

UNIT II B.Sc IVth Semester, Biotechnology

THE CLONAL SELECTION THEORY

FM Burnet initially published this theory in an Australian medical journal (Burnet, 1957) and later expanded his thoughts in a book based on a series of lectures presented at Vanderbilt University in Nashville (Burnet, 1959). Burnet postulated that antigen selects the antibody to be produced from a population of preexisting molecules. In Burnet's hypothesis the primary unit of selection for antigen was the lymphocyte-expressing antibody on its surface rather than antibody circulating in the peripheral blood. The idea that potential antibody-forming lymphocytes possessed cell surface receptors to which antigen bound was reminiscent of Ehrlich's side-chain theory. Postulates that differentiate Burnet's clonal selection theory from Jerne's natural selection hypothesis and from other models of antibody formation include the following:

- Antigen-binding receptors exist on the surface of antibody-forming lymphocytes prior to introduction of antigen. Thus when antigen binds to these cell surface receptors the lymphocyte is activated and produces antibody of that same binding specificity.
- An individual lymphocyte expresses receptors, all of which are the same specificity. Accordingly, a lymphocyte, once selected, produces large quantities of antibody, all of which possess the same antigen specificity.
- Potential antibody-forming lymphocytes go through a developmental stage during which they are exquisitely sensitive to elimination if they interact with self-antigens. This interaction results in the destruction of these lymphocytes and the development of self-tolerance (the ability to differentiate self from non-self). While Burnet was unable to demonstrate the induction of immunological tolerance, the formulation of the clonal selection theory correctly predicted the mechanism responsible for tolerance induction and helped explain the findings published by Billingham et al. (1953)

References:

A Historical Perspective on Evidence-Based Immunology. 2016, Pages 47-54 Chapter 6 - The Clonal Selection Theory of Antibody Formation. Edward J.Moticka.

Allotypes and Idiotypes

Allotypes In addition to class and subclass categories, an immunoglobulin (Ig) can be defined by the presence of genetic markers termed allotypes. These markers are different in different individuals and are thus immunogenic when injected into individuals whose Ig lacks the allotype. Like the blood group antigens (ABO), they are determinants which segregate within a species

(the Ig of some members of the species have them, others do not). Allotypes are normally the result of small amino acid differences in Ig L- or H-chain constant regions. For example, the Km (*Inv*) marker is an allotype of human L-chains and is the result of a leucine vs valine difference at position 191. The *Gm* markers are allotypes associated with the IgG H-chains. Allotypes are inherited in a strictly Mendelian fashion, and usually have no significance to the function of the antibody molecule. Idiotypes Antigenic determinants associated with the binding site of an antibody molecule are called idiotypes and are unique to all antibodies produced by the same clone of B cells. That is, although all antibodies have idiotypic determinants, these determinants are different for all antibodies not derived from the same clone of B cells. Thus, the number of different idiotypes in an individual is at least as numerous as the number of specificities. Antibodies are produced against these idiotypic determinants when they are injected into other animals. In fact, one's own idiotypes may be recognized by one's own immune system. That is, the amino acid sequence associated with the combining site of an antibody (call this idio type, D) is immunogenic even in the individual in which it is produced. An immune response produced against this idio type (anti-D) can eliminate the B cells producing the antibody with this idio type and thus decrease the antibody response to the antigen which initially triggered production of this idio type. Furthermore, an anti-idio type immune response (antibody or T-cell-mediated) expresses its own idio type which in turn can be recognized as foreign and an anti-idio type immune response made against this idio type. Jerne (who shared the Nobel prize with Kohler and Milstein in 1984) described a *Network Theory* which proposes that a series of idio type–anti-idio type reactions are partially responsible for regulation of the immune response.

GENERATION OF ANTIBODY DIVERSITY

Antibody genes: Three unlinked gene groups encode immunoglobulins – one for κ chains, one for λ chains and one for H-chains, each on a different chromosome (Table 1). Within each of these gene groups on the chromosome there are multiple coding regions (exons) which recombine at the level of DNA to yield a binding site. In a mature B cell or plasma cell, the DNA encoding the V region for the H-chain of a specific antibody consists of a continuous uninterrupted nucleotide sequence. In contrast, the DNA in a germline cell (or non B cell) for this V region exists in distinct DNA segments, exons, separated from each other by regions of noncoding DNA (Fig. 1). The exons encoding the V region of the H chain are: V segment (encoding approximately the first 102 amino acids), D segment (encoding 2–4 amino acids), and J segment (encoding the remaining 14 or so amino acids in the V region). For L-chains there are only V (encoding the first approximately 95 amino acids) and J segment (encoding the remaining 13 or so amino acids) exons. In each gene group, there are from 30–65 functional V segment genes. The D and J regions are between the V and C regions on the chromosome and there are multiple different genes for each but fewer in number than those encoding the V segment. Thus, DNA segments that ultimately encode the binding site of antibodies have to be moved over distances (translocated) on the chromosome to form a DNA sequence encoding the V region

(gene ‘rearrangement’). The DNA sequences encoding the C region of the L- and H-chains are 3’ to the V genes, but separated from them by unused J segment genes and noncoding DNA. Furthermore, each gene group usually has one functional C gene segment for each class and subclass. Thus, the H-chain gene group has nine functional C region genes, one each encoding μ , δ , $\gamma 1$, $\gamma 2$, $\gamma 3$, $\gamma 4$, ϵ , $\alpha 1$, $\alpha 2$. For the L-chain gene groups, there is one gene segment encoding the C region of L-chains, but four encoding λ L-chain C regions.

Table 1. Genes for human immunoglobulins

Ig polypeptide	Chromosome
H-chain	14
κ -chain	2
λ -chain	22

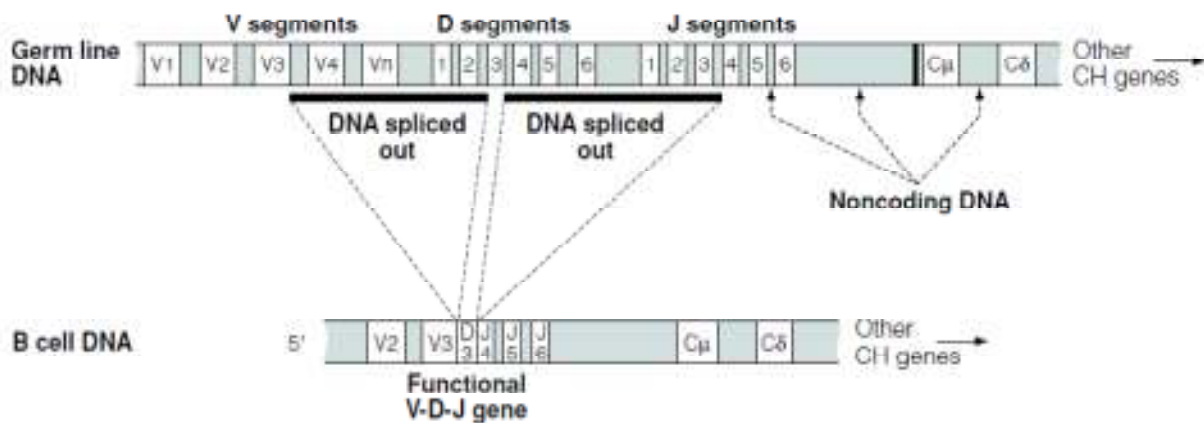


Fig. 1. H-chain genes and translocation. In the germ line, and therefore in a cell destined to become a B cell, the H-chain gene loci contains many V segment genes. In a developing B cell, one of these V segments recombines with one of many D segments, which has already recombined with one of several J segments, to produce a functional VDJ gene. In each B cell, the rearranged gene is transcribed, spliced and translated into a H-chain protein.

Gene rearrangement: During its development, a single B cell randomly selects one V, one D and one J (for H-chains), and one V and one J (for L-chains) for rearrangement (translocation). Gene segments encoding a portion of the V region are moved adjacent to other gene segments encoding the rest of the V region to create a gene segment encoding the entire V region, with the intervening DNA removed. Gene rearrangement in B cells requires the products of two recombination-activating genes, RAG-1 and RAG-2, which appear to be only expressed together

in developing lymphocytes. These enzymes break and rejoin the DNA during translocation and are thus critical to the generation of diversity. The H-chain gene group is the first to rearrange, initially moving one of several D segment genes adjacent to one of several J segment genes. This creates a DJ combination, which encodes the C terminal part of the H-chain V region.

A V segment gene then rearranges to become contiguous with the DJ segment, creating a DNA sequence (VDJ) encoding a complete H-chain V region (*Fig. 1*). This VDJ combination is 5' to the group of H-chain C region genes, of which the closest one encodes the μ chain. A primary mRNA transcript is then made from VDJ through the μ C region gene, after which the intervening message between VDJ and the μ C region gene is spliced out to create an mRNA for a complete μ H-chain. After the H-chain has successfully completed its rearrangement, one of the V region gene segments in either the λ or κ gene groups (but not both) translocates next to a J segment gene to create a gene (VJ) encoding a complete L-chain V region (*Fig. 2*). For κ chains, the DNA sequences encoding the C region of the L-chains are 3' to the V genes, but separated from them by unused J segment genes and noncoding DNA (*Fig. 2(a)*). For λ chains, since the J segment genes are each associated with a different $C\lambda$ gene, translocation of a V gene segment to a J gene segment results in a V region next to a particular $C\lambda$ gene (e.g. $C\lambda 2$ as shown in *Fig. 2(b)*). It is important to emphasize that in each B cell, only one of two L-chain gene groups will be used. A primary mRNA transcript is then made from VJ through the L-chain C region gene, after which the intervening message between VJ and the C region gene is spliced out to create an mRNA for a complete L-chain.

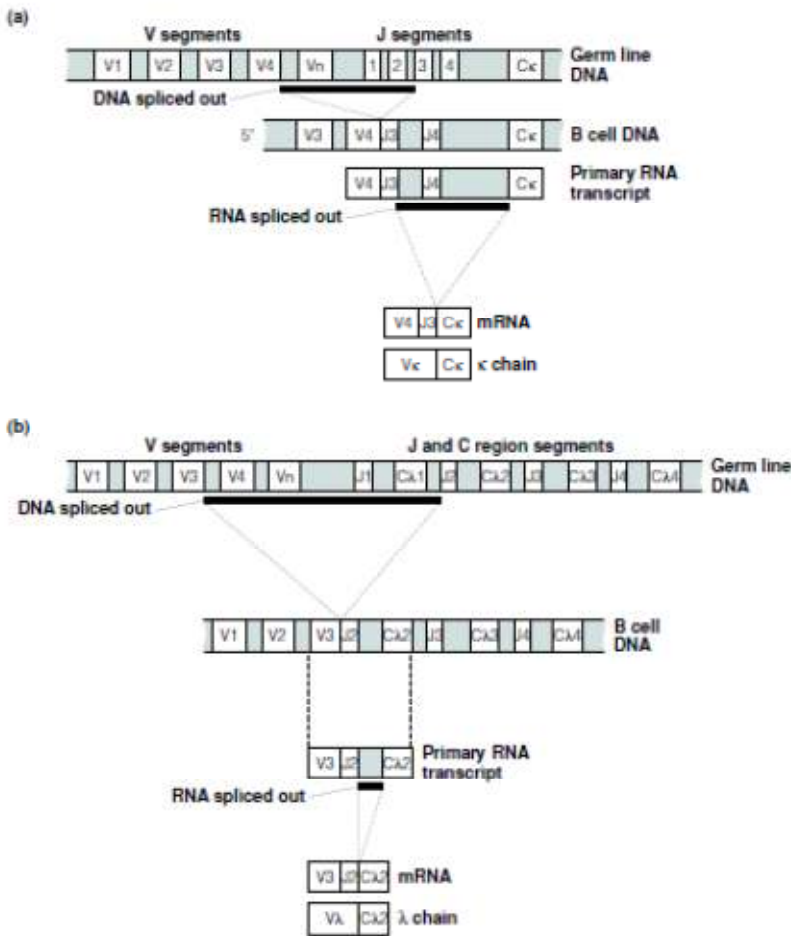


Fig. 2. L-chain genes and translocation. During differentiation of a B cell, and after rearrangement of the H-chain genes, one of the two L-chain groups rearrange. In particular, either (a) a germ line Vc gene combines with a J segment gene to form a VJ combination; or (b) a germ line Va gene combines with one of the J segment C λ gene combinations to form a VJ C λ combination. The rearranged gene is then transcribed into a primary RNA transcript which then has the intervening noncoding sequences spliced out to form mRNA. This is then translated into light chain protein.

Allelic exclusion: After successful rearrangement of the Ig DNA segments, the cell is committed to the expression of a particular V region for its H-chain and a particular V region for its L-chain and *excludes* other H- and L-chain V region rearrangements. This process is referred to as **allelic exclusion** and is unique to B and T cell antigen receptors. If an aberrant rearrangement occurs on the first chromosome the process will continue, i.e., the process does not stop if the cell does not get it right the first time. The process stops, however, if the cell gets it right or runs out of chromosomes to rearrange. In fact, following successful V_H gene rearrangement on one chromosome there is active suppression of further rearrangement of the other V_H gene segments. Similarly, following successful V_L gene rearrangement there is active suppression of further rearrangement of other V_L gene segments. Thus, each B cell makes L-chains all of which contain a V region encoded by the same VJ region sequence and H-chains all of which contain a V region encoded by the same VDJ sequence. Each B cell will therefore express antibodies on its

surface, all of which have exactly the same specificity. This cell and all of its progeny are committed to express and produce antibodies with these V regions.

Synthesis and assembly of Heavy L-chains: After successful rearrangement of both L- and H-chain DNA, L- and H-chain mRNA is produced and translated into L- and H-polypeptide chains that combine in the endoplasmic reticulum (ER) to form an antibody molecule, which is transported to the plasma membrane as the antigen-specific receptor for that B cell. Since the gene encoding the H-chain also contains coding sequences for a transmembrane domain, the H-chain produced contains a C terminal amino acid sequence which anchors the antibody in the plasma membrane. In plasma cells, the part of the mRNA encoding the H-chain transmembrane domain important for its membrane expression on B cells is spliced out. Thus, the antibody produced by a plasma cell does not become associated with the membrane, but rather is secreted.

Differential splicing and class switching As indicated above, the first antibody produced by a B cell is of the IgM class. Soon thereafter the B cell produces both an IgM and an IgD antibody, each having the same V regions and thus the same specificity. This is the result of the differential cleavage and splicing of the primary transcript. In particular, a primary transcript is made which includes information from the VDJ region through the C' region (*Fig. 3*). This transcript is differentially spliced to yield two mRNAs – one for an IgM H-chain and the other for an IgD H-chain. In a mature B cell both are translated and expressed on the B cell surface with L-chain. B cells expressing IgM and IgD on their surface are capable of switching to other H-chain classes (IgG, IgA or IgE). This isotype (class) switching requires stimulation of the B cell by T helper cells and in particular requires binding of the CD40 ligand (CD154) on T cells to CD40 on B cells. In addition, the cytokines produced by the T helper cell influence the constant region gene to which class switching occurs. Th2 cells producing IL-4 induce B cells to class switch to IgE; IL-5, which is also produced by Th2 cells, induces B cells to class switch to IgA; IFN- γ produced by Th1 cells induces class switching to IgG1 (*Fig. 4*). These signals induce translocation of VDJ and its insertion 5' to another constant region gene (*Fig. 5*). Class switch is guided by repetitive DNA sequences 5' to the C region genes and occurs when these **switch regions** recombine. The intervening DNA is cut out and the resulting DNA on the rearranged chromosome in the B cell which has class switched, and in plasma cells derived from this B cell, no longer contains C μ , C δ or other intervening H chain C region genes. A primary transcript is made and the RNA between the VDJ coding region and the new H-chain coding region is spliced out to give an mRNA for the new H-chain.

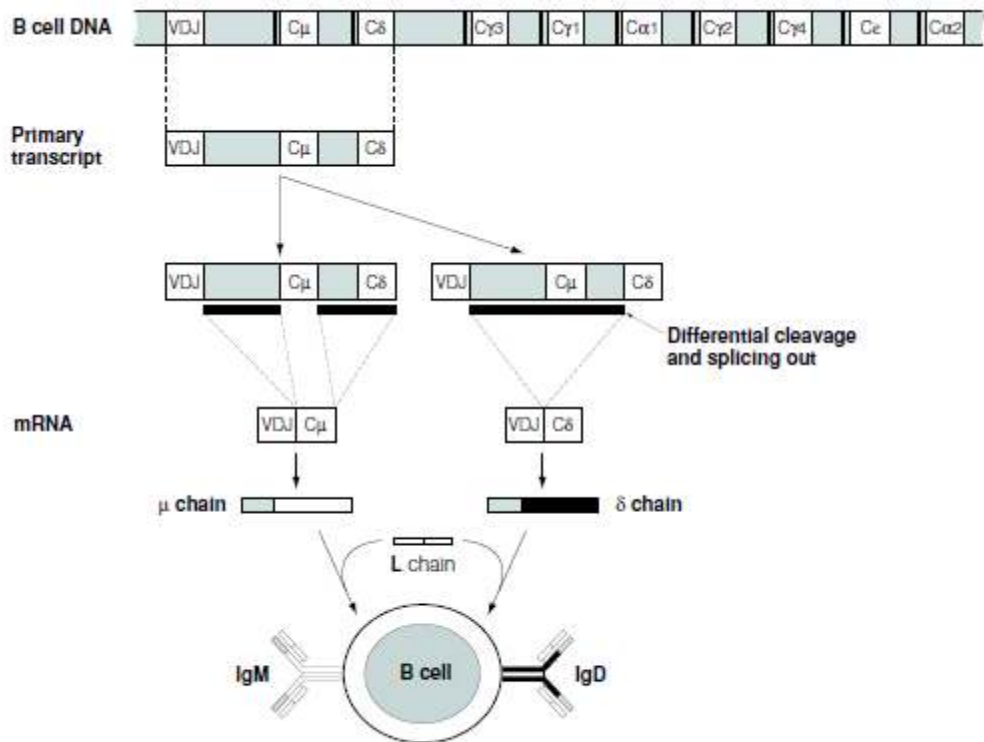


Fig. 3. Expression of IgM and IgD on a mature B cell.

Ways of creating diversity: Ig diversity (the generation of antibodies with different specificities) is created by several antigen-independent mechanisms. In addition, in B cells that have been stimulated by antigen and received T cell help, Ig genes undergo increased mutational events that may increase the affinity of the antibody produced by the B cell. Overall, diversity is generated by:

Antigen-independent events

- at the DNA level as a result of multiple germ line V, D and J heavy and V and J light chain genes,
- at the DNA level as a result of random combination of V, D and J segments or V and J segments,
- at the DNA level as a result of imprecise joining of V, D and J segments,
- at the protein level as a result of random selection and pairing of different combinations of L- and H-chain V regions in different B cells.

Antigen-dependent events

- at the DNA level as a result of somatic mutation in the V region, which may create higher-affinity antibody-binding sites.

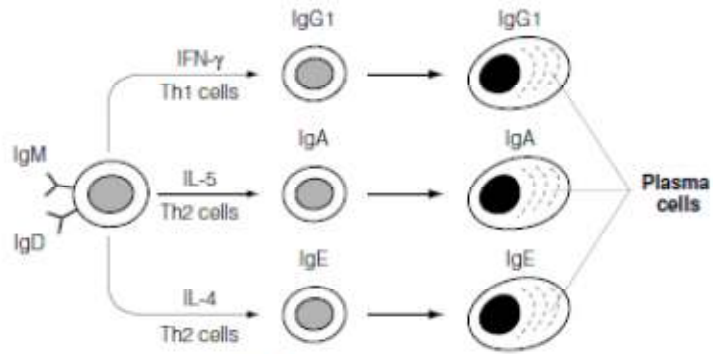


Fig. 4. Generation of antibody class diversity.

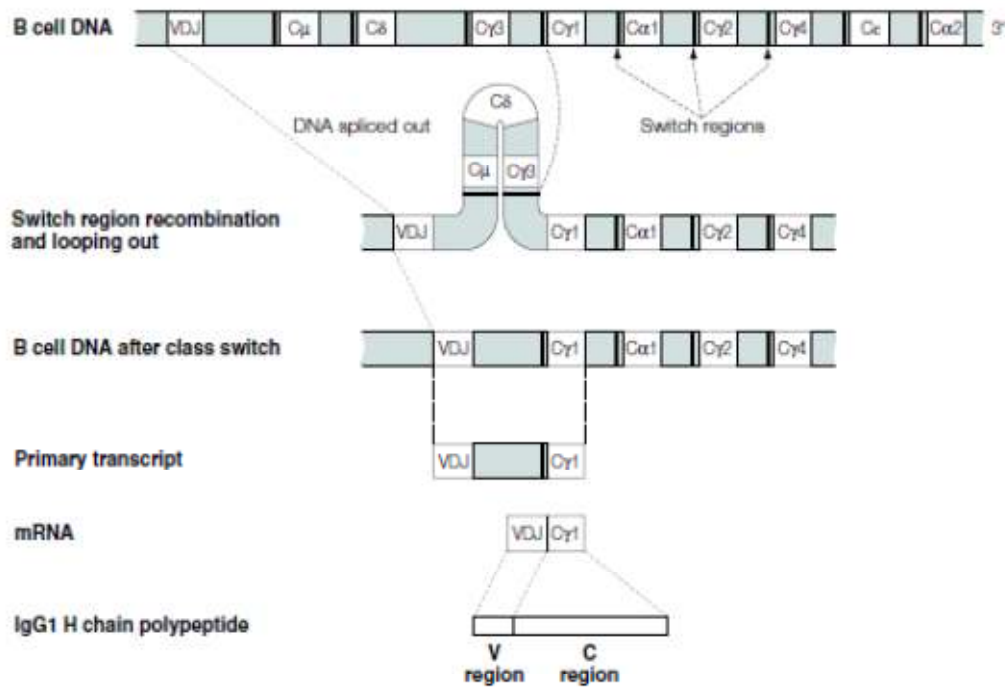


Fig. 5. Class switching.

Although rearrangement of the gene segments that will make up the V region genes occurs in an ordered fashion, they are chosen at random in each developing B cell. As these events occur in a vast number of cells, the result is that millions of B cells, each with a different antigen specificity, are generated. Additional diversity is created during recombination of V and J (L-chain) and V, D and J (H-chain) gene segments due to imprecise joining of the different gene segments making up the V region. That is, for example, although translocation of a V gene segment to a J gene segment could occur with all three nucleotides of the last codon of the V segment joining with all three nucleotides of the first codon of the J segment, it is also possible that one or two nucleotides at the 3' end of the V segment could replace the first one or two nucleotides of the J segment. Such a difference in the position at which recombination occurs can change the amino acid sequence in the antigenbinding area of the resulting V region of the

antibody, and thus change its specificity. Furthermore, after antigen stimulation of the B cell, the DNA of its L and H-chain V regions becomes particularly susceptible to somatic mutation and undergoes affinity maturation (see below). Diversity is also generated as a result of the fact that any L-chain can interact with any H-chain to create a unique binding site. Thus, for example, an L-chain with a particular VJ combination for its binding site could be produced by many different B cells and interact with the different H-chains (i.e. different in their VH region) generated in each of these B cells to create many different specificities. In sum, almost unlimited diversity is created from a limited number of V region gene segments. The diversity almost certainly exceeds the amount of diversity needed to bind the immunogens of microbes. However, the vast majority of the different B cells generated will never encounter antigen to which they can bind, and thus will not be stimulated to further development. And yet, such apparent wastefulness is justified by the fact that this mechanism of creation of diversity ensures that there are B cells, and thus antibodies, reactive with virtually all antigens that will be encountered. When an antigen to which this antibody binds is encountered, the B cell is triggered to divide and to give rise to a clone of cells, each one of which makes, at least initially, the originally displayed antibody molecule.

B cell development and selection

Gene segments encoding the variable parts of the V regions of antibodies rearrange during the pro-B cell stage (*Fig. 6*). Since rearrangement occurs in millions of different ways in these developing cells, many B cells, each with a different specificity, are generated. This generation of diversity occurs in the absence of foreign protein and yields large numbers of mature B cells, of which at least some have specificity for each foreign substance or microbe. The first genes to rearrange encode the variable part of the H chain of the antibody which together with the genes of the constant part of the molecule (and in particular genes which code for the μ H chain) are transcribed first in the differentiation process and appear in the cytoplasm. At this stage the genes in these pre-B cells which code for the variable region of the L chains rearrange. The transcribed H- and L-chains combine, giving rise to a functional IgM antigen receptor which is then expressed on the surface of the cell (immature B cell). It is during this stage that B cells with high affinity for self antigens are induced to die by apoptosis (negative selection). As in the thymus, the majority of the B cells die during development from production of antigen receptors which cannot be assembled or those directed against self antigens. During an antibody response to an antigen, the overall affinity of the antibodies produced increases with time. For example, antibodies produced in the secondary response usually have higher affinity for (tighter binding to) the antigen than those produced in the primary response. This is partly due to clonal selection and the presence of significantly more antigen-binding B cells at the time of the secondary response than during the primary response. If the quantity of antigen is insufficient to stimulate all B cells that could bind the antigen, i.e. when antigen is limited, B cells with the highest affinity antigen receptors will compete most successfully for the antigen. These cell populations are stimulated and give rise to plasma cells making their higher affinity antibody, thus increasing

the affinity of the total pool of antibody. These higher-affinity antibodies are also usually more efficient at effector functions than those produced in the primary response.

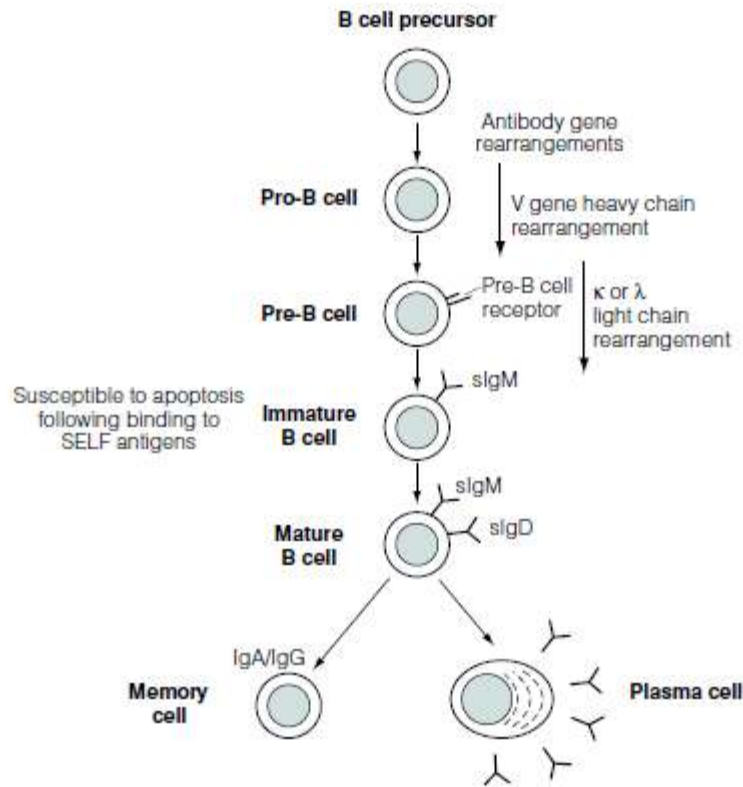


Fig. 6. Life history of a B cell. B cell precursors develop into pro-B cells which begin to rearrange their H-chain V genes. During the pre-B cell stage the translated heavy chain peptide assembles with surrogate light chain to form the pre-B cell receptor. This is thought to mediate further development of the B cell. During the pre-B cell stage, κ or λ light chain genes rearrange with one class of L-chain being transcribed and translated into protein. The κ or λ light chain then associates with new μ heavy chain to replace the surrogate light chain resulting in expression of surface IgM – the cell's functional antigen receptor. This immature B cell is susceptible to apoptosis/energy on contact with self-antigen. Mature B cells acquire surface IgD in addition to IgM and migrate to the secondary lymphoid organs and tissues where they respond to foreign antigens by proliferation and development into memory and plasma cells.

Affinity maturation: After class switch to IgG, IgA or IgE, the DNA of the L- and H-chain V regions of B cells stimulated by antigen and T cells becomes particularly susceptible to somatic mutation. This results in changes in the nucleotides of the DNA and thus corresponding changes in the amino acid sequence of the V regions of the antibody expressed by the B cell. As a result, the B cell may have a different specificity and not bind to or be stimulated by the original antigen. However, it often happens that at least some mutations result in amino acid changes which increase the tightness of binding of the antibody on the B cell to its antigen. These B cells will compete more efficiently for antigen than the original B cell, and will differentiate into plasma cells producing a higher-affinity antibody (**affinity maturation**), resulting in an overall increase in the affinity of the antibody population to that antigen. Typical antibodies have

binding constants of 10^{6-7} M^{-1} . After successive immunization with limiting antigen they are usually 10^{8-9} M^{-1} but may be as high as 10^{12} M^{-1} .

References:

Instant Notes in Immunology. Peter Lydyard, Alex Whelan, Michael Fanger. Taylor & Francis, 2004. ISBN: 1135325731, 9781135325732