Blimp-1 controls plasma cell function through the regulation of immunoglobulin secretion and the unfolded protein response

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Plasma cell differentiation requires silencing of B cell transcription, while it establishes antibody-secretory function and long-term survival. The transcription factors Blimp-1 and IRF4 are essential for the generation of plasma cells; however, their function in mature plasma cells has remained elusive. We found that while IRF4 was essential for the survival of plasma cells, Blimp-1 was dispensable for this. Blimp-1-deficient plasma cells retained their transcriptional identity but lost the ability to secrete antibody. Blimp-1 regulated many components of the unfolded protein response (UPR), including XBP-1 and ATF6. The overlap in the functions of Blimp-1 and XBP-1 was restricted to that response, with Blimp-1 uniquely regulating activity of the kinase mTOR and the size of plasma cells. Thus, Blimp-1 was required for the unique physiological ability of plasma cells that enables the secretion of protective antibody.

The production of antibodies is an essential arm of the immune response that provides both immediate protection against a current infection and long-term immunity to re-exposure to the same pathogen. The antibody-secreting cell compartment consists of short-lived proliferating plasmablasts (PBs), which are generated early in an immune response, and long-lived post-mitotic plasma cells (PCs), which reside in specialized niches in the bone marrow (BM)1,2. These long-lived PCs have been shown to maintain high titers of protective antibody for decades after exposure to pathogen or immunization3. Thus, understanding the factors that control the production, function and long-term survival of PCs is critical for both improved vaccine design and the development of novel approaches for targeting pathogenic PCs in diseases such as multiple myeloma and systemic lupus erythematosus.

To achieve the dual goals of maintaining an extremely high rate of immunoglobulin secretion while ensuring their own long-term survival, PCs show a highly specialized morphology with an enlarged cytoplasm and a tightly arranged endoplasmic reticulum (ER). PCs also constitutively activate the unfolded protein response (UPR), a specialized sensing mechanism for detecting and dealing with large amounts of protein that pass through the ER4.

The differentiation of activated B cells into PCs requires coordinated changes in the expression of many hundreds of genes, including the silencing of B cell–associated transcripts, such as those encoding the transcription factors Pax5, Bach2 and Bcl-6, and the activation of a suite of PC-specific genes5,6. This developmental program is guided by the following triad of transcription factors: IRF4 (encoded by Irf4), Blimp-1 (encoded by Prdm1) and XBP-1 (encoded by Xbp1). IRF4 not only has high expression in PCs but also is essential for PC development, at least in part due to its regulation of Prdm1 (refs. 7–9). Blimp-1 is expressed in all antibody-secreting cells and is also required for their differentiation beyond an early point10–12. Blimp-1 has so far been thought of as a transcriptional repressor that silences several important B cell genes, including Pax5 (ref. 13), Myc14, Cital15, Bcl6, Spib and Id3 (ref. 16), although there is only limited understanding of its targets in PCs17. XBP-1, an important component of the UPR, was initially proposed to be essential for PC formation18; however, subsequent evidence has suggested that XBP-1 is required more specifically for immunoglobulin production19–25. As a consequence of the important functions of IRF4 and Blimp-1 early in the differentiation process, there is little current knowledge about the function of these factors in long-lived PCs23,34. Here we used a genetic approach to investigate the functional consequences of the loss of IRF4, Blimp-1 or XBP-1 in mature post-mitotic BM PCs.

RESULTS

Inactivation of IRF4 and Blimp-1 in PCs

To assess the importance of IRF4 and Blimp-1 in mature BM PCs, we crossed mice carrying loxP-flanked alleles encoding either transcription factor (Irf4fl/fl or Prdm1fl/fl)25,26 with Rosa26-CreERT2 mice (which have tamoxifen-inducible expression of Cre recombinase from the ubiquitous Rosa26 locus)27. This system allowed tamoxifen-inducible

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Figure 1 Inactivation of Irf4 and Prdm1 in PCs. (a) Frequency of Irf4/+/CreERT2 (Irf4/+) or Irf4/−/CreERT2 (Irf4−/) PCs, identified as CD138+/B220+/GFP− (left), or Prdm1+/+/CreERT2 (Prdm1+/+) or Prdm1+/−/CreERT2 (Prdm1+/−) PCs, identified as CD138+/Blimp-1+/GFP− (right), among total BM cells from Rag2−/− host mice given B cells of the those genotypes, assessed at various times (horizontal axis) after treatment of host mice with tamoxifen to induce inactivation of Irf4 (reported by GFP expression) or Prdm1 (experimental plan, Supplementary Fig. 1a). (b) Frequency of BM PCs from Prdm1+/+/CreERT2 or Prdm1+/−/CreERT2 mice various days (horizontal axis) after treatment with tamoxifen (experimental plan, Supplementary Fig. 1b). Each symbol (a, b) represents an individual mouse; small horizontal lines indicate the mean. *P < 0.05 and **P < 0.005 (paired t-test). Data are pooled from three experiments.

inactivation of Irf4 or Prdm1 in pre-existing PCs. To facilitate the tracking of PCs, Prdm1fl/fl and Prdm1+/+ control mice also carried a Prdm1fl/+ reporter allele that expresses green fluorescent protein (GFP) but no functional Blimp-1 (Prdm1fl/+CreERT2 or Prdm1+/+/CreERT2)11, while Irf4fl/fl mice carried an internal GFP-encoding cassette that reports gene inactivation26. We transferred B cells from those mice into B cell– and T cell–deficient Rag2−/− mice to generate a large population of PCs and induced Cre activity by administration of tamoxifen to the host mice 14 d later. Although inactivation of Irf4 occurred equivalently in Irf4fl/fl/CeERT2 and Irf4fl/fl/CeERT2 B cells (approximately 15% GFP+ cells for each genotype; data not shown), GFP+ PCs were lost from the BM as early as 2 d after treatment with tamoxifen (Fig. 1a and Supplementary Fig. 1a), which demonstrated that Irf4 was indispensable for PC survival. In contrast, inactivation of Prdm1 by an identical strategy resulted in a PC population that was stable for many weeks after treatment with tamoxifen (Fig. 1a and Supplementary Fig. 1a). To confirm that result, we induced inactivation of Prdm1 in naive Prdm1fl/+CreERT2 mice and again found that BM PCs persisted without Blimp-1 (Fig. 1b and Supplementary Fig. 1b). We observed a similar difference in the dependence of PC survival on Irf4 and Blimp-1 in the spleen (Supplementary Fig. 1a–d). Although both models showed a significant reduction in PC numbers at the latest time points after inactivation of Prdm1, the data derived from the two genotypes were not strictly comparable, as the Prdm1+/−/CreERT2 B cells continued to produce new PCs throughout the course of the experiment, while the Prdm1fl/+ mice lacked this capacity (Fig. 1a,b and Supplementary Fig. 1c). Consistent with that conclusion, we observed no significant change in the proportion of spleen or BM cells in tamoxifen–treated Prdm1fl/+ mice that displayed a PC phenotype, either at steady state or after immunization with a protein antigen in alum, over the time frame examined (Fig. 1a,b and Supplementary Fig. 1c–e). We assessed the efficiency of the ablation of Prdm1 by transferring Prdm1fl/+CreERT2 or Prdm1+/−/CreERT2 B cells into Rag2−/− host mice and treating the recipients with tamoxifen 2 d after transfer, before PCs could be formed. This approach completely blocked PC differentiation (Supplementary Figs. 1f and 2a), which mimicked the conditional removal of Prdm1 from activated B cells in vitro12,28. PCR genotyping of purified B cells and PCs confirmed efficient inactivation of the Prdm1 locus in both cell types (Supplementary Fig. 2b). These data demonstrated that Blimp-1 was not essential for the long-term survival of BM PCs.

Blimp-1-regulated genes in PCs

The long-term persistence of Blimp-1-deficient PCs enabled us to investigate the effect of the loss of Blimp-1 on the transcriptome of long-lived PCs. We sequenced RNA from BM PCs purified from Prdm1fl/+CreERT2 and Prdm1+/−/CreERT2 mice 21 d after treating the mice with tamoxifen. Analysis of the frequency of Prdm1 transcripts spanning exons 5–6, which are removed by Cre-mediated excision of the loxP-flanked exon 5, revealed an 87% reduction in frequency of full-length transcripts in Prdm1fl/+CreERT2 cells, compared with their abundance in Prdm1+/−/CreERT2 cells (Supplementary Fig. 2c). 465 genes were expressed differentially in Blimp-1-deficient (Prdm1fl/+CreERT2) PCs relative to their expression in control (Prdm1+/−/CreERT2) PCs (Supplementary Fig. 2a), with 170 genes activated by Blimp-1 and 295 genes repressed by Blimp-1 (normalized average expression of ≥4 RPKM (reads per kilobase of exon model per million mapped reads) in at least one sample; false-discovery rate (FDR), ≤0.05; Supplementary Table 1). Cross-referencing of those differentially expressed genes with their binding of Blimp-1, as assessed by chromatin immunoprecipitation followed by deep sequencing with a biotin-tagged Prdm1 allele29 in PBs generated in vitro, revealed that 28% of genes activated by Blimp-1 (47 of 170) and 41% of genes repressed by Blimp-1 (120 of 295) were bound by Blimp-1 and were thus potential direct targets of Blimp-1 (Supplementary Table 1). Partitioning of the proteins encoded by the differentially expressed genes into functional categories showed that the largest group of genes activated by Blimp-1 encoded proteins involved in metabolism and nutrient transporters, whereas many genes repressed by Blimp-1 encoded receptors and signaling molecules (Fig. 2b). This analysis also suggested a role for Blimp-1 in inhibiting B cell function, including antigen presentation (through its effect on genes encoding major histocompatibility complex class II molecules), pathogen recognition (through its effect on Tlr9) and signaling through the activating receptors (through its effect on Cd79b and Bank1). Blimp-1 also appeared to be important for the transcription of genes encoding products related to metabolic activity of the PCs, most probably related to their immunoglobulin-secreting function (through its effect on Tps2, Syvn1 and Fkbp11). Notably, very few genes encoding products involved in the cell cycle or survival were affected by the loss of Blimp-1. For example, Myc, a known target of Blimp-1–mediated repression that encodes a central regulator of proliferation in B cells that is normally repressed in PCs14, was not re-expressed in Blimp-1-deficient PCs. The expression of genes encoding products essential to PC survival, such as Mcl1 (encoded by Mcl1)30, Bim (encoded by Bcl2l11) or BCMA (encoded by Tnfrsf17)31, remained similarly unchanged (Fig. 2c and data not shown).

As Blimp-1 has been linked to silencing of the B cell transcription program after PC differentiation16, we investigated whether the BM PCs reverted to the B cell stage without Blimp-1. Several B cell–associated genes, including Cd22, Spib, Cd79b and Citta, which are usually silenced in PCs, were re-expressed in PCs in the absence of Blimp-1 (Fig. 2c). Most notably, Pax5 and Bcl6, which encode two of the main regulators of the B cell transcriptome and are known targets of Blimp-1 repression13,16, were not re-expressed in Blimp-1-deficient
Figure 2  Transcriptional analysis of Blimp-1-deficient PCs. (a–c) Whole-genome RNA-seq analysis of PCs sorted from the BM of Prdm10CreERT2+/gfp or Prdm10CreERT2fl/gfp mice 21 d after tamoxifen treatment. (a) Scatterplot of differential expression, showing genes with significantly increased expression (blue) or decreased expression (red) in the absence of Blimp-1 (FDR < 0.05; normalized average expression of ≥ 4 RPKM in at least one sample). (b) Functional classification and quantification of proteins encoded by target genes repressed (blue) or activated (red) by Blimp-1 in PCs. Numbers above or below bars indicate total number of genes in each category. (c) Expression of published gene signatures for wild type follicular B cells (FoB) and BM PCs from Prdm10CreERT2 mice (+/gfp PC) or Prdm10CreERT2fl/gfp mice (fl/gfp PC); right margin, positions of some genes of interest. (d) Flow cytometry analyzing expression of the Blimp-1-regulated surface molecules major histocompatibility class II (MHCI11), CD22 and CD93 on Prdm10CreERT2 mature B cells (B220+CD19hi) and Prdm10CreERT2fl/gfp PC. CD138+ (Blimp-1-GFP+) obtained from the BM of mice 21 d after treatment with tamoxifen. (e) Overlap and differences in Blimp-1-activated target genes (Supplementary Table 1) and the PC gene signature. Data are from two experiments with samples pooled from multiple mice (a–c,e) or are representative of at least three experiments (d).

PCs (Fig. 2c and data not shown), which demonstrated that the PCs were not reverting to the more developmentally immature B cell fate in the absence of Blimp-1. The absence of the expression of Pax5 and Bcl6 might also explain the only partial reactivation of B cell genes, such as those encoding the lectin CD22 and major histocompatibility complex class II molecules, in Blimp-1-deficient PCs, compared with their expression in wild-type B cells (Fig. 2c.d). Blimp-1 was also required for the normal expression of some PC genes, including the gene encoding the C-type lectin transmembrane receptor CD93 (Fig. 2c.d). To extend our analysis, we compared the genes activated by Blimp-1 with a published signature of 301 PC genes and found that 88% of the signature genes (264 of 301) were expressed independently of Blimp-1 (Fig. 2e). Combined with the results of published studies10–12,16,20,23, these results demonstrated that although Blimp-1 was essential for the establishment of the full PC gene-expression program, once formed, PCs maintained their unique transcriptome largely independently of Blimp-1.

Control of PC size and ultrastructure by Blimp-1
During our analysis of survival kinetics, we noticed that the PCs in which Blimp-1 was deleted were smaller than and had less granularity than that of Blimp-1–sufficient PCs and had a continuum of reduced expression of the PC marker CD138 and Blimp-1–GFP relative to that of Blimp-1–sufficient PCs (Fig. 3a). Transmission electron microscopy of cellular ultrastructure also revealed that Blimp-1–deficient BM PCs had severe disruption of their distinctive dense ER (Fig. 3b), a finding substantiated by staining with a fluorescent dye specific for the ER (Fig. 3c). In contrast, staining for secretory granules was increased in Blimp-1–deficient PCs relative to this staining in Blimp-1–sufficient PCs (Fig. 3c), suggestive of impaired lysosomal trafficking. As an independent measure of secretory activity of the PCs, we measured cell-surface exposure of the lysosome-associated protein CD107a. Blimp-1-deficient BM PCs had much less staining of CD107a than did their Blimp-1–sufficient counterparts (Fig. 3c), again indicative of impaired fusion of lysosomes with the plasma membrane. Thus, Blimp-1 was needed to maintain the characteristic PC morphology and cytoplasmic organization.

Control of immunoglobulin secretion by Blimp-1
As the fundamental function of PCs is to produce immunoglobulin, we assessed the secretory capacity of Blimp-1–sufficient and Blimp-1–deficient PCs by enzyme-linked immunospot assay. We treated Prdm10CreERT2+/gfp and Prdm10CreERT2fl/gfp mice with tamoxifen, then purified splenic and BM PCs from the mice 14–28 d later and assessed their secretion of immunoglobulin M (IgM) and all IgG. The secretion of both immunoglobulin isotypes was reduced in the spleen and, most strikingly, in the BM in the absence of Blimp-1 (Fig. 4a). To determine if this defect resulted from a decrease in the transcription of immunoglobulin-encoding genes, we analyzed our RNA-sequencing data and found that with the notable exception of those encoding IgM and IgG3, immunoglobulin-encoding transcripts were not affected by the loss of Blimp-1 (Fig. 4b).

The immunoglobulin heavy-chain isotypes are produced in two different isoforms that differ in their use of polyadenylation sites32. The longer membrane-bound receptor form has high expression in B cells, while the shorter secreted form predominates in PCs (Supplementary Fig. 3a). Closer inspection of the expression of exons encoding the immunoglobulin heavy-chain constant regions revealed an increase in the expression of transcripts encoding the transmembrane form in Blimp-1-deficient PCs compared with that in Blimp-1–sufficient PCs,

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which thus lowered the ratio of transcripts encoding the secreted form to those encoding the membrane-specific form in Blimp-1-deficient PCs compared with that observed in Blimp-1-sufficient PCs (Fig. 4c,d).

We then assessed protein expression by intracellular staining and flow cytometry. The proportion of PCs expressing IgG or IgA or the immunoglobulin K- or λ-chain was similar in Blimp-1-deficient PCs and Blimp-1-sufficient PCs (Fig. 4e and data not shown). In line with the reduced expression of transcripts encoding IgM in the absence of Blimp-1, the frequency of IgM+ PCs was substantially diminished (Fig. 1b). Intra- and extracellular flow cytometry staining of IgA and IgM confirmed that whereas expression of total protein was decreased in the absence of Blimp-1, expression of the membrane-specific form was maintained (Fig. 4f). The elongation factor Ell2 has been shown to have a major role in this alternative polyadenylation process13,14. Ell2 was a direct target of Blimp-1 (Supplementary Table 1), and its expression was reduced in Blimp-1-deficient PCs relative to that in Blimp-1-sufficient PCs (Fig. 4g). These data suggested that Blimp-1 contributed to the efficient transition from a membrane-specific form of the immunoglobulin heavy chain to a secreted form in PCs, at least in part through direct control of Ell2 expression.

Regulation of the UPR by Blimp-1

The partial re-expression of the membrane-specific immunoglobulin heavy-chain at the expense of the secreted isofrom in Blimp-1-deficient PCs could explain only in part the secretion defect observed after loss of Blimp-1. Therefore, we assessed expression of the main regulators of the UPR that control protein synthesis, folding and post-translational modifications, and expansion of the secretory apparatus (Supplementary Fig. 3b). Notably, expression of XBP-1, ATF4 and ATF6, the three main transcription factors known to implement the downstream components of the pathway (Fig. 3c). The requirement for Blimp-1 for the full expression of the key regulators of the UPR ‘translated’ into significant down-modulation of the expression of the majority of genes encoding the downstream components of the pathway (P = 0.001; Fig. 5c, Supplementary Table 2 and Supplementary Fig. 4a). Notably, the function of Blimp-1 in the UPR was much broader than the regulation of genes encoding the key transcription factors of the UPR, as Blimp-1 directly bound to 38% of UPR genes expressed in PCs (45 of 119 genes), encoding products that represented all branches of the pathway (Supplementary Table 2). Together these data demonstrated that Blimp-1 as a central transcription factor of the UPR in PCs.

To investigate the overlap between Blimp-1 and XBP-1 in controlling the UPR and the PC transcriptome more generally, we generated mice with inducible deletion of Xbp1 through use of the CreERT2 system (Supplementary Fig. 2d). As reported19,21,22, inactivation of Xbp1 had no effect on the size of the PC population (Fig. 6a). RNA-sequencing analysis revealed that 632 genes were expressed differentially in XBP-1-deficient PCs versus XBP-1-sufficient PCs (Fig. 6b and Supplementary Table 3) and, notably, Prdm1 was not down-modulated but instead was slightly upregulated in the absence of XBP-1 (with a change in expression of 1.680-fold; FDR = 0.047). The comparison of genes regulated by Blimp-1 or XBP-1 showed only a modest overlap of 36 genes (31 activated and 25 inhibited; approximately 10% of total genes regulated by either factor) (Supplementary Fig. 4b), which meant it was unlikely that the Blimp-1-mediated control of PC function was achieved predominantly through regulation of XBP-1, a conclusion in agreement with published studies of PCs derived in vitro20. Of note, XBP-1 deficiency in BM PCs resulted in a global decrease in all transcripts encoding the immunoglobulin heavy-chain complex (Igh) (Fig. 6c). Reduced immunoglobulin expression was confirmed at the protein level (Fig. 6d), and this correlated with reduced secretory function, as measured by expression of CD107a (Fig. 6e) and the size of enzyme-linked immunospots for IgA and IgM (Fig. 6f and data not shown). Overall analysis of the UPR pathway revealed significant down-modulation (P = 0.0005; Fig. 6g) similar to that observed without Blimp-1 (Fig. 5c); however, in contrast to the activity of Blimp-1, the activity of XBP-1 was focused on particular processes of the pathway.
notably protein folding and targeting of proteins to the ER, with almost no effect on other effectors, such as the transcription factors (Supplementary Table 2 and Supplementary Fig. 4a). Loss of XBP-1 also had very little effect on the expression of B cell and PC signature genes, beyond those encoding components of the UPR (Supplementary Fig. 4c). This analysis revealed that while the functions of Blimp-1 and XBP-1 overlapped in the control of IgM expression and some aspects of the UPR, most of their functions in PCs were unique.

Requirement for Blimp-1 for full activity of the kinase mTOR

A characteristic phenotype of the Blimp-1-deficient PCs was their smaller cell size, a feature that is under the control of the mTOR (‘mammalian target of rapamycin’) pathway.\textsuperscript{38} The kinase mTOR is part of two complexes, mTORC1 and mTORC2; the former regulates cell size, organelle biogenesis and protein synthesis (Supplementary Fig. 3c). Analysis of the RNA-sequencing data revealed that the expression of genes encoding the core components of either complex, such as mTOR, Raptor and Rictor, was not affected by loss of Blimp-1 in PCs (data not shown). In contrast, assesment of the activity of the mTORC1 complex through analysis of the phosphorylation of mTOR itself (at Ser2448) and one of its downstream targets S6 (at Ser235 and Ser236) revealed considerable upregulation of the pathway activity in PCs, compared with its activity B cells, and that this was dependent upon Blimp-1 (Fig. 7a). As a control, we assessed phosphorylation of the mTORC2 target Akt (at Ser473) and found this was dependent upon Blimp-1 (Fig. 7b). The supply of amino acids, in particular of leucine, is a crucial regulator of mTORC1 activity,\textsuperscript{39} and we determined that the expression of several carriers of amino acids was lower in Blimp-1-deficient BM PCs than in Blimp-1-regulated genes, and the latter was also directly bound by Blimp-1 (Fig. 7c). Expression of the transferrin receptor CD71 (encoded by Tfrc), another carrier known to modulate mTORC1 activity, was also decreased in absence of Blimp-1 (Fig. 7b). Furthermore, genes encoding two members of the sestrin family, Sesn1 and Sesn3, were targets of Blimp-1-mediated repression; they
were bound by Blimp-1, and their expression was augmented in Blimp-1-deficient PCs (Fig. 7b,c). Members of this family are activators of the AMP-activated protein kinase (AMPK), which inhibits mTORC1 through the phosphorylation of one of its components, Raptor41. In keeping with the possibility of increased AMPK activity, phosphorylation of two targets of AMPK, acetyl-CoA carboxylase (at Ser79) and Raptor (at Ser792), was significantly enhanced following loss of Blimp-1 (Fig. 7a). These data suggested that Blimp-1 acted at multiple points to positively regulate mTORC1 activity, through activation of amino acid carriers, including CD98, and repression of the expression of the negative regulatory members of the sestrin family, and thus prevented AMPK activity.

Figure 5 Blimp-1 controls the UPR. (a) Flow cytometry analyzing intracellular staining of the active spliced form of XBP-1 (XBP-1s) in BM PCs from Xbp1fl/flCreERT2 B cells and in PCs obtained from the BM of Prdm1f/+gfpCreERT2 and Prdm1f/flgfpCreERT2 mice 35 d after treatment with tamoxifen (left), and mean fluorescence intensity of each (right). * P < 0.05 and ** P < 0.01 (paired t-test). (b) Binding of Blimp-1 to Atf6 (top) or Era1 (bottom) in lipopolysaccharide-stimulated Prdm1B612/B612 Rosa26gfp/Wnt1CrePbs, showing Blimp-1-binding regions (small horizontal lines below plots) identified by peak calling with model-based analysis of ChIP-seq data algorithm. Below, exon-intron structure (vertical lines, exons), with arrow indicating direction of transcription, and a scale bar (left) in kilobases (kb). (c) Gene-set–enrichment analysis (bottom) of genes (shaded rectangles; horizontally ranked by moderated t-statistic) upregulated (pink; t > 1), downregulated (blue (t < −1) or not altered (gray) in Prdm1f/flgfpCreERT2 BM PCs relative to their expression in Prdm1f/+gfpCreERT2 BM PCs, obtained from mice 21 d after treatment with tamoxifen; vertical black lines indicate 119 genes encoding components of the UPR (‘UPR gene’), identified by gene annotation and filtered for expression in PCs. Top, enrichment for the position of genes encoding components of the UPR (‘UPR gene’). Data are from two or more experiments (a; mean and s.d.) or two experiments (b,c).

Figure 6 Loss of XBP-1 leads to diminished IgM expression and UPR activity. (a) Frequency of BM PCs (CD138+Blimp-1−GFP+ throughout) in Xbp1f/+Prdm1f/+gfpCreERT2 mice (Xbp1f/+), or Xbp1f/m Prdm1f/+gfpCreERT2 mice (Xbp1fl/m) 35 d after treatment with tamoxifen (to induce inactivation of Xbp1). (b) Scatterplot of differential gene expression in Xbp1f/m Prdm1f/+gfpCreERT2 PCs versus Xbp1f/m Prdm1f/+gfpCreERT2 PCs obtained from mice 21 d after treatment with tamoxifen (as in a), showing genes with significantly increased expression (blue) or decreased expression (red) in the absence of XBP-1 (FDR <0.05, normalized average expression of ≥4 RPKM in at least one sample). (c) Normalized expression of the constant region–encoding exons of genes in immunoglobulin-encoding loci (as in Fig. 4b) in cells as in b. (d) Intracellular staining of IgM in IgA−IgG− cells, IgA in IgM−IgG− cells, and IgG in IgA+IgM− cells, from mice treated as in b. (e) CD107a expression at the surface of BM PCs in cells as in a. (f) Enzyme-linked immunospot assay of the secretion of IgM by isolated PCs as in b, presented as IgM+ cells per 500 sorted Blimp-1−GFP+CD138+ PCs (left), surface area of the spots identified (middle), and wells of an assay with antibody to IgM (right). (g) Gene-set–enrichment analysis of genes encoding components of the UPR in cells as in b (ranked and presented as in Fig. 5c), upregulated (pink; t > 1), downregulated (blue (t < −1) or not altered (gray) after loss of XBP-1 (vertical black lines and plot above, as in Fig. 5c). * P < 0.05, ** P < 0.01 and *** P < 0.005 (paired t-test.). Data are from one experiment representative of two experiments (a; mean and s.d.) or from two experiments (b–g; mean and s.d. in c–f).
DISCUSSION

IRF4, Blimp-1 and XBP-1 are the best characterized transcription factors active in mature PCs. By removing each one in fully differentiated PCs in vivo, we have demonstrated specific roles for these factors in the PC biology. PC survival critically relied on IRF4, potentially through the regulation of key survival molecules such as Mcl-1 (ref. 30). Ablation of the PC lineage at early even points after activation of IRF4 was in agreement with published studies of various myeloma cell lines31 and precluded any further investigation of the downstream targets of IRF4 with these in vivo models. In contrast, we determined that mature PCs survived acute loss of either Blimp-1 or XBP-1. These findings were contrasted to published studies concluding that both factors are essential for PC survival18,28. The main point of difference among these studies was that the others tracked mainly the abundance and specificity of antibody as a surrogate for PC frequency18,28 and thus the loss of antibody production in both mutant mouse strains gave the misleading impression of the disappearance of PCs. The use of a Blimp-1–GFP reporter provided a superior tool for us to track the PCs at high resolution, compared with monitoring CD138 or immunoglobulin secretion15. The survival of Blimp-1- or XBP-1-deficient PCs was also compatible with published reports showing that both proteins might act as tumor suppressors in multiple myeloma32,43.

Blimp-1 has long been known to be essential for the developmental transition from activated B cell to PC12. Blimp-1 is thought to act by repressing genes encoding the main regulators of the B cell program, including Pax5, Bcl6, Spib and Cita, as well as silencing Myc to facilitate the post-mitotic state of mature PCs24. By comparison of our transcriptomic data with a published PC signature6, it was apparent that Blimp-1 was not needed to maintain the core identity, as defined by their transcriptional program, of already mature PCs. Blimp-1 does serve some role in repressing B cell transcription, as various B cell genes, including those encoding CD23, CD22, Spi-B, Id3 and TLR9, but not those encoding Pax-5, Bcl-6 or Myc, were re-expressed in Blimp-1-deficient PCs; this suggested that while some targets required active Blimp-1-mediated repression, the repression of others was maintained indirectly through other mechanisms in fully mature PCs. Our current findings, together with published studies10,12,16,20,29, suggest a model in which Blimp-1 is essential for establishment of the full PC transcriptome, but once it is established, PC identity is maintained independently of Blimp-1.

Immunoglobulin-encoding transcripts account for approximately 70% of the total mRNA in long-lived PCs6, which allows each cell to produce close to 2 ng of antibodies per day44. To reach and stably maintain this secretory capacity, PCs require specialized machinery and metabolic activity. Our data demonstrated that Blimp-1 had a central role in this process through the enforcement of the UPR and mTOR pathways. In PCs, the UPR has been proposed to function not as a stress response but as a preemptive physiological pathway implemented during the early differentiation that precedes the high expression of immunoglobulins45. In support of that conclusion, we demonstrated that XBP-1, not Blimp-1, maintained the expression of immunoglobulin-encoding transcripts in mature PCs. Blimp-1 was intimately involved in the UPR, directly regulating Atf6 and Ern1, both of which encode products required for full XBP-1 expression and UPR function, as well as directly regulating 38% of the downstream genes of the UPR. Blimp-1 was also needed for maintenance of the characteristic cytoplasmic morphology of PCs, a function also linked to the inability to fully activate XBP-1 (refs. 21,46).

Beyond the UPR, the targets of Blimp-1 and XBP-1 were largely independent, suggestive of distinct functions in PC biology. The regulation of activity of the mTOR pathway by Blimp-1 represented one such unique role. mTOR has a function complementary to that of the UPR47, as it modulates protein synthesis or organelle biogenesis in response to environmental stimuli and, in particular, in response to nutrient availability. Moreover, enhanced mTOR activity can promote immunoglobulin production independently of XBP-1 (ref. 46). Our data showed that Blimp-1 directly fostered this pathway through the UPR and the mTOR function, as well as directly regulating 38% of the downstream genes of the UPR. Blimp-1 was also needed for maintenance of the characteristic cytoplasmic morphology of PCs, a function also linked to the inability to fully activate XBP-1.
Together our studies have demonstrated that while the survival and identity of long-lived PCs is largely independent of Blimp-1, this multifunctional transcriptional regulator is essential for the molecular and cellular changes that support the extremely high and sustained rates of antibody secretion that are essential for protective immunity.

METHODS
Methods and any associated references are available in the online version of the paper.

Accession codes. GEO: raw sequence reads, read counts and normalized expression, GSE70981.

Note: Any Supplementary Information and Source Data files are available in the online version of the paper.

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AUTHOR CONTRIBUTIONS
J.T. performed most experiments; W.S., Y.L. and G.K.S. performed the technical assistance; and J. Leahy for animal care. Supported by the National Health and Medical Research Council of Australia (G.K.S. and S.L.N.; 161646, 575790 and 1054925 to S.L.N.; 1054618 to G.K.S.; 1023454 to G.K.S. and W.S.; and 1049416 to A.K.), the Sylvia and Charles Viertel Foundation (A.K.), the Multiple Myeloma Research Foundation (S.L.N.), Boehringer Ingelheim (Busslinger laboratory) and the European Research Council (291740-LymphoControl for the Busslinger laboratory), and made possible through Victorian State Government Operational Infrastructure Support.

COMPETING FINANCIAL INTERESTS
The authors declare no competing financial interests.

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ONLINE METHODS

Mice. *Prdm1*^lop^ mice, *Blimp-1*^lox/lox^ mice, *Irf4*^d^ mice, *Xbp1*^d^ mice and *Rosa26-CreERT2* mice were bred and maintained at the animal facilities of the Walter and Eliza Hall Institute. *Prdm1*^lop^ mice carried a *loxP*-flanked exon 5, while *Prdm1*^lox/lox^ mice expressed an allele truncated after exon 5 (of a total of 7 exons). The PCR analysis for exon 5 loss has been described previously. All mice were on C57BL/6 background. Animal experiments were conducted according to the protocols approved by the Walter and Eliza Hall Institute ethics committee. Immunization was a single intraperitoneal injection of 100 µg NP-KLH precipitated onto alum (ThermoScientific). For cell transfer experiments, total splenocytes (5 × 10⁶) or isolated B cells (2 × 10⁶) were injected intravenously into Rag1<sup>−/−</sup> recipients. Cre-mediated deletion of *loxP*-flanked alleles was triggered by the administration of tamoxifen (Sigma-Aldrich, 0.2 mg/g) by oral gavage on 2 consecutive days.

PB generation in vitro. B cells were isolated from splenocytes by positive selection using the B220- or CD19-coated beads (Miltenyi Biotec) and cultured as previously described. Cultures were seeded at 1 × 10<sup>6</sup>/ml with optimal concentrations of CD40L (100 ng/ml; R&D Systems), interleukin 4 (10 ng/ml; R&D Systems) and interleukin 5 (5 ng/ml; R&D Systems). Cultures were treated with 4-hydroxytamoxifen (Sigma-Aldrich) at 100 nM.

Flow cytometry and cell sorting. Single-cell suspensions were stained with antibodies to the following surface molecules: CD138 (281.2; BD Biosciences), CD19 (1D3; in-house), B220 (RA3-6B2; in-house), IgM (I141; eBiosciences), CD98 (RL388; BioLegend), CD93 (AA4.1; BD Biosciences), major histocompatibility complex class II (M5; eBiosciences), CD22 (OK-97; BioLegend), CD107α (1D4B; BD Biosciences), CD71 (B2; BD Biosciences). Cells were stained with lyso tracker deep red or ETRacker Red (Molecular Probes) according to manufacturer's instructions. For intracellular transcription factor and immunoglobulin measurement, cells were fixed and permeabilized using the eBiosciences transcription factor staining buffer set and BD Cytofix/Cytoperm solution, respectively, then stained with antibodies specific for the following: YB-1 (Q3-695; BD Biosciences), ATF4 (D4B8; Cell Signaling), ATF6 (ab37149; Abcam), IgM (715-475-140; Jackson ImmunoResearch), IgG (715-475-151; Jackson ImmunoResearch), IgA (ma661; eBiosciences), immunoglobulin κ-chain (187.1; BD Biosciences) and immunoglobulin κ-chain (TB28-2; BD Biosciences). For detection of phosphorylated proteins, cells were prepared using BD Phosphoflow lyse/fix buffer and perm buffer III, and labeled to specific antibodies that recognized the following: S6 phosphorylated at Ser473 (M89-61; BD Biosciences). Stained cells were analyzed using an Accuri C6 flow cytometer. For detection of phosphorylated proteins, cells were prepared using BD Phosphoflow lyse/fix buffer and perm buffer III, and labeled with specific antibodies that recognized the following: S6 phosphorylated at Ser473 (M89-61; BD Biosciences), ACC phosphorylated at Ser79 (D7D11; Cell Signaling), Raptor phosphorylated at Ser792 (2083; Cell Signaling) or Akt phosphorylated at Ser473 (M89-61; BD Biosciences). Stained cells were analyzed on a FACSCanto II or Fortessa X20 (BD Biosciences). PCs were enriched from BM cells with biotinylated antibody to CD138 (8B12; in-house) and anti-biotin microbeads (Miltenyi Biotec) and sorted with FACSDivas or FACSaria cytometers (BD Biosciences).

Enzyme-linked immunospot assay. Multiscreen HA plates (Millipore) were coated with antibody to mouse IgM (1021-01) or IgG (1030-01). Cells were incubated on the plates overnight at 37 °C. After incubation with biotinylated goat anti-mouse IgM (1021-08) and IgG (1030-08), followed by streptavidin–alkaline phosphatase, spots were developed using the substrate VectorBlue (Vector Labs). All antibodies used for coating and labeling were obtained from Southern Biotech.

Whole-transcriptome analysis. RNA was isolated from ex vivo–sorted Blimp-1–GFP*^CD19^* PCs from previously tamoxifen-treated *Prdm1*^lop^CreERT2, *Prdm1*^lop^CreERT2, *Irf4*^d^ mice, *Xbp1*^d^ mice and *Xbp1*^d^ mice were 75–100 bp single-end sequencing on an Illumina HiSeq 2500 instrument at the Australian Genome Research Facility. Raw RNA-seq data previously generated for follicular B cells (GSE60927) were also included in this analysis. Sequence reads were aligned to the GRCh38/mm10 build of the *Mus musculus* genome using the Subread aligner<sup>48</sup>. Only uniquely mapped reads were retained. Genewise counts were obtained using featureCounts<sup>49</sup>. Reads overlapping exons in annotation build 38.1 of NCBI RefSeq database were included. Immunoglobulin-encoding genes were excluded from the gene expression analysis and were analyzed separately. Genes were filtered from downstream analysis if they failed to achieve a CPM (counts per million mapped reads) value of at least 1 and at least one library. Counts were converted to log<sub>2</sub> counts per million, quantile normalized and precision weighted with the 'voom' function of the limma package<sup>51,52</sup>. A linear model was fitted to each gene, and empirical Bayes moderated t-statistics were used to assess differences in expression<sup>53</sup>. Genes were called 'differentially expressed' if they achieved a FDR of 0.05 or less. The gene set enrichment plots were generated with the 'barcodeplot' function in limma. Gene-set–enrichment analysis was carried out using the 'roast' method in limma with 999 rotations<sup>44</sup>. One-sided P values are reported.

For the analysis of immunoglobulin-encoding genes, sequence reads were re-aligned to the genome using the Subjunc aligner with the '-all-junctions' option to disable the requirement for the presence of donor and receptor sites when mapping exon-spanning reads<sup>49</sup>. Mapped reads were then assigned to all exons using featureCounts. Exon-spanning reads were assigned to all their overlapping exons. RPKM (reads per kilobase of exon length per million mapped fragments) values for exons encoding immunoglobulin constant regions were computed on the basis of exon lengths and the total exon counts in each library. The Subjunc mapping data were also used to identify the frequency of splicing between *Prdm1* exons 5–6 and 4–6 and thus the efficiency of Cre-mediated deletion of the *loxP*-flanked exon 5 in PCs.

Transmission electron microscopy. BM Blimp-1–GFP*^CD138^* PCs were purified by flow cytometry and prepared for transmission electron microscopy as previously described<sup>21</sup>.

Analysis of Blimp-1 binding by chromatin immunoprecipitation followed by deep sequencing. CD138<sup>+</sup> PCs were generated by lipopolysaccharide-induced differentiation of mature B cells from *Prdm1*^Bio/Bio^Rosa26^Bcr/Bl/A^ mice as described in detail elsewhere<sup>25</sup>. Chromatin from 1 × 10⁶ PBs was prepared using a lysis buffer containing 0.25% SDS before chromatin precipitation by streptavidin precipitation (Bio-Chip), as described<sup>25</sup>. The precipitated genomic DNA was quantified by real-time PCR, and absorbed 1–5 ng of DNA pre-cleared before chromatin immunoprecipitation was used for library preparation and subsequent Illumina deep sequencing. Peak calling and gene assignment is described in detail elsewhere<sup>29</sup>.

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Erratum: Blimp-1 controls plasma cell function through the regulation of immunoglobulin secretion and the unfolded protein response

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In the version of this article initially published, the label along the vertical axis of the left plot in Figure 3c was incorrect, as was the corresponding text in the legend, and there was an incorrect space between the horizontal axis and curves in the right histogram of Figure 5a. The correct label for Figure 3c is ‘ER-Tracker’. The errors have been corrected in the HTML and PDF versions of the article.