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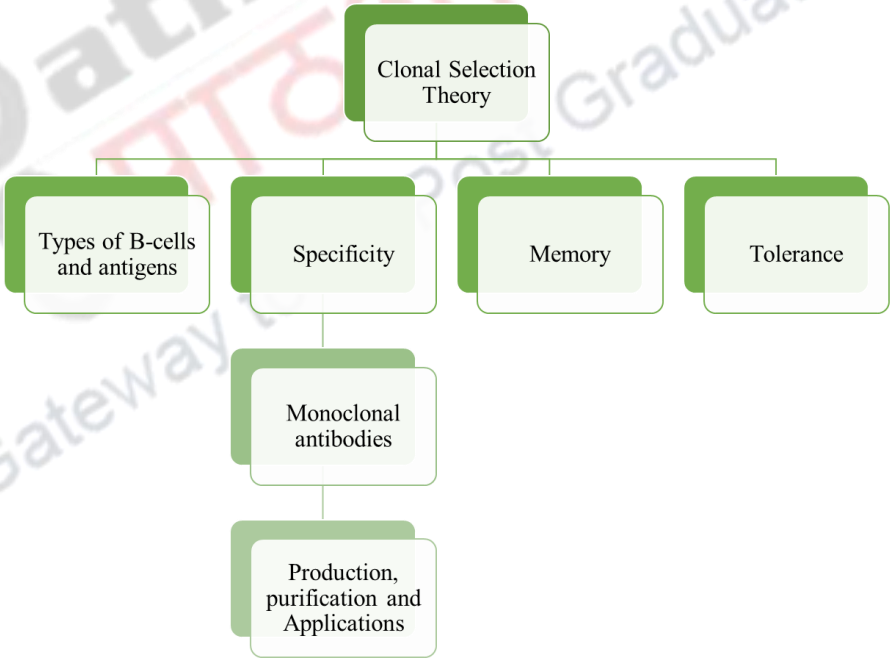
Description of Module	
Subject Name	Biochemistry
Paper Name	Immunology
Module Name/Title	Clonal Selection Theory

Objectives

- ✓ To learn how clonal selection theory explains the features of the immune system
- ✓ To learn about two different types of B-cells which are able to take care of different types of antigens.
- ✓ To understand how antibodies have some similarities with enzymes. In fact, catalytic ab can be produced using transition state analogs as epitopes.
- ✓ To learn about hybridoma technology and understand how we can produce mAb, humanized mAb and Fv molecules.

Concept Map



3. Description

We have mentioned that clonal selection theory argues that immune responses are based upon ‘ready made’ rather than ‘made to measure’ or ‘custom made’ operating system.

The success of the clonal selection theory was based upon its ability to explain all the hall marks of immunity (specificity, memory and tolerance) with some simple postulates.

Neils Jerne is credited with bringing in the idea of selectivity to explain immune system functions. Sir Mac Farlane Burnet gave the final shape to clonal selection theory.

Actually, Jerne resurrected the notion of selection. His ideas were a cause of right trigger at the right time. For Burnet, suddenly, everything fell into right place: the zigsaw was complete.

It is not correct to pit Jernes’s network theory against clonal selection theory. Doing that is simplistic but creates confusion.

The existence of Jerne’s networks continues to find evidence. The network idea is not necessarily incompatible with clonal selection theory.

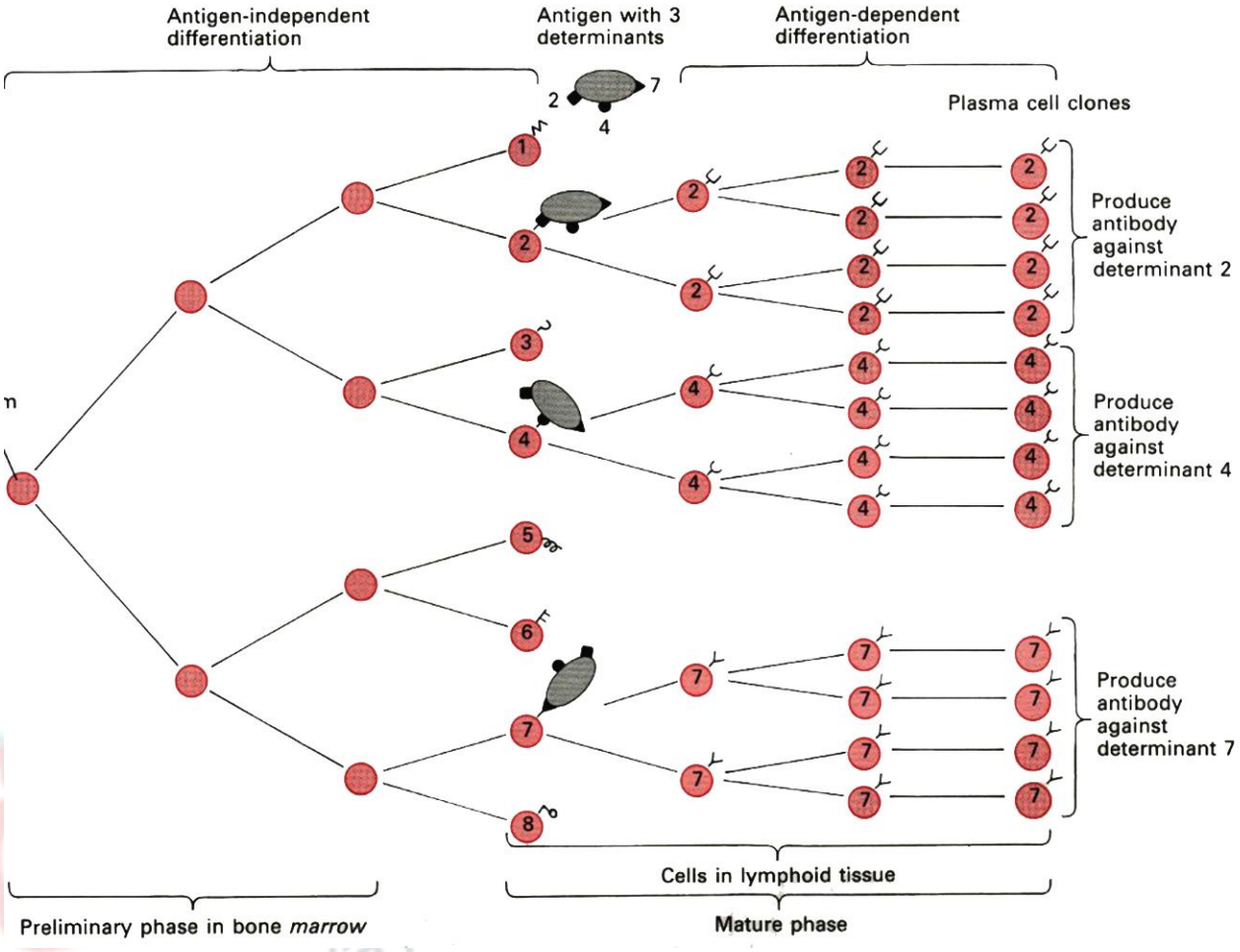


Figure 1

According to clonal selection theory, all "kinds" of B-lymphocytes are continuously made by the body. These lymphocytes have IgM of different specificities on their surface. This event takes place in bone marrow.

Any single cell has IgM which has only one binding specificity. In fact, as we will see, many other surface molecules on the B-lymphocytes also take part in the overall involvement of the B-lymphocyte later on. Thus the role of B-lymphocyte in secreting antibody should not be viewed as an isolated single role. For example, T_h cells help in B-cell response. That T_h cell "cooperation" is specific in

nature. It is mediated by B-cell surface molecules. None of these B-cell surface molecules diminish the specificity of the B-cell towards a particular antigenic determinant/epitope.

An invading pathogen will have several antigens. Some may be surface ones, others become accessible upon its phagocytosis. Each antigen has several epitopes.

For any antigenic determinant, a subset of B-lymphocyte populations will have IgM receptors which will bind to the antigenic determinant. This is the ‘selection’ step some ‘clones’ of B-lymphocytes are selected.

The members of the selected subset of B-lymphocytes do not bind to the same antigenic determinant with same binding constant. The ‘fine tuning’ to select lymphocytes out of this subset which have binding constants is in the “optimum range” of values takes place much later and is called affinity maturation.

A population of cells with a common immediate progenitor cell is called a “clone”. The set of diverse B-cells pre exist before this selection is made and existence of these B-cell variants is independent of the subsequent selection event. Hence clonal selection theory is analogous to Darwinian concept of natural selection.

B-cells produced by bone marrow are circulating cells of the blood and lymph (the fluid in the tissues). They have a lifetime. They are released and circulate in the blood/lymph and they die after a while. New cells are, in the meanwhile, being released by the bone marrow.

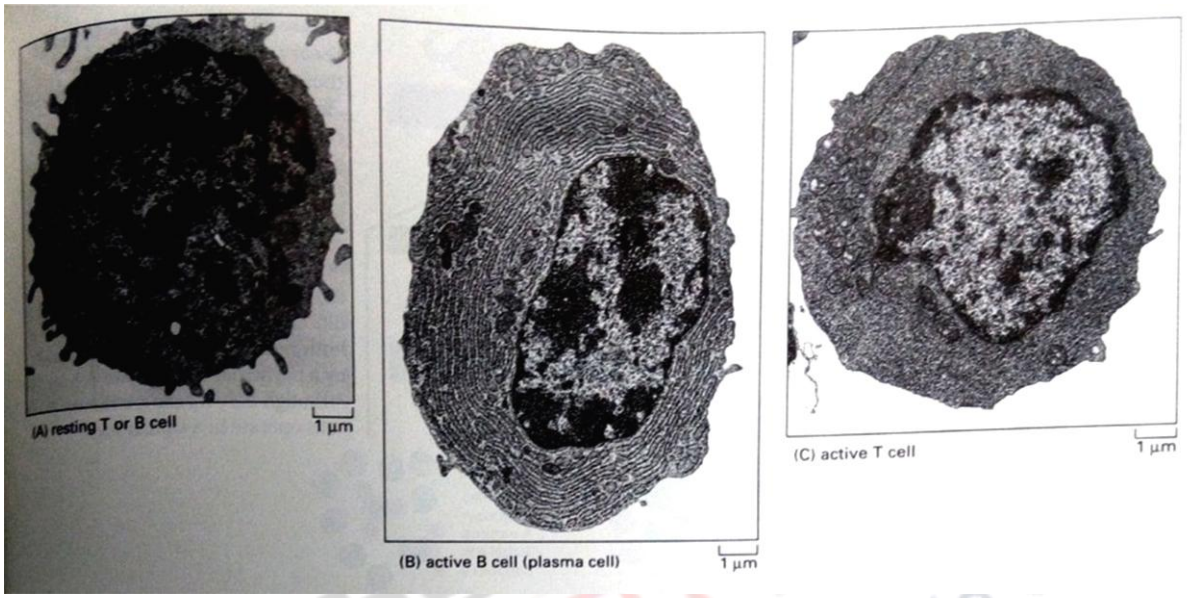


Figure 2

During their circulation, if a B-cell "recognizes" an antigen/epitope, the cell begins its phase of maturation. Their phase consists of:

- ✓ Growth
- ✓ Proliferation to produce its clones
- ✓ Producing a long lived progeny called memory cells

This pre-existing population is one mechanism which is responsible for generation of antibody diversity. This component of diversity is genetic in nature as it is defined by nature. Another component is somatic in nature (we will look at both mechanism in detail in next module).

Most of the discussion on clonal selection theory takes place around B-cells and humoral response. The reasons are purely historical. Our knowing nature of B-cell receptors preceded the identification of T-cell receptor by several years.

There was a period, when scientists thought that there is only one kind of lymphocyte: B-lymphocytes. Identification of T-cells, learning about types of T-cells and their roles etc came up much later.

Acceptance of clonal selection theory facilitated our placing the new information along with the known bits neatly. In fact, participation of T-cells in the case of specific antigen is according to clonal selection theory. They also proliferate and also create memory cells.

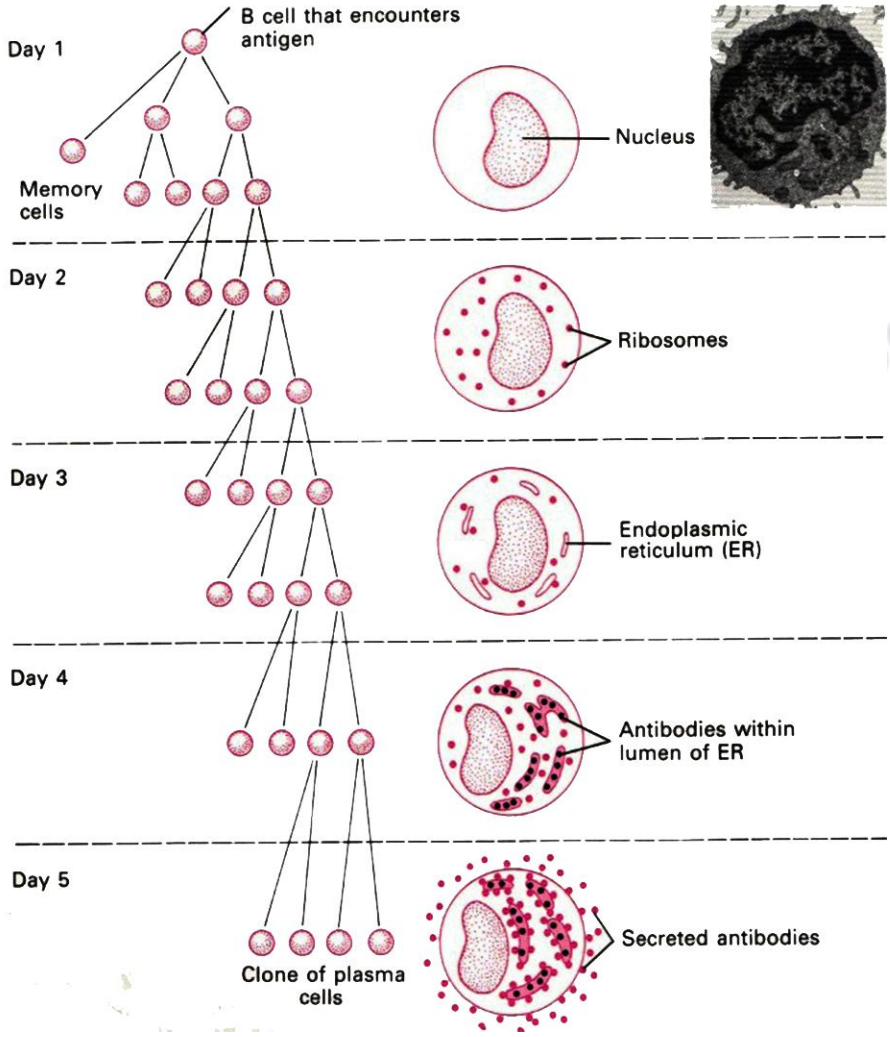


Figure 3

In the maturation phase, proliferation is accompanied by distinct changes in the B-cell population. The cells become bigger in size, protein synthesizing and secretory mechanism appears in abundance. In about 5 days after the encounter, the selected clone of cells are secreting ab.

Some of these become memory B-cells. These are specific to the antigenic determinant and persist for the lifetime **if** they do not encounter the antigen.

The memory B-cells can make IgG or IgA and respond if second encounter takes place. The memory cells, in experimental systems are responsible for the secondary response. They are also the basis for immunization (Vaccination)

The B-cells which are specific to self antigens are eliminated early in their life cycles. This is responsible for immune system distinguishing between self and non-self.

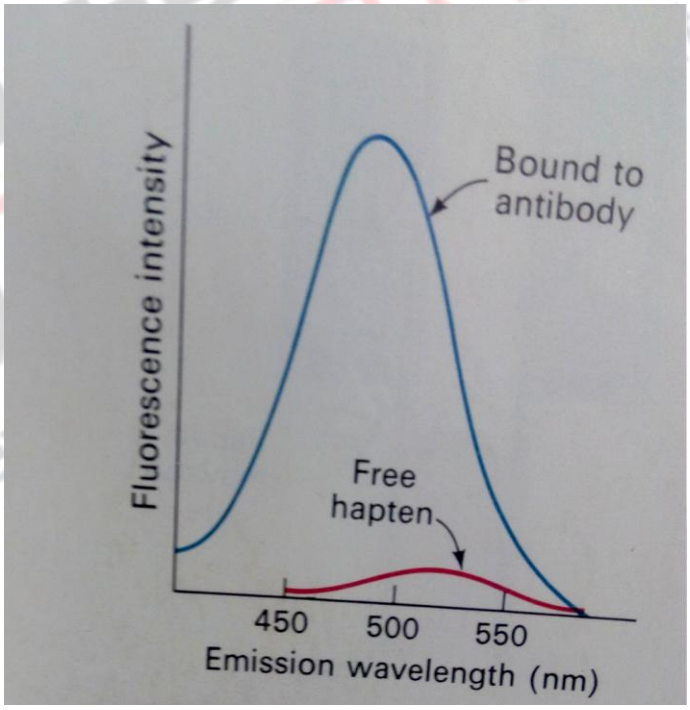


Figure 4

Antibodies (immunoglobulins) share many similarities with enzymes. The ΔG° of binding of many haptens is in the range of -6 to -15 Kcals/mole, which overlaps with corresponding values for many enzyme-substrate and enzyme-coenzyme system.

Antibody against dextrans reaches its maximum of binding constant with hexamer of glucose. In lysozyme, $(NAG)_6$ binding provides the similar picture. The binding site length, thus, can be estimated to be ~25A◦ for immunoglobulin.

The fluorescent emission spectra of the bound fluorescent hapten compared with free haptan indicate that binding site has nonpolar environments

The difference is that enzymes are catalysts. However, during their role as receptor, antibody upon antigen binding does not form a just a complex, it sends signals down the line. Even in free state immune complexes have tremendous physiological importance.

Also, Pauling predicted in 1948 that an antibody raised against transition state intermediate of a reaction, will catalyse that reaction. Decades later, catalytic antibodies were discovered. 38C2 was the first commercially available catalytic antibody which catalyses aldolases type reactions.

Two different types of B-cells exist. B1 cells arise very early during oncological development.

The B-1 are distinguished by:

- ✓ Mostly produce IgM antibodies encoded by germ line
- ✓ Mature independent of bone marrow
- ✓ The antigens which B-1 cells recognise are polysaccharides and lipids from microbes
- ✓ These B-cells do not require T_h cells for proliferation and differentiation after recognising antigen.

Whenever one talks of B-cells, (unless otherwise mentioned), generally the reference is to B-2 type cells.

Whatever we have discussed so far in earlier modules generally has been in reference to B-2 type cells.

B-2 are the mainstream lymphocytes which primarily mediate humoral response.

The characteristics of B-2 type cells are:

- Produced in bone marrow
- Require T_h cells for proliferation and differentiation
- Differentiate into plasma cells which produce IgG, IgA and IgE antibodies.

Thymus independent antigens

The need for two types of B-cells has its origin two types of antigen:

- Thymus independent antigens (T-I antigen)
- Thymus dependent antigens (T-D antigen)

Activation of B-1 cells to some antigens does not require T-cell involvement.

In such cases, B-1 cells respond to T-I antigens by producing IgM. These IgM are relatively low affinity antibodies to the antigen.

Activation of B-1 cells by T-I antigens

Humans and mice who have T-cell deficiency still are able to make antibodies towards many bacterial antigens.

These T-I antigens stimulate naïve B-cells without any specific T-cell. However T-cells do influence B-cell response to T-I antigen cytokines such as IL-5. This has been verified by the fact that animals without T-cells have fewer of B-1 cells.

In fact T-I antigens in turn can be divided into two classes as these stimulate B-cells in different ways.

T-I-1 antigens

These antigens do not select any clones of B-cells. So, their effect of B-cells is rather nonspecific in nature. This is called polyclonal activation.

These antigen are sometimes called B-cell mitogens.

Lectins are important mitogens for lymphocytes. Some also act on B-lymphocytes.

An important mitogen for B-1 cells are lipopolysaccharide (LPS).

LPS can activate both B-cells and dendritic cells. For B-cell activation 100x greater concentration of LPS is required.

At these high concentration, it does not require any specific ag-receptor binding for proliferation of B-cells and secretion of antibodies.

At low concentration (10^3-10^5 times less than for polyclonal activation), only those B-cells get activated which have specific receptors for the TL-1 antigens. This is antibody response to specific epitopes of TL-1 antigen

This response is physiologically relevant during early stages of infection when only low concentration of TL-1 antigen is present, and T-cells response is not available. Their priming and proliferation is yet to take place.

TI-1 antigen do not induce isotype switching, do not induce affinity maturation or results in memory cells as all these require T-cell help.

TI-2 antigens

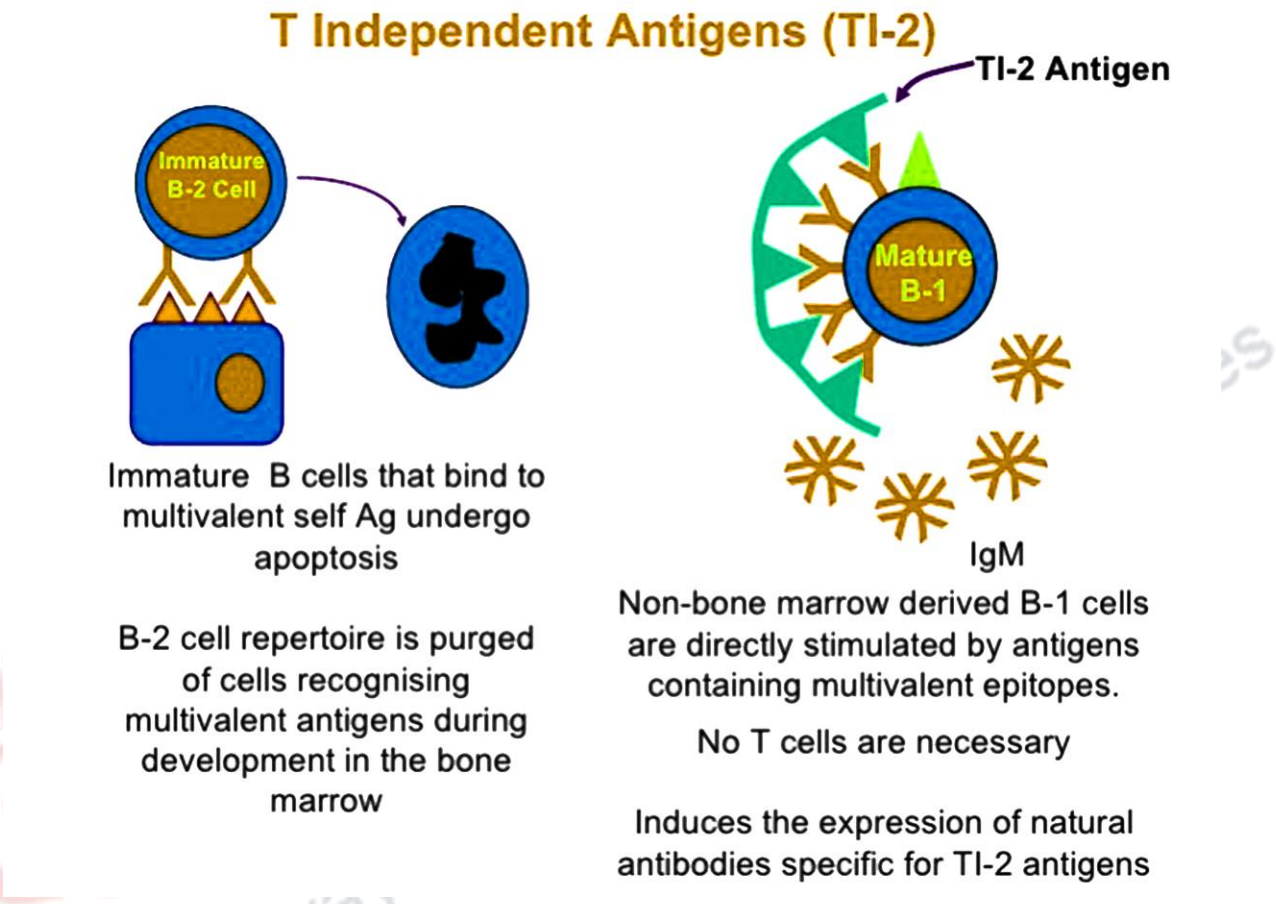


Figure 6

These are polysaccharides which have repetitive structural units and are part of bacterial capsules.

Immature B-cells are inactivated by such antigens. Hence TI-2 antigens only induce response by mature B-cells.

Infants are more prone to some infections as they only have immature B-cells.

B-1 cells (also known as CD-5 B-cells) respond to TI-2 antigens. These CD-5 B cells are autonomously replicating B-cells.

Marginal zone B-cells are non-circulating B-cells which line the border of white pulp of spleen. These increase with age and are responsible for increasing physiological response to TI-2 antigens with age.

The epitope density in TI-2 antigens is critical for their stimulation of B-cells. These antigens act by cross linking of receptors of mature B-cells.

Too extensive crosslinking results in unresponsive (anergic) B-cells.

Insufficient crosslinking does not activate B-cells.

While nude mice (with no Thymus) show response to TI-2 antigens, total depletion of T-cells abolishes the response. Adding T-cells in vivo does augment the response.

The role of T-cell involvement is not clear but is believed to be of nonspecific kind.

These responses by B-cells are physiologically important against some bacterial infections.

Common bacterial pathogens cannot undergo phagocytosis because of polysaccharide capsules. The IgM and IgG antibodies act as opsonins and induce phagocytosis.

These bacterial are called pyogenic as their infection leads to pus formation. Pus consists of dead and damaged neutrophils which had reached infection site.

The overall picture about T-I antigens and B-1 cells can be summarized as follows:

- B-1 cells have limited range of receptors. B-1 cells are not present in lymph nodes, constitute 5% of spleenic B-cells and are important in mucosal immunity. These respond to common microbial antigens. These sometime produce antibodies.
- Even B-1 cells seem to require two types of signals first via T-I antigen binding. The second via mitogenic component (T-I 1 antigens) or cytokine (T-I 2 antigens) for adequate response.

B-cell receptor complex (BCR)

Most of the B-cells in adult animals are of B2 type.

These have a receptor complex (BCR) on their surface after they have matured. These are present all over secondary lymphoid tissues such as lymph node follicles, spleen and Peyer's patches.

The naïve/virgin B-cells, which are yet to encounter any antigen express some variant of leucocyte common antigen.

Upon encountering a thymus dependent antigen, B-cells become plasma cells. These are present in red pulp part of the spleen, lymph node medulla, MALT and at the site of inflammation.

CD system: this is **c**luster of **d**ifferentiation (CD) system of nomenclature.

Leukocytes are distinguished in their cell surface antigens. The most unambiguous way of identifying these antigens is by use of monoclonal antibodies.

A marker designated by its CD number may be specific for a type of cell or for a phase in its differentiation.

Many CD markers are of course common to many cells but in different proportions. So, the CD marker peptide characterizes a cell type.

Immunoglobulins (Ig)/antibodies (Ab) were first detected in sera of immunized animals. Subsequently, it was found that Ig also have a membrane bound form which is anchored in the B-cell membranes.

Assigning two roles to different forms of the same Ig is a neat trick by nature. As both Ag-Ab interaction in sera and binding of Ag to the Ig receptor is a specific process, the single molecular recognition process serves both functions. The binding site(s) to the antigen in both forms of Ig (the soluble secreted Ab) and the cell surface bound receptor is identical.

While the receptor form of the immunoglobulin is a transmembrane protein, the cytoplasmic domain is only a tripeptide. This is too inadequate to discharge the function of signal transduction which is required for B-cell to become a plasma cell.

Having only a tripeptide as the cytoplasmic domain in Ig reflects the economy in design. Ig is specific for each antigen. The signal transduction mechanism can be common. While designing the anchored

form of Ig, only minimum structural components are added to soluble form of Ig (Ab) which are cleaved off while producing the secreted Ab which does not need anchoring.

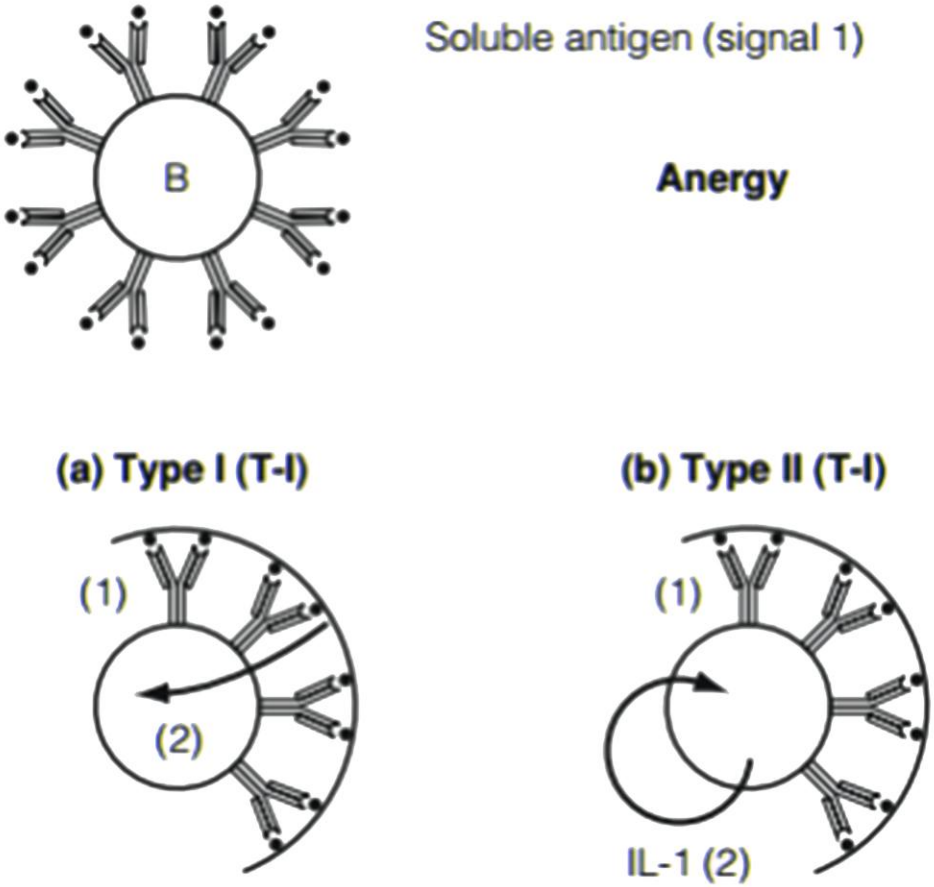


Figure 7

The surface Ig are associated with two polypeptides: Igα (CD79a) and Igβ (CD79b). The polypeptides, common to mature B-cells are markers for such cells. CD79 handles signal transduction.

A part from CD79 polypeptides, immunoreceptor tyrosine activation motifs (ITAMs) are intracytoplasmic segments. These are also part of other immune receptors. ITAMs can be phosphorylated by tyrosine kinases. This leads to B-cell activation.

Immunoreceptor tyrosine inhibiting motifs are present and can also be phosphorylated. Their phosphorylation leads to inhibition of B-cell activation.

Igα and Igβ are 20 kD polypeptides. These polypeptides are present on all Ig bearing B-cell forms including Pre-B cells. This indicates that signal transduction mechanism are put in place early during the development of B-cells.

The binding of Ag to the Ig receptor signals that new genes have to be switched on by nuclear transcription factors. Also, some genes which are required to be expressed only in resting B-cells have to be switch off.

Igα and Igβ are also required for appearance of Ig chains on the cell surface. Transfections of a B-cell by cDNA for H-chain and L-chain resulted in H-chain and L-chain remaining inside the cell. Simultaneous transfection with cDNA of Igα and Igβ chains resulted in the assembly of Ig+Igα+Igβ complex on the B-cell surface.

Igα and Igβ have cytoplasmic domains which are adequate size to carry signal transduction.

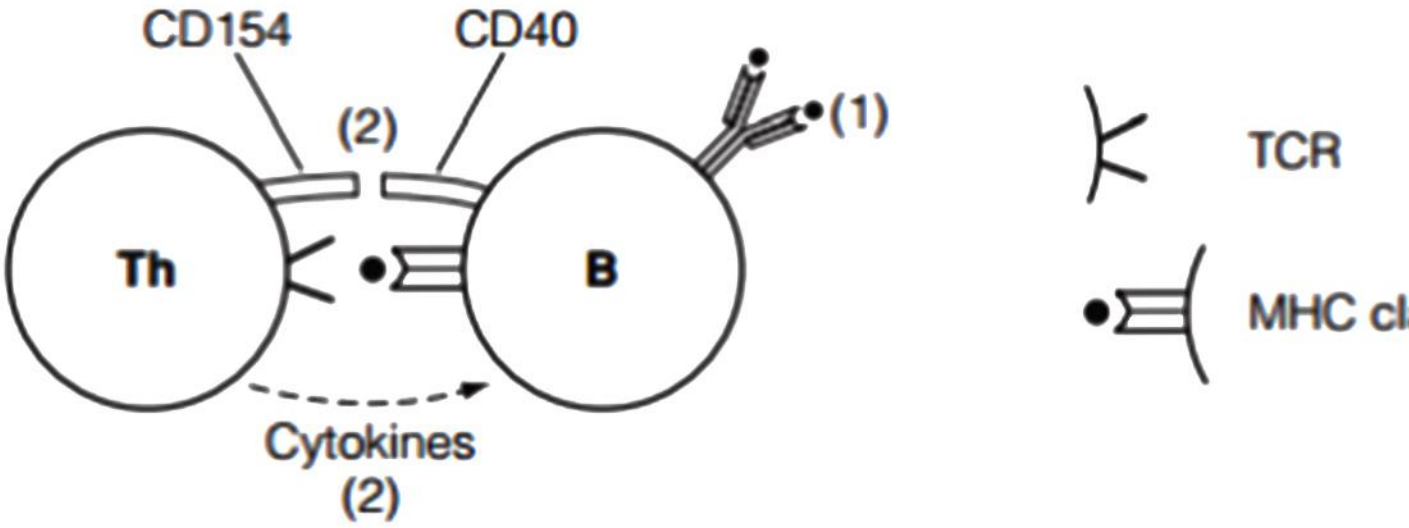


Figure 8: T cell activation of B cells

Binding of Ag to B-cell surface Ig provides the first signal. This signal alone is not enough in the case of T-D antigens. If a second signal is not available, B-cell becomes anergic i.e. it is turned off and does not follow the activation pathway.

T_h cell provides the second signal. Thus signal has two components. Firstly CD40 on the B-cell binds to CD154 on the T_h cells. Secondly T_h cells release cytokines after CD40-CD154 interaction.

This B-cell-T-cell cooperation is very critical for B-cell to become plasma cell, proliferate and start secreting Ab.

This important process of B-cell and T-cell interaction is best discussed in detail after we have discussed T-cells and nature of T-cell receptor. We will cover all this in next few modules and devote one separate module of B-cell-T-cell cooperation.

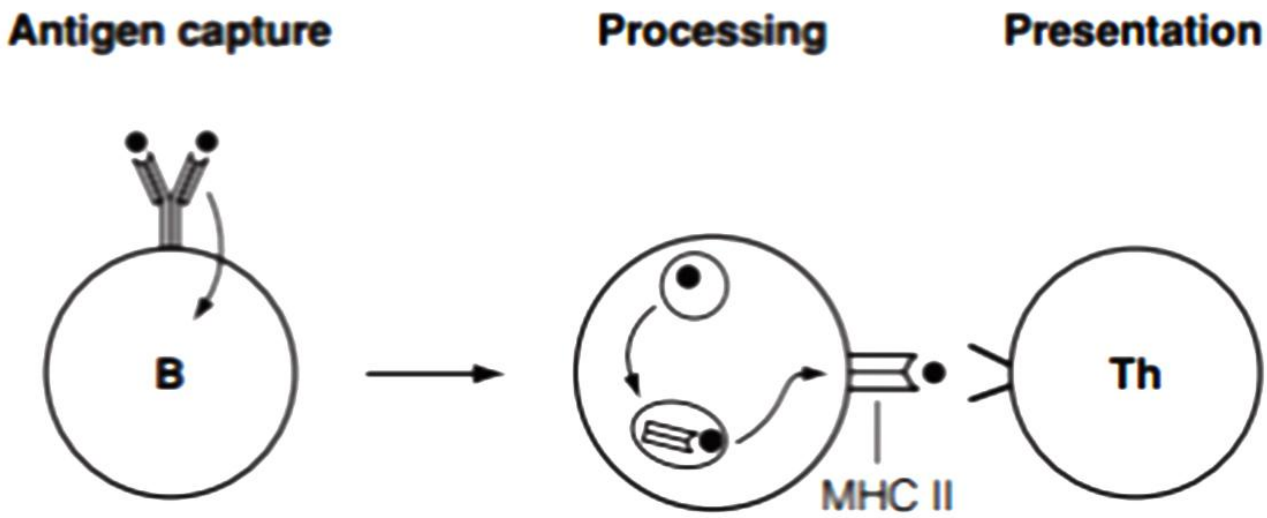


Figure 9: Activation of B cell to T cell help

B-cells are also antigen processing cells. While other APC endocytose the antigen via other receptors, in the case of B-cells, Ag bound to the Ig receptor itself undergoes endocytosis.

After undergoing degradation, the peptides from the antigen again appears on the surface but along with another cell surface molecules called MHC class II.

T-cells specific for the peptide antigen binds to the peptide-MHC complex.

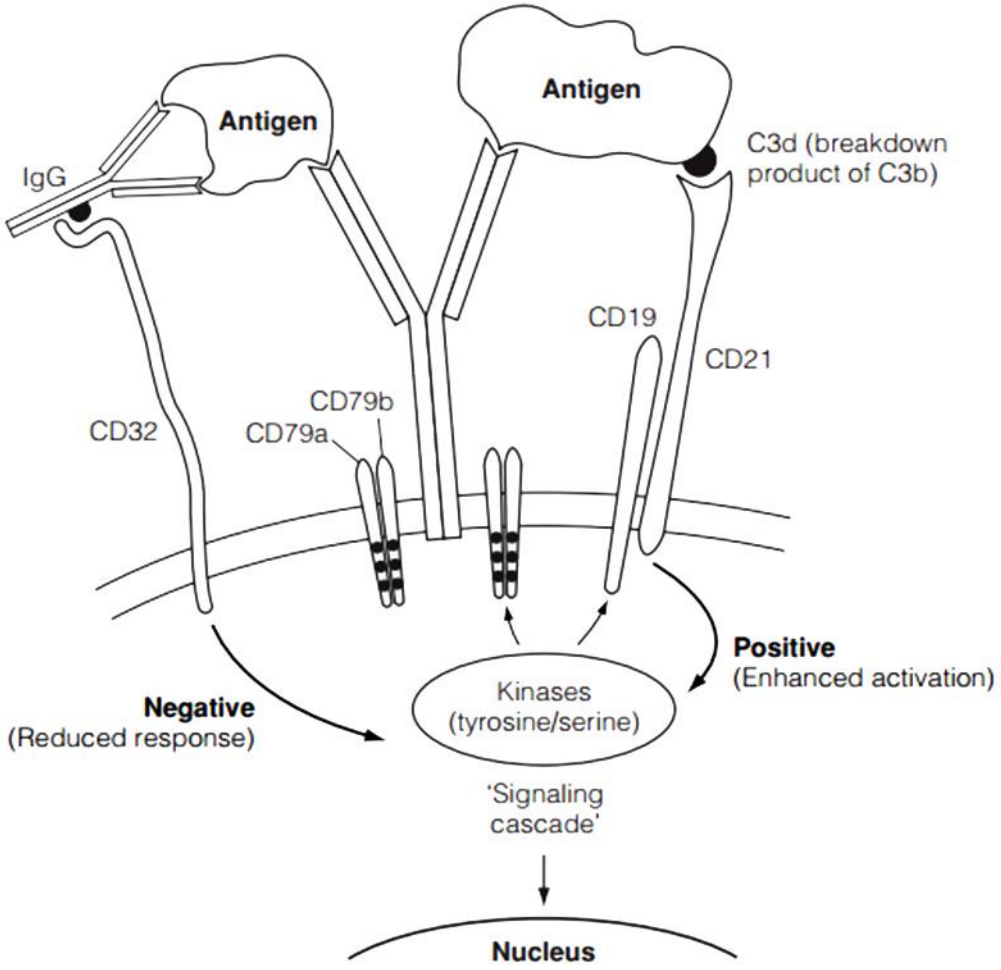


Figure 10: Activation of B-cells via the BCR and co-receptor complex

Thus the clonally selected populations of B-cells and T-cells expand.

ITAMs associated with Igα (CD7aa) and Igβ (CD79b) are now phosphorylated by protein tyrosine Kinases.

Some other surface molecules are also part of the BCR complex. CD21 is a complement receptor that can bind to C3d complement component. This happens if the complement has been activated.

CD81 is essential for “lipid raft” formation. This phenomenon and associated phosphorylation- and dephosphorylation are common in B-cell and T-cell activation and we will discuss these in details in the context of T-cells.

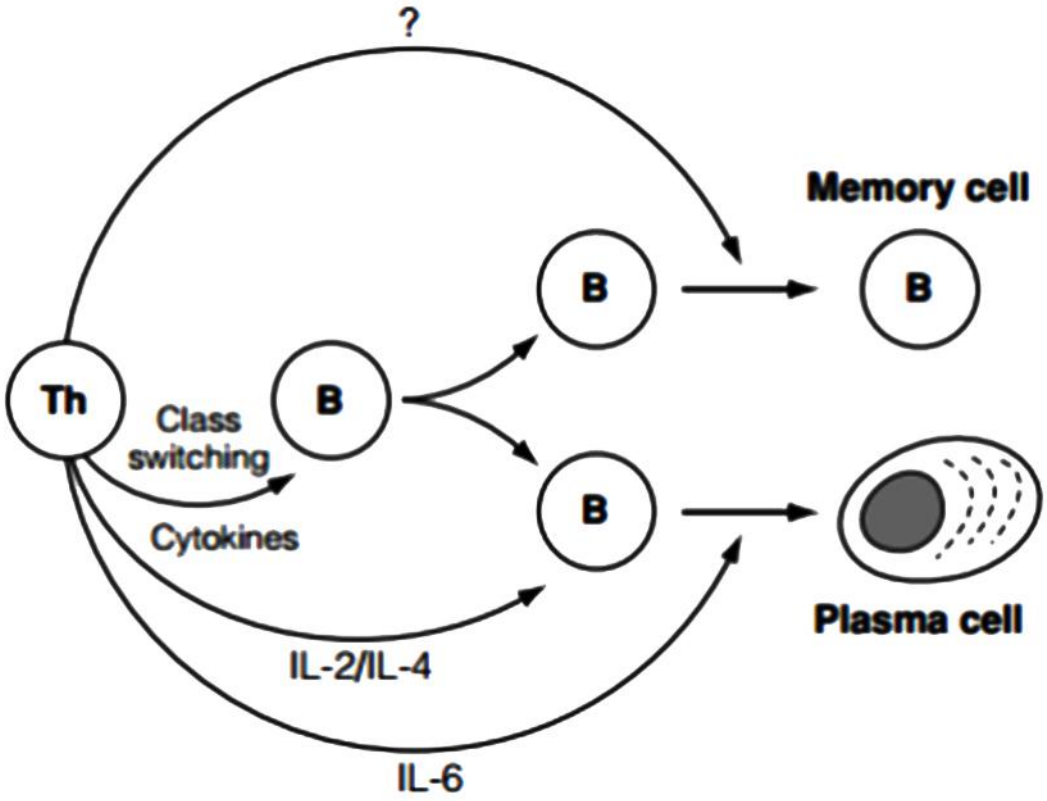


Figure 11: The role of cytokines in maturation of B-cells into memory and plasma cells

The second signal via T-cells is essential otherwise B-cell after binding to the antigen undergoes apoptosis.

CD40 (B-cell) interaction with CD40L (on T-cell) is the second necessary signal. It also causes class switching to produce IgG.

At this stage, FcγR II (CD32) binding to Ig can provide a negative feedback and prevent B-cell activation.

In case of T-I antigens, their binding does not lead to CD40 on B-cells getting digested. So, T-I and T-D antigens induce B-cell responses very differently.

Memory cells

Memory B-cells carry IgG or IgA which are of high affinity than IgM or IgD receptor on naïve cells.

The responses of naïve B-cells upon encounter with antigen and those of memory cells are different. These can be examined by again exposing an immunized animal to the same antigen.

The secondary (or tertiary etc) response consist of largely IgG and result from memory cells. Memory cells also have higher level of MHC class II molecules than naïve B-cells. It should be made clear that in initiating secondary response, memory cells have become plasma cells. In fact secondary response arises from early and more vigorous conversion to plasma cells.

Memory cells can migrate to lymphoid tissues just like naïve cells. However some memory cells remember where they were stimulated due to imprinting in the microenvironment which switches chemokine receptors by interaction with local dendritic cells. Thus, memory cells produced in a mucosal site migrates to other mucosal sites by this mechanism.

Affinity maturation

The increase in affinity is called affinity maturation. Some affinity maturation occurs during the primary response after isotypic switching and somatic hyper mutation.

During primary response many B-cells with receptors of wide range of affinities towards the antigens clonally selected. The average affinity of antibodies produced by such a response is low

As antigen concentration decreases due to the immune action, only B-cells with high affinity receptors are able to bind antigen and are thus clonally selected to expand.

Thus, only the high affinity memory B-cells are involved in the secondary and subsequent responses.

In fact, there is enough evidences that secondary (and further) responses are mediated by memory cells only.

The term “original antigenic sin” arises due to this. This refers to the phenomenon that humans make antibodies against any variant of influenza virus only against those epitopes which were present on the influenza virus to which they were earlier exposed. Remember, memory cells are antigen/epitope specific.

The advantage in this design is that why waste effort in triggering naïve B-cells when memory can mount a quick and efficient response.

A variant virus with completely new set of epitopes, however, is able to trigger naïve B-cells.

While we will occasionally refer to mAbs but will not discuss them in later modules. So, it is necessary to point out the various applications of mAb in broad terms:

- ✓ Ligands in affinity chromatography
- ✓ Vaccines
- ✓ In vitro diagnostic reagents.
- ✓ In vivo diagnostic tools

- ✓ Catalysis in the form of catalytic antibodies

Industrial process for purification of monoclonals in turn depend upon affinity chromatography mostly with protein A as the affinity ligand !

Summary

- Outline of clonal selection theory.
- Antibodies, enzymes and catalytic antibody
- B1 cells, B2 cells and Types of antigens
- Effector and memory B-cells.
- Hybridoma cells and mAb.