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Plasma cells: you are what you eat

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Summary

Plasma cells are terminally differentiated B lymphocytes that constitutively secrete antibodies. These antibodies can provide protection against pathogens, and their quantity and quality are the best clinical correlates of vaccine efficacy. As such, plasma cell lifespan is the primary determinant of the duration of humoral immunity. Yet dysregulation of plasma cell function can cause autoimmunity or multiple myeloma. The longevity of plasma cells is primarily dictated by nutrient uptake and non-transcriptionally regulated metabolic pathways. We have previously shown a positive effect of glucose uptake and catabolism on plasma cell longevity and function. In this review, we discuss these findings with an emphasis on nutrient uptake and its effects on respiratory capacity, lifespan, endoplasmic reticulum stress, and antibody secretion in plasma cells. We further discuss how some of these pathways may be dysregulated in multiple myeloma, potentially providing new therapeutic targets. Finally, we speculate on the connection between plasma cell intrinsic metabolism and systemic changes in nutrient availability and metabolic diseases.

Keywords

plasma cell; metabolism; glucose; glutamine; multiple myeloma

1. INTRODUCTION

The distinguishing feature of adaptive immunity is “immunological memory”, in which long-lived effector populations persist long after antigen is cleared. In this regard, both B and T lymphocytes exhibit similar characteristics and comparable stages of activation, clonal expansion and contraction, and differentiation. This is advantageous for researchers in lymphocyte biology, as mechanistic insights in one lineage can usually be extended to the analogous populations arising in the other. Plasma cells, however, are an exception to this rule, as they lack a similar counterpart in the T cell lineage. While this presents the difficulty of drawing parallels, it also provides a clean slate to examine and explore unique metabolic properties of these cells.

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Declaration of interests

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Plasma cells are terminal effectors within the B cell lineage and devote most of their transcriptional and metabolic resources to synthesize antibodies ^{1, 2}. As a consequence, relatively small numbers of cells can produce large quantities of protective antibodies ³. Thus, the specificity and longevity of plasma cells are the major determinants of durable humoral immunity ⁴. The resultant antibodies pre-exist and provide sterilizing immunity against pathogens upon secondary exposures to physiological inocula. The longevity of this protection was highlighted in a 26-year longitudinal study in humans, whose aim was to estimate the durability of the antibody response against previously administered vaccines and antecedent infections. The authors estimated the half-life of antibody production to range between 11 years up to an entire lifetime, depending on the specific vaccine or infection ⁵. This parameter is not to be confused for the half-life of an antibody molecule, which ranges from a few days to several weeks depending on the isotype ⁶. This sustained level of lifelong immunoglobulin production indicates that plasma cells are either continuously generated through activation of upstream B cells or are a fixed pool of cells that persist without replenishment. Non-specific and periodic activation of memory B cells by toll-like receptor ligands was proposed to generate plasma cells and maintain durable antibody-mediated immunity ⁷. However, more persuasive evidence for the latter mechanism came from reports in mice showing that antigen-specific plasma cell numbers in the spleen gradually waned post immunization, while the numbers of bone marrow resident plasma cells increased and then remained constant for the entire duration of the assay ^{8, 9}. Further, serum antibody titers were unaffected in humans or mice treated with depleting antibodies against CD20, which is expressed by upstream B cells but not by plasma cells. Moreover, human studies have found no correlation between memory B cell numbers and serum antibody titers ^{5, 10–12}. Finally, other researchers found no evidence that inoculation of mice with TLR ligands led to meaningful activation of memory B cells *in vivo* ¹³. These data demonstrate that the durability of antibody production is directly linked to plasma cell lifespan, and not replenishment from re-stimulated memory B cells. Thus, in the context of the findings by Amanna and colleagues, we conclude that the different half-lives of antibody production after vaccination or infection are due to heterogeneity in the lifespan of the plasma cells ⁵. Defining pathways that promote plasma cell longevity is a major goal for vaccine development, especially for immunizations that lead to very transient protection against infections ^{14, 15}.

Plasma cell lifespan and anatomical location are properties often used to distinguish antibody-secreting cell subsets. The shortest-lived plasma cells persist only for several days after their formation and are mostly localized to the extrafollicular regions of secondary lymphoid organs ^{16, 17}. In contrast, the longest-lived plasma cells are largely bone marrow-resident and persist for the entire lifespan of the organism ^{8, 9, 18}. Many plasma cells of intermediate lifespans have recently been found in the spleen, bone marrow, and intestine. In humans, CD19 expression marks relatively short-lived plasma cells in both the bone marrow and the intestine ^{19–21}. In mice, plasma cell subsets of varying lifespans can be distinguished by expression of B220, B-lymphocyte induced maturation protein 1 (BLIMP1), CD93 (also known as AA4.1), major histocompatibility class 2 (MHCII), CXCR3, and uptake of the fluorescent glucose analog 2-deoxy-2-(7-Nitro-2,1,3-benzoxadiazol-4-yl)amino)-D-glucose (2NBDG) ^{1, 22–25} (Figure 1). Plasma cells can also be classified based on their non-antibody

producing functions, exemplified by Lymphocyte-Activation gene 3 (LAG3) expression. LAG-3⁺ plasma cells were found to secrete Interleukin-10 (IL-10) and exhibit a more natural regulatory phenotype as compared to LAG3[−] plasma cells²⁶. These multiple phenotypes should be considered and integrated with our explanation of plasma cell longevity and heterogeneity.

In addition to neutralization of pathogens, antibodies also perform other effector functions, which include activation of complement cascades, recruitment of phagocytes, triggering of mast cells and basophils during hypersensitivity reactions, passive protection of developing fetuses and neonates, as well as regulating bacterial populations and biofilms in mucosal surfaces such as the gut^{27–29}. Yet aside from their otherwise protective roles, self-reactive antibodies are associated with many autoimmune disorders. Plasma cells can also become transformed to cause multiple myeloma (MM), a bone marrow-associated malignancy³⁰. In this review, we discuss the recent findings surrounding the transition from B cells to the plasma cell fate and the metabolic changes that accompany it. We also discuss plasma cell metabolism as a function of both intrinsic and extrinsic signals that shape the function of these cells during health and disease, and finally offer insights and future directions in light of recent work.

2. METABOLIC CHANGES DURING B CELL ACTIVATION AND DIFFERENTIATION

Much of the literature regarding B cell and plasma cell metabolism has been recently reviewed^{31–33}. We will re-summarize many of these conclusions below and in Figure 2, but we place the majority of our emphasis on the potential meaning and caveats of our own findings, and future directions for the field.

2.1 Antigen-inexperienced B cells

B cell development is a process that initiates in the bone marrow and continues in the spleen across phenotypically distinguishable CD19⁺ subsets³⁴. Naïve B cells are composed of antigen-inexperienced follicular (also known as B2), marginal zone, B1a, and B1b subsets. The average lifespan of a naïve B2 B cell is approximately 13 weeks, as compared to marginal zone B cells (22 weeks), the other mature antigen-inexperienced B cell population in the periphery³⁵. The transcription factor Paired Box 5 (PAX5) is crucial for the maintenance of B cell identity, and by modulating its target genes, is responsible for a large fraction of the transcriptional changes that mediate lineage commitment and maturation^{36, 37}. Despite this critical role in establishing and maintaining B cell identity, Pax5 is frequently mutated in B cell malignancies³⁸. To explain the tumor-suppressive role of PAX5, a series of elegant papers demonstrated that in pre-B Acute Lymphoblastic Leukemia (ALL), PAX5 suppresses glucose uptake and ribonucleoside synthesis^{39, 40}. Mutating PAX5 alleviates some of these metabolic constraints, thereby allowing pre-B ALL cells to divide indefinitely. Presumably PAX5 plays a similar role in naïve B cells, thereby ensuring minimal baseline metabolic activity⁴¹. Naïve B cells also rely on B cell receptor (BCR)- and B-cell activating factor (BAFF)-mediated signals through the mammalian target of rapamycin (mTOR) pathway, which has been shown to contribute to increases in cell size,

mitochondrial activity, protein synthesis, and general activity of B cells prior to antigen-induced proliferation^{42–44}. In addition to naïve B cells, marginal zone B cells are also mature antigen-inexperienced splenic B cells and are crucial participants in responses to T-independent antigens^{45, 46}. However, little is documented about the metabolic status of these cells, save that B cell-specific ablation of tuberous sclerosis 1, a subunit of the mTORC1 inhibitor complex, led to a dramatic loss of marginal zone B cells and affected downstream T-independent responses⁴⁷.

2.2 Activated B cells

Naïve B cell metabolic activity is triggered upon B cell receptor signaling and/or Toll-like receptor activation. The B cell activation program induces changes in cell size, increased uptake of nutrients, and increased rate of transcription and translation^{48–50}. In addition to B cell receptor engagement, B cells also receive additional signals such as those through the Toll-like receptors, as well as costimulatory and cytokine signals provided through T cell help. This two-signal mode of activation reflects a similarity between T and B lymphocytes, and in the absence of this second signal, B cells undergo mitochondrial dysfunction and superoxide-induced cell death⁵¹. Co-stimulation also favors uptake of nutrients necessary for anabolism to support activation. Glucose uptake increases concomitantly with upregulation of the glucose transporter GLUT1 (SLC2A1) and is catabolized through glycolysis⁵². The resultant lactate allows for restoration of NAD⁺ and redox balance⁵³. Alternatively, pyruvate derived from glycolysis can be carried into mitochondria to contribute carbons to the tricarboxylic acid cycle⁵³. Glucose can also be oxidized through the pentose phosphate pathway, which generates ribonucleosides that are critical for subsequent proliferation⁵². Through the tricarboxylic acid cycle, glucose-derived pyruvate can be converted into citrate, which further feeds into lipogenic pathways through activity of the enzyme ATP-citrate lyase⁴⁹. These pathways feed into the formation of cholesterol, phosphatidylcholine, phosphatidylinositol, ceramides, and other fatty acids, which in turn serve as substrates for endoplasmic reticulum (ER) and plasma membrane expansion as well as organelle biogenesis⁴⁹.

In addition to glucose, glutamine uptake also increases after B cell activation. This is likely achieved by the PDK1- and Akt- dependent induction of *Slc3a2* expression, a common subunit of multiple amino acid transporters^{54, 55}. In addition to glutamine, this transporter is crucial for the uptake of multiple large neutral amino acids, which are substrates for protein synthesis and feed into other metabolic pathways⁵⁶. While SLC3A2 pairs with SLC7A5 to form CD98, it can also pair with SLC1A5 to make up the ASCT2 transporter, both of which facilitate the uptake of large neutral amino acids by B cells⁵⁷. Glutamine can feed into the TCA cycle as α -ketoglutarate, thereby acting as an anaplerotic substrate to replenish TCA cycle intermediates⁵³. Through the TCA cycle, glutamine can be used to generate other amino acids such as glutamate and aspartate, citrate for use in lipogenic pathways, and succinate which is oxidized to provide electrons for respiration and ATP generation²³. The uptake of both glucose and glutamine are tightly regulated processes and are controlled by expression of the microRNA let-7, which suppresses expression of Hexokinase-2 and c-Myc⁵⁸. In addition to these nutrients, leucine uptake promotes mTORC1 activation in B cells⁵⁹. Thus, activation signals promote nutrient uptake to allow B cells to expand and divide.

After exposure to the antigen and initiating activation programs, B cells migrate towards the interface between the T and B cell zones in the secondary lymphoid organ to recruit help from T cells ⁶⁰. T cells in turn, through recognition of the peptide-MHC-II complex on the surface of B cells, provide help to B cells in the form of costimulatory interactions involving CD154-CD40, ICOS-ICOSL, OX40-OX40L, LFA-2-ICAM-1 as well as through secretion of cytokines and growth factors ⁶¹. These initial interactions enable B cells to subsequently undergo proliferate and form foci at the outer edges of the B cell follicles ⁶². Some of these cells may undergo isotype switching and differentiate into short-lived plasma cells and contribute to the early humoral response while others can form memory B cells ^{63, 64}. Alternatively, some B cells migrate to the centers of B cell follicles and establish germinal centers (GCs) ⁶⁵.

2.3 Germinal centers

Depending on the infection or immunization, GCs can be detected as early as 3 days post-immunization and can persist for many weeks ^{66–69}. The GC is organized into a dark zone, consisting of highly proliferative B cells, and a light zone comprised of non-dividing B cells ⁷⁰. Within the germinal centers, B cells express activation-induced cytidine deaminase (AID), which is responsible for both somatic hypermutation and immunoglobulin isotype-switching ⁷¹. Dark-zone GC B cells proliferate rapidly while accumulating somatic mutations in antibody receptor-encoding genes ^{72, 73}. These cells then migrate to the light zone where they compete among themselves for antigen, which is endocytosed and subsequently presented through MHCII to T cells in an attempt to procure survival signals ⁷³. Only a small fraction of these cells are selected in the light zone and subsequently return to the dark zone undergo more rounds of proliferation, class switching, and affinity maturation.

Much of the proliferative burst in the dark zone has been shown to rely on c-Myc, as its ablation leads to complete abrogation of GCs ^{74, 75}. c-Myc is induced in GC B cells by the action of BCR and CD40 signals ⁷⁶. Signals through the B cell receptor and CD40 also induce mTOR activation, thereby permitting B cells to re-enter cycles of proliferation ^{76, 77}. c-Myc also promotes glycolytic activity by upregulating Hexokinase and Pyruvate kinase in activated cells while modestly increasing enzyme expression of the downstream tricarboxylic acid cycle and pentose phosphate pathways ⁷⁸. In T cells, c-Myc also leads to CD98 upregulation and upregulation of Glutaminase 2 (Gls2), suggesting therefore that it also participates in glutamine metabolism ⁷⁸. It is possible that a similar metabolic regulation is also at play in GC B cells. Intriguingly though, c-Myc is inhibited by the GC-promoting transcription factor B cell lymphoma 6 (BCL6) and is undetectable in DZ B cells ^{74, 75, 79}. To mediate its effects throughout the dark zone, c-Myc activates the expression of AP4, which maintains expression of many c-Myc targets ⁷⁹. c-Myc downregulation is a crucial event leading to the expression of Glycogen synthase kinase 3 (GSK-3), which in turn promotes glycolysis, mitochondrial biogenesis, and ROS formation in proliferating B cells ⁸⁰.

The rapid proliferation and nutrient consumption within GC B cells potentially render them susceptible to starvation-induced cell death. Thus, autophagy is another key pathway

upregulated in GC B cells. Interestingly though, B cell activation transiently downregulates mTORC1-dependent canonical autophagy while promoting upregulation of the non-canonical pathway⁸¹. The large numbers of proliferating cells may also induce a state of low oxygen availability within the GC, observed by hypoxia-marking dyes and expression of HIF1 α . Genetic deletion of VHL, an inhibitor of HIF1 transcription factors, reduces cellular proliferation, AID expression, and dampens mTOR signaling in GCs⁸².

GCs can output memory B lymphocytes and plasma cells. Early hapten studies reported that GC-derived memory B cells tend to express antibodies of lower affinity than do plasma cells, suggesting the existence of an affinity threshold that distinguishes these fates within the GC⁸³. Our own studies have found that the isotype-switched memory B cell compartment is significantly more diverse than long-lived plasma cells³. This allows for memory B cells, but not long-lived plasma cells to mount recall responses to divergent and heterologous pathogens. Mechanistically, GC B cells fated for the memory B cell lineage, identified by expression of several markers, may be actively selected for low affinity^{84–86}. This is mediated in part by BACH2, a critical transcription factor necessary for the memory B cell lineage, which is repressed when cells receive strong T Follicular helper (Tfh) cell-mediated signals⁸⁶. Reciprocally, GC B cells that receive Tfh signals induce high levels of Interferon Regulatory Factor 4 (IRF4) to promote the plasma cell fate (discussed more below). The active selection of low affinity GC cells into the memory compartment is puzzling, as it is unclear how such cells would be selected without also recruiting autoreactive or irrelevant B cells. In an elegant genetic system in which isotype switching is mediated by Cre recombinase but AID-mediated somatic hypermutation is prevented, memory B and plasma cell formation is largely unperturbed⁸⁷. These data argue against an absolute final affinity threshold that must be reached. Since each GC is an autonomous unit with different clonal compositions, it remains possible that a relative affinity threshold still exists⁸⁸. In this case, cells with relatively high antibody affinities within a given GC might be recruited into the plasma cell compartment, irrespective of the absolute on- and off-rates. Regardless, long-lived plasma cells derived from GCs are formed relatively late in the response, whereas memory B cells are formed early⁶⁶. Thus, distinct signals must be provided in early versus late GCs. In helminth infections, Tfh cells within GCs express IL-21 initially, but gradually lose IL-21 and express IL-4 instead⁸⁹. Of note, IL-4 promotes glucose uptake by B cells, suggesting that part of the signaling differences between early and late GCs may be metabolic in nature⁹⁰. Indeed, many of the transcriptional differences between early and late germinal centers are in genes involved in metabolism⁶⁶.

2.4 Memory B cells

Memory B cells are composed of several subsets that differ in their ability to generate new GCs and plasma cells. In both mice and humans, IgM+ memory B cells tend to generate GCs preferentially, whereas IgG+ memory cells are skewed toward the plasma cell fate^{67, 91, 92}. These lineage biases are likely intrinsic to the memory cell transcriptional program rather than the strength of BCR signals, as CD80 and PDL2 expression can functionally separate subsets irrespective of the antibody isotype⁹³. While metabolic pathways in memory T cells have been studied extensively, memory B cell metabolism remains largely unexplored with a few recent exceptions⁹⁴. Like their GC and plasma cell counterparts,

memory B cell responses depend on autophagy. Memory B cells deficient in the autophagy gene ATG7 are present in normal numbers initially after immunization or infection with influenza, but then wane rapidly^{95, 96}. This diminution results from oxidative stress rather than apoptosis or necrosis. Another recent study showed that toll-like receptor ligands and Interferon- α caused human IgD⁺ memory B cells to engage glycolysis and the mTORC1 pathway *in vitro*. Compared with naïve B cells, this led to enhanced differentiation by memory B cells into plasmablasts⁹⁷. It is possible that these functional differences are caused by enhanced sensitivity to toll-like receptor ligands by memory B cells.

2.5 Plasma cells

The exit signals from the GC reaction that promote plasma cell formation are not fully known. Recent studies have suggested that high-affinity B cells in the light zone receive strong CD40 signals, downregulate BCL6, and exit the GC reaction as IRF4⁺ CD69⁺ cells that eventually differentiate into plasmablasts⁹⁸. Expression of IRF4 is important for the formation and function of GCs^{99–101}. Its subsequent upregulation then enables IRF4 to associate with low affinity DNA binding sites and promote expression of BLIMP-1^{99, 100, 102}. BLIMP-1 expression then silences GC B cell transcriptional programs while simultaneously promoting expression of genes required for plasma cell differentiation and function^{59, 103}. Initially, plasmablasts are short-lived, immature, proliferating cells, but gradually acquire a more mature plasma cell phenotype based on BLIMP-1 expression²⁴. Plasma cells formed in the earliest stages of the response tend to be largely short-lived and persist only for a few days in the periphery, though there is substantial heterogeneity in turnover rates^{16, 22, 23}. Some plasma cells produced towards the end of the GC response migrate to the bone marrow and persist there in survival niches, durably secreting high-affinity antibodies^{8, 9, 18, 66, 104}.

Once a plasma cell is formed, much of its metabolism is diverted from organelle biogenesis and anabolism towards antibody synthesis and glycosylation. Through much of B cell activation and its subsequent stages, glucose is taken up via the transporter GLUT1, and it is likely that it remains the chief glucose transporter in these cells⁵². Genetic ablation of GLUT1, however, does not fully ablate plasma cell formation, suggesting that glucose uptake might depend on additional as yet unidentified sugar transporters⁵². GLUT6 (*Slc2a6*) and the solute carrier SLC50A1 are candidates to perform this function as they are highly expressed in both murine splenic and bone marrow plasma cells^{23, 105}. Yet GLUT6 remains largely uncharacterized, whereas SLC50A1 has primarily been implicated in sugar efflux rather than import^{106, 107}. Primary human plasma cells represent even more of a mystery, as none of the known glucose transporters are highly expressed at the transcript level, despite clear evidence of glucose import and catabolism^{19, 105}. Irrespective of its mode of entry, much of the glucose taken up by plasma cells is shuttled into hexosamine biosynthetic pathways in plasma cells, which provides sugars for protein glycosylation^{48, 105}.

Glycosylation patterns influence how antibodies interact with Fc receptors, thereby conferring distinct effector functions¹⁰⁸. Glycosylation primarily occurs at Asn297 as an N-linked glycan in the constant region of the heavy chain, while a secondary site also exists in

some antibodies in the variable region of the heavy chain. While mutating this residue to a glutamine has little impact on antibody formation, inhibiting glycosylation completely using tunicamycin results in plasma cell death *en masse* by promoting protein misfolding and an excessive unfolded protein response^{109, 110}. It is intriguing to note that conditions of inflammation or priming antigen can modulate glycosylation patterns on the surface of the antibody molecule^{111–113}. The signals to plasma cells and downstream mechanisms of differential glycosylation remain unknown.

Plasma cells secrete thousands of antibody molecules per second, and this depends upon an expanded ER. This expansion is primarily driven by the Inositol-requiring enzyme 1 α (IRE1 α) pathway, the most well-studied of the unfolded protein response (UPR) signals in plasma cells¹¹⁴. IRE1 α is a ribonuclease and splicing factor, responsible for the removal of 26 internal nucleotides in XBP-1 transcripts, leading to the formation of spliced X-Box Binding Protein 1 (sXBP1)¹¹⁵. In turn, sXBP1 promotes mitochondrial and ER biogenesis, and its deletion in B cells leads to reduced ER expansion and antibody secretion^{114, 116, 117}. This deletion however does not impact plasma cell numbers, suggesting that antibody secretion is not essential for differentiation^{118, 119}. sXBP-1 expression also shuttles glucose towards hexosamine biosynthesis by regulating expression of key enzymes like Glutamine Fructose-6-phosphate transferase 1 (GFAT1), Glucosamine-phosphate N-acetyltransferase 1 (GNPNAT1) and phosphoglucosyltransferase 3 (PGM3)¹²⁰. In plasma cells, the IRE1 α pathway and sXBP1 targets were found to be equivalent across long-lived 2NBDG+ and short-lived 2NBDG– subsets, suggesting that this mode of UPR is important for antibody secretion but does not correlate with longevity²³. A second pathway downstream of the UPR is cleavage of ATF6 α , leading to a functional transcription factor¹²¹. Expression of ATF6 α targets was also similar across plasma cell subsets, and its genetic deletion had no effect on plasma cell longevity or antibody secretion^{23, 122}. The third pathway activated by the UPR is phosphorylation of eif2 α ¹²¹. Based on *in vitro* cultures, this pathway was proposed to be inactive in plasma cells^{123–125}. However, analysis of plasma cells directly *ex vivo* showed robust activation of this pathway, with slightly elevated p-eif2 α levels in the B220+ subsets²³. Accordingly, ablation of EIF2AK3/PERK, the kinase that phosphorylates eukaryotic translation initiation factor 2 α (eif2 α) during ER stress, led to a selective loss of B220+ plasma cells²³. However, within the B220+ plasma cells, 2NBDG+ cells were similarly sensitive to PERK deletion as were shorter-lived 2NBDG– subsets²³. Moreover, B220– plasma cells were unaffected by the loss of PERK²³. Thus, despite previous findings demonstrating that ER stress-dependent pathways effect *in vitro* plasma cell death, the differences in lifespan between plasma cell subsets *in vivo* are not explained by alterations in the UPR^{126–128}.

Interestingly, long-lived plasma cells show equivalent rates of antibody expression as do their short-lived counterparts but have a greater rate of immunoglobulin secretion²³. These differences are again unlikely to be explained by ER stress responses, given that UPR pathways are activated similarly across all subsets²³. Against our predictions, short-lived plasma cell antibody secretion could not be consistently enhanced by provision of exogenous glycosylation sugars. This suggests that a shortage of these intermediary metabolites do not explain diminished secretion rates, though we cannot be certain that these glycosylation sugars were properly incorporated via the hexosamine salvage pathway¹²⁹.

Instead, provision of additional extracellular amino acids enhanced mitochondrial respiration, intrinsic amino acid biosynthesis, and antibody secretion. This is partly driven by glutamine, which is used for mitochondrial anaplerotic reactions and for glutamate and aspartate synthesis²³. Stable isotope tracing experiments demonstrated that 20-30% of succinate, which is oxidized to fumarate to generate electrons for respiration, is contributed by glutamine²³. Yet it is unlikely that glutamine is responsible for the entire effect of extracellular amino acids, as we have preliminarily observed no survival or secretion defects in glutaminase-deficient plasma cells. Mechanistically, it seems unlikely that these additional amino acids promote antibody secretion by enhancing the rate of translation, as immunoglobulin transcripts and proteins are produced similarly across plasma cell subsets²³. Instead, it may be that enhanced energy production, mitochondrial anaplerosis, or effects on the secretory apparatus indirectly facilitate antibody release¹³⁰.

A major unresolved question is how these metabolic programs are initiated and maintained in long-lived plasma cells. A number of signals, such as TLR ligands, BCR ligation, and cytokines such as IL-4 promote glucose uptake when B cells become activated^{48, 52, 77, 80, 90}. Yet whether these same signals instruct a metabolic program as plasma cells are generated is unknown. Moreover, it is unclear whether extrinsic niche factors maintain this metabolic program in plasma cells, or whether a self-sustaining metabolic feedback loop is initiated during ontogeny. As these questions are resolved, new strategies to enhance or antagonize plasma cells for therapeutic purposes should become clear.

3. A METABOLIC EXPLANATION FOR PLASMA CELL LONGEVITY

Recent studies have demonstrated surprising transcriptional similarity between short- and long-lived plasma cells, both in humans and in mice. In human cells, unbiased comparisons of short-lived tonsillar plasma cells with long-lived bone marrow plasma cells revealed only 7 transcriptional differences¹³¹. Comparisons of refined populations of short-lived CD19+ plasma cells with long-lived CD19– plasma cells in the human bone marrow also revealed relatively few differences, only a fraction of which were consistent across studies^{19, 23}. In the mouse, transcriptional profiles were again similar when splenic (ostensibly) short-lived plasma cells were compared with bone marrow plasma cells¹. Our own studies using bulk and single-cell RNA-seq demonstrated that except for genes involved in proliferation and, unexpectedly, neutrophil effector pathways, the transcriptomes between 2NBDG+ and 2NBDG– mouse plasma cells were surprisingly similar^{23, 105}. We were able to corroborate differences in cellular proliferation across subsets with incorporation of the thymidine analog 5-Bromo-2'-deoxyuridine (BrdU), and it may be possible that neutrophil degranulation pathways may play longevity-independent effector roles in the plasma cell subsets²³. Genes such as BLIMP-1, Myeloid cell leukemia 1 (MCL1), CD28, and B-cell maturation antigen (BCMA), which are essential for the long-term survival of plasma cells, were similarly expressed transcriptionally across all mature mouse plasma cells irrespective of their longevity^{1, 23, 105, 132–135}. In previous work we and others demonstrated that the transcription factor ZBTB20 promotes plasma cell longevity^{136, 137}. Reciprocally, ZBTB32, a related family member, antagonizes plasma cell longevity¹³⁸. In light of the lack of obvious transcriptional differences between short- and long-lived plasma cells, the direct effects of ZBTB20 might actually occur in upstream germinal center reactions, perhaps to

impose a stable metabolic program in plasma cell precursors¹³⁹. Similarly, ZBTB32 is expressed in upstream memory B cells rather than in plasma cells themselves^{93, 138}. We certainly cannot exclude the possibility that very subtle alterations in transcription below our limit of detection cause large changes in plasma cell lifespan. However, if such functionally important and consistent transcriptional changes do exist, it will be difficult to uncover these differences using currently available methods. It is likely, therefore that differences in post- or non-transcriptional regulation, such as metabolism, explain the variability of plasma cell lifespan across subsets.

3.1 Mitochondrial spare respiratory capacity as a determinant of plasma cell longevity

Previous studies in other cell types demonstrated mitochondrial spare respiratory capacity as a metabolic correlate of longevity^{140, 141}. SRC is the difference between the maximum possible ability of a cell to synthesize ATP through the mitochondrial pathway and its basal rates. Spare respiratory capacity is by definition revealed by treatment with uncouplers such as 2,4-Dinitrophenol (DNP) or Carbonyl cyanide-4-trifluoromethoxyphenylhydrazone (FCCP), which are ionophores that permeabilize the inner mitochondrial membrane. These ionophores thus force cells to respire maximally in a futile effort to restore the electrochemical gradient, thereby uncoupling ATP synthesis from respiration¹⁴². The property of spare respiratory capacity was shown in long-lived cerebrocortical neurons and was proposed to act as a bioenergetic buffer to promote longevity¹⁴⁰. The physiological importance of spare respiratory capacity, however, has been difficult to demonstrate for several reasons. First, spare respiratory capacity is defined *in vitro* after addition of uncouplers such as FCCP. Because cells would never be exposed to such treatment *in vivo*, the pathways exposed by such pharmacological manipulations *ex vivo* might never be used under physiological conditions. Second, genetic perturbations remained to be discovered that would allow for a specific ablation of spare respiratory capacity while leaving basal respiration intact. Thus, when our initial observations demonstrated much greater spare respiratory capacity in long-lived plasma cells than in short-lived plasma cells, much work remained to prove the importance of this property *in vivo*.

Fortuitously, we discovered that treatment of long-lived plasma cells with UK5099, a drug that prevents pyruvate from entering the mitochondria, specifically ablated spare respiratory capacity but left basal respiration intact^{105, 143}. Even more fortuitously, our colleagues had recently generated mice conditionally deficient in MPC2, an essential subunit of the mitochondrial pyruvate carrier¹⁴⁴. The heterodimeric complex of MPC1 and MPC2 is the target of UK5099, and each subunit is critical for carrying cytoplasmic pyruvate into the inner matrix of the mitochondria^{145, 146}. Deletion of *Mpc2* led to a progressive loss of bone marrow plasma cells *in vivo* as well as antigen-specific antibodies, but not upstream B cells¹⁰⁵. Thus, to our knowledge, this study was the first to formally demonstrate that the *ex vivo* property of spare respiratory capacity was important *in vivo* for cellular longevity.

Based on available evidence, apoptosis is likely the dominant mechanism that leads to short-lived plasma cell death upon bioenergetics crises. Mice carrying transgenes of *Bcl2* maintain plasma cell numbers after immunization¹⁴⁷. Reciprocally, plasma cells that lack *McI1* die rapidly¹³³. Yet despite the dominance of apoptosis as a death effector mechanism in plasma

cells, other pathways may also contribute. Indeed, plasma cells lacking pro-apoptotic *Bim* are not fully protected from death¹⁴⁸. Several studies have proposed ER stress-dependent death, though these pathways are equally active in short- and long-lived plasma cell subsets^{23, 126, 127}. Aside from apoptosis, the most widely accepted forms of intentional cell death are necroptosis, pyroptosis, and ferroptosis^{149–151}. External methods of clearance mediated by phagocytes are another mechanism of clearance, which is used to remove aging red blood cells¹⁵². An unusual form of oxidative death has been described in *Atg7*^{-/-} memory B cells, but to our knowledge these alternate death effector mechanisms have not been studied in primary plasma cells⁹⁵.

3.2 Nutrient uptake by plasma cells

Our findings with UK5099 and genetic knockouts demonstrated that mitochondrial pyruvate import is the basis for spare respiratory capacity in long-lived plasma cells. The spare respiratory capacity differences between short- and long-lived plasma cells could not obviously be linked to intrinsic differences in mitochondrial function or numbers, yet this issue still needs to be explored further. Mitochondria from long-lived plasma cells are larger and appear more fused than those in short-lived plasma cells, and mitochondrial fusion has been shown to be critical for mediating memory T cell function^{105, 153}. Regardless, pyruvate is derived from glycolysis, as inhibiting glyceraldehyde-3-phosphate dehydrogenase (GAPDH) dramatically reduced spare respiratory capacity¹⁰⁵. Consistently, halving the glucose concentrations in the assay media led to a decrease in spare respiratory capacity. These data implicate increased glucose uptake by long-lived plasma cells relative to short-lived plasma cells as the primary basis for spare respiratory capacity.

Consistent with this conclusion, *in vivo* uptake of the fluorescent glucose analog 2NBDG correlates well with plasma cell longevity. Bone marrow long-lived plasma cells imported high amounts of 2NBDG whereas splenic plasma cells were more heterogeneous^{23, 105}. BrdU pulse-chase experiments clearly demonstrated enhanced longevity by 2NBDG+ plasma cell subsets relative to their 2NBDG- counterparts²³. 2NBDG+ subsets of plasma cells showed higher spare respiratory capacity than did their 2NBDG- counterparts, further supporting the conclusion that longevity is linked to the ability to import extracellular glucose¹⁰⁵. Moreover, 2NBDG+ subsets in the spleen exhibited high rates of antibody secretion, higher levels of autophagosomes, as well as longer half-lives as compared to their 2NBDG- counterparts²³. We also found that each plasma cell subset had mostly distinct immunoglobulin repertoires, were generated contemporaneously through the course of an NP-Ovalbumin (OVA) immunization, and showed similar expression of CD93, a marker of mature plasma cells, indicating that these subsets are formed independently of each other^{23, 25}. When long- and short-lived plasma cells were isolated and cultured *ex vivo*, 2NBDG uptake by long-lived plasma cells remained higher than in short-lived plasma cells. These data suggest an intrinsic ability of long-lived plasma cells to robustly import nutrients, rather than changes in the nutrient content in the local microenvironments of long- and short-lived plasma cells^{23, 105}. Moreover, the data suggest that the decision to commit to longevity (or not) may be determined sometime before or during plasma cell differentiation, and in part involves establishment of a sustainable metabolic program. One caveat to these studies is the concern over the specificity of 2NBDG and its transporters¹⁵⁴. In preliminary observations,

we have found that while radioactive glucose uptake is much higher by bone marrow long-lived plasma cells relative to other plasma cell subsets, similar trends are not readily apparent between splenic 2NBDG+ and 2NBDG- subsets. One technical caveat we have noticed anecdotally is that plasma cells assayed immediately after having been fluorescence activated cell sorted display very poor glucose import rates, perhaps due to stresses imposed by the process ¹⁵⁵. These cells recover after a rest period *ex vivo*. Given the minimal expression of known glucose transporters in mouse and human plasma cells, as-yet unknown genes and pathways likely promote extracellular sugar import.

3.3 Methods to assess plasma cell metabolism

Chemical inhibitors that disrupt anabolic pathways and dyes that mark nutrient uptake and mitochondrial function have proven very useful in perturbing and quantifying metabolic pathways. These dyes provide an essential bridge between classical metabolism experiments performed on bulk populations *in vitro* and single-cell resolution studies that can be performed *in vivo*. Yet as highlighted by our preliminary 2NBDG results, some degree of caution is warranted when using dyes with unclear specificities and inhibitors with problematic off-target effects. The precise targets of most of the widely used Mitotracker and MitoSox dyes have not been defined, and the use of these dyes can be confounded by non-specific over-labelling or by cellular drug efflux pumps. To highlight these issues, hematopoietic stem cells were thought to possess very little mitochondrial mass based on mitochondrial dye stains ^{156–162}. Yet recent studies demonstrated instead that hematopoietic stem cells have substantial mitochondrial mass as visualized by electron microscopy and show robust labeling with Mitotracker dyes when pre-treated with verapamil, an inhibitor of ABC drug transporters ¹⁶³. As another example of how drug manipulations should be treated with caution, we performed pharmacological experiments with plasma cell subsets and etomoxir, an inhibitor of fatty acid oxidation, to define the carbon sources that drive basal respiration. Our results suggested that approximately 50% of basal respiration is derived from long-chain fatty acids ¹⁰⁵. New information in the field, however, has demonstrated clear off-target effects of etomoxir. In addition to acting on Carnitine palmitoyltransferase 1 (CPT1) proteins, which allow long-chain fatty acids to pass the outer mitochondrial membrane, etomoxir can also inhibit 1,2-Diacyl-sn-glycerol acyl transferase, a key enzyme in the *de novo* synthesis of glycolipids and at high concentrations, it inhibits the electron transport chain ^{164, 165}. High concentrations of etomoxir also inhibited mitochondrial adenine nucleoside transporter and depleted intracellular Coenzyme A levels in macrophages ¹⁶⁶. Further, genetic ablation of CPT2, which carries long-chain fatty acids into the inner matrix of the mitochondria, was unable to recapitulate findings obtained on etomoxir treatment of cells, implying therefore an inconsistency between pharmacological and genetic data ^{165–169}. Preliminary data from our group also points towards no defects in mouse plasma cell frequencies upon genetic ablation of CPT2, suggesting therefore that long-chain fatty acids may not have as pronounced a role in plasma cell biology as we had initially anticipated. These findings thus serve as a needed warning in the use of pharmacological inhibitors alone to dissect biological phenomena.

While the use of genetic models potentially offers a more specific solution to dissecting pathways crucial for plasma cell function, they too present their own intrinsic challenges.

For example, deletion of CPT1A is readily compensated by endogenous expression of the functionally redundant gene products CPT1B and CPT1C¹⁶⁸. Moreover, germline knockouts of essential genes often result in embryonic lethality of the strain, as is the case with the *Mpc2*-knockout mouse¹⁷⁰. Plasma cells present their own unique challenges, as no lineage-specific Cre recombinase lines exist. Using the estrogen receptor to the Cre recombinase provides a tamoxifen inducible murine model of gene deletion. The latter system was beneficial for our studies in which we were able to genetically ablate *Mpc2* in mixed bone marrow chimeras months after reconstitution and subsequent vaccination against West Nile Virus (WNV)¹⁰⁵. Alternative approaches have used mice in which the Cre expression cassette has been driven by B cell-specific promoters, such as those of CD79 α , CD23, CD19, and the γ 1-heavy chain promoter^{171–174}. A shortcoming of these Cre-mediated strategies is that deletion of genes of interest occurs ubiquitously (as in the Rosa26-CreERT2 system) or in the lineages preceding plasma cell differentiation (in the B cell promoter-Cre systems). An experimental challenge then becomes how to distinguish between defects in upstream B cell activation and plasma cell formation versus maintenance. A needed solution is to generate new mouse lines in which Cre recombinase expression is restricted to plasma cells. Resolving this dilemma would provide an important tool for plasma cell research.

3.4 Cell extrinsic factors

While cell intrinsic metabolic differences functionally distinguish short-lived from long-lived plasma cells, cell-extrinsic differences also play a role. A Proliferation Inducing Ligand (APRIL) enhances plasma cell survival *in vitro*, and genetic ablation of the APRIL receptor BCMA leads to a loss of plasma cells *in vivo*^{132, 175, 176}. These signals promote the expression of MCL1, the critical BCL2-family anti-apoptotic factor expressed in plasma cells^{132, 133}. A number of other extrinsic stimuli, such as IL-6 and Tumor Necrosis Factor α , and engagement of CD28 and CD44 also promote plasma cell survival *ex vivo*^{134, 177, 178}. Genetic knockout studies, however, have provided mixed results, suggesting that plasma cell-intrinsic signaling by these factors might not all be essential *in vivo*^{133, 179}. A number of accessory cell types such as eosinophils, basophils, megakaryocytes, regulatory T cells, and mesenchymal cells have all been proposed to enhance plasma cell survival^{178, 180–184}. The necessity of some of these cell types and secreted cytokines has been disputed, but a common proposed feature of these populations is their ability to secrete pro-survival cytokines and in turn support plasma cell longevity^{185–189}. The integration of these signals with primary plasma cell metabolism, however, has been understudied with a notable recent exception. Human plasma cells co-cultured with primary mesenchymal stromal cells (MSCs) survived in *in vitro* cultures for up to 8 weeks, versus those cultured in media alone, that died within one day¹⁹⁰. Functional secretome analyses revealed that fibronectin-1 and the 14-3-3 zeta/delta proteins promote plasma cell survival, suggesting a role for the extracellular matrix proteins¹⁹⁰. Culturing these cells under physiological oxygen tension and with MSC supernatants further enhanced survival, suggesting an as yet unexplored role for hypoxia in plasma cell survival. A deeper understanding of the complex interactions between these cells and systems will undoubtedly provide a more complete basic understanding of the factors that promote plasma cell longevity, as well as malignancies.

4. MULTIPLE MYELOMA

Multiple myeloma (MM) is a genetically heterogeneous cancer driven by malignant plasma cells in the bone marrow. MM is marked by immunodeficiency, anemia, renal failure, and bone lytic lesions. The use of drugs like Thalidomide, Lenalidomide, Bortezomib, and Dexamethasone in combination or coupled with autologous hematological cell transfers or stem cell therapy have reported some amelioration of the disease and its symptoms, but MM remains incurable^{191, 192}. While it is difficult to culture myeloma cells from patients in routine cell culture media, a model for studying this disease exists in mice, which involves transplantation of the spontaneously arising 5TMM cells from aged C57BL/KaLwRij mice into syngeneic younger recipients^{193, 194} (discussed more recently in¹⁹⁵). Human xenograft models for multiple myeloma do exist, but these models are complex and most work is performed *in vitro* and on cell lines^{196, 197}.

While extracellular survival factors and niches may be limiting for normal long-lived plasma cell survival and numbers, MM growth and maintenance becomes independent of such constraints. It is possible that to achieve this independence, MM adopts distinct survival signals from their normal counterparts. In addition to cytokines such as IL-6 and BAFF that promote normal plasma cell survival, other factors such as Receptor Activator of NF- κ B (RANK), Vascular Endothelial Growth factor (VEGF), Colony stimulating factor-1 (Csf1), Fibroblast Growth factor-2 (FGF-2), angiopoietin-1, and IL-8 can promote MM survival^{198, 199}. MM may also intrinsically engage iNOS-cGMP signaling, c-Myc, and Src and ERK kinases to persist and grow^{200–202}. Through secretion of IL-10 and Transforming Growth Factor β (TGF- β), regulatory T cells may also promote MM persistence either directly or by preventing rejection²⁰³. Many of these signals and cytokines effect metabolic changes in other systems. Whether they mediate similar changes in MM remains to be fully explored.

As the critical metabolic pathways of plasma cells become defined, it will be important to determine if these same pathways can be targeted to eliminate MM. Long-lived plasma cells support durable immunity, but their bystander ablation as part of MM therapies would not be immediately life-threatening and likely an acceptable side effect. Nonetheless, an ideal therapy would target MM selectively while sparing non-malignant plasma cells. As noted above, spare respiratory capacity is a distinguishing feature of long-lived plasma cells, but such properties to our knowledge have not yet been reported for MM. In unpublished data, we have found that the 5TGM1 MM cell lines exhibit similar spare respiratory capacity and fluorescent glucose analog uptake as do primary plasma cells, but it remains unclear whether these MM cells are dependent on this pathway to form tumors *in vivo*. MM cells in general do exhibit enhanced nutrient uptake over non-transformed primary cells, which is a hallmark of cancerous cells. These cells are heavily reliant on glucose, and consequently are susceptible to chemotherapeutic strategies involving 3-Bromopyruvate and 2-Deoxyglucose, known inhibitors of key glycolytic enzymes^{204–206}. MM is characterized by high expression of Hexokinase-II and Pyruvate kinase M2, both of which are key enzymes in the breakdown of glucose leading to the formation of pyruvate^{207, 208}. This tendency towards aerobic glycolysis, or the Warburg effect, is a unique feature of highly proliferative cells and is likely to shunt the excess of pyruvate towards lactate formation, which in turn is excreted through the monocarboxylate transporters MCT1 and MCT4²⁰⁹. Under normal circumstances, long-

lived plasma cells use glucose mainly for antibody glycosylation, and to a lesser extent for ATP^{23, 105}. Thus, the dependence on aerobic glycolysis in MM is a marked shift from the normal metabolic phenotype of long-lived plasma cells and is likely to promote the malignant transformation of these cells. Further, glycolysis can activate MCL1, an anti-apoptotic factor promoting myeloma survival²¹⁰. Despite the importance of glucose in the metabolism of MM cells, the transporter responsible for its uptake is inconsistent between human and mouse cell lines. GLUT1 and GLUT6 are the chief glucose transporters in primary and MM murine cell lines, but human MM lines show elevated levels of the glucose transporters GLUT4, GLUT8, and GLUT11 with minimal expression of GLUT1^{105, 210}.

The importance of glutamine in plasma cell metabolism was drawn from studies carried out in MM cell lines. Inhibition of glutaminolysis results in MM death²¹¹. This may be another key difference between MM and normal long-lived plasma cells, since as mentioned above, genetic ablation of glutaminolysis has not revealed obvious defects in plasma cell numbers or secretion thus far. c-Myc is an important factor contributing to the tumorigenic phenotype of MM cells and enhances expression of glutamine transporters as well as favoring glutaminolysis^{211, 212}. In addition to being a key source of fuel, glutamine uptake also triggers the mTOR pathway and stimulates cell proliferation through a STAT3-dependent mechanism²¹³. Glutamine also can upregulate HIF1 α and can possibly mediate survival of the tumor cell under conditions of hypoxia, a condition typically associated with the MM bone marrow²¹³. In human MM cell lines, glutamine was preferentially shuttled through the tricarboxylic acid cycle as compared to glucose, and led to the synthesis of the oncometabolite 4-hydroxyglutarate²¹⁴. In MM cells, glutamine uptake was also shown to prevent dissociation of the pro-apoptotic BIM from its complex with BCL2, thus preventing cell-intrinsic apoptotic pathways²¹⁵. To summarize, it appears that MM cells are more heavily dependent on glycolysis and glutaminolysis than are their normal long-lived plasma cell counterparts, and these pathways may represent Achilles heels to target the disease.

5. NUTRITIONAL INFLUENCES ON PLASMA CELL FUNCTION

As mentioned earlier, the metabolic phenotype and longevity of plasma cells likely results from a complex interplay of cell-intrinsic and -extrinsic factors. There is a growing body of literature suggesting that plasma cell generation and function may be impacted by metabolic changes that occur at the organismal and systemic levels. To highlight this point, consider the property of spare respiratory capacity in long-lived plasma cells. It remains unclear under what physiological conditions this spare respiratory capacity is engaged. Despite many efforts to define specific plasma cell survival factors, such as APRIL, IL-6, CD28 engagement, and many others, we have yet to identify an extrinsic stimulus that requires spare respiratory capacity to promote plasma cell survival. Therefore, we have instead speculated that the fluctuations in systemic nutrients over the course of a day, or even a lifespan, place metabolic stresses on cells¹⁰⁵. Perhaps spare respiratory capacity protects against these stresses to prevent bioenergetic crises, especially for cells as metabolically active as long-lived plasma cells. Nonetheless, this explanation remains speculative and not entirely satisfying in the absence of experimental evidence. Therefore, a key direction will be to connect metabolic changes at the systemic level to the specific plasma cell metabolic

pathways and their functional outcomes. We highlight some possible directions and relevant literature below.

5.1 Caloric restriction

A restriction in dietary calories positively correlates with longevity across many organisms. Yet these studies are invariably carried out in controlled, sterile environments, and negative impacts on immunological responses should be weighed. One of the first reports of the effects of caloric restriction on the immune system involved sheep red blood cell immunizations of rats restricted to 25% of their *ad libitum* diet. It was found that rats on the 6-week caloric restriction regimen exhibited marked reductions in lymphocyte numbers as well as reduced serum IgM and IgG titers in response to immunization²¹⁶. Further, offspring of pregnant dams on caloric restriction exhibited similar reduced antibody responses, even if they were weaned on a normal diet. These diminished responses to T-dependent antigens also reflected poorer activation and proliferation in mouse B cell cultures and had similar effects on macrophage and T cell function in mice on caloric restriction²¹⁷. In a model of WNV infection, mice on caloric restriction had fewer Interferon- γ -producing T cells and exhibited higher mortality as compared to mice fed *ad libitum* diets²¹⁸. A recent study involving non-obese human volunteers monitored over two years recapitulated the data from mice, where volunteers on caloric restriction showed reduced inflammation as well as markedly low circulating white blood cell and lymphocyte counts²¹⁹. The caloric restriction-induced lymphopenia, however, is limited to peripheral organs as bone marrow lymphocyte numbers were unaffected in aged mice on caloric restriction²²⁰. The effects of caloric restriction on the immune system may be dependent on the age of the individual too, as old mice on caloric restriction showed restored lymphocyte cellularity when fed *ad libitum*, in contrast to the findings reported by Chandra on neonates^{216, 220}. In models of autoimmunity such as the (NZMxNZB) F1 model of lupus and the MRL/Mp-lpr/lpr model of glomerular nephritis, caloric restriction delayed the onset in disease symptoms and enhanced the lifespan of the mice^{221, 222}. Thus, the available evidence suggests that caloric restriction induces a systemic inhibition of immune cell function. As infectious disease remains one of the major causes of death worldwide, caloric restriction may not be effective in enhancing lifespan in the real world²²³.

While these studies indicate that there is a plasma cell phenotype associated with caloric restriction, their primary readout is serum immunoglobulin levels, which may not be a specific indicator of changes in cellular kinetics or function of antibody-secreting cells. Impacts on T cells, dendritic cells, stroma, and a myriad of other cell types might indirectly lead to altered antibody responses. While a more formal demonstration of the impact of caloric restriction on the B cell response is needed, our findings surrounding the metabolic phenotypes of plasma cells is in accordance with the data presented. As discussed previously, plasma cells exhibit a dependence on glucose and glutamine for their optimal function, and limiting these nutrients therefore, as in caloric restriction, might have a deleterious effect on the longevity and persistence of these cells^{23, 105}. It is also noteworthy that treating mice with Rapamycin, the inhibitor of the mTOR complex, was shown to extend the lifespan of treated rodents, in a manner akin to CR²²⁴. Given the central role mTOR plays in B cell activation, nutrient uptake, class switch recombination, and the overall

transition from B cells to plasma cells, it is possible that the effects of caloric restriction may act through alterations of B cell-intrinsic mTOR signaling. The effects of mTOR signaling in B cell differentiation are stage-dependent, as treatment with rapamycin or through genetic deletion of Raptor resulted in reduced GCs, class switching, and promoted short-lived plasma cell formation^{225, 226}. In already differentiated plasma cells, mTOR inhibition led to reduced antibody synthesis, but did not affect the longevity of plasma cells²²⁵. The metabolic changes that specifically occur in plasma cells during caloric restriction still remain to be defined and may help provide mechanistic explanations for immune system dysfunction during caloric restriction.

5.2 Obesity

Reciprocally, obesity and diabetes might provide an overabundance of nutrients, leading to different systemic changes in the immune response. While obesity predisposes to many diseases, it has also been shown to impair immune responsiveness, thus creating an obstacle in the treatment of infectious diseases. In the recent 2009 H1N1 Influenza A pandemic, 91% of the reported deaths in the state of California were of individuals who had a body-mass index (BMI) greater than 30, and almost 51% of hospitalized patients were categorized as obese to morbidly obese, indicating a strong correlation between obesity and poor responsiveness to the virus²²⁷. Other reports also correlated higher BMI with poor antibody responses to the Hepatitis B (HBV) vaccine in adults and Tetanus Toxoid (TT) vaccination in children. Obese children actually showed higher anti-influenza antibodies in their serum 4 weeks after vaccination as compared to vaccinated non-obese children²²⁸. At 4 months, however, seroprotection and seroconversion rates against A/H1N1 and A/H3N2 were identical between both groups of children while obese children showed higher antibody titers against the B strain relative to the non-obese vaccinated children²²⁸. Similar findings were recapitulated in obese North American adults, who showed higher seroprotection and seroconversion at a month post vaccination as compared to lean participants. Problematically, though, a greater drop in anti-Influenza antibodies was observed among obese individuals at 12 months post vaccination compared to their non-obese counterparts²²⁹. Further, individuals with type 2 diabetes showed similar antibody kinetics in response to the trivalent-inactivated influenza vaccine as compared to healthy individuals, suggesting that these antibody response kinetics are specific to obesity²³⁰. Thus, obesity correlates with an elevated antibody response at early time points, but also correlates with transient anti-influenza antibody responses. As the measurements in all these studies were serum enzyme-linked immunoassays, we speculate that obesity favors the formation of short-lived plasma cells which are responsible for the heightened antibody response, but by an unknown mechanism does not permit either the formation or establishment of the long-lived plasma cell compartment.

There are several possibilities as to how obesity could influence the formation of the long-lived plasma cell compartment. First, it is likely that obesity and its associated systemic inflammation may alter the composition of the bone marrow, thereby rendering it unsupportive to the establishment of the long-lived plasma cells. Evidence for this possibility has come from experiments in murine models of obesity, including high-fat diet fed mice or leptin-leptin receptor deficient animals. Bone marrow resident mesenchymal

stem cells from mice on a high-fat diet as well as from mesenchymal stem cell-specific *lepR^{fl/fl}* mice expressed gene signatures associated with adipogenesis, suggesting that obesity can induce an altered differentiation route for these multipotent cells^{231, 232}. Diet-induced obesity also reduced the proliferative and self-renewing capacity of hematopoietic stem and progenitor cells in the bone marrow, in turn gradually impeding B lymphopoiesis^{233–235}. This gradual lymphopenia may help explain poor immune responses in aged individuals^{227, 229, 230}. Obesity might thus prevent normal stem cell differentiation pathways in the bone marrow and in turn limit the formation of cell types that provide plasma cells essential survival factors. Consequently, plasma cells may be deprived of essential survival factors, thereby compromising durable immunity. A second possibility for poor long-lived plasma cell formation is that the elevated levels of sugars and lipids due to obesity may influence plasma cell metabolism directly. For example, excessive glucose can be shunted into sorbitol, which is associated with diabetes and cellular toxicities²³⁶. Exercise is widely promoted as a conservative but effective intervention for obesity. Yet limited data suggest that exercise has minimal effects on B cells and plasma cell function in both mice and humans^{237–239}. As above, measuring the impact of obesity and exercise on plasma cell-intrinsic metabolism will be critical for moving the field from observations of phenomena to mechanistic studies.

6. CONCLUSIONS

While our findings and those from many others in the field of immunometabolism have defined specific metabolic pathways for defining cellular differentiation outcomes and function, these are just stepping stones towards a more comprehensive understanding of the B cell response. Our work highlights the importance of nutrient uptake in the longevity of the plasma cell, and the cell-intrinsic mechanisms required to balance energy uptake and utilization in plasma cells and their subsets. An unexpected set of findings by our group and others demonstrate that these metabolic pathways come with very few transcriptional consequences, suggesting that plasma cell subsets may differ from each other at the level of their proteomes. *In vivo*, plasma cell behavior is influenced by the local environment, and almost certainly by the systemic metabolic state. To define how these extrinsic cues influence plasma cell metabolism, longevity, and function, approaches that integrate cell-intrinsic, microenvironmental, and systemic parameters are necessary. This approach will be beneficial in our understanding of complex diseases that are based on metabolic dysregulation and provide guidance for the design of vaccines.

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Longevity

Ki67:	+/-	-	-	-	-
MHCII:	+	-	-	-	-
CXCR3:	+	-	-	-	-
BLIMP1:	low	high	high	high	high
CD93:	+/-	+/-	+/-	+/-	+/-
B220:	+	-	+	-	-
2NBDG:	low	low	high	high	high
location:	spleen	spleen	spleen	spleen	BM

Figure 1: Mouse plasma cell subsets and markers.

BM: bone marrow. +/- indicates that a fraction of the cells are positive for the marker.

Figure 2: Metabolic cues direct plasma cell longevity and function.

Schematic representation of the metabolic differences between short- and long-lived plasma cells (SLPCs and LLPCs). **(A)** Despite exhibiting similar transcriptomes, LLPCs take up higher levels of glucose and glutamine than do SLPCs under homeostatic conditions. Nutrients can also be provided through autophagy, which is also higher in LLPCs. These nutrients collectively are catabolized and directed to mitochondria for ATP generation, or used in synthesis and glycosylation of antibodies. ER stress is equivalent between plasma cell subsets, but SLPCs degrade antibody molecules more than LLPCs. As a result, LLPCs secrete more antibody molecules than SLPCs, despite equivalent rates of protein and antibody synthesis. **(B)** Under limiting nutrient availability and low ATP generation, LLPCs can expand their basal respiratory capacity to compensate. SLPCs are unable to perform this function and as a result, initiate pathways of programmed cell death.