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Plasma cells: the programming of an antibody-secreting machine

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Abbreviations: Abs, antibodies; ASC, antibody-secreting cell; BM, bone marrow; ER, endoplasmic reticulum; SLOs, secondary lymphoid organs; UPR, unfolded protein response.

ABSTRACT

Antibodies are an essential component of our immune system, underpinning the effectiveness of both the primary immune response to microbial pathogens and the protective and long-lived immunity against re-challenge. All antibodies are produced by relatively rare populations of plasmablasts and plasma cells, collectively termed antibody-secreting cells (ASCs). It is now apparent that ASCs are unique in the body in terms of their gene expression program and metabolic pathways that enable these cells to have an extraordinary rate of immunoglobulin gene transcription, translation, assembly and secretion. In this review we will discuss the cellular, metabolic and molecular specialization that allows ASCs to maintain such high rates of antibody production, in some cases for the life of the individual. Throughout the review we will link these exquisite cellular and molecular adaptations to the major regulators of ASC gene expression, in an attempt to define how the ASC phenotype and function is genetically programmed.

INTRODUCTION

The production of antibodies (Abs) is essential for human health and underpins the protection provided by virtually all current vaccines. Antibody-secreting cells (ASCs) are a specialized cell type that represents the end-stage of the B-cell differentiation program. In a healthy state, ASCs are widely distributed throughout the body, occupying primary and secondary lymphoid organs (SLOs), the gastrointestinal tract and mucosal sites. ASCs can also be observed in non-lymphoid organs in autoimmune settings, such as in the salivary gland in Sjögrens syndrome [1] or during

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the rejection of transplanted tissue [2]. This wide anatomical distribution allows ASCs to display specialization resulting from their environment and ontogeny, with many IgA-secreting ASCs being found in the gut associated lymphoid tissues; while the peritoneum, at least in the mouse, is a source of IgM secreting ASCs.

All ASCs derive from activated B cells, which come in three major types; follicular B cells, marginal zone B cells and B1 cells [3]. The lineage of B cells impacts on the anatomical location, the diversity of the B-cell receptor expressed and the type of antigen typically encountered. ASCs are conventionally classified as either plasmablasts or plasma cells. Plasmablasts are short-lived, cycling cells that are produced in large numbers early in an immune response. Plasmablasts can be derived from all of the three mature B cell types via an extrafollicular differentiation pathway. Thus, in a primary response, plasmablasts generally lack somatic hypermutation and produce relatively low affinity Abs. Plasmablasts can be elicited by either protein (T-cell-dependent) or non-protein antigens (T-cell-independent e.g. polysaccharides), with the latter response enriched for marginal zone and B1-cell-derived ASCs. Plasmablasts also numerically dominate secondary responses to infection, vaccination or autoantigen during active autoimmune pathology. These plasmablasts derive from memory B cells that have previously undergone affinity maturation in the germinal center reaction and produce Abs with much higher affinity for their targets [3].

Plasma cells are generally considered to be post-mitotic, or at least believed to divide very infrequently [4], and a subset of plasma cells have the capacity to be extremely long-lived [5-7] (Figure 1). The canonical plasma cell derives from follicular B cells via the germinal center, although all mature B-cell subsets and activation routes (T-cell-dependent or independent) can produce plasma cells. Plasma cells are found in the spleen, lamina propria of the gut and the bone marrow (BM), with the BM population being highly enriched for long-lived plasma cells. As long-lived plasma cells represent the terminus of the B-cell lineage they show the most pronounced specialization, having a characteristic cellular morphology, which is visible under light and electron microscopy, a distinct gene expression program and ability to maintain an extremely high rate of Ab production throughout the life of the host [3].

Due in part to their rarity (comprising <1% of the total cellularity of primary and secondary lymphoid organs and blood) and their absence of B cell markers, including CD20, ASCs have often been problematic to identify and further characterize. ASCs are classically defined in humans by the increased expression of CD38, CD27 and CD138, while in mouse CD138 is routinely used in conjunction with the down regulation of B220 or in transgenic mice with the expression of a Blimp-1-GFP reporter allele [8]. Recently a number of alternative strategies have been developed to identify mouse ASCs using the cell surface proteins, Sca-1,

TACI or CD98 in combination with CD138 [9-12], which together offer the promise of more exact identification of ASCs in a variety of tissues and immunological settings.

In this review we will focus on the cellular and genetic processes by which ASCs can initiate and maintain their extraordinary feat of Ab production. We will discuss the homing and adhesion processes that enable ASCs to occupy suitable niches; the metabolic re-organization that ASCs undergo to facilitate and maintain Ab production in the long term; and the survival mechanisms that enable this longevity. Throughout the review we will attempt to link these important cellular processes to the unique gene regulatory network that controls the ASC program.

The antibody-secreting cell transcriptome

An important step in understanding the biology of plasma cells has been to accurately define their transcriptional output. Such studies have assayed the ASC gene expression program in both mice and human [10, 13]. Importantly these studies have also compared naturally occurring ASCs with those elicited in cell culture using stimuli that mimic T-cell-dependent and -independent differentiation. These approaches have allowed the identification of gene expression signatures that can be used to define ASCs in a variety of contexts, as well as providing a wealth of new candidate markers to identify ASCs and also identify possible novel regulators of ASC biology. As discussed below, many components of the signature are genes which control aspects of ASC physiology, Ab secretion, homing and survival.

One clear outcome of these global gene expression studies has been to highlight the stark transcriptional divide between all B cell subsets and all ASCs. This is a consequence of the distinct gene regulatory network in operation in B cells and ASCs. B cells rely on a core program driven by the transcription factor PAX5 [14]. Other factors such as BCL6, EBF1, BACH2, IRF4, IRF8, PU.1, SpiB, E proteins and Ikaros family members often act in specific B-cell stages to modulate gene expression in B cells, resulting in the expression of a swathe of immunologically relevant B-cell specific genes, including CD79a/b, CD19, CD20, and AID [3]. B-cell transcription factors also act to inhibit premature ASC differentiation. Once B cells become activated and initiate the differentiation process, these B cell transcription factors and their target genes are rapidly down regulated, transferring control to a small group of ASC-associated transcription factors, Blimp-1 (encoded by *Prdm1*), IRF4 and XBP1, whose expression in uniquely elevated in ASCs from mouse and humans [10, 13]. How the B-cell program is destabilized and ASC differentiation initiated is a complex area involving multiple regulatory steps and transcription factors, and has been reviewed elsewhere [15].

IRF4 and Blimp-1 are each required for the differentiation of B cells to ASCs [16-18], with *Prdm1* being a direct transcriptional target of IRF4 [17]. Until recently there was little

understanding of the function of either factor in ASCs beyond their early role in differentiation; however, two studies have now provided a wealth of information about IRF4 and Blimp-1 function in committed ASCs [19, 20]. Using inducible gene inactivation models, it was shown that IRF4 is essential for ASC survival, whereas Blimp-1 is dispensable for the survival of long-lived ASCs and is instead required for both the correct processing of *Igh* mRNA and the physiological adaptations required for Ab secretion [20]. Blimp-1 and IRF4 co-regulate many genes involved in ASC metabolism [19, 20]. XBP1, whose expression is maintained at an extremely high level in ASCs through a non-canonical splicing event [21], was initially reported to be required for ASC differentiation; however, subsequent studies using conditional mutagenesis have shown that XBP1 is specifically required for the secretory function of ASCs [20, 22-24]. As outlined below, XBP1 and Blimp-1 are important upstream regulators of the unfolded protein response (UPR) in ASCs.

Antibody-secreting cell migration

In a conventional immune response, ASC differentiation occurs in SLOs, from where the ASCs migrate via the blood to settle in those tissues where they find a supporting niche i.e. the BM and lamina propria (Figure 1). This process is exemplified by the clonal relatedness of IgA^+ ASCs from the gut and BM in both humans and mice [25, 26]. Exit from SLOs is controlled by the sphingosine-1-phosphate receptor-1 (S1PR1) [27]; however, unlike the situation in T cells, S1PR1 expression in ASCs is not regulated by the transcription factor Klf2 [28]. Nonetheless Klf2-deficient mice show abnormal ASC egress, which has been attributed to deregulated integrin β7. Two other adhesion molecules have been implicated in ASC exit from SLOs, the integrin β2 and VLA4 (α4β1). Integrin β2-deficient ASCs remain tethered in the SLOs but, if they make it to the circulation, are able to reach the BM [29]. Integrin activation is an important component of ASC-homeostasis as ASCs with aberrantly activated VLA4, through loss of the tyrosine phosphatase Shp1, cannot egress from the spleen [30], while ASCs lacking the co-chaperone Mzb1, which is required for VLA4 activation, show impaired plasmablast differentiation and BM homing and/or retention [31]. Once in the circulation, ASCs are dispatched to different tissues depending on the chemokine receptor they express: CXCR4 directs them to the BM [32], CCR9 to the small intestine, CCR10 to either the colon, mammary gland or lung [33], and CXCR3 to inflamed tissue [34]. On a smaller scale, plasmablasts within SLOs rely on CXCR4 to leave the lymphoid follicles [35].

How the expression of these receptors is regulated in ASCs remains largely unknown. The transcription factor c-Myb is essential for the migration of antigen-specific ASCs to the BM [36]. c-Myb controls the responsiveness to CXCL12 (the ligand of CXCR4) but not the expression of CXCR4 itself, suggesting that c-Myb's target(s) are downstream of the receptor. Interestingly,

a mutation affecting the desensitization of CXCR4 also leads to the loss of long-lived ASC in the BM [37]. One player identified in the desensitization process is Rgs1, a regulator of chemokine-receptor signaling, whose absence induces abnormal trafficking of ASCs [38]. Looking at the other ASC chemokine receptors, it transpires that responsiveness to the ligands of CXCR4, CXCR3 or CCR9 varies during maturation with terminally-differentiated plasma cells showing a decreased ability to migrate as compared with plasmablasts [34, 39]. It is interesting to note that Blimp-1 inhibits the expression, of *Klf2*, *Rgs1*, and several chemokine receptors (*Cxcr5*, *Ccr7*, *S1pr1*) and adhesion molecules (*Cd22*, *Itgb7*, *Sell*) that are involved in all steps of migration, from the egress out of the SLOs through to settlement in the BM [19, 20]. Thus the up-regulation of Blimp-1 through maturation likely controls the migratory abilities of ASCs [8].

The distinct metabolism of antibody-secreting cells

Unfolded protein response. The specialized nature of ASCs is reflected in their unique transcriptome, morphology and metabolism. More than 70% of the transcripts in ASCs encode the immunoglobulin heavy (IgH) and light (IgL) chains [10], and the endoplasmic reticulum (ER) and Golgi apparatus are hypertrophied to accommodate the very high rate of proteosynthesis in ASCs. A major adaptation of ASC metabolism resides in the re-deployment of the UPR pathway, which is usually a stress response, to a more physiological role. The UPR is a three-pronged pathway, with each arm overseen by a specific transcription factor, namely Atf4, Atf6 and Xbp1 [40]. In most cells, the UPR is induced by the accumulation of unfolded proteins in the lumen of the ER, which triggers an expansion of the secretory apparatus capacity. If the response is not sufficient to deal with the ER stress, the Perk/Atf4/Chop axis is induced, leading to apoptosis. In ASCs, it has been suggested that this Perk/Atf4/Chop branch is selectively inactivated during differentiation, most likely to enhance the potential to deal with huge amount of proteins [41]. Notably, UPR implementation precedes up-regulation of *Igh/Igl* transcription in ASCs, highlighting that the UPR pathway is part of the ASC differentiation process, and not a stress-induced response. The first arm of the UPR to be activated during differentiation is controlled by Ire1/Xbp1; Ire1 being the endonuclease that catalyzes the activating non-canonical splicing of *Xbp1* [21]. Xbp1 is dispensable for ASC commitment, but Xbp1-deficient cells fail to enlarge their ER and secrete little Ab [23, 24, 42]. Inactivation of Xbp1 in mature ASCs induces the loss of both *Igh/Igl* transcripts and secretory capacity [20]. The decrease in *Igh/Igl* transcripts could be partially the result of their degradation by Ire1, which is up-regulated upon Xbp1 loss, as *Igh/Igl* mRNA levels are partially restored in Ire1/Xbp1 double-deficient ASCs [43]. Interestingly, the three arms of the UPR are interconnected because *Xbp1* and *Hspa5* (encoding the chaperone BiP that regulates the triggering of all three branches) are transcriptional targets of Atf6 [21].

Blimp-1 has also been shown to play an important role upstream of each arm of the UPR [20]. Inactivation of Blimp-1 in BM plasma cells induces a reduction in cell volume, disruption of the ER structure in, and a loss of Ig secretion [20], all resulting from UPR down-regulation. Blimp-1 regulates the expression of *Atf6* and *Ern1* (encoding Ire1), each of which impact on Xbp1 activation (Figure 2). Moreover, the production of the mRNA encoding the secreted isoform of the IgH is significantly decreased, as the expression of elongation factor *Ell2*, that favors the secreted form over the membrane-bound one, is regulated by Blimp-1 [19, 20]. Thus Blimp-1 activity sits at the apex of the UPR pathway and controls the Ab-secretory function of ASCs.

Autophagy. ASCs have high autophagic activity that increases during maturation (Figure 1), as illustrated by the expression of some key genes such as *Atg5*, *Atg9*, *Atg13* or *Map1lc3b* [10, 44]. Autophagy acts as a safeguard, allowing ASCs to limit their ER size and Ab secretion. This homeostatic mechanism was uncovered in Atg5-deficient ASCs which display enlarged ER, increased Blimp-1 expression and higher rates of Ab production [44]. It is notable that the autophagy in ASCs seems to specifically target the ER, sparing the mitochondria and ribosomes. Defective autophagy results in low ATP levels, indicating that normally it generates metabolic substrates via a recycling process. Importantly, autophagy-deficient BM ASCs were outcompeted by the autophagy-competent ones, highlighting that the stress from unrestrained Ab production resulted in a form of metabolic burnout [44].

mTOR signaling. One mechanism by which autophagy is regulated is through inhibition driven by the mTORC1 complex (Figure 2). This involves inhibitory phosphorylation of ATG13 and ULK1, two of the three members of the autophagy initiation complex [45, 46]. In addition to autophagy, mTORC1 regulates protein, nucleic acid and fatty acid synthesis, as well as glycolysis and organelle biosynthesis, in response to nutrient and mitogenic stimuli. This control is critical to allow the cell to adapt its metabolism to the environmental supply of nutrients/stimuli. mTORC1 is particularly active in ASCs, and its activity is positively regulated by Blimp-1 through the transcriptional control of both the leucine transporter CD98 (amino acid transporter) and two members of the Sestrin family (Figure 2), which are inhibitors of AMPK, which is itself an inhibitor of mTORC1 through the phosphorylation of Raptor [20]. The ablation of mTORC1 activity by the deletion of Raptor inhibits ASC differentiation but does not affect their survival [47] and, as expected, autophagy is enhanced upon loss of Raptor. Raptor also impacts both the UPR pathway, through genes such as *Hpa5*, *Atf4* and *Atf6*, and the secretory capacity of ASCs. Most of this phenotype resulting from Raptor-deficiency could be the consequence of the observed down-regulation of Blimp-1. Strikingly, over-active mTOR, through inactivation of the negative regulator Tsc1, rescues many aspects of the Xbp1-deficient phenotype, including Ab

secretion and ER structure [48]. In the regulatory loop involving mTORC1, autophagy and Blimp-1, the mechanism underlying the negative regulation of Blimp-1 expression by autophagy remains to be fully elucidated (Figure 2).

Metabolic pathways. The metabolic adaptation of ASCs to their Ab secretory function is also reflected in their sugar utilization, 90% of which is used for Ab glycosylation [49]. Furthermore, modifications of the glycosylation patterns have been observed in Xbp1-deficient cells [50]. To adapt to the high demand, plasma cells express higher cell surface concentrations of the glucose transporter GLUT1 than plasmablasts enabling increased glucose uptake (Figure 1). The regulation of this increase is post-transcriptional as *Slc2a1* (*Glut1*) mRNA levels are similar in both populations [49, 51]. ASCs also acquire distinct metabolic pathways upon maturation, as long-lived plasma cells can engage pyruvate-dependent respiration while plasmablasts cannot. This respiration uses the long chain fatty acid as the major source of carbon, as glucose is diverted to the hexosamine pathway. However glycolysis-derived pyruvate can also be used by mitochondrial respiration to adapt to supply variations and maintain homeostasis. This backup resource is essential given that the blockade of mitochondrial pyruvate import impairs long-lived plasma cell survival [49]. Interestingly, lamina propria IgA$^+$ ASCs appear distinct, as they can be distinguished from splenic ASCs by an elevated glycolytic activity [52]. In general, most of the metabolic differences observed between short and long-lived ASCs are thought to be post-transcriptional [51], however the transcription factor Zbtb32 has been demonstrated to play a specific role in secondary Ab responses. ASCs differentiated from Zbtb32-deficient memory cells show elevated expression of the genes encoding the mitochondrial ribosomal proteins that are required for translation of electron transport chain proteins [53]. Thus Zbtb32 functions to restrain the respiratory capacity of ASCs elicited in a recall response.

Epigenetic regulation. The epigenetic regulation of ASC differentiation and biology has been addressed through the study of the requirement of Ezh2, the catalytic subunit of the PRC2 complex, an essential transcriptional silencer depositing the H3K27me3 mark on chromatin. The expression of *Ezh2* is up-regulated during ASC differentiation and H3K27me3 is associated with genes of the B-cell transcriptional program [54]. Ezh2 can physically interact with Blimp-1 [19] and ablation of *Ezh2* allows the de-repression of well-known Blimp-1 targets including *Spib* and *Tlr9*. Ezh2-deficiency in ASCs also resulted in reduced levels of the UPR pathway, oxidative respiration, glycolysis capacity and Ab secretion [54]. Overall these observations highlight the broad impact of Ezh2 activity on ASC metabolism.

Antibody-secreting cell homeostasis and survival

The lifespan of an ASC can differ drastically, from a few days to decades (Figure 1), and much effort has been exerted to identify the pathways leading to such divergent fates [5, 7, 49]. The relevance of each pro-survival signal likely depends on the maturation stage of the ASC.

Perhaps the best-characterized survival pathway in ASCs involves BCMA, which is the receptor shared by APRIL and BAFF, and is required for the maintenance of long-lived plasma cells in the BM [55]. Human mucosae are also particularly rich in APRIL, where it promotes IgA-switching and plasma cell survival [56]. Inflamed tissues can also support the persistence of long-lived plasma cells, but this process remains poorly understood. BCMA activation sustains the expression of the short-lived anti-apoptotic molecule, Mcl-1 [55, 57]. Loss of Mcl-1 results in rapid death of ASCs [57]. The regulation of Mcl-1 is not yet fully understood, although with the recent development of Mcl-1-specific inhibitors, Mcl-1—induced cell death is now a key target for therapies aimed at killing both myeloma and normal ASCs. IRF4 and Zbtb20 have also emerged as regulators of ASC survival. IRF4 is essential for all normal and malignant ASC survival from an early point, although the mechanism by which IRF4 acts remains unclear [20, 58]. One direct target of IRF4 is Zbtb20, which seems to have restricted functions given that Zbtb20-deficient ASCs have a survival defect in an alum-adjuvanted immunization model but not when TLR ligands were used as adjuvants [59]. Zbtb20 is also required for maximal expression of Mcl-1 [60]. The similarly dramatic loss of ASCs upon both IRF4 and Mcl-1 deficiency suggests that *Mcl1* is controlled by IRF4, although such an interaction has not been demonstrated.

In addition to the APRIL:BCMA axis, several cell-surface proteins promote aspects of ASC longevity, including Syndecan-1/CD138 [61], CD93 [62], CD28 [63], CD44 [64] and CD37 [65]. CD28 expression triggers an intrinsic survival signal, possibly through the activation of the Vav/PLCγ/NF-κB pathway and the up-regulation of BCMA. This protection is specific to long-lived BM plasma cells and, at least in malignant ASCs, is mediated by CD28's engagement of CD80/86 on myeloid cells and subsequent IL-6 secretion [66]. Although the above-mentioned examples indicate that progress is being made, we currently still lack a holistic understanding of the processes controlling ASC homing and longevity.

Conclusion

In recent years the application of large scale genomic profiling to ASCs has led to a leap in the knowledge of this rare but essential cell type. It is now apparent that ASCs have a transcriptome that is distinct from all other lymphocytes, a realization that highlights the potential to develop truly ASC-specific therapies. ASCs are Ab factories and, as discussed in this review, much has also been learnt about how ASCs achieve the feat of maintaining the secretion of enormous quantities of Ab over periods of time that extends to decades in humans. This process requires a

highly specialized cellular structure, metabolic program and the ability to control the stress that is induced by the large protein load. These unique cell biological and metabolic processes offer glimpses of therapeutic approaches to kill either autoreactive or malignant ASCs, or to potentially limit the production of Abs while leaving the ASC intact.

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Conflict of interest:

The authors declare no commercial or financial conflict of interest

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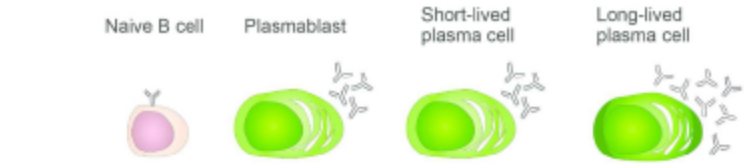
Figure legends



Blimp-1	-	++	++	+++
Lifespan	++	+	+	+++
Proliferation	-	++	-	-
Location	BM, SLO, blood	SLO, Blood	SLO	BM, LP
Isotype	IgM/D	All isotypes	IgM>IgG	IgG>IgA>IgM
Ab secretion rate	-	++	++	++++
Glucose uptake	-	++	++	+++
Autophagy	+	++	++	+++
mTORC1 activity	-	+	+	+++
UPR	-	+++	+++	+++

Figure 1. Summary of the cellular and metabolic properties associated with antibody secreting cell (ASC) maturation. Attributes of naïve B cells and the three populations of ASCs (plasmablasts, short-lived plasma cells and long-lived plasma cells) are shown with – indicating a lack of the component/process and +, ++, +++, ++++ indicating the level of expression/activity). Ab, antibody; BM, bone marrow; LP, lamina propria; SLO, secondary lymphoid organ; UPR, unfolded protein response.

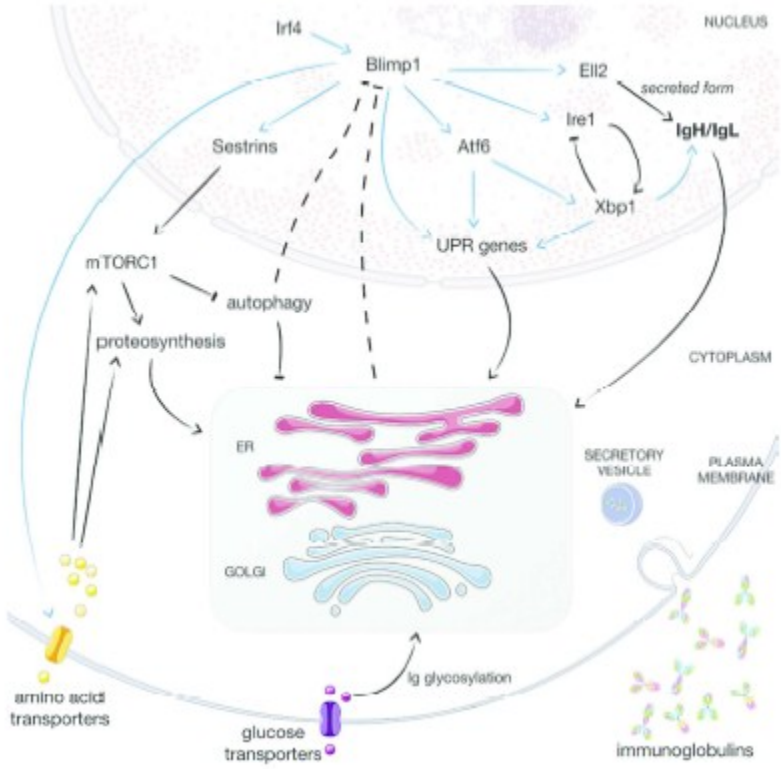


Figure 2. Transcriptional regulation of immunoglobulin secretion. Schematic describing the current understanding of regulatory interactions between Blimp-1, mTORC1, UPR and autophagy that ultimately modulate the expansion of the secretory apparatus allowing high-level immunoglobulin production. Blimp-1 expression is induced by Irf4 and is central to ASC metabolism and immunoglobulin secretion. Blimp-1 oversees the UPR pathway via the transactivation of many genes including *Atf6* and *Ern1* (encoding Ire1), whose combined action leads to the active spliced Xbp1. Blimp-1 (through the expression of *Ell2*) and Xbp1 foster the extremely high expression of the secreted isoform of the *Igh* gene. Blimp-1 sustains the high activity of mTORC1 by enhancing the expression of two members of the Sestrin family and amino acid transporters such as CD98. mTORC1 in turn supports the expansion of the cell volume, in particular the endoplasmic reticulum (ER) and Golgi, by fostering proteosynthesis and limiting autophagy. Both autophagy and the expansion of the secretory apparatus act as negative regulators of Blimp-1 expression through unknown mechanisms. Arrows indicate a positive regulatory effect, with those in blue indicating transcriptional regulation. T-bars depict negative regulatory interaction. Dashed line designates a described regulatory interaction whose molecular mechanism remains unknown.

