09/26/05; 9 AM
Shiv Pillai

B Lymphocyte Development and Activation

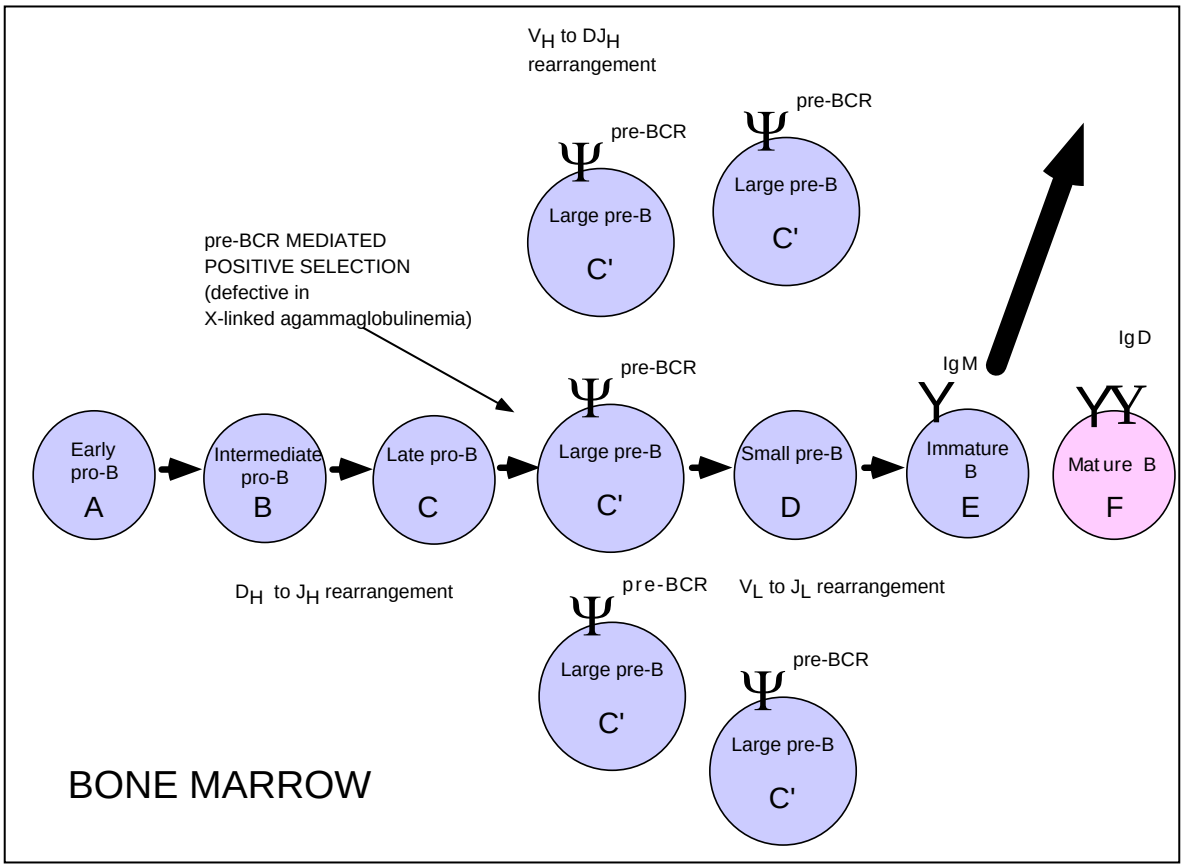
Recommended Reading: Abbas et al., 5th edition, Chapters 7 and 9; Janeway et al., 5th edition, Chapters 6 and 9

B cell development is initiated in the fetal liver prior to birth and continues in the bone-marrow subsequently. The B-1 lineage develops primarily from fetal liver derived stem cells. Conventional B cells are derived from bone-marrow derived HSCs and are sometimes referred to as B-2 cells.

While the initial steps of B lymphoid commitment and development depend upon signals from stromal cells, unique B lineage specific selection events drive the developmental program after immunoglobulin gene rearrangement is initiated. Approximately one-third of developing pre-B cells in the bone-marrow make in-frame V to DJ_H rearrangements and these cells are positively selected (positive selection I). In the bone-marrow pre-B receptor signaling mediates both positive selection I as well as allelic exclusion at the IgH locus. Once both heavy and light chain genes are rearranged and expressed, immature B cells may also be positively selected in response to antigen receptor mediated signals (positive selection II). If these cells express self-reactive antigen receptors, they may be subject to receptor editing) or they may be selected against by means of a deletional process.

Cell selection and allelic exclusion mediated by the pre-B receptor

A rearranged immunoglobulin heavy chain gene encodes two forms of heavy chain protein, a membrane form and a secreted form. mRNAs specific for these two forms are generated as a result of differential splicing and polyadenylation. It is the membrane form of the immunoglobulin heavy chain that is involved in signal transduction, as part of the pre-B receptor in pre-B cells, and the antigen receptor in B cells.



<

Figure 1. A summary of the early satges of B cell development in the bone marrow including the pre-BCR

Pre-B cells which have made in-frame rearrangements at the heavy chain locus after V to DJ_H rearrangement and which can therefore synthesize a μ protein are positively selected. This selection event depends on signals from the pre-B receptor. The pre-B receptor mediated survival signal is dependent on the presence of the membrane immunoglobulin heavy chain, surrogate light chains, the Igα/β heterodimer, Syk and (in man, but not critically so in mice) Btk. This latter kinase is defective in X-linked agammaglobulinemia, an inherited disease in which the pre-B to B cell transition is blocked. Formal evidence suggesting that Src family kinases are involved in the generation of the pre-B receptor mediated survival signal is lacking, presumably because of the large number of Src family kinases that can associate with the pre-B and B cell receptors. However since both Syk and Btk are activated by Src-family kinases, it is extremely likely that Src family kinases associated with the pre-B receptor play a role in generating a signal for survival and

allelic exclusion. The pre-B receptor is largely intracellular although it can be detected with some difficulty on the surface of pre-B cells. No ligand has been identified for this receptor and it is likely that it signals constitutively, being triggered by the act of pre-antigen receptor assembly.

It is formally possible that a yet to be identified ligand exists for the pre-B receptor. However truncated immunoglobulin heavy chains lacking ligand binding regions can participate in the generation of pre-B survival and progression signals. Given the fact that pre-antigen receptors are generated, not to respond (like most receptors) to external clues, but to monitor if the "right" kind of rearrangement has taken place within a given cell, it is not unreasonable to suspect that such a receptor does not require an external ligand for signal initiation. The fully assembled receptor, because of some structural feature of the surrogate light chains, may be constitutively "on" or may respond to a ligand made by the pre-B cell itself

Clonal deletion and receptor editing during B cell development in the bone-marrow

A proportion of the B cells that are generated each day express antigen receptors that are directed against self-antigens. B cells bearing receptors that are cognate for multivalent self-antigens (polysaccharides, membrane proteins, plasma membrane glycolipids) need to be either eliminated or tolerized in some other manner. At the immature B cell stage, crosslinking of the B cell receptor by a multivalent ligand might induce signals that could lead to the induction of apoptotic death. Such a view has been supported by studies in which "monoclonal mice" have been generated which express specific immunoglobulin heavy and light chain transgenes that form a receptor directed against a multivalent antigen (a membrane anchored protein for example). Initial studies of this sort suggested that at the immature B stage a strong crosslinking stimulus could lead to the elimination of these B cells thus leading to the generation of tolerance. It has now become apparent that B cells which receive a strong crosslinking stimulus at the immature B cell stage may be given "another chance" or chances to reform themselves. They do this by undergoing further rearrangements primarily of light chain genes and also by presumably deleting their existing rearranged light chain genes. If a given B cell has already made a productive Vκ to Jκ rerrangement and is triggered to edit its receptor, the next Vκ to Jκ rearrangement that it makes will delete out and replace the previously rearranged VκJκ unit. As a

result this cell may now make a receptor that no longer is triggered by multivalent self and will both evade elimination as well as have the potential to be a useful citizen in a future pathogen directed immune response.

Is deletional tolerance a “physiologically” relevant phenomenon in the B lineage? At present the jury is still out on this issue - the weight of the more recent evidence favors the view that multivalent self- antigens that can access the bone marrow probably induce tolerance by provoking antigen receptor signal mediated receptor editing. Other modalities of tolerance induction, such as anergy and follicular exclusion may be relevant at least for a subset of soluble self-antigens and these will be discussed in a later section in this chapter.

Positive selection II and peripheral maturation

For most students of the immune system positive selection of T cells during thymic development seems to make intuitive sense when one considers the need to select MHC restricted T cells. It has not been readily apparent why a positive selection phenomenon might be of relevance for recently generated B cells. Of the large number of B cells generated in the bone marrow every day (about 10^8) cells only about 10-15% actually make it to the periphery. Most newly generated B cells have a life-span that can be measured in days but a proportion of naive B cells become long-lived cells which may recirculate in the periphery for 6-8 weeks. The possibility has been raised in some studies that a proportion of newly generated B lymphocytes might in a non-random way (based on receptor specificity) be selected to develop in the periphery and to become long lived B cells.

It is now clear that at the immature B cell stage, in addition to negative selection or receptor editing, B lymphocytes are also positively selected. The strength of BCR signaling determines in part whether a B cell matures into a marginal zone B cells, a follicular B cell, or a B-1 B cell.

T-independent B cell activation

Multivalent antigens, exemplified by bacterial polysaccharides, can directly trigger B cells with little or no T cell help. While this category of antigens can invoke an immune response and antibody secretion the B cell is not generally triggered to go through the processes of isotype switching and affinity maturation. As a result the antibodies generated by T-independent antigens tend to be mainly of the IgM class and of relatively low affinity.

Signal Two for T-independent immune responses is provided by Toll Like Receptors which respond to specific Pathogen Associated Molecular Patterns.

T-dependent pathways of B cell activation

Protein antigens are internalized by B cells (by receptor mediated endocytosis) cleaved into peptides and specific peptide-MHC class II complexes are presented to cognate T cells. An activated T cell may trigger a B cell by a number of means the most critical receptors on the B cell being CD40 and the IL-4 receptor. T-dependent responses result in the formation of germinal centers (described below) in secondary lymphoid organs and it in these specialized expansions of B cells that the processes of isotype switching and somatic mutation occur.

Signal Two for T-dependent immune responses is provided by CD40, which is triggered by the trimeric membrane bound CD40 ligand that is expressed on the surface of activated helper T cells.

Diversification and recombinational events that occur in germinal centers: somatic mutation and isotype switching

Mature B cells may encounter cognate protein antigens (T-dependent) or their receptors may be crosslinked by multivalent T-independent antigens. Protein antigens are bound by the antigen receptor, processed and presented in an MHC class II context to helper T cells. The CD40 ligand on activated T cells delivers a growth signal to B cells via the CD40 molecule on B cells. Additional signals are provided by T cell derived cytokines. T dependent signals are delivered in the germinal centers of lymph nodes and contribute to clonal expansion, the initiation of isotype switching and somatic mutation. In patients with X-linked hyper IgM syndrome, T-B collaboration is abrogated because of an inherited absence or defect in the CD40 ligand.

Isotype switching

While signals via CD40 are essential for expansion, somatic mutation and isotype switching, specific signals from distinct cytokine receptors may influence isotype switching from IgM to specific heavy chain loci. The generation of interleukin-4 by helper T cells may for instance help drive class switching to IgE, while a high local concentration of TGF-β may drive switching to IgA. Signal transduction leads to the transcriptional induction of DNA binding proteins which identify switch sites upstream of heavy chain constant regions and help mediate a deletional recombination event which brings the

rearranged VDJ unit from its location adjacent to the μ locus to a site next to the heavy chain locus targeted for switching.

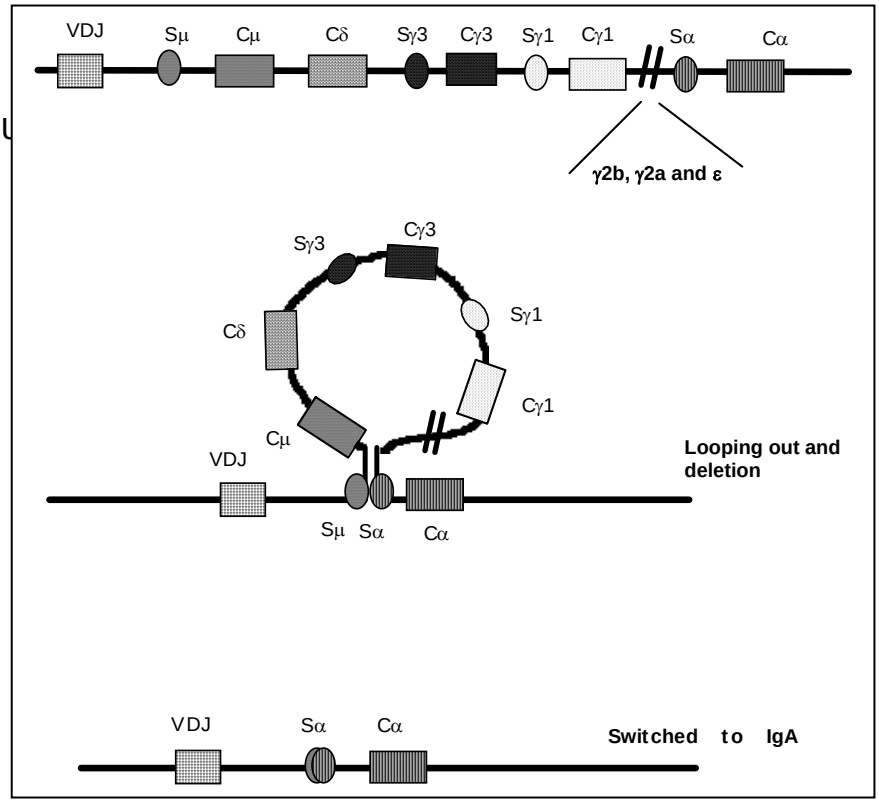


Figure2. A schematic view of isotype switching

ps
tr
ea
m
of
ea
ch
co
ns
ta
nt
re
gi
on
is
a
re
gi
on of DNA known as a switch (S) region. S regions contain multiple repeats of GAGCT and TGGGG. Just upstream of each switch region is a I region promoter and an I exon. When a heavy chain isotype is targeted for class switching, a transcript is initiated from the I region 5' to the targeted constant region. The transcript reads through the I exon, the switch region and the constant region exons and is terminated and polyadenylated at the 3 ' end of the constant regions. This transcript is spliced (removing the switch region and other introns). It has been postulated that the spliced RNA might be a structural component of the switch recombinase. Very little is known at present about this recombinase. However AID (discussed below) is an essential component of the switch machinery.
<

Cell selection in germinal centers and affinity maturation

Somatic mutation occurs in the dark zone cells in germinal centers of lymph nodes. In this process, V regions and flanking sequences are mutated. The mutational process starts from the 5' end of the rearranged V gene. The 3' border of the segment that is mutated is variable, and mutation can spread into the adjacent intron. There is a

strand bias in the process. As a result of this process a number of B cell clones with altered affinity for the original immunogen are generated. Clones with the highest affinity for antigen are selected and expand during the process of affinity maturation.

A protein known as AID (Activation induced cytidine deaminase) has been shown to be required for both class switching and somatic mutation. AID is now known to be a DNA deaminase. AID is induced in a T-dependent manner in activated B cells and works in conjunction with a single stranded DNA binding protein called RPA to deaminate Cs in single stranded DNA (generated during transcription), The conversion of Cs in hotspots to Us is a critical event in both somatic mutation and isotype switching. Conversion of a C to a U may result in the mutated residue being fixed as a T following replication. Alternatively the U residue may be removed by uracil N-glycosylase to generate an abasic site which may then be repaired in an error prone manner generating all other possible substitutions.

B cell activation and disease

Genetic defects in the CD40L/CD40 pathway contribute to inherited hyper-IgM syndromes in which T dependent B cell activation is defective and germinal center formation, isotype switching, and affinity maturation fail to occur.

Approximately 90% of all human lymphomas arise from activated B cells, and the majority of these tumors are derived from post-germinal center B cells. The process of somatic mutation can be somewhat imprecise, leading to the generation of oncogenic point substitutions in certain genes such as the Bcl-6 gene.

Summary:

- B cells are derived from hematopoietic stem cells. Initial development occurs in an antigen-independent manner in the bone marrow (or the fetal liver) and depends on specific stromal factors.
- The pro-B cell is the earliest committed step in the B cell lineage. D to J recombination of the Ig heavy chain marks the transition to the late pro-B stage while a proper, in-frame V to DJ recombination event marks the transition to the pre-B cell stage.
- The pre-B cell expresses the pre-BCR consisting of the membrane-bound Ig heavy chain with surrogate light

chains (VpreB and λ5). Expression of the pre-BCR is required for positive selection I which involves the survival and proliferation of pre-B cells. The pre-BCR associates with the Igα/Igβ heterodimer and signals via Syk and Btk. Btk is mutated in patients with X-linked agammaglobulinemia, a disease in which the pro-B to pre-B cell transition is blocked.

- Signaling via the pre-BCR is required for allelic exclusion at the heavy chain locus. Allelic exclusion may be regulated by both the inactivation of RAG activity as well as accessibility to the heavy chain gene locus. Phosphorylation of RAG-2 may be critical for its ubiquitination and subsequent proteasomal degradation. Signaling via the pre-BCR may induce κ or λ light chain gene rearrangement as well. A productive light chain rearrangement marks the transition to the immature B cell stage.
- Crosslinking of the BCR on the surface of immature B cells by self-antigen in the bone marrow may induce apoptosis. This is known as clonal deletion or negative selection. However, self-reactive B cells may be given a second chance via receptor editing in which rearrangement of light chain genes or at the heavy chain locus can occur to produce a new BCR.
- In positive selection II, the BCR is “tickled” to induce the emergence of immature B cells into the periphery 2-3 days after the pre-B cell stage. These immature cells initially express surface IgM but little or no IgD. Transition to the mature B cell stage requires BCR signaling: Syk is required for follicular entry while Btk is needed for peripheral maintenance.

Maturation of the B cell correlates with higher IgD expression.

- B cells are activated in the periphery via antigen-dependent mechanisms. T_H cells are required for the B-cell response to protein antigens (“thymus-dependent antigens”). Within 1-3 weeks after exposure to thymus-dependent antigen (i.e., protein antigens), activated B cells migrate from focal aggregates into follicles to generate germinal centers. It is in germinal centers that isotype switching and somatic mutation occur. CD40-CD40L interactions are required to form germinal centers. Boys with defects in the X-linked CD40L gene present with the X-linked hyper IgM syndrome. Similar clinical phenotypes are observed in patients with recessive defects in the CD40 gene and in patients with a defect in the activation induced cytidine deaminase (AID) gene.
- Isotype switching involves recombinational events between switch regions. The nature of the switch recombinase is not known. AID is required for isotype switching in an incompletely understood manner. CD40 signaling is required and cytokines induce accessibility of specific loci.
- Somatic mutation occurs in proliferating activated B cells (centroblasts) in the dark zone of germinal centers. AID is required for somatic mutation and is now known to function as a DNA deaminase. Once in the light zone, somatically mutated B cells interact with antigens bound to follicular dendritic cells and most of these B cells (now called centrocytes) will undergo apoptosis. Only those centrocytes that express high-affinity BCRs are selected by antigen on FDCs (follicular dendritic cells) in the apical light zone

(affinity maturation). These cells differentiate to form memory cells and plasma cells.

- T cells are not required for the B-cell response to multivalent antigens such as pneumococcal polysaccharides or LPS (“thymus-independent” antigens). Isotype switching and affinity maturation do not occur in T-independent responses. While specific follicular B cells may also respond to T-independent antigens, both B-1 cells and Marginal Zone B cells represent pre-activated populations that respond rapidly to T-independent antigens.
- The close connection between B cell activation and disease will be discussed.

Selected Reviews

Goodnow, C. C. (1996). Balancing immunity and tolerance:deleting and tuning lymphocyte repertoires. Proc.Natl.Acad.Sci. USA *93*, 2264-2271.

Rajewsky, K. (1996). Clonal selection and learning in the antibody system. Nature *381*, 751-758.

Pillai S, Cariappa A, Moran ST. Positive Selection and Lineage Commitment during Peripheral B lymphocyte Development. Immunological Reviews. 2004 197, 206-218

Objectives / Study Questions

1. Describe the pre-B receptor and outline its functional roles during B cell development. Distinguish between allelic exclusion and isotypic exclusion.
2. What is receptor editing? At what stage of development is clonal deletion of B cells presumed to occur?
3. What are T-dependent and T-independent antigens? What provides Signal Two for T dependent antigens?
4. What is the major site at which marginal zone B cells are found? What do these cells do?
5. What are B-1 cells?
6. How does isotype switching occur?
7. What is the germinal center reaction. What changes might a B cell undergo in a germinal center?

8. How does AID promote somatic hypermutation?