LECTURE: 11

Title: B- LYMPHOCYTES PRODUCTION AND MATURATION

LEARNING OBJECTIVES:

The student should be able to:

- **Identify the site of B-lymphocyte production.**
- **Describe the anatomical feature of the bone marrow in relation to the mechanism of B-cell maturation.**
- **Describe the sequence of B-lymphocyte maturation and antigen-induced differentiation, such as:**

- **Lymphoid stem cell**
- **Pro-B-cell**
- **Pre-B-cell**
- **Immature B cell**
- **Mature B cell**
- **Activated/blast B cell**
- **Memory B-cell**
- **Plasma cell (AFC).**

- **Describe the development stages of B-cell antigen receptor.**
- **Identify the main surface immunoglobulins (sIg) that exhibited first, and become part of the B-cell surface membrane.**
- **Describe the allelic exclusion of Ig genes.**
- **Explain the mechanisms of heavy and light chain assembly.**

LECTURE REFRENCE:

1. TEXTBOOK: JONATHAN M. AUSTYN AND KATHRYN J. WOOD
Principles of Cellular and Molecular Immunology . Chapter 8. pp. 442-447.

2. TEXTBOOK: ROITT, BROSTOFF, MALE
IMMUNOLOGY. 6th edition. Chapter 8. pp. 131-145.

3. TEXTBOOK: ABUL K. ABBAS. ANDREW H. LICHTMAN. CELLULAR AND MOLECULAR IMMUNOLOGY. 5TH EDITION. Chapter. 6 .pg 189-214.

4. TEXTBOOK: MALE, COOKE. OWEN, TROWSDALE, CHAMPION
ADVANCE IMMUNOLOGY. Chapter 1. pp 1.1-1.16. Chapter 9. pp. 9.1-9.15.

5. TEXTBOOK: JOHN CLANCY, JR. BASIC CONCEPTS IN IMMUNOLGY. PP 33-38.

B-LYMPHOCYTE PRODUCTION AND MATURATION

INTRODUCTION

B lymphocytes are generated from the common lymphoid progenitors, which are originated from the differentiation of the haematopoietic stem cells. The yolk sac, fetal liver, and the adult liver are the sites in the body where these cells are generated, and developed. These cells (B- lymphocytes) are named (B), because of their origin (B one marrow). B-cell is differentiated into the plasma cell (antibody-forming cell), these later cells produces glycoproteins which are called immunoglobulins (antibodies).

B- LYMPHOCYTES DEVELOP IN THE BONE MARROW

B lymphopoiesis is generally defined as the generation of a pool of mature B cells with a large diversity of receptors for many different antigens. Each B cell's antigen receptor (immunoglobulin, Ig) must display monospecificity, so that each B cell can produce antibody that expresses single specificity. Cells that are committed to the B cell lineage in the bone marrow express cell surface glycoproteins CD45Ra (B220) and CD19.

Two events hallmark the formation of B lymphocytes in the marrow before their exit to peripheral lymphoid tissues: **sequential somatic gene rearrangement** of the heavy can light chain genes for immunoglobulin and expression of a characteristic pattern of cell surface and intracellular molecules that regulate differentiation **(Figure-1)**.

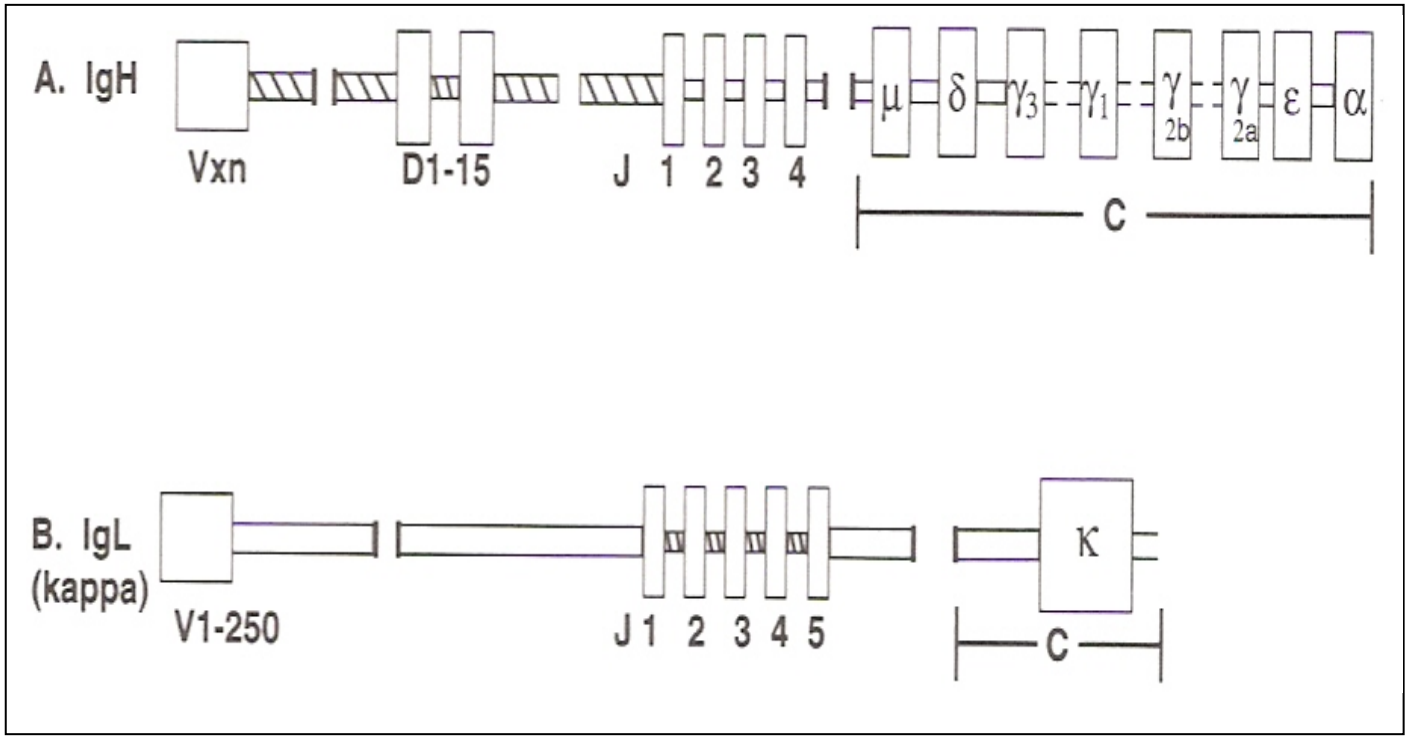

Figure-1 Organization of Ig genes

The expression of cell surface molecules has allowed the recognition of distinct stages of B cell differentiation. However, it should be realized that the process is a continuous one so that division into stages is somewhat arbitrary **(Table-1)**.

STAGAE	CHROMOSOMAL Ig GENE REARRANGMENT		Ig PROTEIN EXPRESSED
	IgH	IgL	
Pre-pro	DJ/GL	GL/GL	None
Early pro	DJ/DJ	GL/GL	None
Late pro	VDJ/DJ	GL/GL	Pre-B cell receptor
Pre	VDJ/DJ	VJ/GL	Igμ
Newly formed	VDJ/DJ	VJ/GL	IgM
Mature	VDJ/DJ	VJ/GL	IgM, IgD

Table-1 Stages in the development of a B-Lymphocyte (* GL: Germline)

B lymphopoiesis occurs throughout the lifetime of human and rodents. Proliferation of ht precursor stages is a key element in regulation of B cell production. Non-haematopoietic fibroblastic like cells, called stromal cells, makes up the micro-environment that controls commitment of stem cells into the B cell lineage and numbers of lymphocytes produced per day. Stromal cells form a reticular network of cell processes that contact millions of haematopoietic precursors of all lineages. At early stages, the B cell precursors (pre-pro-B cells) must interact physically with the stromal cells in order for proliferation and maturation to occur. Later stages (late pro-B cells) merely need the soluble growth factors produced by stromal cells. Stromal cells produce several necessary growth factors and cell-cell adhesion molecules. One key growth factor for B lymphopoiesis is interleukin-7. Mice that lack either interleukin-7 or its receptor are vastly deficient in B cells and serum antibodies.

REARRANEMENT OF IMMUNOGLOBULIN GENES

The earliest identifiable stage of B cell differentiation is the pre-pro B cell. In this stage, the process of Ig gene rearrangement begins. The first rearrangement entails the joining of the **D segment** to the **J segment** of the Ig heavy chain gene **(IgH)**. Subsequent rearrangements bring the **V region** juxtaposed to the DJ portion **(Figure-2)**.

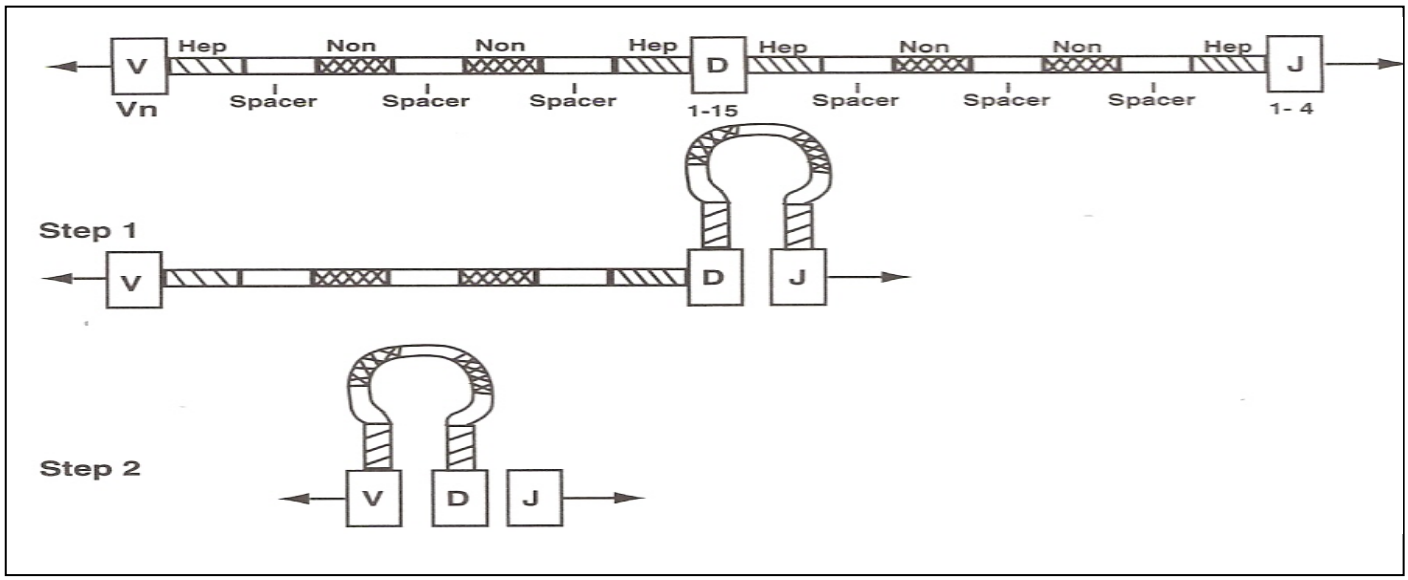

Figure-2 VDJ joining

A productive rearrangement of the IgH gene must occur before rearrangement of the Ig light chain gene **(IgL)** will begin. The pro-B cell stage is highlighted by continued IgH rearrangement, joining the V region with the already joined DJ segments. While the DJ rearrangements take place on both chromosomes, VDJ rearrangement usually occurs only on one chromosome. If the rearrangement is not productive, for example due to introduction of anew stop codon or frame shift, rearrangement then proceeds to the second chromosome. By the end of ht pro-B stage, IgH rearrangement is complete. By the time the cells reach the pre-B cell stage, Igμ protein can be observed in the cytoplasm, and rearrangement of one of the two immunoglobulin light chain (IgL) genes initiates. The method of rearrangement is similar, but only one joining is needed to baring the V region sequence to the J segment. The rearrangement begins first on the kappa gene, and if this sis unproductive, then the lambda genes are rearranged. **The process of immunoglobulin gene rearrangement has been studied extensively. Ig rearrangement is a highly ordered process that is similar to T cells, but ensures that only B lymphocytes will produce immunoglobulin.**

RECOMBINATION ENZYMES RAG-1 AND RAG-2

Two proteins are necessary for the rearrangement process, RAG-1 and RAG-2 (recombinase-activating genes 1 and 2), appear at the pre-pro- B cell stage. RAG-1 and RAG-2 genes are active during all phases of Ig gene rearrangement, but these genes are not expressed in mature B cells that have completed rearrangement. However, Ag-activated B cells in the germinal center reexpress RAG genes as the cells undergo affinity maturation. Terminal deoxynucleotidal transferase (TdT) is another enzyme that is involved in IgH rearrangement and is expressed early in the formation of B cells, but is not necessary for rearrangement to take place. When TDT is present, random nucleotides are added to open N regions during the rearrangement of D and J segments, thus increasing the diversity of the sequences. The expression of TDT diminishes by the late pro-B cell stage, prior to light chain rearrangement. Consequently, IgH but not IgL shows N region diversity.

THE PRE-B CELL RECEPTOR: AN Ig EXPRESSED BY EARLY B CELLS

Two transiently expressed proteins were discovered recently that form a light chain-like molecule termed the surrogate light chain. The surrogate light chain is composed to two gene products, lambda 5 and V-pre-B, which respectively are homologous with the lambda IgL and V region genes. Surrogate light chains can combine with the μ heavy chain protein in pro- and pre-B cells, forming a structure referred to as the pre-B cell receptor. The combination of the IgH protein and the surrogate light chains may be involved in stimulation proliferation of cells in the late pro-B cell stage of differentiation. Few B cells form in the absence of the pre- B cell receptor. After kappa or lambda light chain proteins are synthesized, the IgL displaces the surrogate light chain from interaction with the IgH protein so that the intact immunoglobulin can be formed and delivered to the cell surface.

SELECTION OF IMMATURE B CELLS

A newly formed B cell displays IgM on the cell surface. At this stage, the B cell is still immature and responds to antigen differently from a mature B cell. Immature B cells can be functionally removed by interaction with self antigen, either by undergoing programmed cell death (apoptosis) or by anergy, in which the cell is rendered nonresonsive in the presence of the antigen. Thus, similar to T cells, **immature B lymphocyte under go** a process of **"negative selection"** to delete cells that are reactive to "self" antigen.

Immature B cells that are not removed by the processes of negative selection leave the bone marrow and migrate to peripheral or secondary lymphoid tissues such as the spleen and lymph nodes. Here further maturation takes place and the newly formed B cells express IgD, in addition to IgM, on the cell surface. The mature B cells are now fully responsive to antigens and interaction with T cells. When they interact with antigen in secondary lymphoid tissue, they as well as some Th cells, help form germinal centers, whereupon somatic hypermutation, selection of cells producing high affinity antibody, class switching, and production of plasmablasts as well as memory cells occurs. The tissue-specific regulation of Ig gene rearrangement occurs on several levels. First, the Ig gene chromatin "opens" and becomes accessible for transcription. The accessibility of the chromatin is necessary for recombination and is controlled by tissue-specific transcription factors. Second, recombination signal sequences, conserved monomer/heptamer sequences, flank the V, D, and J segments of the heavy chain gene and V and J segments of the light chain genes. The juxtaposition of these sequences brings the two recombining segments into close proximity and makes the intervening DNA into a loop that can be cleaved by recombination-specific enzymes. The limited tissue expression of two of the enzymes necessary for recombination, RAG-1 and RAG-2, is a third level of regulation. Thus, only in B cells are fully rearranged Ig heavy or light chain genes observed **(Figures 1, and 2)**.

IMMUNOGLOBULIN GENES ARE FULLY REARRANGED ONLY IN B CELLS

Tissue-specific transcription factors regulate accessibility of the Ig gene chromatin. Ig gene segments are flanked by unique recombination signal sequences. Expression of recombination enzymes is highly regulated in lymphoid cells. The monospecificity of each newly formed B cell is achieved by the sum processes of cellular differentiation. This phenomenon is called allelic exclusion, referring to the fact only the Ig on one allele (chromosome) are transcribed and translated into protein. This assures that one B ell will react with only one antigenic determinant and will produced antibody to only that antigen. The somatic recombination of Ig heavy and light chain gene segments accounts for the extreme diversity of the B cell antigen repertoire. In the heavy chain gene, and of four J region segments may combine with any of 15 D segments, and the VD product may combine with any of more than 200 V genes. Diversity is expanded by the imprecise joining of the DNA or even the addition of random nucleotides by TDT during rearrangement. Even more diversity is added by the order of rearrangements. The heavy chain genes rearrange prior to the light chain genes. Once a productive heavy chain rearrangement is achieved, a phase of rapid cellular division occurs, which produces several daughter cells expressing identical Ig heavy chains. The daughter cells each produce different light chain gene rearrangements and , subsequently, proteins that combine with the heavy chain proteins to yield many unique antigen-binding specificities. In this manner, as many as 10^{11} different antibody specificities can be generated in the primary repertoire from newly formed B cells. However, it should be realized that further selection for high affinity antibody-producing cells will occur in the germinal centers of secondary lymphoid tissue.

SOURCES OF ANTIBODY DIVERSITY

♦ Multiple D and J genes can combine into many different patterns with up to 200 V genes.
♦ Rejoining of the DNA cleavage sites may yield codons for new amino acid sequences.
♦ TdT adds nucleotides to the N regions of the DNA that are cleaved during recombination.
♦ Combining of identical IgH proteins with different IgL chains yields multiple specificities.

DIFFERENTIATION

The development pathway of the B cell can be divided into two phases:

A. Antigen-Independent phase: this phase has several stages these are:

1. Stem cell starts to rearrange its germ line V, D, J genes to generate the gene for a heavy chain of the immunoglobulin. After the rearrangement of these genes, the H chain of IgM (μ) is Synthesized in the cytoplasm, and the presence of the major histocompatibility class II (MHC II) is expressed on the cell surface, the cell is called Pre-B cell.

2. Genes for the light (L) chains are rearranged, and a complete light chain is synthesized. This combines with the μ-chain to make a monomeric form of IgM which is inserted into the cell's membrane. This monomeric surface IgM (sIgM) now acts as a receptor and permits the cell to recognize and respond to pathogens. However, these B cells are considered to be an immature B cells, and contact with antigen leads to the shutdown or deletion of this clone, rather than expansion and differentiation.

3. Next stage of maturation, a B cell expresses both sIgM and sIgD receptors on its surface. δ Heavy chain is combined with light chains to form sIgD receptor. This cell is considered as a mature B cell (having both sIgM, and sIgD receptors). At this stage the B cell is considered to be a mature B cell and fully capable of responding to stimulation by specific antigen.

B. Antigen-Dependent phase: the stage in differentiation triggered by specific antigen, with the help of the T-cell, the mature activated B-cell is differentiated into plasma cell "antibody producing cell (APCs), and resting mature memory B cell. APCs can make Ig class switching, such as switching from IgM to IgG or IgA, or IgE under the influence of T-cell factors. All these switched Igs will have same specificity.

RESTING B CELLS

The cytoplasm of the resting B lymphocytes is filled with scattered single ribosomes. Activated B cells are found with developing endoplasmic reticulum. These B cells do not display Gall bodies or LGL morphology.

B-LYMPHOCYTE TRAFFICKING

B cell trafficking has not been studied as extensively as T cell trafficking, but the two processes seem rather similar. Like virgin T cells, virgin B cells also have general adhesion molecules that admit them to the complete range of secondary lymphoid organs. How ever, experienced B cells don't tend to be as migratory as experienced T cells. Most just settle down in secondary lymphoid organs and in the bone marrow, produce antibodies, and let these antibodies do the traveling.

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