Available online at www.sciencedirect.com

ScienceDirect

Current Opinion in Immunology

Regulation of metabolic supply and demand during B cell activation and subsequent differentiation

Takeshi Egawa[1] and Deepta Bhattacharya[2]

B cell activation and differentiation are associated with marked changes in proliferative and effector functions. Each stage of B cell differentiation thus has unique metabolic demands. New studies have provided insight on how nutrient uptake and usage by B cells are regulated by B cell receptor signals, autophagy, mammalian target of rapamycin, and transcriptional control of transporters and rate-limiting enzymes. A recurring theme is that these pathways play distinct roles ranging from survival to antibody production, depending on the B cell fate. We review recently published data that define how these pathways control metabolic flux in B cells, with a particular emphasis on genetic and *in vivo* evidence. We further discuss how lessons from T cells can guide future directions.

Addresses
[1] Department of Pathology and Immunology, Washington University School of Medicine, Saint Louis, MO 63110, USA
[2] Department of Immunobiology, University of Arizona College of Medicine, Tucson, AZ 85724, USA

Corresponding author: Bhattacharya, Deepta (deeptab@email.arizona.edu)

Current Opinion in Immunology 2019, **57**:8–14

This review comes from a themed issue on **Lymphocyte development & activation**

Edited by **Wasif Khan**

https://doi.org/10.1016/j.coi.2018.10.003

Introduction

The primary role of B cells is to produce antibodies against infectious pathogens. To accomplish this seemingly simplistic goal, a highly regulated process is initiated to clonally expand relevant B cells, followed by a massive contraction to narrow the response to the minimal set of cells required to maintain protection against re-infection. In a conventional T cell-dependent (TD) response, naïve B cells are initially activated through cognate interactions between antigen and the B cell receptor (BCR). This triggers activation signals, internalization and processing of antigen to display to cognate CD4+ T cells, and co-stimulation through CD40:CD40L interactions. After a period of limited clonal expansion, B cells then differentiate either into plasma cells (PCs), germinal center (GC) B cells, or directly into IgM+ memory B cells [1]. PCs formed early in the response are localized to the extrafollicular regions and mostly live for a period of a few days [2]. GC B cells engage multiple rounds of rapid proliferation-coupled with isotype class switching, somatic hypermutation (SHM) of their immunoglobulin genes, and selection by CD4+ follicular helper T cells for high affinity variants [3]. Rare memory B cells and PCs emerge from the GC through selection processes that remain incompletely understood. Together, these cell types maintain long-term immunity against the offending pathogen and its relatives, sometimes for life [4]. The metabolic requirements of each of these stages differ [5,6]. We discuss below a number of recent studies that help explain how stage-specific metabolic requirements are communicated to B cells, and then how the resultant nutrients are used to support function.

Metabolic demands of initial B cell activation

Because of the difficulties in following the very early stages of B cell activation *in vivo*, most work in this area has come from *in vitro* studies. As much of the historical work has been reviewed recently [5], we focus instead on studies published within the past few years. Naïve B cells minimally proliferate and thus have relatively few bioenergetic requirements [7], such that lineage-determining transcription factors such as Pax5 and Ikaros suppress glucose uptake and ribonucleotide synthesis [8••,9]. BCR-activated B cells must thus markedly change their metabolic profiles to expand, proliferate, and generate ATP, much of which is driven by enhanced glucose uptake through the transporter GLUT1 [10–12]. BCR-induced metabolic changes are dependent on PKCβ, which is required for the metabolic switch to glycolysis and for downstream mTOR activation, mitochondrial function, and heme biosynthesis [11,13].

As with T cells, antigen receptor-stimulated B cells use second signals such as Toll-like receptor- or CD40-engagement to become fully activated, thereby reducing the chances of autoimmunity. In the absence of these secondary signals, B cells accumulate mitochondrial calcium and produce excessive amounts of superoxides, eventually leading to activation-induced cell death [14]. Costimulatory signals such as TLR activation enhance uptake of glucose and glutamine, which are used as carbon sources for the pentose phosphate pathway to support proliferation, *de novo* lipogenesis to expand the endoplasmic reticulum [15], and for mitochondrial

respiration and anaplerosis [16]. Nutrient uptake and the extent of B cell activation is tempered by the expression of the let-7 microRNA cluster, which suppresses glucose and glutamine transporter expression. In the absence of let-7, excessive antibodies are produced in response to T-independent antigens *in vivo*. These studies collectively provide a valuable baseline that begins to explain how B cells integrate antigen-specific signals with metabolic programs to support subsequent differentiation (Figure 1).

Germinal center metabolism

Following initial T-dependent activation, antigen-specific B cells and CD4+ T cells form GCs, which are specialized anatomical structures located within the centers of B cell follicles. GCs are divided into two distinct areas, light- and dark-zones (LZ and DZ), in which B cells exist in distinct proliferative and metabolic states [17,18]. In the LZ, B cells are mostly in a non-proliferative state where they compete with one another for CD4+ T cell help by presenting peptide on MHC class II. Only a small fraction of LZ B cells are selected by T cells to enact a proliferative program as they migrate to the DZ to divide for several days. These proliferating B cells are the templates for AID-mediated SHM, and are thus critical for the emergence of protective antibodies with affinity-enhancing mutations. This proliferative program is enabled by an anabolic response driven by the transcription factor c-MYC, which not only regulates cell cycle progression but also promotes expression of transporters of nutrients and enzymes for glycolysis and glutaminolysis [19–21]. GC B cells require both BCR and CD40 signals for c-MYC induction, thus enforcing a stringent selection checkpoint dependent on CD4+ T cell help [22]. As a counterexample, either BCR or CD40 engagement is sufficient for naive B cells to induce c-MYC and proliferation. Despite the absolute requirement for c-MYC in GC B cells, its expression is restricted to a small fraction of LZ B cells and becomes undetectable in proliferating DZ B cells [17,20,21]. This paradox is explained by a MYC-induced transcription factor AP4, which maintains many c-MYC target genes, including nutrient transporters and metabolic enzymes after down-regulation of c-MYC [23•]. Activity of mTORC1 correlates well with engagement of the MYC-AP4 pathway. mTORC1 is activated upon selection of GC B cells in the LZ and is required for cell growth, sustained expansion, and dwelling of selected B cells in the DZ [24••]. Thus, c-MYC-dependent and mTORC1-centered metabolic programs, both of which are activated by T cell help, independently or synergistically support durable proliferation of DZ B cells.

While cell-intrinsic programs triggered in selected GC B cells by T cell-derived help enact metabolic pathways to meet the demands of DZ B cell proliferation, environmental factors specific to the GC microenvironment also modulate GC B cell differentiation. One such example is restriction of mTORC1 activation and AID expression in LZ B cells by hypoxia [25]. Hypoxic conditions in the LZ induce elevated activity of HIF transcription factors. HIFs in turn inhibit activity of mTORC1 and expression of AID, and thereby restrict proliferation of GC B cells and isotype class switching [25]. As a result, overexpression of HIFs through genetic deletion of their inhibitor VHL leads to diminished GC responses. Given that oxygen availability is normally controlled by proximity to blood vessels, it is unclear how hypoxia is preferentially induced in LZ versus. DZ cells, or whether the reciprocal deletion of HIF transcription factors would be sufficient to cause exaggerated GC responses. The enzyme glycogen synthase kinase 3 (GSK3) is also important for GC B cell responses. Through restriction of c-MYC, GSK3 protects proliferating B cells from deprivation of nutrients, such as glucose and amino acids [26]. Since GCs contain a high density of activated cells with elevated glycolytic and mTORC1 activity, GC B cells may have adapted or been desensitized to nutrient deprivation in the microenvironment through GSK3-mediated pathways.

The engagement of autophagy is another notable metabolic program in GC B cells. Compared to resting B cells, autophagy is upregulated in GC B cells, which is consistent with the demand for nutrients to support rapid proliferation [27]. Interestingly, B cells lacking WIPI2, a component in the autophagy pathway, differentiate preferentially to ASCs at the expense of GC B cells, and this altered B cell differentiation is associated with a specific increase in non-canonical autophagy and elevated mitochondrial activity. Although the detailed mechanism is unknown, a shift between distinct autophagy pathways or autophagy-independent function of WIPI2, may control the checkpoint between GC B cells and PCs. These recent findings are collectively summarized in Figure 2.

Metabolic programs to support plasma cells and memory B cells

The major goal of the GC reaction is to output affinity-matured memory B cells and antibody-secreting PCs, the principal effectors within the B cell lineage. A model of LPS-induced differentiation *in vivo* has shown that PCs are formed by a series of transcriptional changes downstream of the canonical PC transcription factor BLIMP1 and EZH2 [28–30]. These transcriptional changes are division-dependent and related to ER stress genes, oxidative metabolism enzymes, and amino acid transporters, thereby leading to mTORC1 activation and enhanced antibody secretion [31,32•]. Other studies have shown that autophagy is also activated and required to regulate ER stress responses, antibody secretion, and energy metabolism [33].