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Editorial overview: B cell shades of diversity and memory

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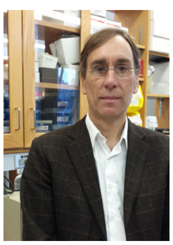

Dr Wasif N Khan received his PhD in Immunology from the University of Umeå, Sweden with Dr Sten Hammarström in 1990, and performed his postdoctoral research at Harvard Medical School with Dr Frederick W. Alt. He then joined Vanderbilt University School of Medicine where he was faculty for over 10 years. He is professor of Microbiology and Immunology with secondary appointments in the Sylvester Comprehensive Cancer Center, Miami Center for AIDS Research, and Diabetes Research Institute at the University of Miami, Miller School of Medicine, Miami, Florida, USA. He contributed significantly to the training of postdoctoral fellows as the Director of the trans-UM postdoctoral programs for eight years and recently stepped down to focus on his research. His current research seeks to understand mechanisms that regulate B cell tolerance and B cell differentiation into antibody secreting cells with as focus on B cell receptor (BCR) coordination with B cell activating factor (BAFF), Toll-like receptors

The field of B cell immunobiology is continuously being infused with exciting new discoveries. These results are providing deeper insights into the somatic processes that generate a diverse naïve B cell repertoire needed for long lasting anti-pathogen humoral immunity. Not surprisingly, technological advances such as next-generation deep sequencing, single cell transcriptomics, and tools for in depth analysis of B cell antigen receptor (BCR) repertoire have enabled this progress. Additionally, metabolomic studies of pre-antigen and post-antigen encounter B cells have helped to elucidate how activated B cells adapt to changes in energy and nutrient requirements required for these cells to undergo clonal expansion and take on new functions. Some exciting examples of these advances may be gleaned from the review articles in this special issue on 'B cell shades of diversity and memory' in the *Current Opinion in Immunology*.

One approach for understanding the underpinnings of B cell activation by antigen is to dissect the kinetics, amplitude and duration of changing intracellular molecular events that occur after BCR engagement, as these changes can influence whether a cell takes on alternate choices such as activation induced cell death versus differentiation into distinct cell fates. For instance, understanding underlying mechanisms is critical for elucidating how normal B cell responses lead to cellular proliferative bursts without causing autoimmune proliferative diseases. Two reviews in this issue by Akkaya and Pierce, and by Egawa and Bhattacharya, provide insights into metabolic fluxes that occur during B cell development, proliferation and differentiation. Both reviews discuss major differences in how B and T cells manage their metabolic needs.

The Pierce group recently reported that BCR initiated increases in metabolism are transient unless the B cells receives a second signal derived by a TLR or a helper T cell. Without this second signal B cell activation wanes within hours. These findings explain why this second signal is obligatory if BCR engagement is to prime proliferation and differentiation, rather than resulting in activation induced cell death [1]. Here, Akkaya and Pierce review this and other recent findings that signify the role of timely and proportionate changes in metabolic activity during quiescent and growth phases in the life of a B cell, spanning from early precursor to memory B cell and antibody producing plasma cell. The authors review the mechanisms controlling oxidative phosphorylation (OxPHOS) and glycolysis under the control of hypoxia induced factor-1a (HIF-1α), AMPK, and Gsk3 in conjunction with other pertinent pathways in B cell. The overall view is that B cells are continually tested for metabolic fitness; this process allows cells to meet their rapidly changing needs, while also ensuring genome stability during differentiation events that require the induction and repair of DNA double-strand breaks (DSB) such as V(D)J recombination and heavy chain isotype class switching.

(TLRs) and helper T cells in normal B cell development and immune response and, in autoimmunity and malignancies.

David Allman is Professor of Pathology and Laboratory Medicine and the Immunology Graduate Program director at the Perlman School of Medicine at the University of Pennsylvania in Philadelphia, Pennsylvania, USA. His research focuses on several stages of B cell development and differentiation including the conversion of naïve and memory B cells into antibody-secreting plasma cells.

Microenvironmental input in germinal centers (GCs) markedly affects changes in metabolic activity in B cells as they undergo several rounds of proliferative cycles in the GC light and dark zones. Egawa and Bhattacharya review current evidence providing insights into BCR regulation of nutrient uptake and usage in B cells including in GCs. The authors review pertinent genetic and *in vivo* data on this process, and also discuss sources of nutrients and mechanisms of multifaceted control of metabolic activity in activated B cells. These processes involve autophagy, mammalian target of rapamycin (mTOR) signaling, and transcriptional control of transporters and rate-limiting enzymes that together ensure metabolic fitness at this key phase of B cell activation. The authors provide an integrated view of metabolic regulation by relevant genetic networks in these processes. Interestingly, certain metabolic modules play distinct roles in promoting survival versus proliferation versus antibody production at distinct phases of B cell activation.

How adaptive immunity changes with age and disease has long been associated with alterations in the B cell antigen receptor repertoire, but it has been difficult to develop a detailed understanding of how these changes translate into age-related deficiencies in immune function. Nielsen and Boyed review new technologies including high-throughput DNA sequencing (HTS) that have tremendously increased the breadth and more importantly the depth of the data sets that can be harvested. This work has transformed biomedical research, in particular research into the unique and complex mechanisms needed for V(D)J recombination to work efficiently. A significant advantage of these new technologies is that very small sample quantities can be employed, allowing investigations into key but unanswered questions. One example centers on differences in the maturation of immune responses in infants versus adults. Nielsen and Boyed review these technologies and implications for answering these and numerous related questions. They also discuss new advances in understanding the capabilities and limitations of the infant immune system; such analyses have proven critical for investigating how infant dietary exposure can affect a wide range of phenotypes including peanut allergy.

DNA double-strand breaks (DSB) form during V(D)J gene rearrangement and heavy chain class switch recombination; both processes are integral to B cell development and function. Recently, Alt *et al.* described a high-throughput genome-wide, translocation sequencing (HTGTS) approach to detect DSBs anywhere in the genome generated by RAG, AID or other enzymes [2]. HTGTS is an unbiased and comprehensive approach to elucidate primary and secondary B cell repertoires, as well as to detect aberrant DSBs that threaten genome stability. The combination of HTGTS with highly multiplexed phenotypic analyses for example, by CyTOF mass cytometry of isolated immune cells or in histological sections of other tissues, will likely be transformative in detection and analysis of human cancers.

The molecular regulation of V(D)J recombination, somatic hypermutation (SHM) and retoire selection remains a subject of intensive research. Wesemann *et al.* discuss several aspects of how the B cell repertoire is shaped in light of several points of freedom and constrains exerted on the selection processes. They highlight inputs of host environment including self-antigens and microbial products that vary in individuals due to varying microbiome. They also discuss a role for polyreactivity and autoreactivity on B cell selection, with the implication that polyreactivity may provide the naïve B cell repertoire with a deliberately abundant storehouse of innateness for the production of antibodies against common pathogens. They also discuss the relevance of polyreactivity to B cell tolerance mechanisms. Regarding the extent of autoreactivity in the human and mouse B cell repertoire, recent elegant work by Weiess and Zhikherman groups, using sensitive reporter systems for BCR signaling, have suggested that all B cells in mice and humans are variably autoreactive and polyreactive [3]. The consequences of these reactivities may depend on the strength of binding to autoantigens in terms B cell survival and positive versus negative selection. Further work is required to reveal the fine differences between the thresholds of autoreactivity that may either help produce a more diverse repertoire or result in autoimmunity.

Autoimmune diseases are diagnosed via detection of secreted autoantibodies, typically these are serum IgG antibodies. Functionally, immunoglobulins can be divided into two interrelated forms that bind the same antigen; surface membrane IgM (or BCR) and secreted antibodies. The principles of repertoire selection and implementation of self-tolerance are established mostly on the basis of membrane IgM–BCR; this form communicates with the intracellular mechanisms that mediates diverse outcomes including apoptosis, anergy and receptor editing. Escape from these mechanisms can result in the production of autoantibodies. Notwithstanding pragmatic issues, there is a disparity in the application of knowledge gained from autoreactive B cell responses mediated by IgM–BCR and the use of secreted autoantibodies for the diagnosis of autoimmune diseases. This disparity is highlighted by recent findings by Jumma's group that demonstrate differences in antigen binding between IgM–BCR and soluble IgG antibodies [4]. The data reveal that most antibodies that have been identified as autoreactive in their soluble form using conventional immunoassays are not autoreactive when expressed as cell surface BCRs. These findings imply that antibody regions outside the antigen binding site influence antibody binding to antigen. These findings warrant further investigations to better understand the molecular basis of the binding differences in membrane bound versus secreted antibodies, as well as to develop assays that more closely mimic binding characteristics of membrane BCR.

In addition, three reviews in this section highlight the capacity of BCR signaling to drive diverse outcomes. The classic view of antigen-driven B cell differentiation holds that responding B cells undergo several rounds of cell division before giving rise to at least three types of daughter cells with diverse functions. These antigen-driven daughter cells include antibody-secreting plasma cells, and GC and memory B cells. This classic model further holds that many additional antigen-specific plasma cells and memory B cells arise from GCs. Plasma cells provide humoral protection by continuously secreting copious amounts of serum antibodies, perhaps for life. In contrast memory B cells recirculate or home to particular tissues poised to generate rapid waves of additional plasma cells upon secondary exposure to the original antigen. Together the reviewed penned here by Cancro and Sanz, together with the review by Smith and Baumgarth, highlight the multiple nuances and general complexity associated with BCR signaling and its impact on B cell selection and function.

Cancro and Sanz review recent findings on a unique subset of memory B cells defined by the expression of the T_H1-affiliated transcription factor Tbet and CD11c. Notably, these cells possess several unique features for memory B cells, and appear to play a unique role in interferon-regulated responses that constrain the cell–cell spread of intracellular microbes. Notably, the CD11c$^+$Tbet$^+$ B cells are hyper-responsive to TLR7 and may play dominant roles in autoimmune diseases, including systemic erythematosus (SLE) [5]. In this regard, these cells are similar to B1 cells in their characteristic high responsiveness to TLR ligands.

The development of B1 B cells is heavily influenced by self-Ag mediated selection processes. Consequently, many B1 B cells appear to experience low chronic BCR signaling throughout their lifetime, and these events condition these cells to take on unique functions including the low-level but constitutive secretion of IgM antibodies into the serum and body cavities. These so-called natural antibodies can play important roles in host defense against a wide variety of bacterial and viral pathogens. Here Smith and Baumgarth provide an up to date review on the unique facets of B1 B cell development and function with a focus on their unique role in host protection. This discussion touches on several issues currently under investigation by many investigators including the selection and role of low affinity and polyreactive antibodies in host protection and homeostasis, and unique immunoregulatory functions played by B1 B cells and related cells.

Completing our consideration of BCR-driven differentiation is a review on other aspects of memory B cells from Tomayko and Allman. These authors discuss recent advances on the characterization of memory B cells, with an emphasis on the notion that memory B cells can take on one of many identities, each with potentially unique roles in host defense. For instance, it is becoming clear that while some memory B cells readily partake in GC responses upon secondary stimulation, others instead rapidly produce a substantial wave of plasma cells without contributing to secondary GC responses. These findings, together with the characterization of Tbet+ memory B cells, suggests that there may be as many as 4 or more unique types of memory B cells.

Coordinated function of key transcription factors, epigenetic modifiers and coding and non-coding transcriptomes including long non-coding RNAs, regulates normal B cell development and activation. In addition, DNA methylome dynamically changes during B cell development with methylation at nearly 5 million CpGs sites, with distinct regulatory regions preferentially demethylated in precursors and GC B cells with impact on immune response. Perturbation in these mechanisms including aberrant DNA methylomes and altered expression of long non-coding RNAs (LncRNAs) can lead to auto-immunity *and B cell lymphomagenesis*. Andrews and Payton review recent advances in the epigenomics of normal B cell development and activation and in B cell cancers, including discussion of the underlying causes of changes in DNA methylation and lncRNAs expression with oncogenic roles in B cell cancers. Specifically, the authors review the most recent discoveries regarding the role of epigenetic regulation in B cells, focusing on latter stages of maturation and activation, particularly in the GC, and development of mature B cell cancers and their mechanisms to subvert epigenetic regulation.

In certain autoimmune diseases as well as chronic infection and cancer, tertiary lymphoid structures (TLS) can form in target organs and appear to influence local immune responses. TLS are similar to secondary lymphoid organs in that they have compartmentalized organization of B and T cells, high endothelial venules and follicular dendritic cells. This organization allows TLS to perform functions similar to GCs, and thus contribute to B cell mediated immunity. However, TLS lack a capsule and lymphatic system. Pitzalis *et al.* review this relatively understudied area, and in doing so provide insights into TLS-associated B cells in cancer, autoimmunity and organ transplantation.

As B cell researchers focus on metabolomic regulation of different B cell states, from their initial development to the antigen-induced induction of memory B cells, we are entering a particularly exciting era of discovery. Ultimately this work will reveal how molecular modules composed of specific genetic networks, intracellular signaling effectors, and metabolic regulators integrate to regulate the complex life and function of a B cell. An important question is whether increased definition of these modules and comprehensive data analysis will lead to insights that will allow precise target-specific interventions that will improve patient diagnosis and treatment.

We thank the authors for contributing their expert reviews reflecting on recent and ongoing investigations, to this special issue of lymphocyte development and activation.

References

1. Akkaya M, Traba J, Roesler AS, Miozzo P, Akkaya B, Theall BP, Sohn H, Pena M, Smelkinson M, Kabat J *et al.*: **Second signals rescue B cells from activation-induced mitochondrial dysfunction and death**. *Nat Immunol* 2018, **19**:871-884.

2. Hu J, Meyers RM, Dong J, Panchakshari RA, Alt FW, Frock RL: **Detecting DNA double-stranded breaks in mammalian genomes by linear amplification-mediated high-throughput genome-wide translocation sequencing**. *Nat Protoc* 2016, **11**:853-871.

3. Zikherman J, Parameswaran R, Weiss A: **Endogenous antigen tunes the responsiveness of naive B cells but not T cells**. *Nature* 2012, **489**:160-164.

4. Iype J, Datta M, Khadour A, Ubelhart R, Nicolo A, Rollenske T, Duhren-von Minden M, Wardemann H, Maity PC, Jumaa H: **Differences in self-recognition between secreted antibody and membrane-bound B cell antigen receptor**. *J Immunol* 2019 http://dx.doi.org/10.4049/jimmunol.1800690.

5. Jenks SA, Cashman KS, Zumaquero E, Marigorta UM, Patel AV, Wang X, Tomar D, Woodruff MC, Simon Z, Bugrovsky R *et al.*: **Distinct effector B cells induced by unregulated toll-like receptor 7 contribute to pathogenic responses in systemic lupus erythematosus**. *Immunity* 2018, **49**:725-739 e726.