

B LYMPHOCYTES

Robert Brink

Garvan Institute of Medical Research, Sydney, Australia

Keywords: Immunoglobulin, antibodies, plasma cells, self-tolerance, antigens, follicular, marginal zone, memory, T-dependent, T-independent, somatic hypermutation, affinity maturation, germinal center, autoantibodies, lymphoma.

Contents

1. Introduction
2. History
3. B cell development
 - 3.1. Rearrangement of Ig Genes in Bone Marrow
 - 3.2. Peripheral Maturation and Localization
 - 3.3. Marginal Zone B Cells
 - 3.4. B1 B Cells
 - 3.5. Memory B Cells
 - 3.6. Plasma Cells
4. Enforcement of self-tolerance during B cell development
 - 4.1. Immature Bone Marrow B Cells
 - 4.2. Immature to Mature B Cell Transition
 - 4.3. Marginal Zone B Cell Development
5. B cell responses to foreign antigens
 - 5.1. Ig Class Switching
 - 5.2. T-Independent (TI) B Cell Responses
 - 5.2.1. TI Type 1 (TI-1) Responses
 - 5.2.2. TI Type 2 (TI-2) Responses
 - 5.3. T-Dependent (TD) B Cell Responses
 - 5.3.1. Initiation Of T:B Cell Co-Operation
 - 5.3.2. Extrafollicular Plasma Cell Response
 - 5.3.3. Germinal Center Response, Somatic Mutation and Affinity Maturation
 - 5.3.4. Memory B Cells and Secondary Responses
6. B cells and Disease
 - 6.1 Autoimmune Diseases
 - 6.2. B Cell Lymphomas
- Glossary
- Bibliography
- Biographical Sketch

Summary

B lymphocytes (B cells) are a specialized group of small migratory cells whose primary role is to produce antibodies in response to foreign organisms or molecules (antigens) that enter the body. Along with T lymphocytes, B cells form the adaptive arm of the immune system that allows vertebrates to respond to a wide range of antigens. Individual B cells express unique antigen receptors in the form of immunoglobulin

molecules consisting of a constant region and an antigen-binding variable region. The processes that generate antigen receptor diversity are largely random and result in the production of many B cells that recognize components of self as well as those that can be used to eliminate invading pathogens. The immune system has therefore developed the property of “self-tolerance” whereby anti-self B cells are prevented from producing autoantibodies by being physically eliminated at various stages during B cell development. B cells that survive due to their lack of self-reactivity circulate through the body and, upon recognizing foreign antigen, commence cell division and ultimately differentiate into antibody-secreting plasma cells. B cell responses to foreign antigens can occur with or without collaboration from T helper cells that recognize the same antigen. Responses that occur without T cell help (T-independent) typically occur in response to molecules associated with pathogens such as bacteria and viruses and provide a rapid wave of antibody production. T-dependent B cell responses, although somewhat slower, provide longer-lasting production of high affinity antibodies and also generate memory B cells that provide effective immunity against re-infection. As well as protecting the host, B cells can contribute to disease through inappropriate activation of anti-self B cells (autoimmunity), production of antibodies that give rise to allergies, and uncontrolled growth leading to B cell lymphomas.

1. Introduction

The ability to recognize and eliminate foreign organisms and structures is a characteristic of almost all life forms. Even relatively simple multicellular organisms such as the flatworm are capable of producing anti-microbial agents in response to infection. In more complex invertebrates such as the fruit fly, phagocytic cells that can recognize and ingest invading microorganisms form an additional level of protection against infection. The evolution of the vertebrates heralded the arrival of a new type of immune cell called the lymphocyte. Lymphocytes brought with them the ability to recognize and eliminate a vastly increased range of foreign structures (antigens) and gave rise to what is commonly referred to as “adaptive” immunity.

The ability of the adaptive immune system to respond to a wide variety of antigenic structures is based fundamentally on the expression of clonal antigen receptors by individual lymphocytes. These receptors exhibit an incredibly diverse range of antigen binding properties, meaning that the lymphocyte repertoire as a whole has the ability to recognize and respond to almost any invading structure that is confronted. The corollary of this, however, is that the frequency of lymphocytes that participate in an adaptive immune response to any given antigen is exceedingly low. The rapid mobilization and proliferative expansion of antigen-activated lymphocytes is therefore one of the crucial properties of adaptive immune responses.

Adaptive immunity in humans and other mammals is based on two major lymphocyte subsets, namely B cells and T cells. These two cell types, although they have much in common, function in fundamentally different ways. The major role of B cells is to produce antibody molecules. Because antibodies are secreted versions of the B cell membrane antigen receptor, the antibodies produced by an antigen-activated B cell can specifically bind to and eliminate the original activating antigen. Before antibodies can be produced however, B cells must differentiate into a specialized cell type known as

the plasma cell. Plasma cell differentiation can occur directly after antigen encounter but often requires cooperation from T cells that have also been activated by the same foreign antigen. The “Immunology and Immunopathology” theme within the EOLSS contains separate chapters that deal in depth with the related topics of “*Antibody Structure and Function*” and “*T lymphocytes*”. These subjects will be touched upon here insofar as they relate to the development, differentiation and function of B lymphocytes but the reader is directed towards these other chapters for more detailed coverage.

2. History

Lymphocytes were first identified by William Dawson in 1770. He found them to be small, rounded cells that were principally located in organs such as the spleen, thymus, and lymph nodes. Remarkably, the true function of lymphocytes was destined to remain unknown until nearly two centuries later. In 1890 it was recognized that protective antibodies appeared in blood serum in response to the introduction of foreign antigens into the body, and that these antibodies were specific for the antigen in question. How this type of immune response occurred and which cell type produced the antibodies became the subject of much conjecture. Clues that lymphocytes might be involved in immune responses did appear in the early 20th century in studies by Murphy and Morton that noted the infiltration of lymphocytes into grafts and tumors that were in the process of being rejected. However, it wasn't until the 1950's that the concept of lymphocytes as the source of antibodies began to emerge. In 1957, MacFarlane Burnet published the theory that each cell capable of producing antibody has a unique specificity that is also expressed as a cell surface antigen receptor (“clonal selection hypothesis”). This theory was rooted in the original and insightful “side chain hypothesis” of Paul Ehrlich nearly 70 years earlier but introduced the idea that a lymphocyte's fate was determined through the interaction of its clonal antigen receptor with the environment.

Soon after the publication of Burnet's theory it was discovered that lymphocytes are not a single type of cell but can be divided into two major subsets. The fact that these subsets were ultimately called B lymphocytes (B cells) and T lymphocytes (T cells) is somewhat of a historical curiosity. B cells were first identified in chickens and named by virtue of their dependence on the avian organ known as the bursa of Fabricius for their development. T cells were so named when it was found that they required the thymus for their development. B cells were later shown to derive from the bone marrow in mammals, fortuitously maintaining the relevance of the label originally obtained from the chicken. It was soon discovered that B cells were the source of antibodies, but that in most cases T cells were also required for antibody production to occur. The recognition in the late 1960's that this occurred by B cells and T cells recognizing different parts of the same immunogen represented a watershed in cellular immunology and forms the basis of how we understand antibody production to occur to this day. This finding also pointed to the emerging importance of molecular analysis in order to understand the complexities of lymphocyte responses.

During the 1970's, molecular biology and genetic analysis revolutionized all spheres of biological study. The genetic complexities underlying clonal antigen receptor expression by lymphocytes were soon discovered and, over the coming decades, many

molecules that regulated all phases of B cell development, differentiation and function were identified. The advent in the 1980's of transgenic mouse models, in which the B cell repertoire could be manipulated to express predominantly a single species of antigen receptor, allowed responses to specific antigens to be followed. This proved especially powerful in revealing how B cells that recognize “self” antigens are controlled so as to prevent autoantibody production. Since the early 1990's, further elucidation of gene function in the immune system has been obtained through the generation of mice engineered to lack expression of specific genes (gene knockout mice) but also through studies of inherited immune disorders that have identified a number of genes critical for the proper functioning of the immune system. Much has been learned and there is more to be discovered about how B cells are made and how they behave. Below is a summary of our current understanding of B lymphocytes based primarily on studies made within human and mouse experimental systems.

3. B Cell Development

3.1. Rearrangement of Ig Genes in Bone Marrow

B cells are in constant production throughout life. In adults, B cells as well as T cells, granulocytes, macrophages, neutrophils, NK cells, platelets and erythrocytes all derive from a relatively small number of multipotent precursor cells called hemopoietic stem cells. Hemopoietic stem cells reside primarily in bone marrow and proliferate there in order to both undergo self-renewal and to produce daughter cells that will ultimately differentiate into one of the hemopoietic lineages. Proliferating precursor cells that are committed to the B lymphocyte lineage can be identified by the expression of a number of cell surface proteins but are characterized in particular by the commencement of DNA rearrangements within the immunoglobulin (Ig) genes that will eventually encode the clonal B cell antigen receptor (BCR) and potentially later encode secreted antibody.

As is discussed in detail in *Antibody Structure and Function*, Ig molecules consist of paired heavy and light chains that are each comprised of a constant region and clonally defined variable region. The sequences of the heavy and light chain variable regions that will ultimately be expressed by the developing B cell are determined by the largely random assortment of V, D and J gene segments from the 5' regions of the Ig genes (Figure 1). Initial gene rearrangements occur at one of the two Ig heavy chain loci. At the pro-B cell stage, a D segment is first rearranged to a J_H segment. Subsequently, one of the many 5' V_H segments is translocated to form a V_HDJ_H variable region exon. Whilst the large numbers of V_H, D and J_H segments available for recombination leads to great diversity of the variable region exons generated in different B cells, this diversity is increased even further by the imprecise nature of the joining process, which often results in the deletion or addition of nucleotides at the junctions between neighboring gene segments. If the variable region exon generated by the initial round of gene rearrangements in the pro-B cell does not encode a functional protein (eg due to stop codon or frame shift), the alternative Ig heavy chain locus undergoes V_HDJ_H recombination. This ordered recombination process is critical to ensuring “allelic exclusion” of Ig expression in individual B cells: in other words the “one cell one receptor” law as originally proposed by Burnet.

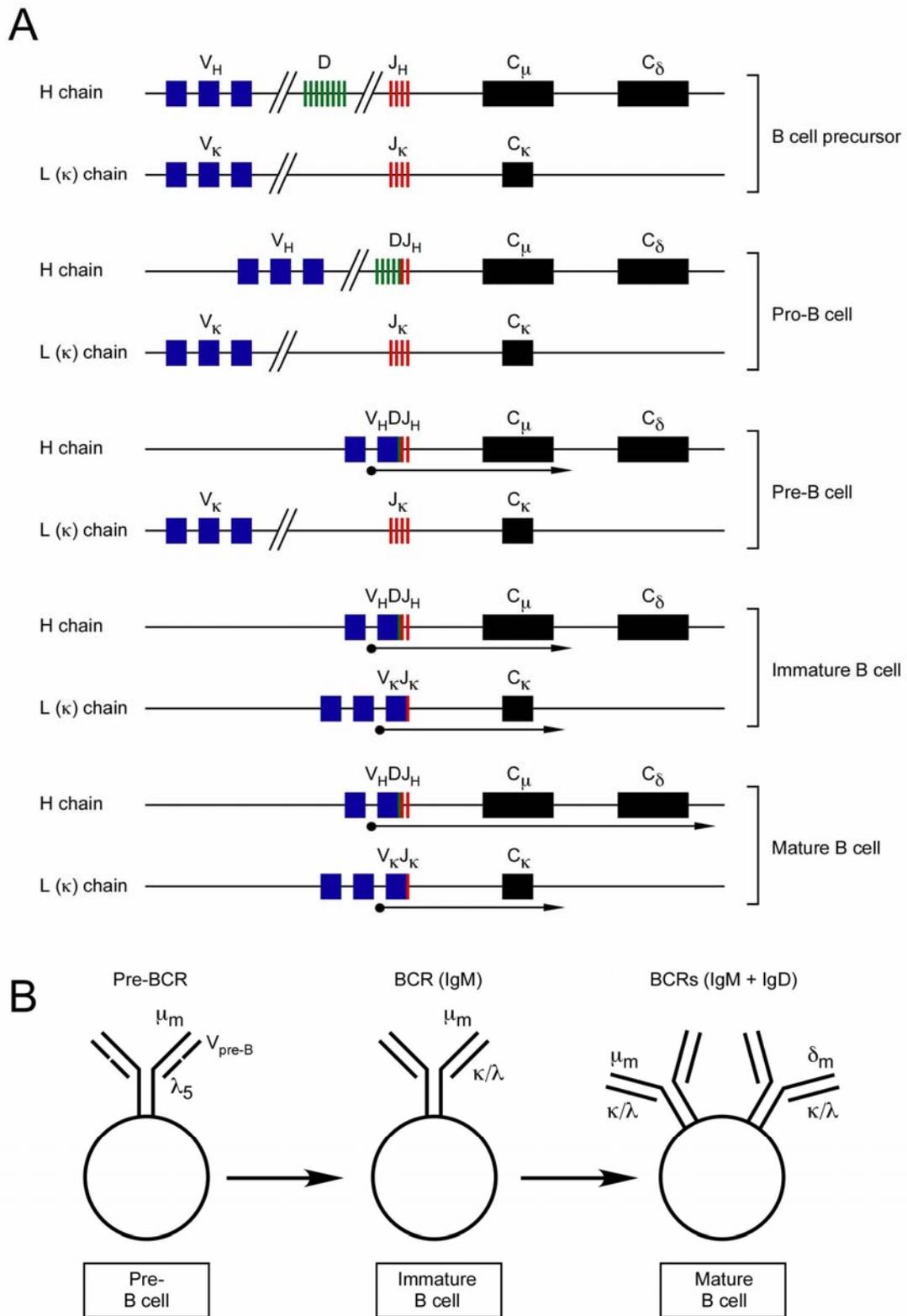


Figure 1. Ig gene rearrangements and cell surface Ig phenotype during B cell development. **(A)** Progressive changes in rearrangement and transcription of Ig heavy (H) and light (L) chain genes during B cell development. For clarity only the κ L chain locus is shown. Multiple V and J gene segments are present in the germ-line

configuration of the Ig genes with additional D gene segments present in the H chain gene. Initial recombination events occur in the H chain locus of pro-B cells, the earliest committed precursors, where one of the D segments is fused to a J_H segment with the resulting loss of the intervening DNA. In pre-B cells a V_H segment is subsequently fused to the DJ_H to form the V_HDJ_H variable region coding exon. Transcription from the promoter associated with the V_H segment through to the μ constant region gene ($C\mu$) (indicated by arrow) facilitates expression of μ membrane (μ_m) heavy chain. Subsequent $V\kappa$ - $J\kappa$ recombination results in the expression of κ light chain driven from the $V\kappa$ -associated promoter. As the B cell matures, transcription of the H chain locus progresses through the δ constant region sequences ($C\delta$) to allow expression of the variable region in both μ_m and δ_m heavy chains. **(B)** Changes in surface Ig expression during B cell development. Following expression of μ_m in pre-B cells, it is expressed on the cell surface in combination with the germ-line encoded pseudo-light chain components V_{pre-B} and λ_5 . Successful rearrangement of either κ or λ light chains results in the expression of bona fide Ig light chain which replaces the V_{pre-B} and λ_5 components of the pre-BCR to produce the IgM BCR expressed by immature cells. Subsequent synthesis of δ_m along with μ_m heavy chain results in the coexpression of IgM and IgD BCRs by mature B cells.

Following successful rearrangement of the Ig heavy chain locus, the developing B cell becomes a pre-B cell. Pre-B cells express μ heavy chains encoded by the rearranged V_HDJ_H exon spliced to the μ heavy chain constant region exons (Figure 1). The μ gene is most proximal of the eight (mice) or nine (human) sets of constant region genes that comprise the 3' portion of the Ig heavy chain locus. Prior to the production of a bona fide Ig light chain, pre-B cells express a cell surface receptor consisting of μ membrane (μ_m) heavy chain in complex with two pseudo light chain proteins called V_{pre-B} and λ_5 (Figure 1B). Expression of the pre-B cell receptor signals the cessation of Ig heavy chain gene rearrangements and the commencement of rearrangements at light chain loci. Ig light chain gene rearrangements occur in a similar fashion to the heavy chain locus except only V and J segments are assembled. Mice and humans both have two light chain genes called κ and λ . Rearrangements typically occur at the κ locus first but proceed to the λ locus if the rearrangements at κ are unsuccessful. Once again, rearrangement of the light chain locus proceeds in an ordered manner and terminates once a successful rearrangement has been obtained in order to ensure that individual B cells express only a single species of heavy and light chain. Failure to express a functional BCR results in the death of the developing lymphocyte. Indeed BCR expression is required for survival throughout the subsequent life of the B cell, apparently due to its ability to constitutively deliver essential survival signals.

3.2. Peripheral Maturation and Localization

The successful completion of Ig gene rearrangements marks the transition from pre-B cell to immature B cell. Immature B cells express antigen receptor in the form of IgM, a complex of μ_m heavy chain with either κ or λ light chain. Immature B cells exit the bone marrow via the blood stream and circulate to the spleen. Here the cells mature further, undergoing a number of changes in gene expression. This includes extended transcription of the Ig heavy chain locus and differential splicing of the rearranged Ig

heavy chain region to δ as well as μ heavy chain constant regions (Figure 1). The results in the coexpression by maturing B cells of cell surface IgD and IgM with identical antigen specificity.

The number of B cells produced in the bone marrow every day greatly exceeds the body's requirements. Thus the majority of the immature B cells survive for only one or two days before they die in the periphery, with only around 10% of immature B cells joining the peripheral pool of mature, long-lived B cells. Mature B cells, which have a half-life of weeks to months, are found mainly in the secondary lymphoid tissues such as spleen, lymph nodes and tonsils. These cells also circulate around the body through the blood vessels and the lymphatic networks that connect secondary lymphoid tissues. Some B cells also recirculate back to the primary lymphoid tissue i.e. the bone marrow.

Lymphocytes are typically grouped into spatially defined structures within secondary lymphoid tissues. In the spleen, lymphocyte-rich areas known as the white pulp lie between broader lymphocyte-free areas called red pulp. Within splenic white pulp and in lymph nodes, B cells are organized into specific areas referred to as primary follicles or B cell follicles. This B cell area is typically located adjacent to a T cell rich zone, meaning that these two lymphocyte subsets are normally spatially separated within secondary lymphoid tissues. The separation of B and T cells is brought about by differential responsiveness to specific chemokine molecules. Chemokines are a family of secreted proteins that play a major role in regulating cellular migration and localization within the immune system. Thus B cells cluster around networks of follicular dendritic cells (FDCs) due to the production by these cells of the B cell chemo-attractant CXCL13 (Figure 2). T cells on the other hand migrate towards a different subset of dendritic cells that produce the chemokines CCL19 and CCL21 (Figure 2). The significance of this separation will become more evident later when we discuss how antigen-activated B and T cells collaborate during T-dependent B cell responses to foreign antigens (see Section 5.3).

3.3. Marginal Zone B Cells

Within the spleen there exists a subset of mature B cells that possesses a distinct phenotype and localization compared to the B cells from the primary follicle. These are known as marginal zone (MZ) B cells since they are located in the area of the same name that lies external to the FDC-rich B cell follicle and inner T cell area (Figure 2). Located between the follicle and the MZ is a narrow marginal sinus through which blood flows (Figure 2). MZ B cells are therefore directly exposed to blood borne antigens and are thought to be the first line of defense against such antigens (see Section 5.2). Unlike follicular B cells, MZ B cells do not appear to circulate around the body but remain within the MZ unless activated. Also distinct from follicular B cells is the ability of MZ B cells to undergo self-renewal via a low but significant level of self-replication. MZ B cells are particularly sensitive to activation by antigen and polyclonal B cell stimuli such as Toll-like receptor (TLR) ligands and express high levels of a number of molecules involved in B cell activation.

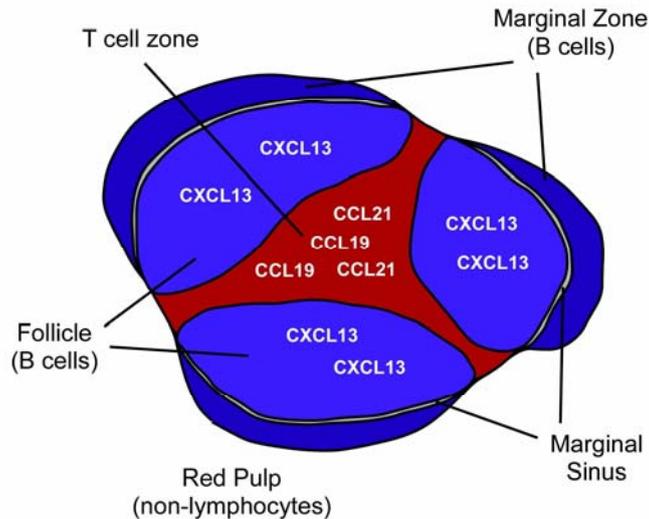


Figure 2. Spatial organization of B and T lymphocytes within the spleen. Lymphocytes gather into defined areas known as the white pulp that lie within broad essentially lymphocyte-free areas called the red-pulp. Within the white pulp, T cells gather in the central area that surrounds a central arteriole (not shown). B cell follicles sit adjacent to the T cell area and these are in turn surrounded by a blood filled space known as the marginal sinus. The marginal zone lies outside the marginal sinus and contains the marginal zone B cell subset. Dendritic and stromal cells also lie within the white pulp and, within the central T cell area, produce the T cell chemoattractants CCL19 and CCL21. Cells within the follicles produce the B cell chemokine CXCL13, thus maintaining the separation between B cells and T cells within secondary lymphoid tissues.

TO ACCESS ALL THE 25 PAGES OF THIS CHAPTER,
 Visit: <http://www.eolss.net/Eolss-sampleAllChapter.aspx>

Bibliography

Dudley D.D., Chaudri J., Bassing C.H. & Alt F.W. (2005) Mechanism and control of V(D)J recombination versus class switch recombination: similarities and differences. *Adv. Immunol.* **86**, 43-112. [Summary of the molecular mechanisms that regulate the gene rearrangements responsible for variable region gene formation and switching of Ig constant region sequences].

Forsdyke D.R. (1995). The origins of the clonal selection theory of immunity as a case study for evaluation in science. *FASEB J.* **9**, 164–166. [A thorough analysis of the evolution of the clonal selection theory of MacFarlane Burnet from the original side chain theory of Paul Ehrlich. Both men were awarded the Nobel prize in Physiology or Medicine in 1960 and 1908 respectively].

Goodnow C.C., Spent, J., Fazekas de St Groth, B. & Vinuesa, C.G. (2005). Cellular and genetic mechanisms of self tolerance and autoimmunity. *Nature* **435**, 590-597. [Excellent summary of the molecular and cellular mechanisms that underpin self-tolerance in both the B cell and T cell repertoires].

Küppers R. (2005). Mechanisms of B-cell lymphoma pathogenesis. *Nat. Rev. Cancer* **5**, 251-262. [Overview of the genesis and characteristics of the various B lymphomas].

Martin F. & Chan A.C. (2006) B cell immunobiology in disease: Evolving concepts from the clinic. *Annu. Rev. Immunol.* **24**, 467-496. [Highlights recent findings regarding the involvement of B cells in autoimmune diseases].

Martin F. & Kearney J.F. (2001) B1 cells: similarities and differences with other B cell subsets. *Curr. Opin. Immunol.* **13**, 195-201. [Summary of the characteristics of lymphocytes in the B1 lineage compared to other B cells].

McHeyzer-Williams L.J. & McHeyzer-Williams M.G. (2005) Antigen-specific memory B cell development. *Annu. Rev. Immunol.* **23**, 487-513. [Summary of the generation of memory B cells in vivo and their involvement in secondary antibody responses].

Pabst O., Herbrand H., Berhardt G. and Förster R. (2004) Elucidating the functional anatomy of secondary lymphoid organs. *Curr. Opin. Immunol.* **16**, 394-399. [Presents the current state as well as the history of our knowledge of secondary lymphoid tissues including the role of chemokines in their cellular organization].

Pillai S., Cariappa A. & Moran, S.T. (2005) Marginal zone B cells. *Annu. Rev. Immunol.* **23**, 161-196. [Summarizes our current understanding of the development and function of marginal zone B cells].

Shapiro-Shelef M. & Calame K. (2005) Regulation of plasma cell development. *Nat. Rev. Immunol.* **5**, 230-242. [Summarises current knowledge of the molecular and cellular regulation of plasma cell differentiation from activated B cells].

Wardemann H, Yurasov S, Schaefer A, Young J.W., Meffre E. and Nussenzweig M.C. (2003) Predominant autoantibody production by early human B cell precursors. *Science* **301**, 1374-1377. [Elegant analysis of the frequency of autoreactive B cells at progressive stages through B cell development – basis of the statistics shown in Figure 3].

Wolniak K.L., Shinall S.M. & Waldschmidt T.J. (2004) The germinal center response. *Crit. Rev. Immunol.* **24**, 39-65. [Detailed analysis of the cell types and sequence of events that make up the germinal center response].

Biographical Sketch

Robert Brink obtained his Honours degree in Science from the University of Sydney in 1987 where he majored in Biochemistry and Genetics. He completed his PhD in 1992 under the supervision of Antony Basten and Chris Goodnow at Sydney's Centenary Institute, where he worked on novel transgenic mouse models of B cell self-tolerance. In 1994 he took up a postdoctoral position in the laboratory of Harvey Lodish at the Whitehead Institute in Boston, where he investigated how the newly identified TRAF family of intracellular proteins function in signal transduction by cell membrane receptors. Upon returning to the Centenary Institute in 1996, he developed a number of genetically modified mouse models designed to elucidate *in vivo* B cell responses and TRAF protein function. In 2006 Dr Brink was recruited to the Garvan Institute of Medical Research in Sydney to head the B Cell Immunology group. In 2010, he was appointed Leader of the Institute's Immunology Research Program. Dr Brink is a Senior Research Fellow of the National Health and Medical Research Council and Associate Professor in the Faculty of Medicine at the University of New South Wales. His major research focus continues to be on the regulation B cell survival and antibody production during protective immune responses and how these processes contribute to diseases such as autoimmunity, allergy and cancer.