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Title:
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Date:
2021-09-01

Citation:
Trezise, S. & Nutt, S. L. (2021). The gene regulatory network controlling plasma cell function. Immunological Reviews, 303 (1), pp.23-34. https://doi.org/10.1111/imr.12988.

Persistent Link:
https://hdl.handle.net/11343/298637

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Article type : Invited Review

The gene regulatory network controlling plasma cell function

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Running title: The regulation of plasma cell function

This is the author manuscript accepted for publication and has undergone full peer review but has not been through the copyediting, typesetting, pagination and proofreading process, which may lead to differences between this version and the Version of Record. Please cite this article as doi: 10.1111/IMR.12988

Keywords: antibody secretion; unfolded protein response; B cell differentiation; plasma cell

SUMMARY

Antibodies are an essential element of the immune response to infection, and in long-term protection upon re-exposure to the same micro-organism. Antibodies are produced by plasmablasts and plasma cells, the terminally differentiated cells of the B lymphocyte lineage. These relatively rare populations, collectively termed antibody secreting cells (ASCs), have developed highly specialised transcriptional and metabolic pathways to facilitate their extraordinarily high rates of antibody synthesis and secretion. In this review we discuss the gene regulatory network that controls ASC identity and function, with a particular focus on the processes that influence the transcription, translation, folding, modification and secretion of antibodies. We will address how ASCs have adapted their transcriptional, metabolic and protein homeostasis pathways to sustain such high rates of antibody production, and the roles that the major ASC regulators, the transcription factors, Irf4, Blimp-1 and Xbp1, play in co-ordinating these processes.

INTRODUCTION

Antibody secreting cells (ASCs) are the highly specialised, terminally differentiated cells of the B lymphocyte lineage, that provide a critical arm of the adaptive immune response. The production of high-affinity antibodies is not only important for the elimination of pathogens during infection, but also provides long-term protection from re-infection. In healthy individuals ASCs are relatively rare in hematopoietic and lymphoid organs, typically comprising less than 1 % of cells, however the frequency of ASCs can rise substantially in response to infection or autoimmune stimulation. For example, ASCs comprise up to 30 % of peripheral B lymphocytes in individuals hospitalised with severe COVID-19 [1]. ASCs are also abundant in some other tissues, such as the lamina propria of the gastrointestinal tract, the lactating mammary gland and the meringues layer of the central nervous system [2].

There are two subsets of ASCs, plasmablasts and plasma cells, which differ in their surface marker phenotype, proliferative capacity, lifespan and the level of expression of the transcription factor Blimp-1. Plasmablasts are a short-lived, proliferating population that express intermediate levels of Blimp-1 [3,4]. It is these characteristics, along with the observation that this population appears shortly after immunization, that has led to the view that plasmablasts are the more immature of the ASC populations [3,5]. In contrast, plasma cells express high levels of Blimp-1, generally do not divide and are capable of living for years, up to decades [3,6-8].

The unique identities of B cells and ASCs are regulated by two mutually antagonistic genetic networks, requiring a profound change in the transcriptional program of a cell transiting between these differentiation states. This irreversible transcriptional switch is facilitated by a reorganisation of the chromatin architecture following B cell activation and during the differentiation process [9,10]. We previously performed a comprehensive transcriptional analysis of all aspects of the B cell differentiation process from naïve B cells through to long-lived BM plasma cells [11]. Strikingly, this study determined that the majority of the ASC transcriptome is devoted to immunoglobulin (Ig) gene expression. This analysis also revealed that despite differences in anatomical location, lifespan and proliferation status, plasma cells from the bone marrow and spleen, as well as splenic plasmablasts share a core transcriptional signature [11]. The expression of this gene signature is also largely independent of Ig isotype, with the exception of genes influencing homing and migration, which are differentially expressed between IgM^+, IgG^+ and IgA^+ ASC populations [12]. In addition to the known regulators of ASC differentiation and function, most notably *Irf4*, *Prdm1* and *Xbp1* (discussed below), genes within this signature encode proteins involved in a diverse range of cellular processes. Notably, key functional groups contained within the ASC signature include genes involved in protein homeostasis through the unfolded protein response (UPR) or endoplasmic reticulum-associated degradation (ERAD) pathway, gene expression and translation, intracellular protein transport, post-translational modification, metabolism, and receptors and signalling pathways *(Figure 1)*. Collectively these gene expression changes extinguish the B cell genetic program and allow

remodelling of the cell, including massively expanding the endoplasmic reticulum (ER) to facilitate the production of large amounts of secreted antibody.

In this review we focus on the gene regulatory network that controls ASC maintenance and function. We will pay particular attention to the adaptations that ASC undergo to provide the capacity to produce required amounts of secreted antibody and where it is known, link the control of these features to the activity of the key regulators of the ASC transcriptional identity.

THE IRF4-BLIMP1-XBP1 TRIAD

Initiation of ASC differentiation

Although the activity of many transcription factors impact on the terminal differentiation of B cells to ASCs, it is Irf4, Blimp-1 and Xbp1 that have received the most focus. These three transcription factors appear to act as hubs through which the other known factors such as Irf8, the ETS family proteins (PU.1, SpiB, Ets1), Bcl6, Bach2, NFκB, AP1 and E proteins (E2A, E2-2) influence the differentiation process (reviewed in [13]).

Irf4 regulates many processes within the B cell lineage from the early stages of development in the bone marrow, through to germinal center formation and the differentiation and survival of ASCs [14-17]. Irf4 is expressed at intermediate levels in activated B cells, with its expression increasing markedly in all ASCs populations in humans and in animal models [18]. Irf4 is essential for the initial steps of ASC differentiation, in part due to its role in promoting the expression of *Prdm1*, the gene encoding Blimp-1, and repressing the expression of the negative regulator of differentiation, *Bcl6* [18-22].

Blimp-1, in contrast, plays no role in the differentiation of B cells prior to the ASC stage, as its expression is restricted to plasmablasts and plasma cells [3]. This absence is essential for normal B cell homeostasis as premature Blimp-1 expression prevents B cell development and immune function [23,24]. Although Blimp-1 is not required for the earliest stages of the terminal differentiation process, it is essential for the generation of ASCs [25,26]. Blimp-1 regulates gene expression by recruiting chromatin-modifying complexes, including the BAF, NuRD and PRC2, to its target genes [27]. During differentiation, Blimp-1 represses the expression of key B cell genes,

including *Bcl6*, *Pax5* and *Id3* and promotes the expression of UPR genes, including indirectly promoting expression of *Xbp1* [27-29]. Xbp1 is a regulator of the UPR and is ubiquitously expressed, but is upregulated in protein-secreting cells, including ASCs, through both transcriptional and post-transcriptional mechanisms [30]. Although initially thought to be essential for ASC generation, it is now clear that differentiation can occur in the absence of Xbp1 [31-34]. Xbp1 does however play a profound role in the secretory capacity of ASCs by driving an increase in cell size and ER content, and by promoting the expression of genes involved in ER homeostasis and secretory protein production [35].

Maintenance of the ASC signature

As outlined above, a great deal is known about the factors driving the B cell to ASC differentiation process, however the block in differentiation that occurs in the absence of Irf4 or Blimp-1 means that our understanding of the functions of these genes in established ASCs is more limited [13,36]. Using a tamoxifen-inducible system that allows for the targeted deletion of genes within mature ASCs *in vivo*, we have shown that while the inactivation of *Irf4* leads to the rapid loss of the ASC compartment, loss of *Prdm1* or *Xbp1* does not influence plasma cell survival [16]. The persistence of Blimp-1 deficient plasma cells allowed us to investigate the role that Blimp-1 plays in maintaining the ASC transcriptome. Interestingly, expression of the majority of ASC signature genes was unaffected by the inactivation of *Prdm1,* and many genes that are directly repressed by Blimp-1 early during the differentiation process were not re-expressed following Blimp-1 inactivation in mature plasma cells. This suggests that while Blimp-1 is required for establishing the ASC genetic network during differentiation, it is largely dispensable for its maintenance in established plasma cells. However, this is not to say that plasma cells were unaffected by the loss of Blimp-1, as the mutant ASCs had an altered morphology and a severe reduction in their antibody secreting capacity. These defects can be explained in part by the role of Blimp-1 in promoting *Ig* gene transcription and in regulating the UPR, which will be discussed in the following sections. The inducible inactivation of Xbp1 also had minimal impact on the maintenance of the ASC signature, with the key exception of UPR genes that are required for protein import and processing within the ER [16].

The rapid loss of the ASC compartment following the inactivation of the *Irf4* gene complicates the investigation of its function in this population. Recent work has demonstrated that overexpression of the pro-survival protein Bcl2 is sufficient to prevent plasma cell death following the loss of Irf4 and, therefore, this system can be used to interrogate Irf4 function in mature plasma cells [37]. Transcriptional analysis of these Irf4-deficient plasma cells revealed a loss of expression of the ASC gene signature, suggesting that Irf4 is required to maintain cell identity. Furthermore, Irf4-deficient ASCs have reduced expression of *Xbp1* and a reduction in ER structure. Interestingly, the expression of *Prdm1* was not altered, suggesting that although Irf4 is required for the initial upregulation of *Prdm1*, it is not essential for its sustained expression. Together these studies demonstrate that there is minimal overlap in the genes regulated by Irf4, Blimp-1 and Xbp1, with the exception of the UPR pathway genes, indicating that these three factors regulate separate elements of the ASC transcriptome in established plasma cells [16,37].

ANTIBODY SECRETION

Plasma cells are highly specialised secretory cells, capable of producing approximately 2 ng of antibody per cell per day [38]. Compared to B cells, where *Ig* transcripts constitute 2 % of the transcriptome, approximately 70 % of the ASC transcriptome is devoted to *Ig* gene expression [11]. Blimp-1 appears to control the upregulation of *Ig* gene expression during differentiation, as *Prdm1*$^{-/-}$ pre-plasmablasts were unable to increase *Igh* and *Igk* expression [27]. This failure to upregulate the *Ig* genes may be due to decreased chromatin accessibility in the 3' enhancer regions of the *Igh* and *Igk* loci, however, minimal Blimp-1 binding was observed in these regions, suggesting an indirect effect [27]. Notably, the transcription of *Igh* was also severely reduced in *Xbp1*$^{-/-}$ plasma cells [16]. ASCs utilize an alternate polyadenylation site in *Igh* genes compared to B cells, resulting in the production of shorter transcripts lacking the transmembrane domain [39]. Ell2 is involved in the regulation of this process as it promotes the use of the distal *Igh* polyadenylation site and consequentially, the production of the form of the *Igh* mRNA encoding the secreted antibody [40,41]. Blimp-1 promotes this switch towards

producing secretory *Igh* transcripts as it directly activates the expression of both *Ell2* and its activator *Eaf2 (Figure 2*, [16,27]).

Following translation by ER-associated ribosomes and translocation into the ER lumen, newly synthesised Ig polypeptides are bound by ER resident chaperones, including Grp78/*Hspa5* (also known as immunoglobulin binding protein, BiP) which facilitate correct protein folding. Antibodies are hetero-tetrameric proteins, consisting of two heavy chains and two light chains, which are linked by disulphide bonds. During import into the ER lumen, Grp78 and co-chaperone Dnajb11 will bind to the unfolded IgH polypeptides and prevent the inappropriate formation of intrachain disulphide bonds [42]. The IgH will remain bound by this complex until it combines with a correctly folded light chain and interchain disulphide bonds chain be formed [43,44]. During this protein-folding process, a high-mannose glycan chain will be added to IgH. This glycosylation step is not only important for facilitating correct folding and processing of the antibody within the ER but these *N*-linked glycans also influence antibody stability, flexibility and effector functions [45,46]. All IgH molecules receive the same core glycan structure, however, this structure can be modified by glycosyltransferases and glycosidases to generate the final glycan chains present on secreted antibodies. The structure and sugar content of these glycan chains can influence the interaction of the Fc domain with complement proteins and Fc receptors on immune cells, ultimately influencing the antibody effector functions [47]. All IgH forms contain at least 1 *N*-glycosylation site, with mouse IgE containing 9 potential *N*-glycosylation sites. The specialised metabolic program that ASCs require to perform these important post-translational modifications will be discussed in detail in the following section.

Both Xbp1 and Blimp-1 have essential roles in supporting increased antibody translation and protein processing within the ER *(Figure 2)*. Xbp1 is a key regulator of the UPR that drives the increase of the cell's ER, mitochondria, ribosome and lysosome content, and induces the expression of many genes required for ER protein homeostasis [16,35,48,49]. Consequentially, *Xbp1*-deficient plasma cells have a reduced ER network and a severely diminished antibody secreting capacity [16,32,33]. Plasma cells lacking Blimp-1 also have a reduced ER network and decreased

expression of genes involved in ER protein processing pathways [16]. Blimp-1 also supports increased rates of antibody translation by sustaining mTORC1 activity in plasma cells through multiple mechanisms *(Figure 2*, [16]). The mTORC1 kinase complex is a master regulator of protein, lipid and nucleotide biosynthesis pathways that responds to growth factors and changes in nutrient levels such as amino acids [50]. The import of amino acids through CD98 is a key regulator of mTORC1 activation and CD98 expression is directly promoted by Blimp-1 in plasma cells [16,50]. mTORC1 signalling promotes antibody production as inhibition of mTORC1 activity with Rapamycin decreases the rates of Ig synthesis and secretion [51,52]. Conversely, plasma cells with overactivation of mTORC1 signalling, through the deletion of its negative regulator Tsc1, have increased rates of antibody synthesis and secretion [53]. Blimp-1 also promotes mTORC1 activity by directly inhibiting the expression of Sestrin family members, *Sesn1* and *Sesn3,* which inhibit mTORC1 activation [16,54,55].

HOW DO PLASMA CELLS MAINTAIN THEIR HIGH RATE OF ANTIBODY PRODUCTION?

Protein homeostasis

Plasma cells perform a massive amount of Ig synthesis, all of which must be correctly folded and post-translationally modified within the ER. This makes plasma cells particularly sensitive to ER stress, and as a consequence, they are dependent on ER stress responses including the UPR and ERAD pathway to maintain protein homeostasis. In keeping with this, many of the genes involved in either the ERAD pathway or the UPR are elements of the ASC gene signature and/or are directly regulated by Irf4, Blimp-1 or Xbp1 *(Figure 3*, [11,16,27,35,37,48,49]). The ERAD pathway ensures that unfolded or misfolded proteins are removed from the ER and are marked with ubiquitin for subsequent proteasomal degradation [46,56]. If proteins are not efficiently folded correctly then the glycan chains that are attached to them following translocation into the ER are susceptible to modification by ERman1 and ER degradation enhancing alpha-mannosidase-like (EDEM) proteins [56]. Proteins carrying these modified glycans are then delivered to Sel1l and the E3 ubiquitin ligase Hrd1, polyubiquitinated and then pulled from the ER into the cytosol by p97/Vcp [56,57]. Once in the cytosol, these polyubiquitinated misfolded proteins are degraded by the cytosolic-proteasome system [58].

When the ERAD pathway is insufficient to limit the accumulation of misfolded proteins within the ER, the UPR can become activated *(Figure 4)*. The UPR is a conserved pathway which allows cells to handle the stress caused by the accumulation of unfolded or misfolded proteins within the ER lumen [59,60]. In most cell types, the UPR acts to alleviate ER stress and restore protein homeostasis, however, if this is not possible it will activate cell death pathways [61]. In addition to its function as a chaperone protein, Grp78 also binds to the ER stress sensor proteins Perk, Ire1a and Atf6a, and keeps them in an inactive state *(Figure 4,* [62]). When the protein folding capacity of the ER is reached and unfolded proteins accumulate within the ER lumen, Grp78 dissociates from ER stress sensors to bind to unfolded proteins, leaving the stress sensors free to activate the UPR [63-65]. There is increasing evidence that the ER stress sensors may also be directly activated by unfolded proteins, however, it is still unclear whether this direct activation can occur while the ER stress sensors are still bound by Grp78 [60]. Atf6a drives the transcription of many ERAD and UPR components, including *Xbp1*, *Hspa5*, *Hrd1* and EDEM genes [66,67]. Ire1a dimerises and splices *Xbp1* mRNA to generate the active form sXbp1 [67,68]. sXbp1 can then transit to the nucleus and drive expression of ERAD and additional UPR genes, and promote the expansion of the ER network, increasing the cells protein processing capacity [35,49,67]. Sustained ER stress, however, can result in the formation of Ire1a oligomers which can induce cell death [69]. Perk also phosphorylates eIF2a, resulting in a global inhibition of protein translation to reduce ER stress [70]. This cellular state favors the translation of Atf4 which induces the expression of the pro-apoptotic Chop [71]. Prolonged Perk activation and Chop expression result in cells undergoing apoptosis in most systems [61].

To maintain such high rates of antibody production, plasma cells require their UPR to be constitutively active without inhibiting protein translation or activating the cell death pathways [59]. The Ire1a and Atf6 arms of the UPR are both engaged during B cell differentiation, however, the Perk/Atf4/Chop arm of the UPR is selectively inactivated *(Figure 4,* [68,72-74]). It was initially proposed that activation of the UPR during differentiation was the result of increased Ig synthesis causing an accumulation of unfolded proteins in the ER [30]. However, increased

expression of UPR components occurs prior to cells increasing their antibody production [51,72,75]. Furthermore, the upregulation and splicing of *Xbp1* occurs normally in plasma cells that are unable to increase Ig synthesis or produce secreted antibody [34]. Interestingly, Blimp-1 appears to play a central role in regulating this specialised UPR. Blimp-1 promotes *Xbp1* expression and processing into its active form by directly regulating the expression of *Ern1*, which encodes Ire1a, and *Atf6* [16]. Consequentially, Blimp-1-deficient plasma cells have reduced levels of sXbp1 and a reduced ER network [16]. Blimp-1 control of the UPR extends further than promoting Xbp1 activation as Blimp-1 also directly binds to many downstream UPR genes [16,27]. Together, this suggests that the activation of the UPR in ASCs is part of the Blimp-1 driven differentiation process, not simply a consequence of increased Ig synthesis.

Autophagy

The ERAD-Ubiquitin-proteasome system is not the only degradation pathway utilised by ASCs to maintain ER homeostasis. Autophagy is a process for the degradation of proteins, organelles and pathogens, which can be used to recycle large portions of the cell. During this process a double membraned vesicle known as an autophagosome is formed around the cellular contents to be degraded, and this autophagosome will then fuse with lysosomes. Plasma cells have increased rates of autophagosome formation compared to plasmablasts, suggesting that they have increased rates of autophagy [76,77]. ASCs use autophagy to limit ER expansion and antibody production, and to provide nutrients through the degradation of cellular components, optimising the balance between antibody synthesis and cellular metabolism. ASCs that lack the key autophagy gene Atg5, and are therefore unable to undergo autophagy, have an expanded ER network and synthesise more Ig than their WT counterparts [78]. However, this increased antibody synthesis comes at a cost as *Atg5*$^{-/-}$ ASCs have a reduction in cellular ATP levels and are more susceptible to apoptosis. Therefore, despite increased antibody secretion per cell, the overall antibody response to immunisation and infection is impaired when ASCs are unable to undergo autophagy [78-80]. Importantly, mTORC1 negatively regulates multiple aspects of the autophagy process, suggesting that plasma cells carefully balance autophagy and mTORC1 activity to maximise their rates of antibody secretion without impairing their survival [81].

Metabolism

ASCs require a specialised metabolic program to be able to sustain their high rates of antibody synthesis and glycosylation. Plasma cells have constitutively high rates of glucose uptake and approximately 90 % of this glucose is used for antibody glycosylation through the hexosamine biosynthesis pathway [82]. Plasmablasts appear to utilise glucose differently as they have lower rates of glucose uptake and, unlike plasma cells, are able to synthesise glycogen. The rate of glucose uptake is linked to the rate of antibody secretion. All ASCs synthesise Ig at similar rates regardless of their rate of glucose uptake, however, cells with lower rates of glucose uptake degrade Ig more rapidly, resulting in reduced antibody secretion [76].

The specialised metabolic program of plasma cells also contributes to their longevity as they have developed a mechanism of buffering against changes in nutrient availability. When cellular ATP levels are lowered, plasma cells are able to redirect glucose away from antibody glycosylation and towards glycolysis, promoting survival at the expense of antibody secretion [82,83]. Blocking mitochondrial pyruvate import, either pharmacologically or through conditional deletion of mitochondrial pyruvate carriers, leads to a reduction in plasma cell life-span. In keeping with this, mutations that decrease either the rate of glucose uptake or pyruvate metabolism pathways, such as *Enpp1* and *Cd28* loss of function mutations respectively, have detrimental effects of plasma cell life-span [84,85]. In contrast, plasmablasts appear largely unable to redirect glucose to pyruvate-dependent respiration and inhibition of mitochondrial pyruvate import does not impact their respiratory capacity or life-span [82].

Blimp-1, Xbp1 and Irf4 have all been implicated in regulating aspects of the ASC metabolic program. Blimp-1 facilitates nutrient uptake by directly promoting the expression of *Enpp1*, which enhances glucose uptake, and the genes encoding both chains of the amino acid transporter CD98 (*Slc3a2*, *Slc7a5* [16,27]). Xbp1 is involved in the regulation of the hexosamine biosynthesis pathway, which generates precursors for antibody glycosylation, as it drives the expression of key enzymes Gfpt1, Gnpnat1 and Pgm3 [86]. Irf4 has been linked to maintaining ASC

mitochondrial homeostasis [37]. However, despite recent advances in identifying the distinct metabolic profiles of ASC subsets, the underlying regulation of these differences remains unclear. Plasma cells express higher cell surface levels of the glucose transporter Glut1 compared to plasmablasts, which presumably contributes to their increased rates of glucose uptake, however, *Glut1* is not differentially expressed between these populations at the transcriptional level [11,82]. Furthermore, despite differences in proliferation, antibody secretion and life-span, there are very few transcriptional differences between ASCs with high and low rates of glucose uptake, and single cell RNA-sequencing clusters do not correlate well with ASC metabolic profiles [76]. Together this implies that these metabolic networks, which have major influences on antibody secretion and cell lifespan, are largely controlled through as yet unidentified post-transcriptional mechanisms.

NON-ANTIBODY SECRETORY FUNCTIONS OF PLASMA CELLS

With the majority of their transcriptome being devoted to Ig expression [11], it can be easy to think of ASCs as being solely dedicated to antibody production, however, there is increasing focus on the influence that ASCs have on other aspects of the immune response . Subsets of ASCs that produce the anti-inflammatory cytokines IL-10 or IL-35 have been observed in a murine model of experimental autoimmune encephalitis (EAE) [87,88]. The IL-35 secretion by ASCs inhibits IFNγ and IL-17 production by T cells, reducing the severity of disease. IL-10 and IL-35 secreting ASCs are also detectable following *Salmonella enterica* infection, however, in this system these immunosuppressive ASCs are detrimental as they impair the anti-bacterial response, leading to increased mortality from infection [87,89]. A population of IL-17 producing ASCs emerges following *Trypanosoma cruzi* infection and the secretion of IL-17 by B lineage cells is required for parasite control, although the specific contribution of ASC-derived IL-17 remains to be determined [90]. The molecular controls of cytokine production by ASCs remains unclear, although it is known Irf4 and Blimp-1 directly control *Il10* transcription in a variety of other immune cell types [88,91].

Although the role of IgA$^+$ ASCs in regulating intestinal homeostasis through the production of dimeric secretory IgA is well known, there is also increasing evidence that these cells have immunosuppressive roles outside of the gut [92]. For example, studies in mouse models and human multiple sclerosis (MS) have implicated ASCs in restraining neuroinflammation [88,93]. Using a mouse model of EAE, Rojas et al. demonstrated that IgA$^+$ ASCs generated in the gut are able to migrate to the CNS during neuroinflammation and reduce disease severity through the secretion of IL-10 [94]. This migration of ASCs from the gut to the CNS during inflammation appears to be conserved in MS, as IL-10 expressing IgA$^+$ ASCs can be found within the meninges of relapsing MS patients. In keeping with this conclusion, relapsing patients also have decreased levels of IgA in their intestinal lumen compared to MS patients in remission and healthy controls [94,95]. Intriguingly, colonisation of mice with the commensal *Trichomonas musculis* promotes the expansion of the intestinal IgA$^+$ ASC pool and reduces EAE severity [94]. This raises the possibility that boosting the population of immunosuppressive gut ASCs may be a potential avenue for reducing the severity of disease in neuroinflammatory conditions.

TARGETING PLASMA CELLS IN DISEASE

Pathogenic plasma cell populations

The ability of long-lived plasma cells to sustain such high rates of antibody production provides an essential element of the adaptive immune response and immunological memory, however, this trait is highly detrimental when the antibodies that these cells produce are self-reactive. The generation of autoantibodies has been linked to the pathogenesis of many autoimmune conditions including systemic lupus erythematosus, rheumatoid arthritis, myasthenia gravis and NMPDAR encephalitis [96-98]. Pathogenic plasma cells are also characteristic of asthma and allergy, as well as being major mediators of the rejection of organ grafts and injected proteins, such as clotting factors in hemophilia [99,100]. Targeting these autoreactive long-lived plasma cell populations remains challenging as they are refractory to the therapies used to target pathogenic B cells [97]. Furthermore, targeting malignant plasma cells in multiple myeloma (MM), which is the second most common hematological malignancy, is similarly difficult [101]. MM patients typically suffer from multiple complications from the excessive plasma cell prevalence

and antibody production including hypercalcaemia, renal insufficiency, anemia and bone destruction, and despite recent improvements in treatment, the median survival for patients ineligible for autologous stem cell transplant is only 5 years [101]. Hence, there is a wide variety of circumstances where targeting ASCs or antibody production *per se* would be of substantial clinical benefit.

Plasma cells in tumors

It is becoming increasingly clear that plasma cells and intra-tumoral antibody production can also have significant effects on the anti-tumor response. The presence of intra-tumoral IgG1 is typically associated with good clinical outcomes in many cancer types as it can drive complement activation, phagocytosis and antibody-dependent cell cytotoxicity (ADCC) [102]. High levels of IgG4, both intra-tumoral and in serum, are commonly associated with poor prognosis and IgG4 antibodies have been shown to interfere with the positive effects of tumor-specific IgG1 [102-105]. Similarly, high abundance of intra-tumoral IgA is a poor prognostic marker in melanoma and bladder cancer [106,107]. Mice that are unable to class-switch to IgA have enhanced anti-tumor responses in hepatocellular carcinoma and metastatic prostate cancer models, suggesting a direct pro-tumor effect of IgA^+ plasma cells in these systems [108,109]. However, this picture is not black and white as high levels of intra-tumoral IgG4 and IgA are associated with a favourable prognosis in lung squamous cell carcinoma and ovarian cancer, respectively [110,111]. Importantly, in many of these systems it remains to be determined whether the presence of certain isotypes directly influences the anti-tumor response, or whether it is simply a reflection of a tumor microenvironment that favors switching to that antibody isotype. The development of pre-clinical mouse models of inducible antibody and ASC deficiency will aid greatly in validating whether this pathway represents a *bona fide* therapeutic target in cancer.

Targeting pathogenic plasma cells

Specifically targeting the long-lived plasma cell population in MM or antibody-driven autoimmune diseases remains challenging. Proteasome inhibitors, such as Bortezomib, can efficiently deplete ASC and MM cells, however, they are not ASC specific, and treatment can

result in adverse effects including neuropathy and cardiovascular toxicity [112]. While there are currently effective therapies to specifically deplete the B cell population, such as the anti-CD20 monoclonal antibody Rituximab, these therapies are unable to target the ASC compartment as the expression of CD20 and many other B cell surface markers are downregulated during the terminal differentiation process [113]. The development of monoclonal antibodies targeting MM surface proteins CD38 (Daratumumab and Isatuximab) and Slamf7 (Elotuzumab) have improved the treatment of MM as they induce the killing of tumor cells through ADCC [114,115]. CD38 and Slamf7 are also expressed on non-malignant ASCs and could potentially be used to target these populations in antibody-driven autoimmune conditions. However, CD38 and Slamf7 expression is also not ASC restricted as CD38 is expressed on red blood cells, T regulatory cells and myeloid derived suppressor cells, while Slamf7 is expressed on the surface of natural killer cells, DCs and $CD8^+$ T cells [114,115]. Multiple therapies targeting BCMA (encoded by *TNFRSF17*), which is more selectively expressed on the surface of ASCs, are currently in clinical trials, and if approved these therapies may allow for the specific targeting of myeloma cells and the plasma cell population [116]. Anti-BCMA antibody-drug conjugates have been developed that can selectively deliver anti-cancer agents to MM cells [117-119]. Bi-specific antibodies that simultaneously bind target cells and immune effector cells are also in development for the treatment of MM, with almost all of these current approaches targeting BCMA and the T cell receptor signalling molecule CD3 [115,120-122]. Targeting BCMA is also the favored approach for targeting myeloma cells using chimeric antigen receptor (CAR) T cell therapies [123-128].

A concern in depleting plasma cells in antibody-driven pathologies, is that current strategies are unable to discriminate between protective ASCs, such as those providing vaccine protection against infections such as measles and tetanus, and their pathogenic counterparts. Identification of specific surface markers expressed by functional subsets may allow for the more precise targeting of sub-populations of ASCs. For example, approximately 80 % of the IL-10^+ plasma cell population that emerges following *Salmonella* infection co-express LAG-3 and CD200 [89]. Therefore, it may be possible to selectively deplete this immunosuppressive subpopulation using therapies that target these markers. As our understanding of ASC subsets

and non-antibody functions increases, it is likely that additional functional subsets, and their associated surface markers, will be identified.

An ideal therapy for the treatment of antibody-driven autoimmune diseases would be to selectively deplete self-reactive long-lived plasma cells based on their antigen specificity, leaving the rest of the plasma cell pool intact. A recent study has made this closer to reality by selectively depleting a population of OVA-specific plasma cells from mice *in vivo* [129]. In this model, all plasma cells are labelled with an OVA-conjugated anti-CD138 antibody. OVA-specific plasma cells will then secrete antibody which binds to the conjugated OVA, effectively labelling themselves. These labelled plasma cells are then vulnerable to depletion through ADCC or complement-mediated pathways. The authors demonstrated that a single dose of their anti-CD138-OVA conjugate was sufficient to deplete approximately 60 % of the OVA-specific bone marrow plasma cell population, leaving other specificities untouched. Such a treatment would also be beneficial for the treatment of allergies or in preventing transplant rejection by selectively depleting allergen- and alloantigen-specific plasma cells respectively, while leaving immunological memory intact.

OUTSTANDING QUESTIONS AND FUTURE AREAS

The differentiation of B cells into ASCs involves dramatic, irreversible changes in the cell's transcriptome, chromatin architecture, morphology and metabolic program. Despite advances in our understanding of how these changes are initiated, how these programs are maintained to allow plasma cells to secrete such massive amounts of antibody throughout their long life-span remains less clear. Our finding that Blimp-1 is required for the establishment, but not the maintenance, of the ASC transcriptional identity suggests that at some stage in the differentiation process ASCs switch from a differentiating to a mature maintenance transcriptional network [16]. When this occurs and how this is controlled are currently unknown. It is also evident that ASCs have developed a specialised protein homeostasis and metabolic program to facilitate high rates of Ig translation and processing, but the question remains: how are ASCs able to maintain such high rates of *Ig* gene transcription? Furthermore, how are these

specialised programs regulated? Studies examining plasma cell metabolism highlight the need to look further than transcriptional regulation in our efforts to understand these processes [76].

There is an increasing appreciation for the diversity that exists within the ASC compartment. This is supported by the recent identification of metabolically and functionally distinct subpopulations, demonstrating that ASCs are neither a homogeneous population, nor solely devoted to antibody secretion [76,89]. Advances in single-cell technologies will likely enable the extent of this heterogeneity to be revealed. However, once these differences are identified, the challenge will be in understanding what drives this variation and whether this knowledge could be used to advance the treatment of diseases induced by pathogenic ASCs or inform how to best engineer vaccines to generate a long-lived protective plasma cell population.

In recent years, there has been great interest in the role that gut-derived IgA^+ plasma cells play in neuroinflammatory conditions such as MS [94,95]. Additionally, gut-derived IgA^+ plasma cells have recently been identified in the meninges in steady state, where they are proposed to express protective anti-microbial IgA as a pre-emptive strike against bacterial dissemination in the CNS [2]. It is likely that future research will pay increased attention to the links between the microbiome, intestinal plasma cells and neuroimmunology. The influence of plasma cells and intra-tumoral antibodies in cancer settings is likely to be another major area of focus. The most pressing unresolved questions in this area are: what drives the association of certain isotypes with a good or bad patient prognosis in different cancer settings and do plasma cells influence the anti-tumor response through both antibody-dependent and -independent mechanisms. Understanding the relationship between plasma cells, intra-tumoral antibodies and anti-cancer responses will inform the most appropriate avenues of treatment for these patients.

ACKNOWLEDGMENTS

This work was supported by the National Health and Medical Research Council (NHMRC) of Australia grants (1155342, 1144905 and 1160830 to S.L.N.), as well as Victorian State Government Operational Infrastructure Support and Australian Government NHMRC IRIIS.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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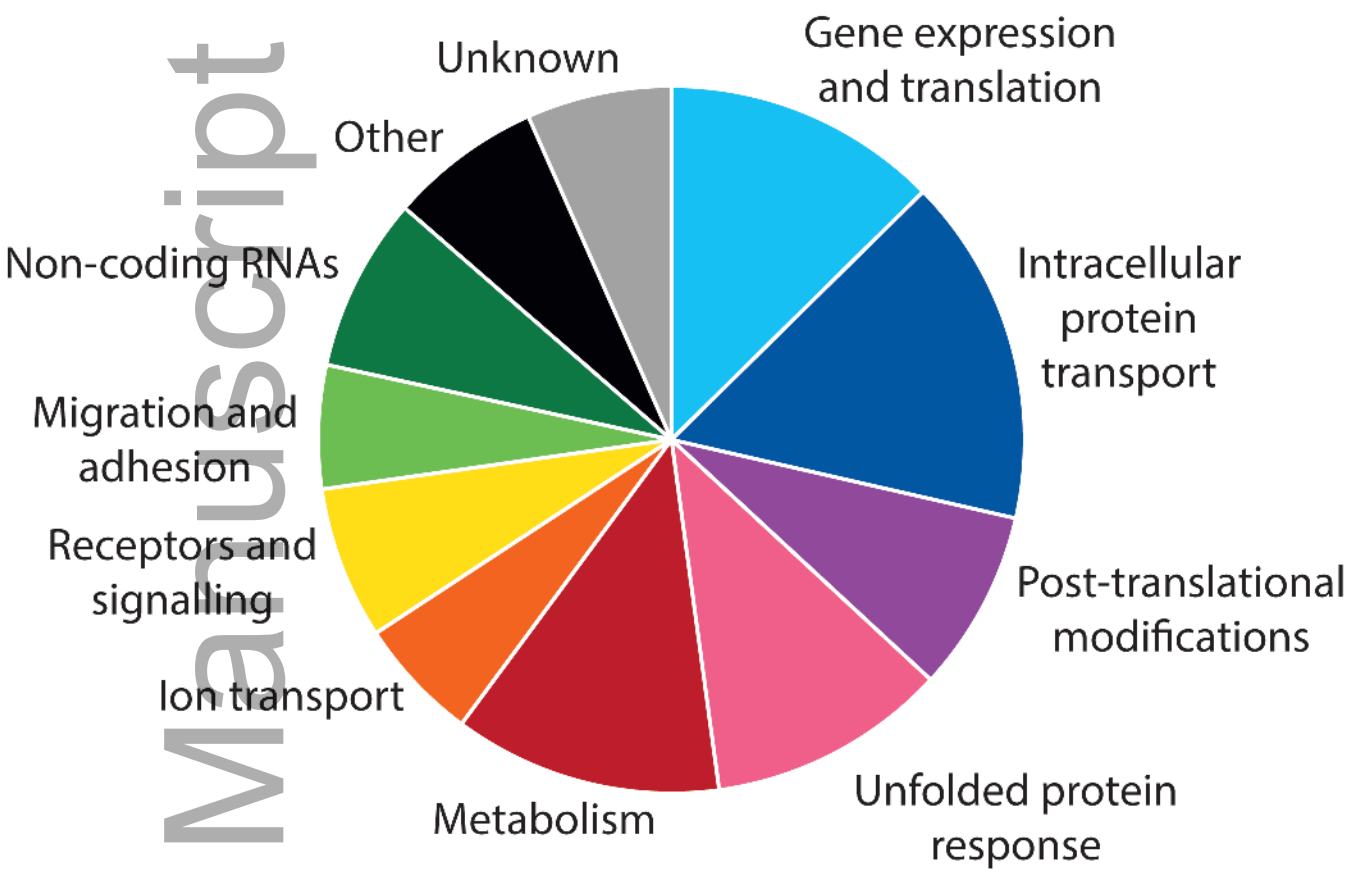
FIGURE LEGENDS

Figure 1. Functional groups within the antibody secreting cell gene signature. Pie chart listing the predicted functions of the 301 genes that compose the mouse ASC gene signature. Data are from [11].

Figure 2. The role of Blimp-1 in regulating antibody secretion. Interactions between the key elements that positively and negatively regulate antibody secretion. Proteins and cellular processes are labelled in black; nutrients are labelled in gray. Lines indicate the type and direction of regulation.

Figure 3. Protein homeostasis within the endoplasmic reticulum. Overview of the ER protein processing pathway with genes that are within the ASC gene signature labelled. Red indicates genes directly regulated by Xbp1 in mouse plasma cell hybridomas [48]. Bold indicates genes directly regulated Blimp-1 in mouse plasmablasts [16,27]. Italics indicates genes directly regulated by Irf4 in human multiple myeloma [130]. Gene function obtained from KEGG pathway: mmu04141.

Figure 4. Activation of the unfolded protein response. (A) In circumstances where the endoplasmic reticulum-associated degradation (ERAD) pathway is sufficient to remove misfolded proteins from the ER and maintain protein homeostasis, the protein chaperone Grp78 is bound to both unfolded proteins and the ER stress sensors Ire1a, Perk and Atf6a. (B) In many cell types, if the concentration of unfolded proteins increases in the ER lumen, Grp78 dissociates from the ER stress sensors, leaving them free to activate the UPR signalling pathway. Ire1a splices *Xbp1* mRNA, allowing the translation of the transcriptionally active form sXbp1. sXbp1 can transit to the nucleus and activate expression of ERAD and UPR genes, leading to ER expansion. Perk phosphorylates eIF2a, causing a block in global translation. This cellular state favors the translation of Atf4. Atf4 induces transcription of Gadd34 and the pro-apoptotic Chop. Prolonged Chop expression leads to cell death. Atf6a transits to the Golgi where it is cleaved, releasing the p50 fragment. p50 then drives expression of Xbp1 and ERAD genes. (C) Due to their need to stably process huge amounts of protein for an extended period, ASCs have a modified UPR. The Ire1a and Atf6a arms of the UPR are active in ASCs, but the Perk/Atf4/Chop apoptosis pathway is selectively deactivated.



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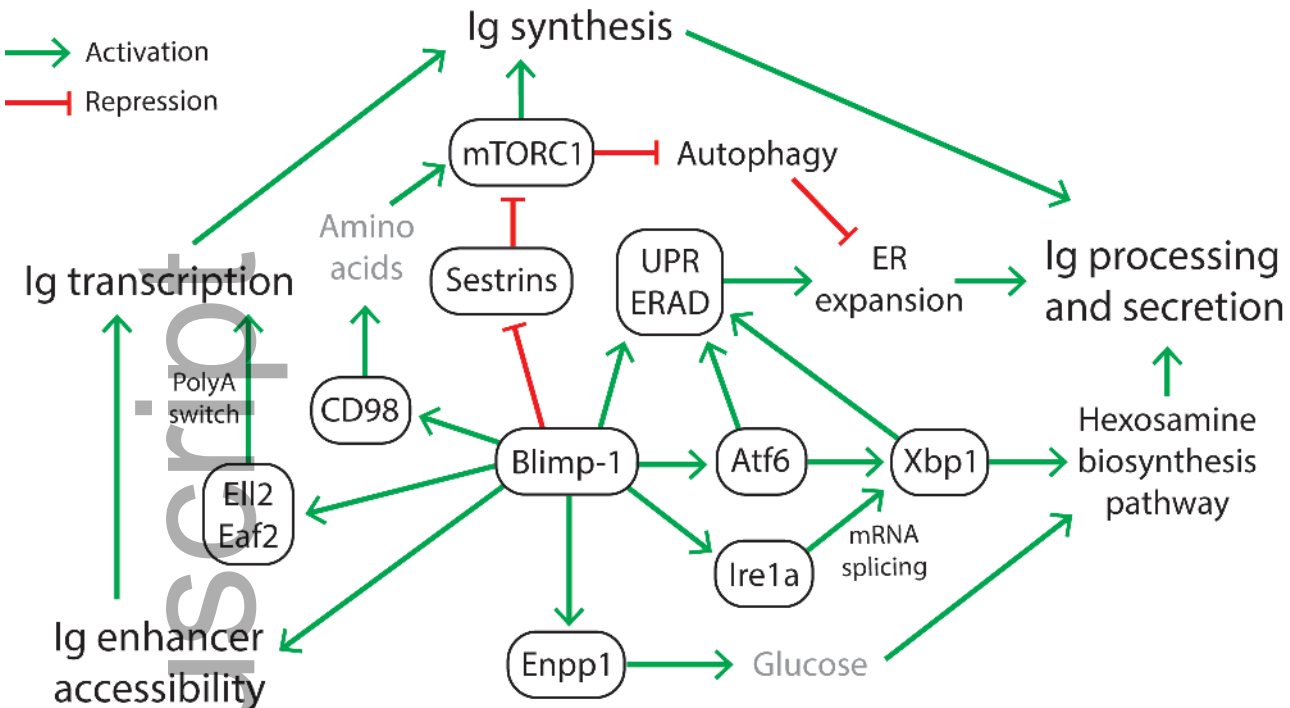

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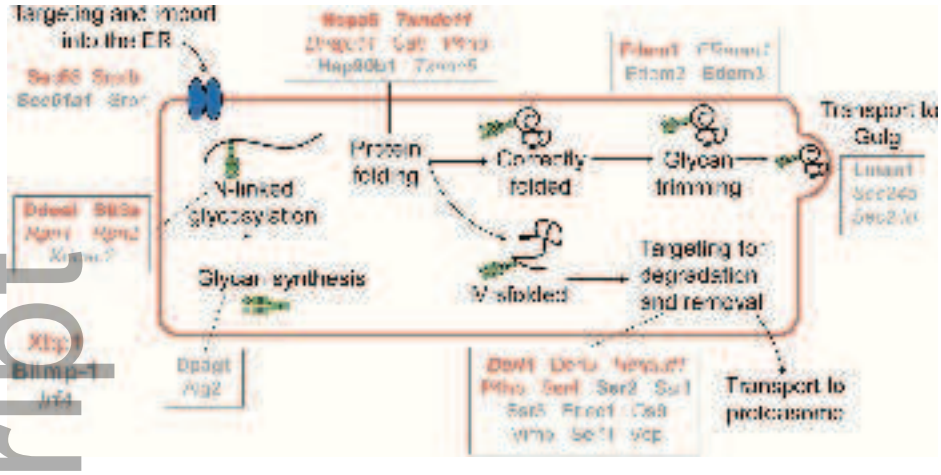

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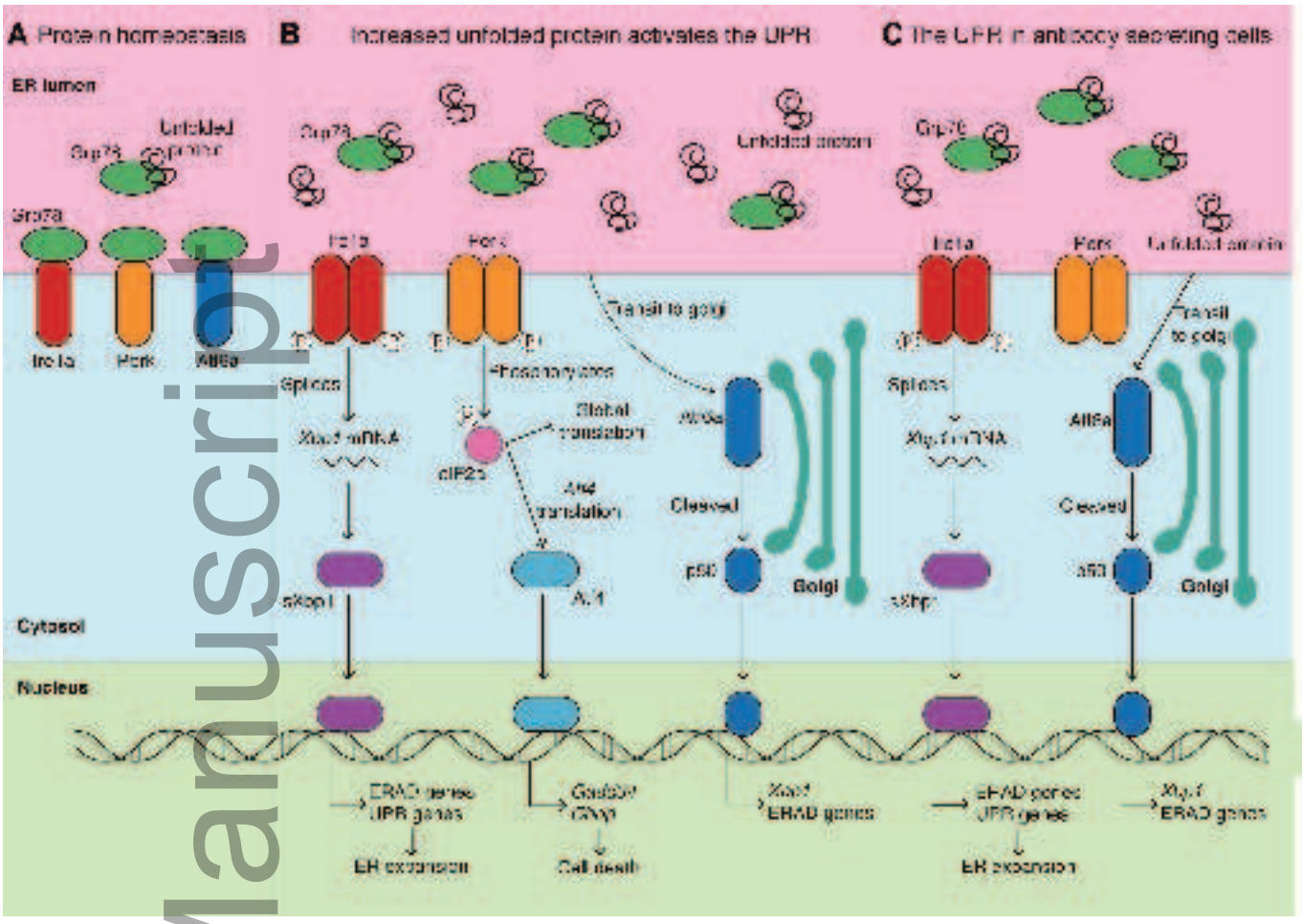

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