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From zero to sixty and back to zero again: the metabolic life of B cells

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Throughout their lifetimes B cells shift metabolic gears to move rapidly from quiescent states to full out proliferative expansion and back again. Here we discuss recent findings that shed light on how B cells rapidly shift gears to metabolically fuel expansion and then just as rapidly down shift during phases of receptor rearrangements to ensure genome stability. We also discuss the link between metabolic activity and fate decisions in B cells.

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Introduction

An adult human produces over a billion new B lymphocytes each day to maintain its defense arsenal against invading pathogens. Because the genes encoding the membrane Ig of the B cell receptor for antigen (BCR) are assembled somatically from smaller gene segments in a highly error prone process, the risk of producing cells that fail to assemble a functional BCR at all or assemble a functional but autoreactive BCR is high [1]. Later in antigen-driven processes in germinal center (GCs) B cells further modify their IgV_H and V_L by somatic hyper mutation, another error prone process that may result in unwanted or dysfunctional B cells. But importantly B cells expressing somatically mutated V_H and V_L serve as a critical source of B cells for selection in affinity maturation of antibody responses. Thus, from the first antigen-independent attempt to assemble an IgM heavy chain in the bone marrow to the antigen-driven production of high affinity B cells in GCs, life for a B cell is a continual test of fitness. For fit B cells a common outcome is proliferative expansion. While quiescence is essential during times of receptor rearrangements to ensure genome stability, proliferation is required for clonal expansion [2]. To expand, quiescent B cells must rapidly increase their metabolic activity to extraordinary levels to support proliferative rates that are faster than that of any other cell in a healthy individual. Alternatively or in addition, fit cells may be activated to differentiate, a process that also involves metabolic reprograming. Thus, through their lifetimes B cells alternate between distinct phases of quiescence, proliferation and differentiation (see Figure 1). Although our understanding of the signals that induce metabolic changes in B cells and the impact of these changes on B cell fates is far from complete, the existing data suggest that links between these exist. In this review we discuss recent discoveries that shed light on how B cells rapidly switch gears between different metabolic states depending on their activation and differentiation states and highlight the link between B cell metabolism and fate determination.

B cell progenitors go through multiple rounds of high and low metabolic activity states during their maturation in bone marrow

The process of producing a BCR involves two distinct Ig rearrangement events. The cells first undergo a V_H-Cμ rearrangement that if successful produces a surface IgM that pairs with a surrogate light chain forming the pre-BCR expressed by early-stage large pre-B cells [3]. Expression of the pre-BCR triggers rapid proliferation and clonal expansion accompanied by increased metabolic activity including high levels of glucose uptake and mitochondrial ROS production [4,5,6•]. Proliferation at this stage is dependent on the ability of cells to boost glycolysis and is highly sensitive to glycolysis inhibitors [7]. This checkpoint is controlled by the activity of a heterodimeric transcription factor called hypoxia induced factor-1 (HIF-1). The alpha domain of this dimer (HIF-1α) is sensitive to O_2 levels [8] and the complex is most stable in the hypoxic environment of the bone marrow [9]. The increased metabolic activity of large pre-B cells resulting in oxygen consumption may further decrease oxygen levels in the microenvironment and thus increase the stability of HIF1α. The activity of HIF1 then increases expression of glucose transporters and glycolytic enzymes to maintain high levels of glycolysis [7]. The control of cell proliferation during the large pre-B cell stages also requires the cytokine IL-7, produced by stromal cells in the bone marrow [10]. IL-7 signaling turns on the PLCγ mediated mTOR activation, a critical pathway is required for progression of B cell development [11]. Additionally IL7 induces the PI3K-Akt pathway, contributing to increased glucose utilization capacity

Figure 1

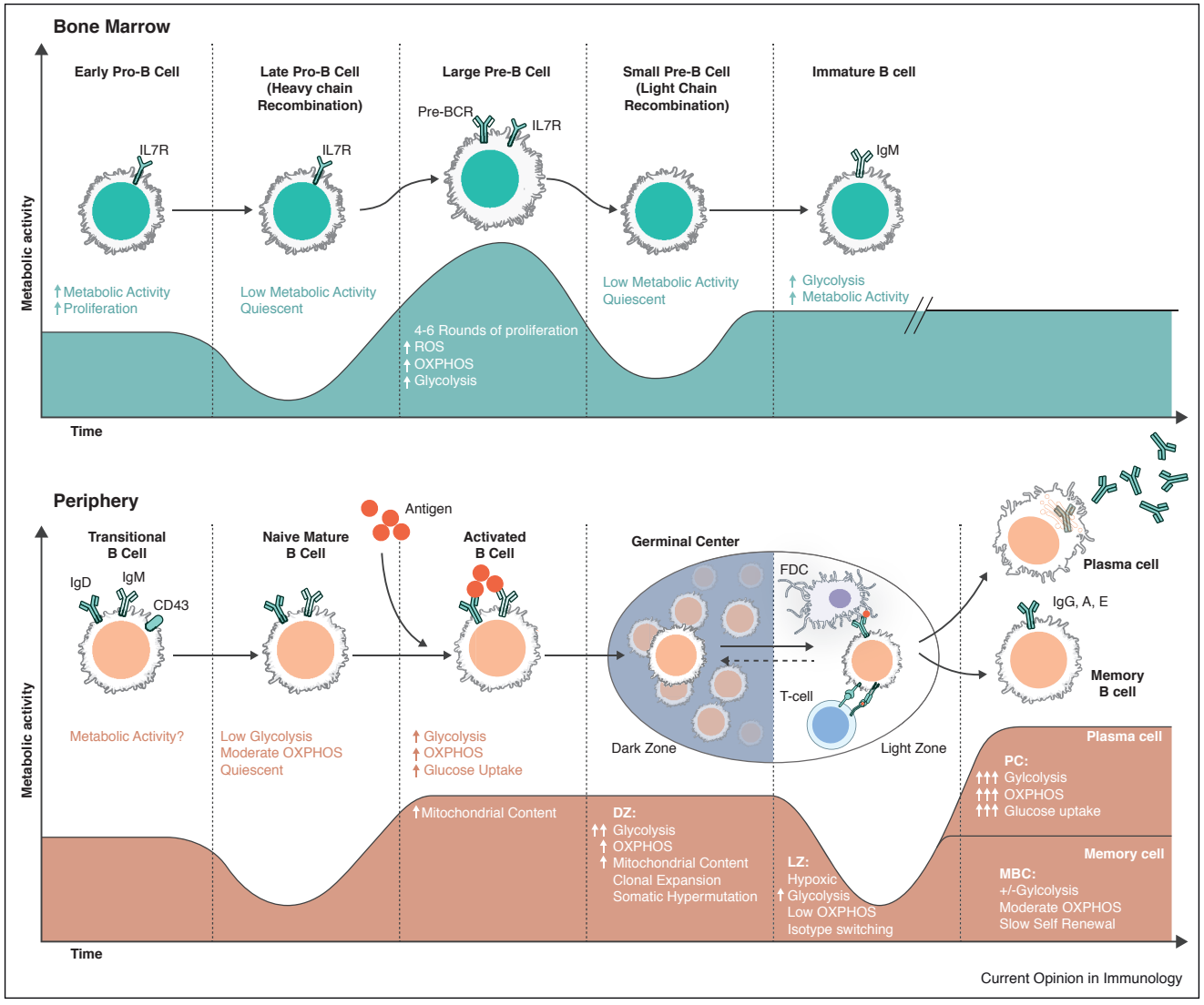

Illustration of changes in metabolic activity during different B cell maturation and differentiation stages.

early after expression of the pre-BCR at the large pre-B cell stage [5,6•,12]. However, later in the development of the pre-B cell the expression of the IL-7 receptor wanes and signals from pre-BCR control further differentiation [5]. Following the initial pre-BCR-dependent metabolic burst and cell proliferation of large pre-B cells, the PI3K-Akt pathway is inactivated inducing metabolic quiescence [13]. Quiescence is characterized by substantial decreases in both Oxidative Phosphorylation (OXPHOS) and glycolysis as large pre B cells differentiate into non-proliferating small pre-B cells [6•]. Upon completion of light chain rearrangement, IgM is expressed on the cell surface and small pre-B cells differentiate into immature B cells. Immature B cells have the second highest glycolysis activity among B cell progenitors in the bone marrow [7]. However, the transition from small pre-B cell to immature B cell is not sensitive to glycolysis inhibitors indicating the activity of alternative energy producing pathways [7]. Immature B cells are tested for fitness through clonal selection and fit immature B cell clones exit the bone marrow and enter the blood stream. These cells mature through transitional stages via unidentified metabolic programs until metabolically quiescent naïve mature B cells differentiate in secondary lymphoid organs.

B1 cells are metabolically more active than their B2 cell counterparts in the periphery

The mature B cell compartment consists of two phenotypically and functionally different cell types, namely B1 and B2 cells. Lymphocyte lineage-specific HIF1α-deficiency in mice result in abnormalities in both B1 and B2 cell

development highlighting the importance of HIF1α-mediated metabolic processes during maturation of both B1 and B2 lineages [14]. Unlike B2 cell pool which is constantly replenished by new cells from bone marrow throughout life, mature B1 cells are generated predominantly in fetal life and depend on self-renewal to maintain the mature B1 cell pool during adult life [15,16]. In line with this basal proliferation activity, a recent study of the CD5-expressing subset of B1 cells (B1a cells) showed that both glycolysis and OXPHOS were more active in B1 cells as compared to B2 cells and this phenotype was maintained by increased transcription of key glycolysis and OXPHOS enzymes. B1 cells appeared to be metabolically adapted to the lipid-rich environment of the pleural and peritoneal cavities in that they increase their capacity for lipid uptake, fat droplet synthesis for storage and active breakdown of fat droplets through autophagy to fuel their active beta oxidation processes that metabolizes fat [17••].

Mature naïve B2 cells spend much of their lifespans circulating between secondary lymphoid organs in search of antigens. Survival of these B cells depends heavily on the presence of a soluble factor secreted by stromal cells called BAFF. While lack of BAFF blocks B2 cell maturation at the transitional cell stage, overactivity of the BAFF-BAFF receptor pathway results in autoimmunity showing that a tight regulation of this axis is required [18–20]. BAFF receptor signaling induces glucose utilization, through the PI3K-Akt pathway and BAFF appears to be necessary for naïve B cells to respond to antigen [21]. However, naïve B cells still depend heavily on OXPHOS and show no proliferative activity suggesting pro-glycolytic and cell cycle reentry inducing activities of BAFF are kept in check by other mechanisms [21–23]. Recently, Jellusova *et al.* showed that glycogen synthase kinase 3 (Gsk3) activity in naïve B cells is responsible for prevention of cell proliferation and maintenance of metabolic quiescence [24••]. The PI3K-Akt pathway is responsible for inhibition of Gsk3 activity [25] yet apperantly the PI3K induction of BAFF is insufficient to overcome metabolic quiescence. The PI3K-Akt pathway only reaches the activity level required for a full scale metabolic boost and cell proliferation following BCR activation [26,27]. This synergy model may also explain why B cells pretreated with BAFF respond better to BCR stimulation.

Antigen stimulated B cells require second signals in order to maintain their metabolic integrity

The metabolic quiescence of naïve B cell ends upon antigen stimulation through the BCR that induces a rapid increase in both OXPHOS and glycolysis [23,28••]. Using specific inhibitors for the kinases downstream of the BCR, Syk, Btk, PI3K and JNK were shown to be essential for the BCR-induced metabolic boost while inhibition of p38 and MEK pathways did not alter the outcome indicating a multilevel control of metabolic activity [28••]. Although both OXPHOS and glycolysis increase proportionally following BCR signaling, the early events following antigen encounter such as spreading and contraction of B cell on antigen-containing membranes and internalization of BCR-bound antigen for antigen processing and presentation depend solely on energy generated by OXPHOS. The reliance on OXPHOS may reflect the skewing of naïve B cells towards OXPHOS. OXPHOS may provide energy until remodeling allows glucose utilization to take place. In support of this hypothesis, although both OXPHOS and glycolysis immediately increase following BCR activation, no metabolic reprograming to increase the expression of glucose transporters or glucose uptake occurs within the first few hours following BCR activation. Remodeling begins later first by transcriptional upregulation of glycolytic enzymes and downregulation of enzymes linked to lipid metabolism and later by upregulation of glucose transporters providing more efficient glucose entry into cells to fuel the increased glycolytic needs of activated B cells. In addition, the mitochondrial mass of cells also increases which keeps OXPHOS up to par with increased metabolic demands on the B cells [28••].

In addition to antigen stimulation through the BCR, B cells are responsive to a variety of innate stimuli which also provide signals for survival, differentiation and proliferation. A metabolic burst similar to that induced by BCR signaling has been described following stimulation of B cells through TLR4 binding its ligand to LPS [22] and TLR9 binding CpG [23,28••]. However, as we showed recently [28••], despite similarities in early metabolic remodeling following both BCR and TLR9 stimuli, BCR and TLR9 signaling have very different outcomes. TLR9 signaling alone can drive survival, differentiation and proliferation and interestingly, the outcome of a B cell's encounter with TLR9 agonists depends on both the chemical structure of the agonist and how it is delieved to the cell [29,30•]. Although antigen stimulation through the BCR activates naïve B cells to begin metabolic remodeling process, metabolic activity is not sustainable in the absence of a secondary signal provided through cognate B cell- T helper (T_H) cell interactions or by TLR signals (see Figure 2). If the secondary signals are not received within the first several hours following antigen encounter, BCR mediated increase in intracellular calcium through store operated calcium entry (SOCE) system results in increases in inner mitochondrial permeability which initiates mitochondrial matrix swelling disturbing mitochondrial OXPHOS leading to excess ROS production. ROS increases activate a feed-forward cycle with ROS and calcium causing increased mitochondrial dysfunction eventually leading to cell death. This metabolic death clock ensures that the initial activation of B cells with antigen only proceeds to

Figure 2

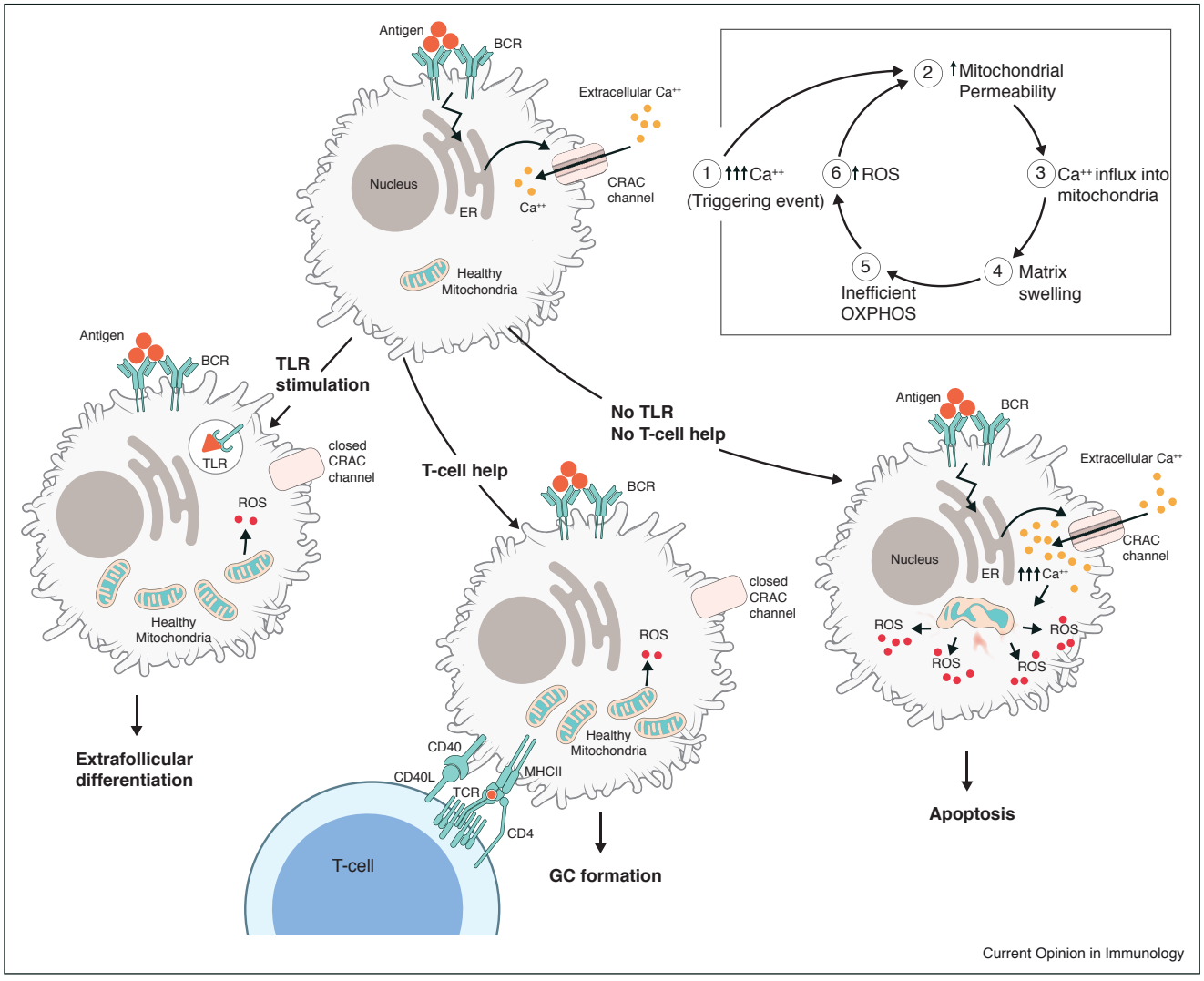

Illustration of cellular changes that follow BCR stimulation. Possible activation-induced B cell fate outcomes are depicted.

full immune activation once the self-reactivity of the B cell is ruled out through positive signals from antigen-specific T_H cells or upon confirmation of infection through TLR stimulation [28••,31]. Although exact signaling pathway second stimuli use in order to prevent mitochondrial dysfunction warrants further exploration, recently Protein Kinase C (PKC-b) activity was shown to be critical in maintaining mitochondrial fitness upon B cell activation [32••].

Based on our rescue model, both T cell help and TLR signaling serve the same purpose, to ensure B cell survival and differentiation of fit B cells. However, current evidence suggests that the fate of B cells that receive second signals from T_H cells and that of B cells receiving second signals through TLR may diverge. T cell help drives entry into GCs and affinity maturation and long-term memory but in contrast TLR signaling may favor differentiation to short-term T-independent plasma cell differentiation [33••].

Microenvironmental factors play major roles in metabolic remodeling of germinal center B cells

B cells that enter GCs cycle between the dark zone in which B cells rapidly proliferate and undergo somatic hypermutation, and the light zone in which B cells that become quiescent compete for antigen for presentation to T follicular helper (Tfh) cells [34]. The tissue microenvironment of the light zone is both oxygen-poor and nutrient-poor and therefore HIF1α, AMPK and Gsk3 are dominant in light zone B cells [24••,35••,36••]. Through the effects of these transcription factors, PI3k signaling is inhibited, mTOR activity is reduced and

cMyc is degraded promoting catabolism over anabolism and proliferation [24••,35••]. HIF1α also induces glycolysis and prevents entry into the TCA cycle through activation of PDK [37]. cMyc is upregulated in light zone B cells that successfully compete for antigen and as a consequence receive both BCR and CD40 signals [38•,39]. This induces a second glycolytic burst which accompanies rapid proliferation and mitochondrial biogenesis and meets the energy needs of light zone B cells that may re-enter the dark zone [24••,40].

After the GC reaction is completed B cells have the potential to differentiate into memory B cells or plasma cells (PCs). Similar to naïve B cells, memory B cells are characterized by metabolic quiescence likely dependent on OXPHOS. However, they differ from naïve cells by their remarkable population stability which is a result of their unique features such as longevity, slow but active self-renewal capacity and ability to survive independently of BAFF [41–44]. Memory B cells also require T_H cells for rapid antigen recall responses [45], however AICD has not been described for memory B cells and B–T interactions are unlikely to be a matter of life and death. Overall, despite similarities to naïve B cells, the metabolic activity of memory B cells is likely governed by unique signaling pathways which warrant further investigation. PCs on the other hand are terminally differentiated and do not proliferate. However, PCs are highly active metabolically in order to produce and secrete high levels of antibody. Recent evidence showed that PCs in general have a very active glucose and amino acid uptake machinery and depend on both glycolysis and OXPHOS for their energy needs [22,46••]. However, PCs are a heterogenous group of cells with different life expectancies, antibody secretion rates and tissue localizations [47]. Therefore multiple factors appear to determine their unique metabolic choices as detailed in a recent review [48••].

Metabolic profiling of B and T cells reveals major differences in how these cells handle their bioenergetic needs

A comparison of the metabolic changes that activated T cells undergo as compared to B cells provides further insight into the link between metabolism and fate determination. Both activated B cells and T cells rapidly increase OXPHOS and glycolysis and later undergo cellular remodeling to increase mitochondrial content and nutrient uptake [28••,49••,50,51•]. However, recent studies provide evidence that substantial differences exist between metabolic reprogramming of these two lymphocyte subsets. For example, although activation-induced increase in OXPHOS and glycolysis occur proportionally in B cells [28••,52], activated T cell metabolism is skewed towards glycolysis [49••,50]. Although glycolysis is an inferior energy producing pathway with only two ATP per glucose molecule compared to 38 ATP produced in OXPHOS, it appears to be a metabolic pathway common to rapidly proliferating cells such as tumor cells and embryonic stem cells. The choice to produce lactate from glucose even in the presence of oxygen is known as aerobic glycolysis or 'Warburg effect'. Aerobic glycolysis spares mitochondria for the generation of biomolecules such as fat biosynthesis rather than energy production. This trend is not pronounced in activated B cells that continue to burn fatty acids in the TCA cycle and also divert glucose into the TCA cycle by promoting pyruvate dehydrogenase activity through transcriptional downregulation of its inhibitors, PDKs, and upregulation of its activators, PDPs [28••]. One reason why activated B cells may prefer to maintain high levels of OXPHOS unlike activated T cell is that B cells differentiate into antibody producing PCs. Biosynthesis of antibodies depends heavily on glycosylation that may require that B cells spare at least part of the available glucose for this purpose rather than burning it in glycolysis. Another major difference between metabolic remodeling of activated B and T cells is their link to cell survival. BCR stimulation initiates a time-bomb leading to AICD that can only be defused by secondary survival signals [28••]. In contrast, our recent studies show that mitochondrial functions appear unaffected in T cells even after prolonged stimulation through the TCR [49••]. Such a difference in the outcome of BCR and TLR signaling may reflect the fact that T cells depend on central tolerance to remove autoreactive cells and therefore, unlike B cells that rely on peripheral tolerance to remove autoreactivity, do not require the function of a metabolic death clock.

Conclusions

B cell metabolism and function are two intermingled concepts that complement each other throughout the life span of the cell. Recent evidence shows how metabolic preferences govern B cell function, survival and differentiation. In light of these observations, it is fair to speculate that abnormalities originating from derailment from normal B cell development track such as hematologic malignancies and autoimmunity have links to dysregulated metabolic checkpoints as well [53]. Therefore, further research focusing on unique aspects of B cell metabolism would certainly benefit long standing pursuit in developing efficient solutions to these problems.

Conflict of interest statement

Nothing declared.

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