress *myc* in other tumors, such as myeloid tumors, where it is induced by IRF8 (73).]

DLBCL: diffuse large B cell lymphoma

There is active interest in determining how forcing expression of Blimp-1 or, alternatively, blocking its activity or knocking down its expression might affect the growth and phenotypic properties of myeloma or other B cell tumors. In a recent study, treatment of myeloma cell lines with 2-methoxyestradiol, which suppresses their growth and induces apoptosis, also led to an elevation of Blimp-1 and XBP-1 and repressed *MYC* and *PAX5* (129). In some myeloma cell lines, the proportion of the beta form of Blimp-1 is elevated in comparison with normal plasma cells (62, 130), which might decrease the overall activity of Blimp-1, but the functional consequences of elevated beta form have not been elucidated clearly. Assuming Blimp-1 is necessary for the Ig secretion program in myeloma, as it is in normal plasma cells, one reasonable prediction is that blocking Blimp-1 in the tumors might block Ig secretion. Whether this would cause apoptosis owing to endoplasmic reticulum stress as observed upon administration of protease inhibitors (131) or to failure of other survival mechanisms (39) or dedifferentiation (101) remains to be determined.

BLIMP-1 IN T CELL BIOLOGY

The understanding that Blimp-1 is also important in the T lymphocyte lineage emerged only within the past 2–3 years. Thus, this work is at an earlier stage. Although the overall effect of lacking Blimp-1 T cells is fatal, owing to spontaneous inflammatory disease, the cellular and molecular effects are more subtle and complex than the effects in B cells.

Regulatory T (Treg) cells: those $CD4^+CD25^+$ regulatory T cells, both constitutive and inducible, that express FoxP3 and suppress T cell responses

LCMV: lymphocytic choriomeningitis virus

Expression of Blimp-1 in T Cells

Blimp-1 protein is expressed in human (32, 132) and mouse (35, 74) T cells, and in both species Blimp-1 levels are significantly higher in antigen-experienced cells. Indeed, the levels of Blimp-1 mRNA and protein in antigen-experienced murine and human T cells are similar to those observed in cultures where plasma cells are generated in vitro by LPS treatment of splenic B cells (76, 132). Although steady-state Blimp-1 mRNA is present in low levels in mouse thymocytes (76, 133) and naive $CD4^+$ and $CD8^+$ T cells (76, 132), levels increase 20–40 times upon stimulation with α-CD3, α-CD28, and IL-2 (35, 76, 132). Induction of Blimp-1 mRNA and protein in vitro is slow, with maximum levels achieved 3–5 days poststimulation (76, 132). T cell receptor (TCR) restimulation in the presence of α-CD28 leads to a further increase in Blimp-1 mRNA expression (132; L. Cimmino & K. Calame unpublished). Blimp-1 mRNA is also found in high levels in ex vivo purified $CD4^+$ or $CD8^+$ effector/memory ($CD62L^{Lo}/CD44^{Hi}$) and memory phenotype ($CD62L^{Hi}/CD44^{Hi}$) T cells, with the first subpopulation showing slightly more abundant Blimp-1 transcripts (**Figure 5**) (76). Finally, Blimp-1 transcripts are observed in high levels in ex vivo purified $CD4^+CD25^+CD62L^{Hi}$, which are mostly composed of $Foxp3^+$ cells, known as naturally occurring, regulatory T (Treg) cells (35, 76).

Prdm1-GFP knockin (**Figure 1*c***) reporter mice (24) have been used to monitor Blimp-1 expression in T cells (24). GFP expression is not detected in the thymus or in naive peripheral T cells, but it was observed in in vitro–activated T cells, in vivo antigen-experienced $CD4^+$ and $CD8^+$ peripheral T cells, and in $CD4^+CD25^+CD62L^{Hi}$ cells, which are primarily $Foxp3^+$ Treg cells (24). Thus, despite the limitations of using GFP as a reporter for Blimp-1 expression, these data are consistent with observations described above showing that within the T cell lineage Blimp-1 is found in highest levels in antigen-experienced cells and in naturally occurring Treg cells. In agreement with these patterns, Blimp-1 is upregulated in vivo in $CD8^+$ antigen-specific T cells upon infection with herpes simplex virus (HSV) (35) and with lymphocytic choriomeningitis virus (LCMV) (R. Rutishauser & S. Kaech, unpublished).

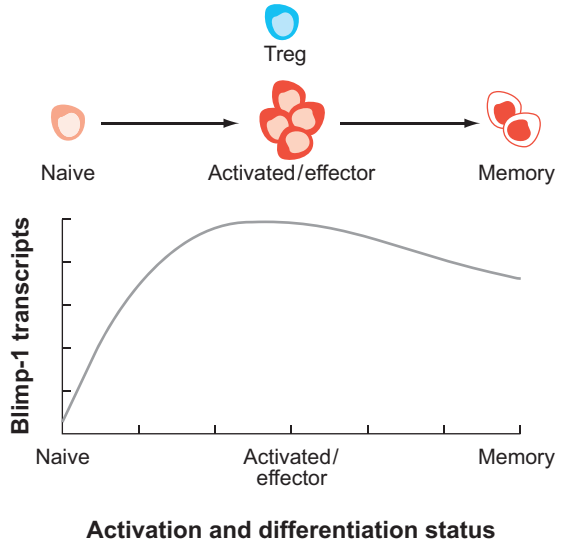

Figure 5

Blimp-1 expression in T cells. Blimp-1 mRNA (represented by arbitrary units on the y-axis) is present in low levels in naive $CD4^+$ and $CD8^+$ T cells but is abundant in ex vivo isolated antigen-experienced ($CD62L^{Lo}CD44^{Hi}$ and $CD62L^{Hi}CD44^{Hi}$) cells. Memory $CD8^+$ T cells and some of the memory $CD4^+$ T cells are contained in the $CD62L^{Hi}CD44^{Hi}$ subpopulation, which expresses slightly lower levels of Blimp-1 mRNA than the $CD62L^{Lo}CD44^{Hi}$ cells. In vitro stimulation (not depicted) of the naive T cells results in a slow increase in Blimp-1 expression, reaching levels similar to that observed in the ex vivo isolated antigen-experienced cells. Ex vivo isolated $CD4^+CD25^+$ naturally occurring, regulatory T (Treg) cells express Blimp-1 transcripts at the same levels as effector nonregulatory $CD4^+$ T cells (35, 76, 132).

Although Blimp-1 expression is consistently elevated in antigen-experienced $CD4^+$ T cells, levels vary: Naive $CD4^+$ T cells stimulated under Th2 conditions (IL-2, IL-4, and anti-IFN-γ) express higher levels of Blimp-1 steady-state mRNA than do cells stimulated under Th1 (IL-2, IL-12, and anti-IL-4) or nonpolarizing conditions (IL-2) (132; L. Cimmino & K. Calame, unpublished). Indeed, Blimp-1 mRNA expression seems to be repressed as cells differentiate under Th1 conditions because at the end of a 6-day culture, Th1 cells express less Blimp-1 steady-state mRNA than do cells stimulated under nonpolarizing conditions (L. Cimmino & K. Calame, unpublished). However, differential expression of Blimp-1 in Th subsets was not observed in the Blimp-1-GFP knockin reporter mice (35). Nonetheless, immunoblotting reveals that Blimp-1 protein is higher in Th2 than in Th1 cells after two rounds of polarization in vitro (L. Cimmino & K. Calame, unpublished). Importantly, these differences are observed before any restimulation at the end of the differentiation rounds, suggesting that the differential levels of Blimp-1 expression are maintained in resting Th1 and Th2 cells and might be related to the maintenance of the respective T helper phenotype.

Blimp-1 and T Cell Development

The pattern of Blimp-1 mRNA expression in T cells—low in thymocytes and naive T cells, high in antigen-experienced T cells—is consistent with the idea that Blimp-1 is more important for T cell function than development; however, conditional deletion of Blimp-1 in T cells (*prdm1*$^{F/F}$Lck-Cre mice) resulted in significant alterations in the thymus (76). The *prdm1*$^{F/F}$Lck-Cre mice have a threefold decrease in the numbers of total thymocytes (76), which can be attributed to a marked reduction in the absolute numbers of $CD4^+$ and $CD8^+$ double-positive (DP) as well as single-positive (SP) $CD4^+$ or $CD8^+$ thymocytes. This defect is observed as early as 4 weeks after birth and progresses with age. There is no significant alteration in the number of double-negative (DN) and γδ thymocytes and no significant differences in the distribution of the various DN thymocyte subsets between control and *prdm1*$^{F/F}$Lck-Cre mice, as determined by CD25 and CD44 expression. Also, developmental stage-specific surface markers (CD5, TCRβ, HSA, CD24, and CD69) and short-term BrdU incorporation are indistinguishable between control and *prdm1*$^{F/F}$Lck-Cre mice, suggesting that the maturation and proliferation of each subset are preserved in the absence of Blimp-1. However, DP thymocytes are more susceptible to cell death, suggesting that Blimp-1 may regulate survival during negative selection of DP thymocytes (76). Nevertheless, the possibility that decreased survival of the *prdm1*$^{F/F}$Lck-Cre mice DP thymocytes is secondary to the immune activation in the periphery cannot be excluded, and more work is required to clarify the role of Blimp-1 in thymocyte development.

Functions of Blimp-1 in T Cells

Mice lacking Blimp-1 in their T cells have provided valuable information about the role(s) of Blimp-1 in the T lineage. Although these CKO mice were created in different ways in two laboratories, most of the observations from the two groups were consistent. However, one key difference is the possible role Blimp-1 may play in $CD4^+$ Treg cells.

Spontaneous inflammatory disease in mice with T cells lacking Blimp-1. Two strategies were used to generate mice with Blimp-1-deficient T cells. One group crossed the *prdm1*$^{F/F}$ mice (**Figure 1*d***) with proximal Lck-Cre or CD4-Cre transgenic mice, generating mice in which Blimp-1 deletion was specifically restricted to T lymphocytes (76; G. Martins & K. Calame, unpublished). The other group used fetal liver cells from *prdm1*$^{gfp/gfp}$ (**Figure 1*c***) embryos to reconstitute the hematopoietic compartment of Rag-1-deficient mice, resulting in the

generation of chimeric mice in which cells from the myeloid and lymphoid lineages were *prdm1*$^{gfp/gfp}$ and Blimp-1 deficient (35). Despite the differences in the two approaches, both studies revealed that lack of Blimp-1 results in profound alterations of T cell function and homeostasis, culminating with the spontaneous development of inflammatory disease (35, 76).

Whereas in the first model (T cell–specific deletion of Blimp-1) the inflammatory disease was concentrated in the colon (35, 76), mice reconstituted with *prdm1*$^{gfp/gfp}$ fetal liver cells also had inflammation in other organs, including lungs and liver (35). Although the nature of this difference has not yet been systematically addressed, it may simply reflect the different approaches used (deletion of Blimp-1 in T cells only versus deletion of Blimp-1 in the lymphoid and myeloid compartments). If so, one would predict that Blimp-1 might be important for regulating the function of hematopoietic cells other than T and B lymphocytes, a possibility that remains to be investigated. It is also possible that the difference in severity of the inflammatory disease observed in the two models is related to differences in the genetic background: The *prdm1*$^{F/F}$Lck-Cre mice were of mixed 129xC57BL/6 background, whereas the *prdm1*$^{gfp/gfp}$ mice where backcrossed into C57BL/6. In other models of spontaneous development of inflammatory diseases (134) the severity and target organs might vary in different genetic backgrounds. Finally, differences in the animal facilities used to house the different Blimp-1 CKOs could also explain differences in the inflammatory phenotype.

The cellular and molecular mechanisms associated with development of colitis in mice with Blimp-1-deficient T cells are not completely understood. T cell dysfunction is associated with colitis in many murine models, which are generally characterized by deregulated activation of effector T cells with excessive production of IFN-γ and decreased production of IL-10 and/or defective development/function of Tregs (135, 136). Current data indicate several probable causes of colitis development in the *prdm1*$^{F/F}$Lck-Cre mice. Blimp-1-deficient CD4$^+$ T cells produce increased amounts of IFN-γ, and their production of IL-10 is significantly impaired (see below), indicating deregulated T cell activation and/or biased Th1 differentiation. There is also partial impairment of Treg function in the absence of Blimp-1, discussed below.

Interestingly, a small population of CD4$^+$ T cells with colitogenic potential can be found in normal mice (137). Apparently, these cells can be driven into the antigen-experienced pool by the presence of commensal bacteria but are normally kept under control by Treg cells in an IL-10-dependent manner (137). Hence, it would be interesting to know if the antigen-experienced cells that accumulate in mice with T cell–specific deletion of Blimp-1 contain these potentially colitogenic cells. If that is indeed the case, the defective production of IL-10 by Blimp-1-deficient Treg cells (see below) could provide a mechanistic explanation for their accumulation. Nonetheless, direct evidence linking the accumulation of the antigen-experienced cells with the spontaneous development of colitis in the T cell–specific Blimp-deficient mice is still lacking, and it is not known if the defect in IL-10 production is associated with colitis in these mice.

T cell homeostasis and attenuation of IL-2 production. The elevated expression of Blimp-1 in peripheral antigen-experienced T cells and the substantial accumulation of these cells in both *prdm1*$^{F/F}$Lck-Cre and *prdm1*$^{gfp/gfp}$ mice, despite the different genetic backgrounds of the mice, provide strong evidence that Blimp-1 is important in T cell homeostasis. Evidence from studies in which Blimp-1 is ectopically expressed (74, 132) or deleted from T cells (35, 76) indicates that Blimp-1 regulates responsiveness and homeostasis of peripheral T cells by attenuating both proliferation and survival. Blimp-1-deficient antigen-experienced CD4$^+$ T cells are less susceptible to apoptosis upon restimulation

in vitro (35; G. Martins & K. Calame, unpublished), and in vitro–differentiated Blimp-1-deficient Th1 cells are more resistant to cell death caused by cytokine withdrawal (35), suggesting that Blimp-1 normally promotes apoptosis of effector T cells.

In addition, it appears that Blimp-1 attenuates proliferation induced upon TCR stimulation of naive T cells. Ectopic expression of Blimp-1 resulted in decreased proliferation of $CD4^+$ T cells (132), and naive $CD4^+$ T cells from the *prdm1*$^{F/F}$Lck-Cre mice proliferate better than wild-type cells when stimulated in suboptimal conditions (76). Stimulation in optimal conditions, including CD28-mediated costimulation and addition of exogenous IL-2, abrogates the proliferation differences between Blimp-1-sufficient and -deficient cells (35, 76), suggesting that regulation of proliferation by Blimp-1 is limited to conditions in which costimulation is absent or weak, such as presentation of a self-antigen in the periphery where the normal result is anergy of the responder T cell (138, 139). Thus, lack of Blimp-1 may cause increased responsiveness to self-antigens. Furthermore, one may speculate that Blimp-1 normally attenuates tonic signaling through the TCR (140). Both possibilities remain to be experimentally evaluated.

Blimp-1-deficient $CD8^+$ T cells seem to respond abnormally to exogenous antigens, as indicated by the increased accumulation of antigen-specific $CD8^+$ T cells after infection of *prdm1*$^{gfp/gfp}$ mice with HSV (35). Also, in a system where P14 TCR transgenic, *prdm1*-deficient or control naive $CD8^+$ T cells were transferred to wild-type recipient mice and the recipients infected with LCMV, there was significantly more accumulation of Blimp-1-deficient cells (R. Rutishauser & S. Kaech, unpublished).

One key mechanism by which Blimp-1 modulates T cell responsiveness is likely to be through repression of IL-2. IL-2, acting in both autocrine and paracrine manners, regulates the initial expansion of naive T cells upon TCR stimulation in vitro (141, 142) and possibly in vivo. Paracrine IL-2 is also important for the in vivo survival of Tregs, which in turn regulate the proliferation of peripheral T cells (142, 143). Multiple observations support the idea that Blimp-1 represses IL-2. Production of IL-2 is elevated in naive Blimp-1-deficient $CD4^+$ T cells following activation in vitro (76), and IL-2 steady-state mRNA levels in naive Blimp-1-deficient $CD4^+$ T cells are elevated relative to controls both before and following activation in vitro (G. Martins & K. Calame, unpublished). Ectopic expression of Blimp-1 significantly decreases IL-2 production in wild-type $CD4^+$ and $CD8^+$ T cells, and IL-2 production declines when Blimp-1 levels are highest after TCR stimulation (74, 132). Moreover, ectopic expression of Blimp-1 inhibited the expression of an IL-2 promoter-GFP reporter (74) in vitro.

However, the relationship between IL-2 and Blimp-1 is additionally complicated and interesting. As detailed below, IL-2 signaling strongly induces *prdm1* transcription (74). Thus, T cell activation induces *il2* transcription, IL-2 signaling induces *prdm1* transcription, and Blimp-1 feeds back to repress *il2* transcription (**Figure 6**). Blimp-1 therefore appears to be a key component of the pathway by which IL-2 downregulates its own expression during later phases of T cell activation (144) (**Figure 6**).

Together these data suggest a model (**Figure 7**) in which Blimp-1 controls T cell function in two different stages: At the initial activation stage it may regulate responsiveness by attenuating IL-2 production and proliferation, and, subsequently, it may enhance the elimination of effector cells via apoptosis. Additionally, Blimp-1 might be required for the proper function of Treg cells, another pathway by which Blimp-1 regulates T cell responses. Thus, Blimp-1-deficient T cells would proliferate more when differentiating into effectors, and the effectors would survive better. This could help to explain the abnormal accumulation of antigen-experienced cells observed in the Blimp-1-deficient mice.

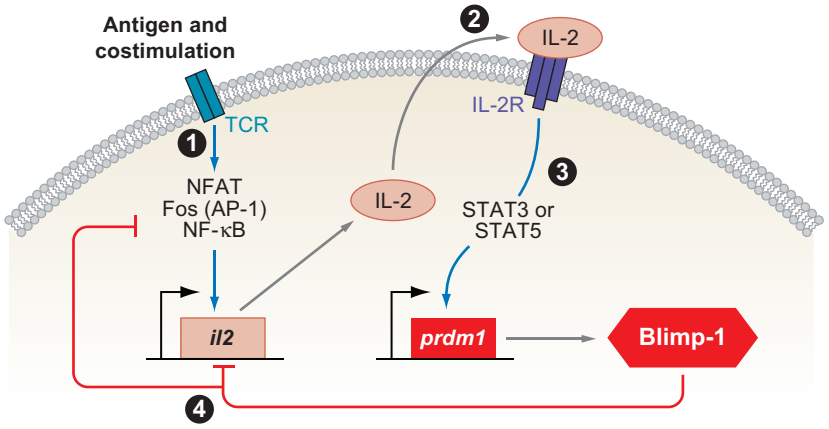

Figure 6

The regulatory feedback loop involving Blimp-1 and IL-2 in T cells. (1) Antigen and costimulation induce *il2* (*pink rectangle*) transcription via NFAT, AP-1, and NF-κB. (2) IL-2 secreted from the cell, in either an autocrine or paracrine manner, binds the IL-2 receptor. (3) IL-2 signaling, probably via activated STAT3 or STAT5, induces *prdm1* transcription and Blimp-1 protein (74). Blimp-1 represses *il2* directly and also represses *fos*, a component of AP-1 which activates *il2* (4) (G. Martins & K. Calame, unpublished).

T helper differentiation. Blimp-1 appears to play a role in Th2 cells by repressing Th1 genes (**Figure 8**). Deletion of Blimp-1 in T cells causes increased production of IFN-γ and decreased production of IL-10 (35, 76). Production of IL-4 was decreased in one model of T cell deletion of Blimp-1 (35) but not significantly altered in another (76). The increased production of IFN-γ by Blimp-1-deficient T cells suggests that Blimp-1 may attenuate Th1 differentiation. Consistent with that, Blimp-1 transcripts and protein are more abundant in Th2 than in Th1 cells differentiated in vitro (132; L. Cimmino & K. Calame, unpublished). Despite these observations, Blimp-1-deficient $CD4^+$ T cells can be polarized into Th1 or Th2 cells in vitro, and IL-4 production seems unaffected, although IFN-γ production is slightly increased in both Th1 and Th2 cells derived from Blimp-1-deficient mice (L. Cimmino & K. Calame, unpublished). It will be important to determine if Blimp-1-deficient Th cells can still polarize normally in an in vivo immune response, where the factors directing Th polarization, such as strength of TCR stimulation and concentration of different cytokines, are more complex than in the in vitro cultures.

Consistent with increased IFN-γ production, Blimp-1-deficient T cells show increased levels of IFN-γ and Tbet steady-state mRNA (L. Cimmino & K. Calame, unpublished). Another important player in the regulation of Th differentiation by Blimp-1 appears to be Bcl-6. Blimp-1-deficient T cells have increased Bcl-6 mRNA levels (L. Cimmino & K. Calame, unpublished), and Bcl-6 proteins can repress Th2 differentiation by interfering with the function of GATA-3 (145), a master regulator of Th2 differentiation (reviewed in 146), and by direct repression of *il5* transcription (147). Thus, alleviating *bcl6* repression could be one mechanism responsible for increased production of Th1 cytokines in Blimp-1-deficient T cells. The idea that Blimp-1 might be required to inhibit Th1

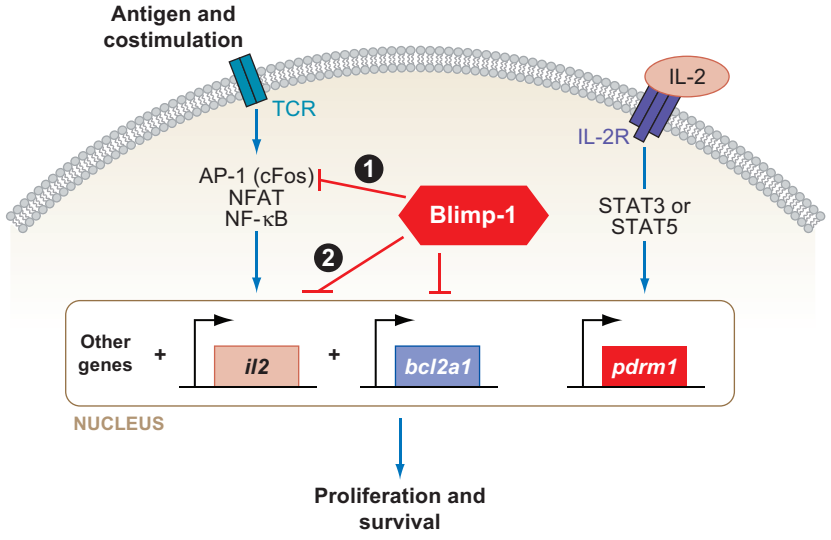

Figure 7

A model for Blimp-1 attenuation of proliferation and survival in CD4+ T cells. TCR stimulation of naive T cells induces IL-2 production and Blimp-1 expression. Blimp-1 attenuates T cell proliferation and survival following primary activation in several ways. (1) Blimp-1 attenuates induction of the *il2* gene indirectly by repressing *fos* and directly by repressing *il2*. (2) Blimp-1 also represses transcription of *bcl2a1* (although it is not known if this occurs directly or indirectly). By interfering with pathways 1 and 2, Blimp-1 might regulate proliferation and survival upon primary TCR stimulation. Pathway 2 could also remain operative in antigen-experienced cells at later stages of differentiation because Blimp-1-deficient Th1 effector cells seem more resistant to cytokine deprivation cell death (35). Additionally, Blimp-1 might repress genes related to activation-induced cell death (AICD) induction (not shown). This latter pathway is more likely to be operative in antigen-experienced cells, at later stages of differentiation, as indicated by the phenotype of mice with Blimp-1-deficient T cells (35, 76).

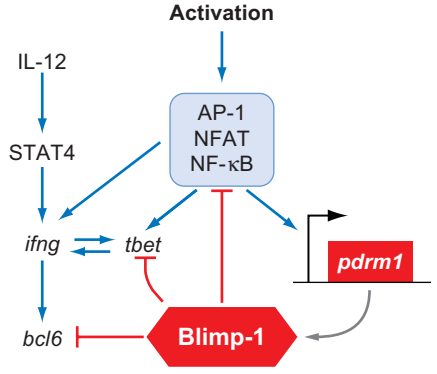

Figure 8

A model for Blimp-1 attenuation of Th1 cell differentiation. Blimp-1 represses *ifng* directly and indirectly via repression of *tbet*. It also represses *bcl6* and *tbet* directly and indirectly via repression of *ifng* (L. Cimmino and K. Calame, unpublished).

differentiation also fits with the observation that ablation of Blimp-1 in T cells results in spontaneous development of colitis, which, in most of the cases, is mediated by IFN-γ-producing cells (135, 136). However, to date there is no direct evidence that a biased Th1 response is a cause of the spontaneous colitis/inflammatory disease observed in mice with Blimp-1-deficient T cells.

Colitis development in the *prdm1*$^{F/F}$Lck-Cre mice might also be associated with the impaired production of IL-10. The decreased production of IL-10 by the Blimp-1-deficient cells is most likely a cell-intrinsic defect because it is observed in conditions in which IFN-γ is minimal or nonexistent, such as when purified CD4+CD25+Foxp3+ Treg cells are stimulated in vitro (G. Martins & K. Calame, unpublished). This finding also

indicates that regulation of IL-10 production by Blimp-1 extends to the Treg cells, and this defect could be one of the triggers of the inflammatory disease observed in these mice, as discussed below.

Differentiation of CD8$^+$ T cells. The expression pattern of Blimp-1 is similar in CD4$^+$ and CD8$^+$ cells (35, 74, 76, 132), but little is known about the role of Blimp-1 in CD8 cells. As mentioned above, *prdm1*$^{gfp/gfp}$ mice are able to mount an efficient CD8$^+$-mediated response to HSV infection, and in this model, Blimp-1-deficient CD8$^+$ T cells performed immediate effector functions, such as IFN-γ production and cytotoxicity, normally. Interestingly, there was significant accumulation of antigen-specific CD8$^+$ T cells by day 55 postinfection in the *prdm1*$^{gfp/gfp}$ mice. Kallies et al. (35) suggested that these were memory cells, but neither their phenotype nor function was evaluated. If these cells are indeed memory cells, these results would indicate that Blimp-1 attenuates the formation of memory cells or enhances their elimination. This would be consistent with the idea that Blimp-1 is important for the elimination of antigen-experienced cells and, in the CD8$^+$ lineage, may limit the transition from effectors to memory cells.

Blimp-1-deficient antigen-specific cells also accumulate in vivo after infection with LCMV, in a transfer system where only the antigen-specific CD8$^+$ T cells were Blimp-1 deficient (R. Rutishauser & S. Kaech, personal communication). In this system, the accumulated cells have a memory precursor effector-like phenotype (CD127^{Hi} KLRG1^{Lo}) characterized by high levels of expression of the IL-7 receptor. Taken together, these results suggest that Blimp-1 might play an important role in the differentiation of CD8$^+$ T cells, but this requires further investigation. It will be important to know if the memory cells that accumulate in the absence of Blimp-1 in both systems can actually provide protection upon infection when transferred to a naive recipient. Also, identification of Blimp-1 target genes in these systems could provide important insights into the molecular mechanisms regulating the differentiation of memory cells in the CD8$^+$ lineage. In this context, an important player could be Bcl-6, which is a target of Blimp-1 in B and T cells and is known to support the survival of memory CD8$^+$ T cells (148, 149).

Treg cell function in vivo. The expression of high levels of Blimp-1 in Treg cells and the inflammatory disease associated with Blimp-1 ablation in T cells suggest a role for Blimp-1 in Treg cell differentiation and/or function. However, CD4$^+$CD25$^+$ Foxp3$^+$ Treg cells develop normally in the absence of Blimp-1 (35, 76). Indeed, *prdm1*$^{gfp/gfp}$ mice show increased frequency of splenic CD4$^+$/FoxP3$^+$ cells (35), and peripheral CD4$^+$/Foxp3$^+$ cells numbers increase with age in the *prdm1*$^{F/F}$Lck-Cre mice (G. Martins & K. Calame, unpublished observations). It is important to determine if this expansion is due to increased IL-2 in the mice. Blimp-1-deficient Treg cells perform normally in in vitro suppression assays and are also able to suppress colitis caused by homeostatic expansion of naive CD4$^+$ T cells in Rag1-deficient mice (35, 76). Nevertheless, Blimp-1-deficient Treg cells cannot suppress acute colitis induced by dextran sodium sulfate (DSS) administration (76) and do not prevent inflammatory disease in Blimp-1-deficient mice (35, 76), demonstrating a functional impairment.

The reason that Blimp-1-deficient Tregs control colitis in the RAG reconstitution system but not in the DSS system is not clear but may be related to the differential requirement for IL-10. IL-10 is associated with resistance to DSS-induced colitis (150, 151) but may not be as critical for preventing colitis induced by homeostatic proliferation of naive CD4$^+$ cells (137). Available data indicate that Blimp-1 is required for IL-10 production by Treg cells (G. Martins & K. Calame, unpublished observations) as well as for nonregulatory CD4$^+$ T cells (35, 76). Because Blimp-1 is a repressor,

it is likely that Blimp-1 represses a gene that negatively regulates IL-10 expression, but this remains to be determined.

Targets of Blimp-1 in T Cells

RNA microarray analyses and gene expression studies using a quantitative reverse transcriptase PCR (qRT-PCR) have begun to reveal genes that are targets of Blimp-1 repression in T cells. This list includes *bcl2a1*, *bcl6*, *il2*, *erg2*, *ifng*, *prdm1*, *tbet*, and *fos*. Some of these targets (*bcl2a1* and *bcl6*) are in common with B cells; others seem to be unique to T cells (**Table 1**). qRT-PCR studies, showing higher levels of mRNA in Blimp-1-deficient T cells compared with controls, combined with ChIP assays, demonstrating binding of endogenous Blimp-1 in primary T cells, have shown that *il2*, *fos*, *ifng*, *tbet*, and *bcl6* are direct targets of Blimp-1-dependent repression in T cells (G. Martins, L. Cimmino & K. Calame, unpublished).

The evidence that Blimp-1 represses IL-2 production was discussed above, and it makes sense that *il2* is a direct target of Blimp-1 repression. It seems likely that, in addition to direct repression of *il2*, repression of *fos* is important for the Blimp-1-dependent attenuation of T cell activation and IL-2 production. mRNA encoding Fos, a member of the AP-1 family of transcription factors, is elevated in Blimp-1-deficient $CD4^+$ T cells following TCR stimulation. Fos is an important activator of IL-2 production upon TCR stimulation in T cells (152, 153). Thus, repression of *il2* transcription by Blimp-1 in T cells seems to occur both directly and indirectly. Although not yet directly tested, Blimp-1 may repress *il2* in Treg as well as in nonregulatory $CD4^+$ T cells (**Figure 9**).

Repression of *ifng*, *tbet*, and *bcl6* is very likely to be important for Blimp-1-dependent repression of Th1 differentiation. *Ifng* is not only directly repressed by Blimp-1 but may also be indirectly repressed by suppression of *tbet*. In turn, decreased IFN-γ production would decrease expression of both *tbet* and *bcl6*. In yet another example of redundant regulation, Blimp-1 also directly represses both of these genes. Thus, Blimp-1 is part of a complicated network of regulation that inhibits Th1 differentiation (**Figure 8**). However, the functional importance of this aspect of Blimp-1's activity remains largely untested.

An interesting common target gene for Blimp-1 in both B and T cells is *bcl2a1*, encoding A1, an antiapoptotic member from the Bcl-2 family of proteins (reviewed in Reference 154). A1 (a and b) are highly expressed by Blimp-1-deficient cells in microarray studies comparing in vitro stimulated $CD4^+$ T cells from control and $prdm1^{F/F}$ Lck-Cre mice (G. Martins & K. Calame, unpublished). Accordingly, A1 transcripts, measured by qRT-PCR, are more abundant in Blimp-1-deficient $CD4^+$ T cells stimulated in vitro. In B cells, *bcl2a1* is repressed by Blimp-1 (50), and enforced expression of A1 rescued Blimp-1-induced cell death (155). In T cells, A1 is expressed upon TCR stimulation (156), and it may play a role in protecting cells from cytokine deprivation–induced cell death (157). A CD2-driven *bcl2a1* transgenic mouse showed an increased number of peripheral T cells, but their activation status was not evaluated. T cells from these transgenic mice also show decreased death upon stimulation with T cell mitogens (157). Similar to other Bcl-2 family members, A1 does not seem to play any nonredundant role in protecting T cells from activation-induced cell death (AICD) (157). This is consistent with the fact that AICD, as assayed following in vitro activation of naive $CD4^+$ T cells, was unaltered in $CD4^+$ T cells from the $prdm1^{f/f}$ mice (76). Nonetheless, Blimp-1-deficient in vitro–generated $CD4^+$ effector T cells are more resistant to cytokine withdrawal–induced cell death (76; G. Martins & K. Calame, unpublished). Thus, derepression of *bcl2a1* in the Blimp-1-deficient T cells might contribute to the accumulation of the antigen-experienced cells in the periphery of the $prdm1^{f/f}$ Lck-Cre mice. Sequence inspections reveal the

AICD: activation-induced cell death

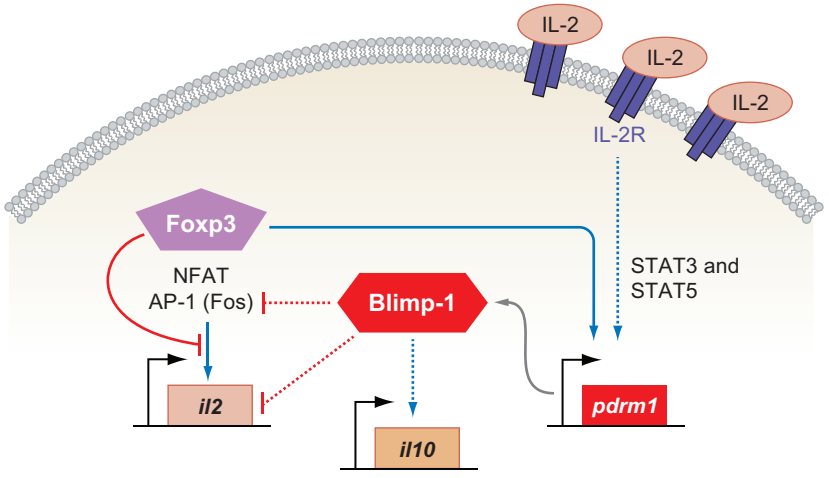

Figure 9

A model for Blimp-1 in Treg cells. Blimp-1 is expressed in high levels in Treg cells, induced by Foxp3 directly and probably also by IL-2, to which Treg cells are highly responsive. Blimp-1 could promote IL-10 expression by repressing a factor that normally inhibits its expression. IL-2 production is normally repressed in Treg cells by Foxp3, which interferes with NFAT induction of the *il2* gene. Direct or indirect repression of *il2* by Blimp-1 may also occur, as discussed in the text. Dotted lines represent regulation that is predicted based on findings in nonregulatory T cells but not yet confirmed in Treg cells.

presence of Blimp-1 putative-binding sites in the promoter region of the murine *bcl2a1* gene (G. Martins & K. Calame, unpublished observations). ChIP studies are needed to clarify if A1 is a direct target of Blimp-1 in T cells.

Regulation of Blimp-1 Expression in T Cells

While several aspects of transcriptional regulation of *prdm1* appear to be conserved in T cells and B cells, the overall regulatory pathways in T cells are distinct, and in T cells there is a clear role for antigen receptor stimulation in Blimp-1 induction.

TCR and IL-2. Blimp-1 mRNA and proteins are induced during T cell activation. In vitro stimulation of naive $CD4^+$ T cells with α-CD3, or α-CD3 and α-CD28, results in Blimp-1 mRNA induction, but maximum levels are induced when exogenous IL-2 is administered together with α-CD3 and α-CD28 (132; L. Cimmino & K. Calame unpublished), suggesting that IL-2 greatly potentiates *prdm1* induction upon TCR stimulation. Indeed, T cells lacking the β-chain of the IL-2 receptor (IL-2R$\beta^{-/-}$) express significantly less Blimp-1 than wild-type cells upon TCR stimulation, and TCR stimulation in the presence of IL-2-neutralizing antibodies resulted in very low/undetectable Blimp-1 protein (74). Therefore, a large portion, but not all, of *prdm1* induction upon TCR stimulation seems to be secondary to induction of IL-2. Furthermore, IL-2 is required to maintain Blimp-1 expression after the initial in vitro activation of $CD8^+$ T cells, a function that IL-15, another common gamma chain cytokine, was unable to perform (74). The importance of IL-2 for Blimp-1 expression is consistent with the late expression of Blimp-1 during T cell activation in vitro, and it is likely to have important regulatory functions because Blimp-1 represses *il2*, as discussed above. The components downstream of IL-2 signaling required for Blimp-1 induction remain to be identified, but STAT5 is probably an important element in this pathway.

Other regulators. High levels of Blimp-1 in naturally occurring Foxp3$^+$ Treg cells are likely to be caused by IL-2 signaling, which is critical for maintenance and proliferation of Treg cells in the periphery (158) (**Figure 9**). In addition, a genome-wide analysis of Foxp3 targets showed that *prdm1* is directly activated by Foxp3 (159), adding further support to the idea that Blimp-1 is required for proper function of Treg cells. Extrapolating from the data in nonregulatory cells, we can surmise that Blimp-1 likely represses *il2* transcription in Treg cells. This would provide another mechanism, in addition to competition with NFAT (160), by which Foxp3 represses *il2* in Treg cells (**Figure 9**). Comparative analysis of gene expression profiles in Blimp-1-sufficient and -deficient Treg cells will be necessary to further elucidate Blimp-1's mechanisms of action in Treg cells.

The fact that Blimp-1 transcripts can be induced, albeit to low levels, in the IL-2Rβ$^{-/-}$ T cells upon TCR stimulation, indicates the existence of IL-2-independent pathways for Blimp-1 induction. Blimp-1 protein levels were increased in these cells upon TCR stimulation in the presence of IL-4 or IL-12, suggesting that these cytokines can also induce Blimp-1 expression (74). Interestingly, in these studies IL-4 seems to be a stronger inducer of Blimp-1 protein than IL-12 (74). In wild-type naive CD4$^+$ T cells, Blimp-1 mRNA is also induced more strongly by IL-4 than by IL-12, and in this same system, administration of exogenous IFN-γ reduces the induction of Blimp-1 transcripts upon TCR stimulation (L. Cimmino & K. Calame, unpublished). Thus, consistent with the expression patterns discussed above, TCR stimulation in Th2-promoting conditions (IL-4) results in strong induction of Blimp-1, whereas Th1-promoting conditions (IL-12 and IFN-γ) result in weak Blimp-1 induction. IL-21 is expressed in T cells as well as B cells (160a), and although IL-21 induces *prdm1* in B cells, there is currently no information as to whether IL-21 induces *prdm1* in T cells. IL-21 is expressed at high levels in inflammatory conditions (161, 162), and if it induces Blimp-1 in T cells, this could be a mechanism for downregulation of T cell function and containment of inflammation.

In addition to being regulated by TCR stimulation and cytokines, preliminary data indicate that *prdm1* is autoregulated by Blimp-1. This was first discovered in epidermal keratinocytes, where a Blimp-1 deficiency results in elevated amounts of transcripts from the deleted allele relative to transcripts from the wild-type allele. In this same system, ChIP assays show that Blimp-1 directly binds the *prdm1* gene (162a), thus indicating that lack of Blimp-1 results in increased transcription of Blimp-1 mRNA. Similarly, in vitro–stimulated CD4$^+$ T cells from *prdm1* CKO mice show an increase in Blimp-1 mRNA as detected by real-time PCR using primers for the region upstream of the deleted region (L. Cimmino & K. Calame, unpublished). CD4$^+$CD25$^+$ T cells from *prdm1*$^{gfp/gfp}$ mice also seem to have increased amounts of *prdm1* mRNA, as demonstrated by semiquantitative RT-PCR (35). The physiological relevance of Blimp-1 autoregulation in T lymphocytes still remains to be addressed, but given the general action of Blimp-1 in attenuating T cell responses, it might be that autoregulation was selected to avoid inappropriate termination of immune responses. In this context, it would be interesting to learn if Blimp-1 autoregulation only occurs when Blimp-1 is expressed in very high levels or, in other words, if *prdm1* itself is among the set of Blimp-1 targets that are susceptible to regulation only when Blimp-1 is available at very high levels.

OVERVIEW

Interestingly, although mice lacking Blimp-1 in their B cells are deficient in antibodies, they survive well in pathogen-free conditions. In contrast, mice lacking Blimp-1 in T cells die of inflammatory disease within a few months. In B cells, Blimp-1 is clearly required and sufficient for terminal differentiation of plasma cells. In T cells, its roles

appear more complicated, probably reflecting the more complicated pathways of activation, effector and memory differentiation, and homeostatic maintenance in this lineage. Whereas a common role for Blimp-1 in both lineages may involve terminally differentiated functions, the apparent roles of Blimp-1 in memory T cells and in attenuating responses to activation appear to be unique and not found in the B lineage. While it is too early to understand fully how the molecular mechanisms of Blimp-1 action and regulation may differ in T and B cells, there appear to be interesting commonalities between the two lineages.

Common Themes

In spite of apparently serving quite different regulatory roles in the life of B and T lymphocytes, there are some aspects of Blimp-1-dependent regulation that are strikingly similar. First, the majority (8 of the 10) identified direct targets of Blimp-1 repression (**Table 1**) are themselves transcriptional regulators with their own, often extensive and important, sets of gene targets. Thus, Blimp-1 acts early in specific transcriptional regulatory cascades. For example, direct target Pax5 both activates genes required for B cell commitment (163) and function (164) and represses genes expressed in other hematopoietic lineages and genes expressed in plasma cells (60). A second common theme is the redundancy in how Blimp-1 regulates some genes. For example, in B cells, *myc* is repressed by Blimp-1 both directly and indirectly by repression of an important activator, E2F (50). AID mRNA is repressed indirectly by Blimp-1-dependent repression of two activators, Pax5 and E2A (50). In T cells, Blimp-1 represses *il2* directly and indirectly by repressing an activator of IL-2 transcription, *fos*. *Tbet* is repressed both directly and indirectly by repression of its strong inducer, IFN-γ. It seems reasonable to suggest that this redundancy reflects the importance of repressing given target genes and/or provides the possibility for multiple ways of fine-tuning of these regulatory networks. A final common theme is that Blimp-1 is often involved in feedback loops. In both B and T cells, Blimp-1 represses *bcl6*, and Bcl-6 represses *prdm1*. Such mutual repression loops can help establish mutually exclusive states—a GC B cell versus a plasma cell in the B lineage and Th1 versus Th2 in the $CD4^+$ T cell lineage. A different feedback loop is present in naive T cells where activation induces IL-2 (**Figure 6**), IL-2 in turn induces Blimp-1, and Blimp-1 then represses *il2* itself as well as *fos*, and Fos is a mediator of activation. In this setting, Blimp-1 is apparently important in the attenuation of the immune response.

Remaining Questions

Important and interesting questions remain to be answered regarding Blimp-1's role in lymphocytes in both a short-term and a long-term sense. In the short-term, a complete understanding of Blimp-1's roles in various T cell subsets and in lymphoid malignancies remains to be established. It will be of interest to identify additional direct and indirect targets to understand more fully the regulatory pathways dependent on Blimp-1, especially in Treg cells and $CD8^+$ T cells, which have not yet been studied in detail. Many groups are currently identifying various signals and transcriptional mechanisms that regulate Blimp-1, and given the importance of Blimp-1 action, understanding its regulation is a key issue. The functional importance of alternate promoter usage to generate the beta form needs further study, as does the possibility that Blimp-1 mRNA or protein may be regulated posttranscriptionally by degradation, covalent modification or other mechanisms. The mechanism of action of Blimp-1 requires more study to identify corepressors that are required for its activity. Structural determination of a repressor-corepressor complex would provide molecular information that might aid in designing small-molecule inhibitors of Blimp-1. The possibility of competition between Blimp-1

and IRF1 or IRF2 also deserves additional study. Finally, it is intriguing to speculate that, although Blimp-1 appears normal in some forms of common variable immune deficiency (CVID) (165), there could be mutations in *prdm1* in other forms of CVID.

In a long-term sense, as more information is obtained on the role, regulation, and mechanism of action of Blimp-1 in both normal and abnormal or malignant lymphocytes, it can be compared with the growing understanding of the roles of Blimp-1 in early embryogenesis in many species including *Drosophila* (166), Xenopus (167), Zebrafish (168–172), Fugu (173), and mouse (22, 36, 174) as well as with results of studies showing roles for Blimp-1 in other adult murine cell lineages such as sebocytes (175) and epidermal keratinocytes (162a). Hopefully, when more data are available, such comparisons will reveal information about how transcriptional regulatory pathways involving Blimp-1 have evolved and may provide insight into the currently unanswered question of why this single transcription factor plays such important and varied roles in multiple cell lineages.

DISCLOSURE STATEMENT

The authors are not aware of any biases that might be perceived as affecting the objectivity of this review.

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