B cell activation and Humoral Immunity

Humoral immunity is mediated by secreted antibodies and its physiological function is defense against extracellular microbes (including viruses) and microbial exotoxins. Humoral immunity can be transferred to other individuals by the transfer of serum (antibodies). Defect in humoral immunity leads to enhanced infections by bacteria and fungi. Antibodies also participate in autoimmune disorders and hypersensitivity.

When an antigen with multiple epitopes gains entry into the body, different clones of B cells recognize and produce antibodies against different epitopes, thus the natural response is said to by polyclonal. However, by using hybridoma technology it is possible to develop a clone of B cells directed against a single epitope, and produce monoclonal antibodies.

Antibodies are produced by plasma cells in the secondary lymphoid organs, but antibodies can perform their effector functions at any site in the body. Once the antibodies enter the circulation or mucosa, they can easily reach sites of infection. Circulating antibodies can recognize antigen present in blood or can pass through the endothelium into tissue spaces and render their effector functions.

The first exposure to a microbe or an antigen, either by infection or by vaccination, leads to the activation of naive B lymphocytes. These B cells differentiate into antibody-producing plasma cells and memory cells. Some of the antibody producing cells migrate to the bone marrow and live in this site for several years, where they continue to produce antibodies even when antigen has been eliminated. It is estimated that over half the IgG found in serum of normal individuals is derived from these long-lived antibody producing cells, which were induced by exposure to various antigens throughout the life of the individual. When the same antigen enters the body again, the circulating antibodies provide immediate protection against infection. At the same time, memory cells too are activated by the antigen and the resulting secondary response provides high level of protection.

Antibody response to protein antigen requires participation of both T cells and B cells. Those antigens which require participation of T cells for immune response are called T-dependent and those which do not require participation of T cells are called T-independent antigens. Since the CD4 T lymphocytes stimulate B cells, they are called helper T cells. Antibody response to non-protein antigens, such as polysaccharides and lipids do not need participation of antigen-specific helper T cells, thus these antigens are said to be T-independent.

Helper T cell-dependent humoral immune responses to protein antigens generate antibodies of high affinity. This is because helper T cells, which recognize protein antigens, provide signals to B cells to produce high affinity antibodies. In contrast, antibodies to T-independent antigens are mainly of low-affinity. Antibody responses to T-independent antigens are simple and mainly consist of IgG and IgM whereas helper T-cell dependent humoral response to protein antigen are highly specialized and consists of immunoglobulins of different classes and subclasses.

Primary and secondary antibody responses to protein antigen differ quantitatively and qualitatively. Primary responses result from the activation of previously unstimulated naïve B cells, whereas secondary responses are due to stimulation of memory B cells. The secondary response develops more rapidly than primary response and larger amounts of antibodies are produced in secondary response. Heavy chain istotype switching and affinity maturation also increase with repeated exposure to protein antigens.
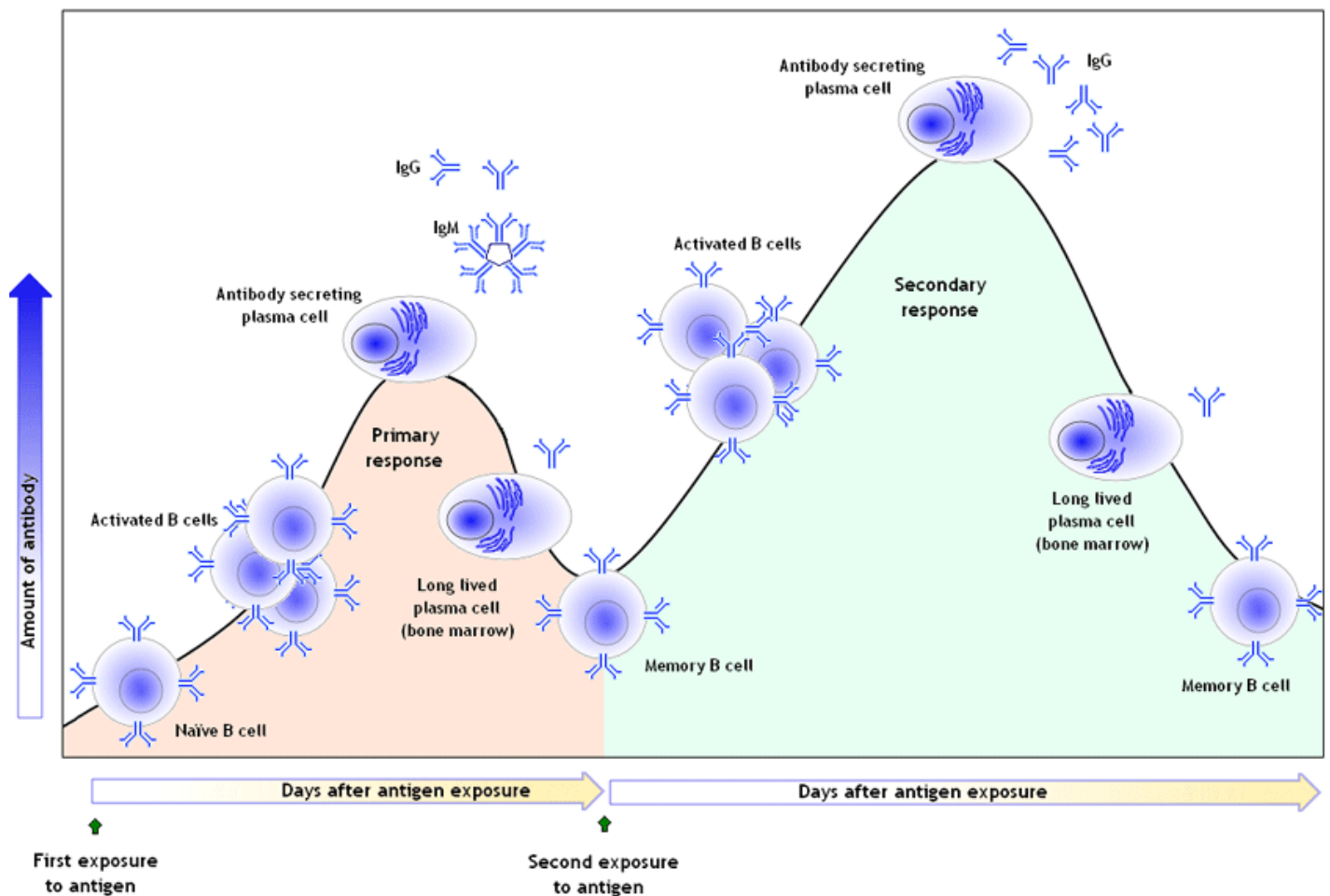
Features of primary and secondary antibody response

| Feature	Primary response	Secondary response
Time lag after immunization	Usually 5-10 days	Usually 1-3 days
Peak response	Smaller	Larger
Antibody isotype	Usually IgM>IgG	Relative increase in IgG
Antibody affinity	Low affinity	High affinity
Induced by	All immunogens	Only protein antigens

Blood-borne antigens are drained into spleen, antigens from skin and other epithelia are drained into lymph nodes while the ingested and inhaled antigens are drained to mucosal lymphoid tissues. The B cells mature and attain capability to recognize antigen in the bone marrow. These cells enter peripheral lymphoid tissues, which are the site of interaction with foreign antigens.

The process of activation of B cells and the generation of antibody producing cells consists of distinct sequential phases. The recognition phase is initiated by the interaction of antigens with a small number of mature IgM and IgD expressing B lymphocytes specific for each antigen. Binding of antigen to Ig surface receptors of B cells initiates the activation phase. Activation leads to series of responses resulting in its proliferation. This in turn results in differentiation, where effectors cells secreting antibodies and memory B cells are formed.

Antigen recognition and B cell activation

The IgD and monomeric IgM surface receptors of B cells binds to specific antigen and initiate the B cell activation. The B lymphocyte antigen receptor serves two roles in B cell activation. First, antigen-induced clustering of receptors delivers biochemical signals to the B cells that initiate the process of activation. Second, the receptor binds protein antigen and internalizes it into endosomal vesicles, which are processed and presented to helper T cells at the surface with MHC II molecules.

The B cell antigen receptor delivers activating signals to the cells when two or more receptor molecules are brought together, or cross-linked, by the multivalent antigens. Two membrane proteins, Igα and Igβ that are linked by disulphide bonds to each other and covalently linked to membrane Ig transduces the signals generated by clustering of surface receptors. These two molecules, together with surface Ig form the B lymphocyte antigen receptor complex. The early signaling events initiated by B cell receptor complex is similar to the events occurring in T cell receptor complex signaling. Cascading signaling events ultimately activates transcription factors that induce the expression of genes whose products are required for functional activation of B cells.
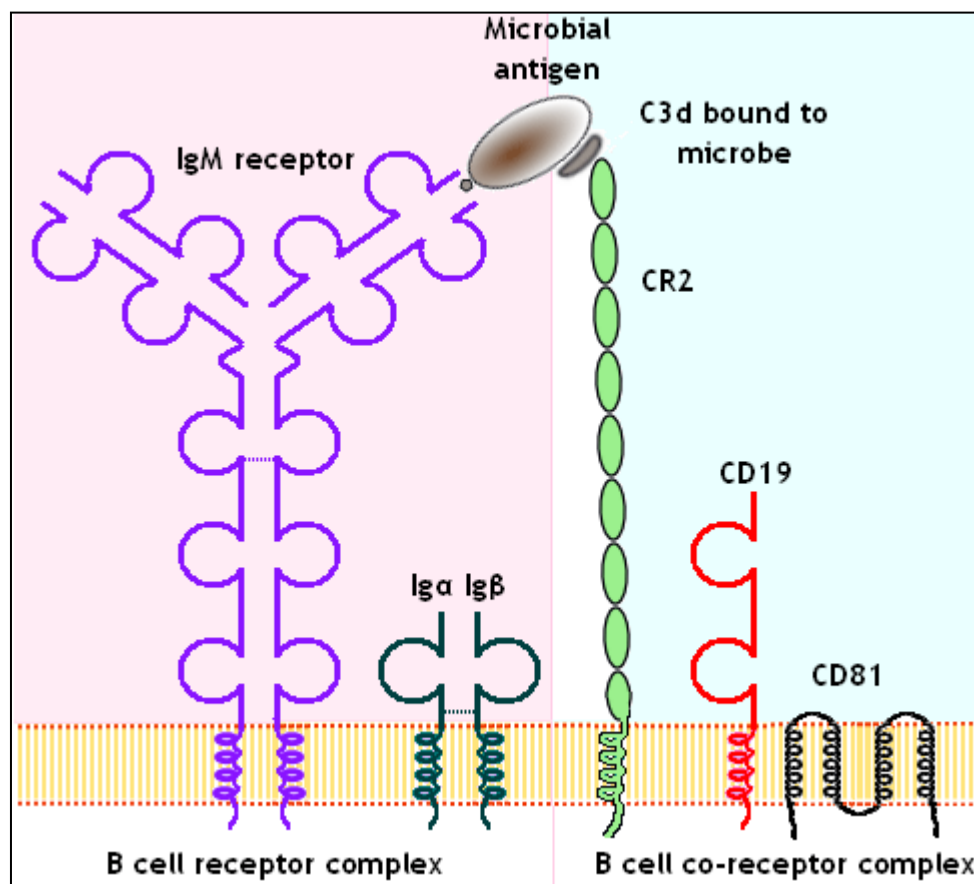
Second signals required for B cell activation is provided by complement proteins. A breakdown component of complement binds to the complement 2 receptor (CR2) on B cells and serves as an important second signal for B cell activation. CR2 is a receptor for the complement protein C3d, which is generated by the proteolysis of C3. C3d is generated following complement activation by either classical or alternate pathway in response to microbial infection. The C3d binds covalently to the microbe or the antigen-antibody complex. This complex of antigen-C3d binds to B cell, with the antigen recognized by surface immunoglobulin and the C3d recognized by CR2. CR2 forms a complex with two more integral membrane proteins- CD19 and CD81. The CR2-CD19-CD81 complex is often called B cell co-receptor complex. Binding of C3d to B cell CR2 leads to augmentation of signaling pathways initiated by antigen binding.

The early cellular events that are induced by antigen-mediated cross-linking of B cell receptor complex prepare the B cell for subsequent proliferation and differentiation. The events that follow are:
1. Entry of the previously resting B cells into cell cycle, which is accompanied by increase in cell size, cytoplasmic RNA and ribosomes
2. Enhanced survival of the B cells as a result of induction of various anti-apoptotic genes
3. Increased expression of class II molecules and co-stimulators, first B7-2 and later B7-1
4. Increased expression of receptors for several T cell derived cytokines

Signaling by the B cell receptor for B cell activation varies with the nature of the antigen. Most T-independent antigens, such as polysaccharides and glycolipids are polymers that display multiple identical epitopes on each molecule. Such antigens effectively cross-link surface receptors and initiate response even though they are not recognized by helper T cells. In contrast, most naturally occurring globular proteins express only one copy of epitope per molecule in their native conformation. Such molecules can not simultaneously bind and cross-link antigen receptors and are unlikely to deliver activating signals. However, such proteins may cross-link receptors if they become bound to previously produced antibodies or complement components. Helper T cells recognize protein antigens and their products are capable of inducing B cell proliferation and differentiation. Therefore, protein antigens may or may not trigger signals from antigen binding, instead the major function of surface receptors is to bind and internalize the protein antigen.

RESPONSE TO T-DEPENDENT ANTIGENS

Antibody responses to protein antigens require recognition of antigen by the helper T cells and co-operation between the antigen-specific B cells and T lymphocytes. The interaction between helper T cells and B cell sequentially involves antigen presentation by B cells to differentiated T cells, activation of helper T cells and expression of membrane and secreted molecules by the helper T cells that bind to and activate the B cells. The net result is the stimulation of B cell clonal expansion, isotype switching, affinity maturation and differentiation into memory cells.


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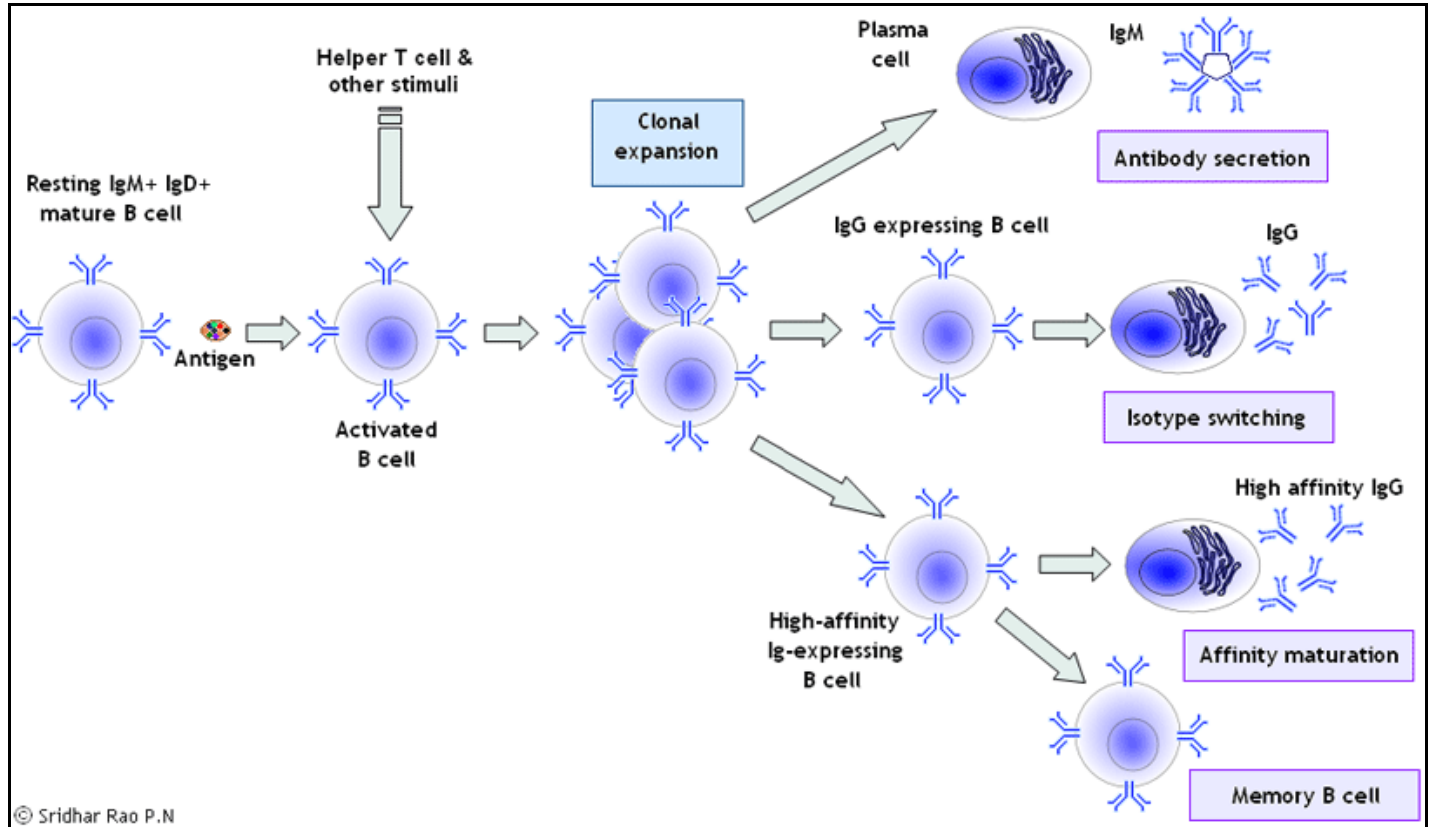
T-dependent antibody responses to protein antigen occur in phases that are localized in different anatomical regions within peripheral lymphoid organs. The early phase that comprises B cell proliferation, initial antibody secretion and isotype switching occur in the T cell area and primary follicles. The late phase occurs in the germinal center within lymphoid follicles and result in affinity maturation and memory B cell production.

Activated B cells and T cells that recognize foreign protein antigen in the peripheral lymphoid tissue come together to initiate humoral immune response. Within one or two days of antigen administration, naïve CD4+ T cells recognize antigen presented by professional APCs in the T cell area of lymphoid organs. B lymphocytes that also recognize the antigen in the follicle get activated and move out of the follicle into the T

cell area. The initial encounter between the antigen-activated T cell and B cell occur at the interface of the follicles and T cell area. This event occurs approximately 3-7 days after antigen exposure.

Antigen-specific B cells bind to native antigen to surface Ig receptors, internalize (receptor mediated endocytosis) and process it in endosomal vesicles. The peptide fragment of the antigen is then presented along with MHC class II proteins on their surfaces. The antibodies that are subsequently formed are specific to conformational determinants of the antigen. A single B cell may bind and endocytose a protein and present multiple different peptides complexed with MHC class II proteins to different T cells, but the resultant antibody response remains specific for the native protein.

Antigen binding to membrane Ig enhances the expression of co-stimulators on the surface. As the internalized antigen is being processed, the B cell also expresses B7-1 and B7-2. Helper T cell that recognizes MHC-peptide complex on B cell also binds to B7 molecule with its CD28 and gets stimulated to proliferate. Once activated by antigen recognition and costimulation, T cells express a surface molecule CD40L that binds to CD40 on the B cell surface. This engagement results in initiation of enzyme cascades that leads to transcription of several genes. Engagement of B cell CD40 to helper T cell CD40L also leads to enhanced expression of B7 molecules on B cell, resulting in more T cell activation. Antigen recognition by B cells enhances the expression of receptors for cytokines.
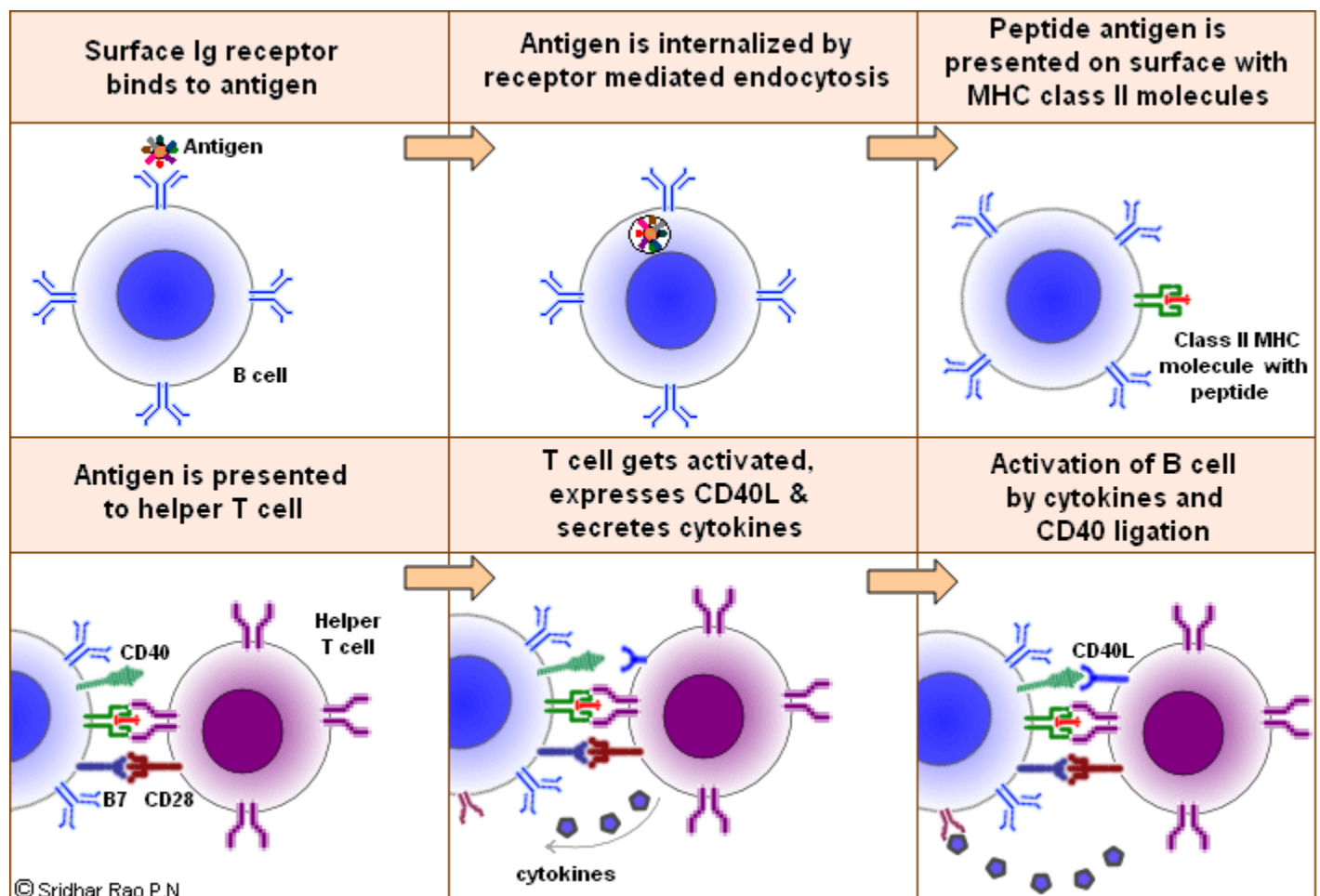
Activated helper T cell secretes cytokines that stimulate B cell proliferation. Cytokines serve two principal functions in antibody responses:

- They provide amplification mechanism by B cell proliferation and differentiation
- They determine type of antibodies produced by promoting isotype switch

B cells that are in direct contact with the activated T cells are exposed to high concentration of cytokines secreted by the T cells. IL-2, IL4 and IL-5, which are secreted by activated helper T cell acts on B cell to

induce proliferation. All the stimuli that B cell receives activate transcription of immunoglobulin genes. Some of the B cells that have proliferated differentiate into effector cells that actively secrete antibodies. The secreted antibodies have same specificity to the surface Ig receptor that captured the antigen, but vary in their carboxyl terminal. Cytokines may also affect RNA processing to increase the amount of immunoglobulin production.

Within the lymphoid tissue, antibody secreting cells are found mainly in extrafollicular sites, such as red pulp of spleen and medulla of lymph node. These cells also migrate to bone marrow at 2-3 weeks after antigen exposure, and bone marrow becomes the principal site of antibody production. Antibody secreting cells do not circulate actively. Many of the antibody secreting B cells change into plasma cells that are morphologically distinct B cells committed to abundant antibody production.
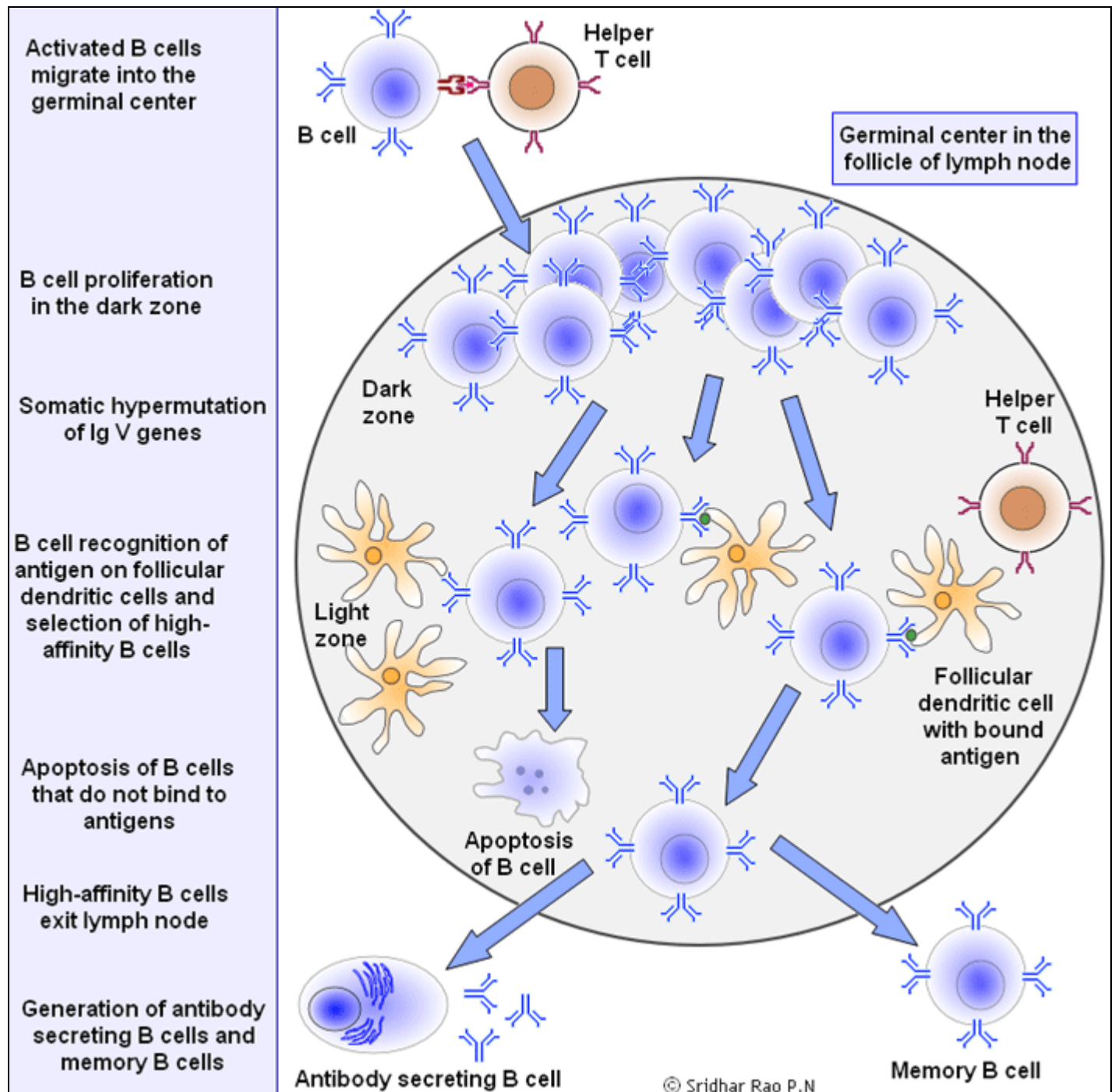
The antibodies that are secreted initially are predominantly of the heavy chain μ (IgM) isotype. In response to CD40 engagement and cytokines, some of the progeny of activated B cells undergo the process of heavy chain isotype switch. This leads to production of antibodies with heavy chains of different classes such as γ (IgG), α (IgA) and ε (IgE). The secretory form of δ heavy chain is rarely made, hence IgD is not found in plasma. Apart from CD40 signaling, cytokines too play an important role in regulating the pattern of heavy chain isotype switch. For example, IL-4 induces switch to IgE. Different cytokines that regulate heavy chain class switching are made by different subsets of helper T cells that are generated in response to distinct types of microbes. TGF-β that is produced by many cell types, in association with T cell derived IL-5 stimulates production of IgA in mucosal lymphoid tissue, resulting in production of local immunity. Cytokines have antagonistic actions too, for example, IFN-γ inhibits IL-4 mediated B cell switching to IgE and IL-4 reduces Ig2a production.
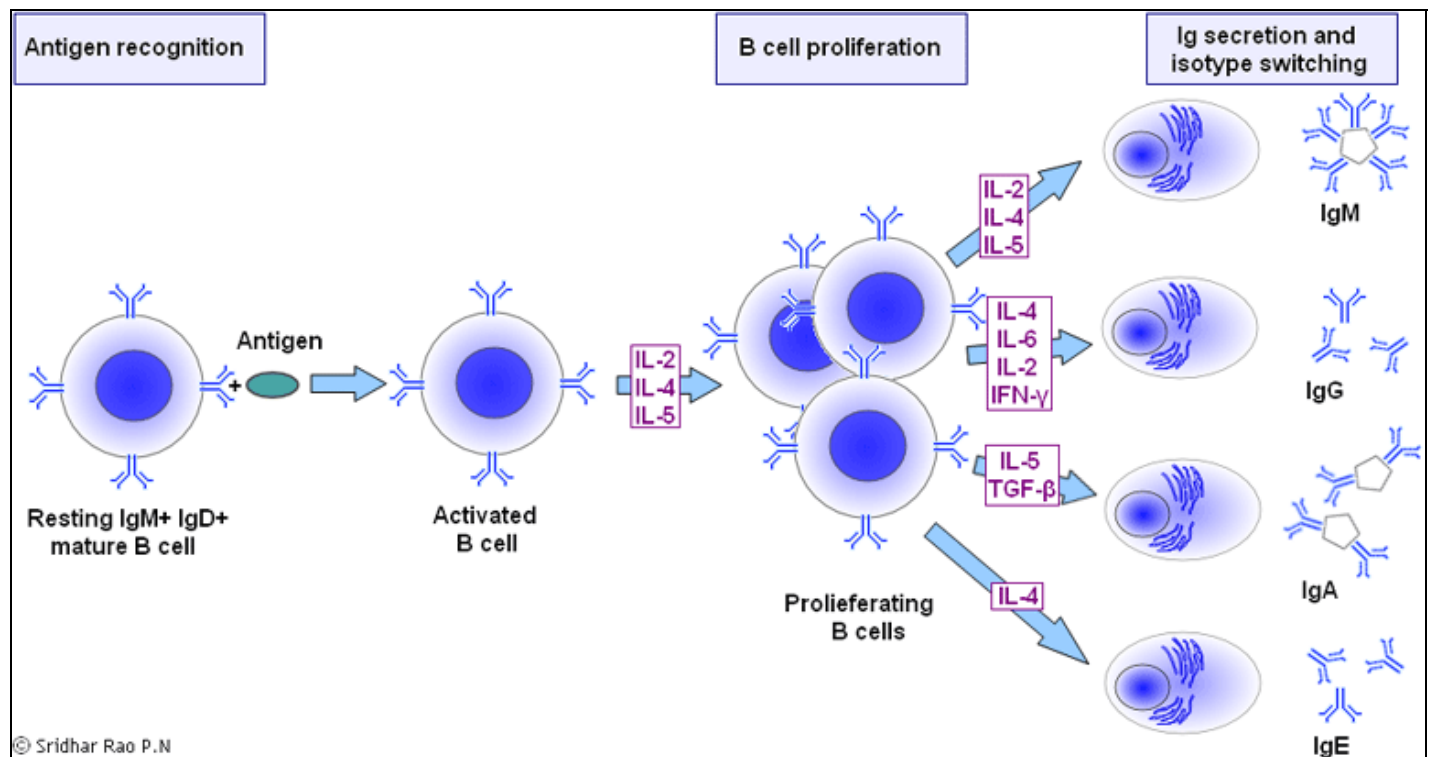
The mechanism of isotype switching is a process called switch recombination, in which the rearranged VDJ gene segments recombines with a downstream C region gene and the intervening DNA is deleted.

The late events in helper T cell dependent antibody response, including affinity maturation and generation of memory B cells occur in the germinal centers of lymphoid follicles. Within 4-7 days after antigen exposure, some of the activated B cells migrate deep into the follicle and begin to proliferate rapidly, forming the germinal center. It is estimated that a single B cell can give rise to a progeny of 5000 cells in 5 days. Each

fully formed germinal center contains cells derived from only one or a few antigen-specific B cell clones. Follicular dendritic cells that are found only in the lymphoid follicles express complement receptors (CR1, CR2 and CR3), Fc receptors and CD40L. All these molecules are involved in stimulation of germinal center B cells. Follicular dendritic cells are not derived from bone marrow and do not express MHC class II molecules. The progeny of proliferating B cells in the germinal center are smaller cells and sometimes called centrocytes. The proliferating B cells accumulate in the basal dark zone of the germinal center, which has few follicular dendritic cells. The small non-dividing progeny of B cells migrate to an adjacent basal light zone where they come in contact with abundant follicular dendritic cells.

Activated B cells migrate into the germinal center

B cell proliferation in the dark zone

Somatic hypermutation of Ig V genes

B cell recognition of antigen on follicular dendritic cells and selection of high-affinity B cells

Apoptosis of B cells that do not bind to antigens

High-affinity B cells exit lymph node

Generation of antibody secreting B cells and memory B cells

Helper T cell

B cell

Germinal center in the follicle of lymph node

Dark zone

Light zone

Helper T cell

Follicular dendritic cell with bound antigen

Apoptosis of B cell

Antibody secreting B cell

Memory B cell

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Affinity maturation is the process that leads to increased affinity of antibodies to a particular antigen as T dependent humoral response progresses. This is the result of somatic mutations in the Ig genes followed by selective survival of B cells producing antibodies with highest affinity. Helper T cells and CD40-CD40L

interactions are required for affinity maturation to proceed and therefore, affinity maturation is seen only to T dependent protein antigens.

B cell

Antigen

Antigen with complement

Fc receptor

CR1/CR2

Follicular dendritic cell
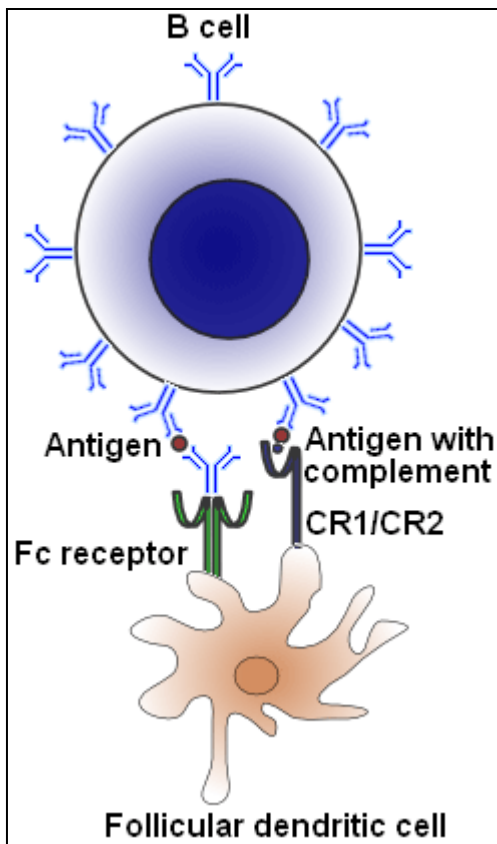
Proliferating germinal center B cells show high rates of point mutations in their rearranged heavy and light chain genes. These somatic mutations generate antibodies with high affinities for the antigen. Follicular dendritic cells in the germinal centers display antigen, and the B cells that bind to these antigens with high affinity are selected to survive. The small B cells in the light zone of the germinal center, which are the cells in which Ig genes have undergone point mutations, require antigen signals to be rescued from programmed cell death. Follicular dendritic cells express receptors for the Fc portion of antibodies and for product of complement components (C3b and C3d). These receptors bind and display antigens that are complexed with antibodies or complement products. Signals generated from antigen binding to surface Ig of B cells block cell apoptosis. Only those B cells, where the mutation has resulted in high affinity Ig receptors are selected to survive. Germinal center, is a site of B cell apoptosis. The net result of this selection process is a population of B cells producing antibodies with significant higher affinities for the antigen than the antibodies produced by the same clones of B cells earlier in the immune response. Some of the survivor B cells migrate from the basal light zone of germinal center to apical zone, where they may undergo additional isotype switching. The cells then exit the germinal center and develop into high affinity antibody secretors, many of which migrate to the bone marrow and continue to secrete antibodies for months to years.

Some of the antigen-activated B cells do not develop into antibody secretors. Instead, they acquire the ability to survive for long periods without antigenic stimulation. These memory cells are capable of mounting rapid antibody responses to subsequent introduction of antigen.

Factors that determine the nature of humoral immure response to protein antigens:

Humoral immune response has the capacity to generate different types of antibodies that combat different infections and functional optimally at different sites. The nature and magnitude of immune responses are influenced by the relative amounts of different cytokines produced at the site of B cell stimulation. During T cell activation, different T cells can differentiate into subpopulations of effector cells that produce different cytokines. Th1 subset secretes IFN-γ, which promotes isotype switching to IgG2a. Th1 subsets are helpful in eliminating intracellular microbes. By secreting IL-4 (isotype switch to IgE, IgG4) and IL-5 (activation of eosinophils), Th2 subsets are responsible for defense against helminthes. The dominant Ig isotype produced during a humoral immune response also depends on the tissue in which the antigen exposure occurs. Orally administered or inhaled antigens tend to stimulate IgA production, because B cells in mucosal lymphoid tissue readily switches to IgA.

RESPONSE TO T-INDEPENDENT ANTIGENS

Many non-protein antigens such as polysaccharides and lipids stimulate antibody production in the absence of helper T cells, and these antigens are called T-independent antigens. Important TI antigens include polysaccharides, glycolipids, and nucleic acids. These antigens are not processed and presented along with MHC proteins and hence cannot be recognized by helper T cells. Most TI antigens are polyvalent, being composed of multiple identical epitopes. Such polyvalent antigens may induce cross-linking of surface Ig molecules on B cell. This leads to activation of B cell without the requirement of helper T cell. TI antigens are classifies into two types, TI-2 antigens are polysaccharides, glycolipids, and nucleic acids where as TI-1

antigen is lipopolysaccharide (LPS). TI-1 antigens can directly stimulate B cells without requirement of any other cell. At low concentration gram negative bacterial LPS stimulates specific antibody production, but at high levels it acts as a polyclonal B cell activator, stimulating growth and differentiation of most B cells without binding to the membrane receptors. LPS is a polyclonal activator in mice but not in humans. In addition, many polysaccharides activate complement by alternate pathway and generate C3d, which binds to the antigen and provide second signal for B cell activation. Responses to TI antigens consist largely of IgM antibodies of low affinity and do not show significant heavy chain class switching, affinity maturation or memory. However, certain non-protein antigens such as penumococcal capsular polysaccharide can induce antibodies predominantly of IgG2 subclass.

Despite no participation from the helper T cells, certain polysaccharide vaccines provide long lasting immunity. The reason for this could be that the polysaccharides are not degraded readily and may persist for long periods in the lymphoid tissue, and continue to stimulate newly formed B cells. It is not known if memory cells are produced against these antigens, but subsequent exposure to the same antigens induces rapid and large secondary response.

REGULATION OF HUMORAL IMMUNE RESPONSE:

The simultaneous engagement of B cell immunoglobulin and Fcγ receptors by antigen-antibody complexes inhibit B cell activation. It is called antibody-feedback since the secreted IgG antibodies itself downregulates antibody production. IgG antibodies inhibit B cell activation by forming complexes with the antigen, and these complexes bind to a B cell receptor for the Fc portions of the IgG called the Fcγ receptor II (or CD32). This binding inhibits events in Ig-mediated signal transduction and terminates the B cell response to the antigen. The simultaneous binding of the antigen-antibody complex to the surface Ig receptor as well as Fcγ receptor results in blocking of signals needed for B cell activation.
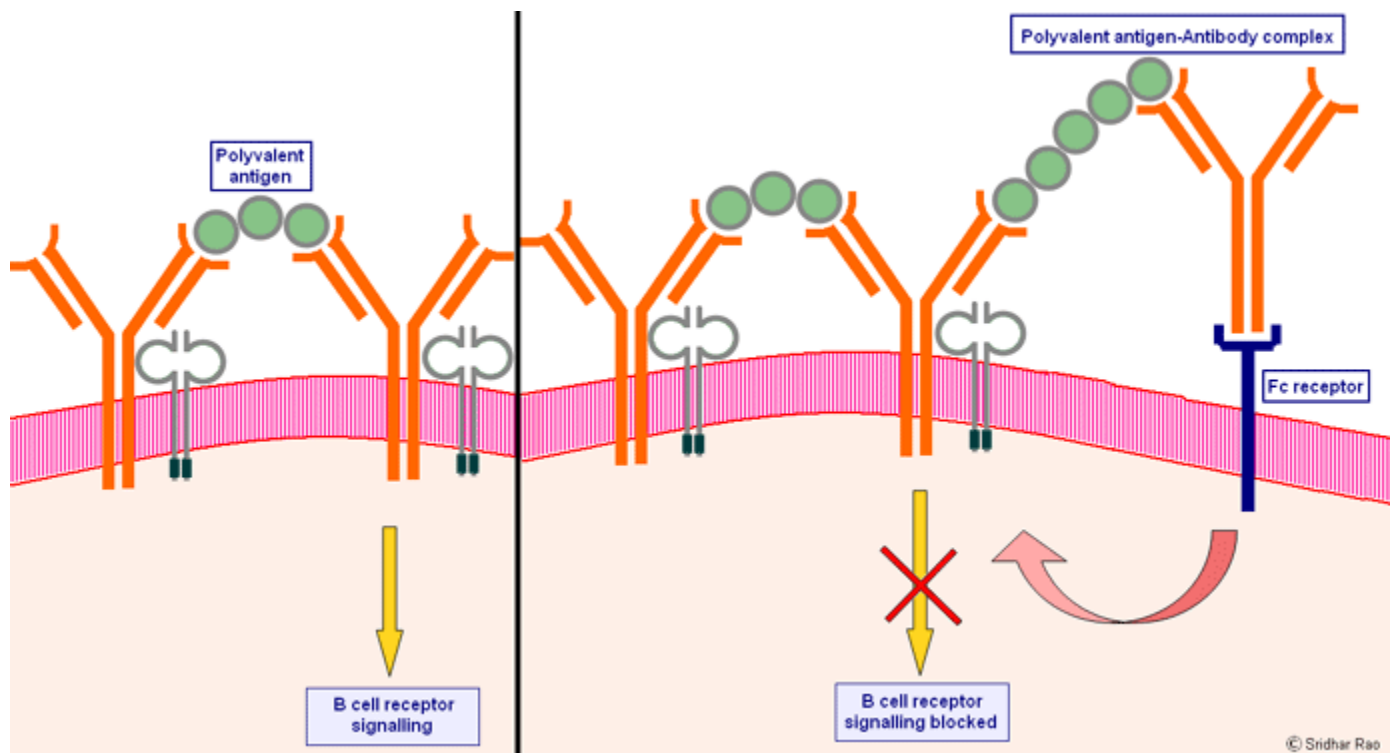
Antibodies are also known to amplify antibody production by activating complement and generating C3d, but antibodies also exert negative feedback to inhibit antibody production. It is assumed that in early infection IgM is produced, which can activate complement but does not bind to Fcγ receptor, whereas in late infection IgG produced as a result of isotype switch can bind to Fcγ receptor and inhibit B cell.