- **B-Cell Activation and Proliferation**

After export of B cells from the bone marrow, activation, proliferation, and differentiation occur in the periphery and require antigen. Antigen-driven activation and clonal selection of naive B cells leads to generation of plasma cells and memory B cells. In the absence of antigen-induced activation, naive B cells in the periphery have a short life span, dying within a few weeks by apoptosis.

- **Thymus-Dependent and Thymus- Independent Antigen Have Different Requirements for Response**

Depending on the nature of the antigen, B-cell activation proceeds by two different routes, one dependent upon TH cells, the other not. The B-cell response to **thymus-dependent (TD) antigens** requires direct contact with TH cells, not simply exposure to TH-derived cytokines. Antigens that can activate B cells in the absence of this kind of direct participation by TH cells are known as **thymus-independent (TI) antigens.** TI antigens are divided into types 1 and 2, and they activate B cells by different mechanisms. Some bacterial cell-wall components, including lipopolysaccharide (LPS), function as *type 1 thymus-independent (TI-1) antigens. Type 2 thymus-independent (TI-2) antigens* are highly repetitious molecules such as polymeric proteins (e.g., bacterial flagellin) or bacterial cell-wall polysaccharides with repeating polysaccharide units. Most TI-1 antigens are polyclonal B-cell activators **(mitogens);** that is, they are able to activate B cells regardless of their antigenic specificity. At high concentrations, some TI-1antigens will stimulate proliferation and antibody secretion by as many as one third of all B cells. The mechanism by which TI-1 antigens activate B cells is not well understood. When B cells are exposed to lower concentrations of TI-1 antigens, only those B cells specific for epitopes of the antigen will be activated. These antigens can stimulate antibody production in nude mice (which lack a thymus and

thus are greatly deficient in T cells), and the response is not greatly augmented by transferring T cells into these athymic mice, indicating that TI-1 antigens are truly T-cell independent. The prototypic TI-1 antigen is **lipopolysaccharide (LPS),** a major component of the cell walls of gram-negative bacteria. At low concentrations, LPS stimulates the production of antibodies specific for LPS. At high concentrations, it is a polyclonal B-cell activator.

TI-2 antigens activate B cells by extensively crosslinking the mIg receptor. However, TI-2 antigens differ from TI-1 antigens in three important respects.

- First, they are not B-cell mitogens and so do not act as polyclonal activators.
- Second, TI-1 antigens will activate both mature and immature B cells, but TI-2 antigens activate mature B cells and inactivate immature B cells.
- Third, although the B-cell response to TI-2 antigens does not require direct involvement of TH cells, cytokines derived from TH cells are required for efficient B-cell proliferation and for class switching to isotypes other than IgM.

Properties of thymus-dependent and thymus-independent antigens

		TI ANTIGENS	
Property	TD antigens	Type 1	Type 2
Chemical nature	Soluble protein	Bacterial cell-wall components (e.g., LPS)	Polymeric protein antigens; capsular polysaccharides
Humoral response			
Isotype switching	Yes	No	Limited
Affinity maturation	Yes	No	No
Immunologic memory	Yes	No	No
Polyclonal activation	No	Yes (high doses)	No

The humoral response to thymus-independent antigens is different from the response to thymus-dependent antigens. The response to TI antigens is

generally weaker, no memory cells are formed, and IgM is the predominant antibody secreted, reflecting a low level of class switching. These differences highlight the important role played by TH cells in generating memory B cells, affinity maturation, and class switching to other isotypes.

- **Two Types of Signals Drive B Cells into and Through the Cell Cycle**

Naive, or resting, B cells are nondividing cells in the G0 stage of the cell cycle. Activation drives the resting cell into the cell cycle, progressing through G1 into the S phase, in which DNA is replicated. The transition from G1 to S is a critical restriction point in the cell cycle. Once a cell has reached S, it completes the cell cycle, moving through G2 and into mitosis (M). Analysis of the progression of lymphocytes from G0 to the S phase revealed similarities with the parallel sequence in fibroblast cells. These events could be grouped into two categories:

1. competence signals
2. progression signals.

Competence signals drive the B cell from G0 into early G1, rendering the cell competent to receive the next level of signals. Progression signals then drive the cell from G1 into S and ultimately to cell division and differentiation.

Competence is achieved by not one but two distinct signaling events, which are designated ***signal 1*** **and** ***signal 2***. These signaling events are generated by different pathways with thymus-independent and thymus-dependent antigens, but both pathways include signals generated when multivalent antigen binds and crosslinks mIg (Figure 11-6). Once the B cell has acquired an effective competence signal in early activation, the interaction of cytokines and possibly other ligands with the B-cell membrane receptors provides progression signals.

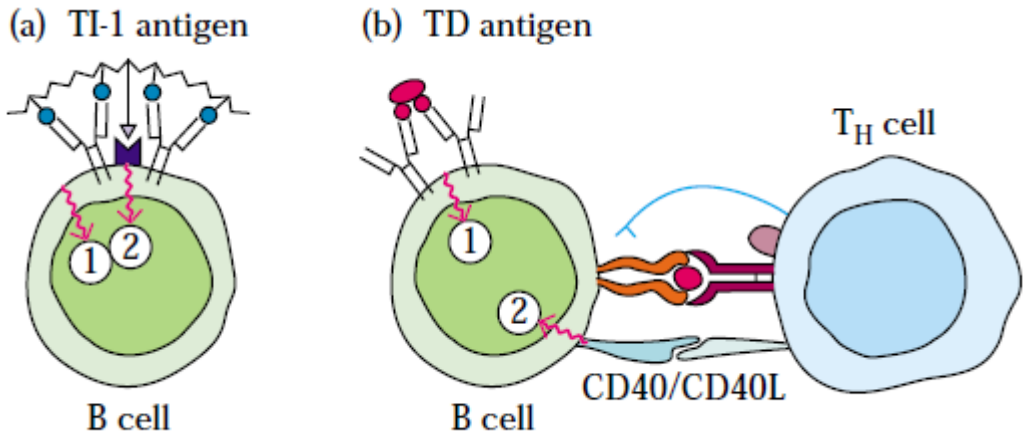

Figure 1: An effective signal for B-cell activation involves two distinct signals induced by membrane events. Binding of a type 1thymus-independent (TI-1) antigen to a B cell provides both signals. A thymus-dependent (TD) antigen provides signal 1 by crosslinking mIg, but a separate interaction between CD40 on the B cell and CD40L on an activated TH cell is required to generate signal 2.

- **Transduction of Activating Signals Involves Ig-α/Ig-β Heterodimers**

All isotypes of mIg have very short cytoplasmic tails. Both mIgM and mIgD on B cells extend into the cytoplasm by only three amino acids; the mIgA tail consists of 14 amino acids; and the mIgG and mIgE tails contains 28 amino acids. In each case, the cytoplasmic tail is too short to be able to generate a signal by associating with intracellular signaling molecules, such as tyrosine kinases and G proteins.

The discovery that membrane Ig is associated with the disulfide-linked heterodimer Ig α/Ig β, forming the **B-cell receptor (BCR),** solved this long standing puzzle. Thus the BCR is functionally divided into the

- ligand-binding immunoglobulin molecule
- the signal-transducing Igα/Ig β heterodimer

The Ig α chain has a long cytoplasmic tail containing 61 amino acids; the tail of the Ig β chain contains 48 amino acids. The cytoplasmic tails of both Ig αand Ig β contain the 18-residue motif termed the **immunoreceptor tyrosine-based**

activation motif (ITAM). Interactions with the cytoplasmic tails of Igα/Ig β transducer the stimulus produced by crosslinking of mIg molecules into an effective intracellular signal. The BCR itself has no PTK activity; this activity is acquired by recruitment of a number of different kinases, from nearby locations within the cell, to the cytoplasmic tails of the signal. Phosphorylation of tyrosines within the ITAMs of the BCR by receptor associated PTKs is among the earliest events in B-cell activation and plays a key role in bringing other critical PTKs to the BCR and in their activation. The antigen-mediated crosslinking of BCRs initiates a number of signaling cascades that ultimately result in the cell's responses to the crosslinking of its surface immunoglobulin by antigen. The crosslinking of BCRs results in the induction of many signal-transduction pathways and the activation of the B cell.

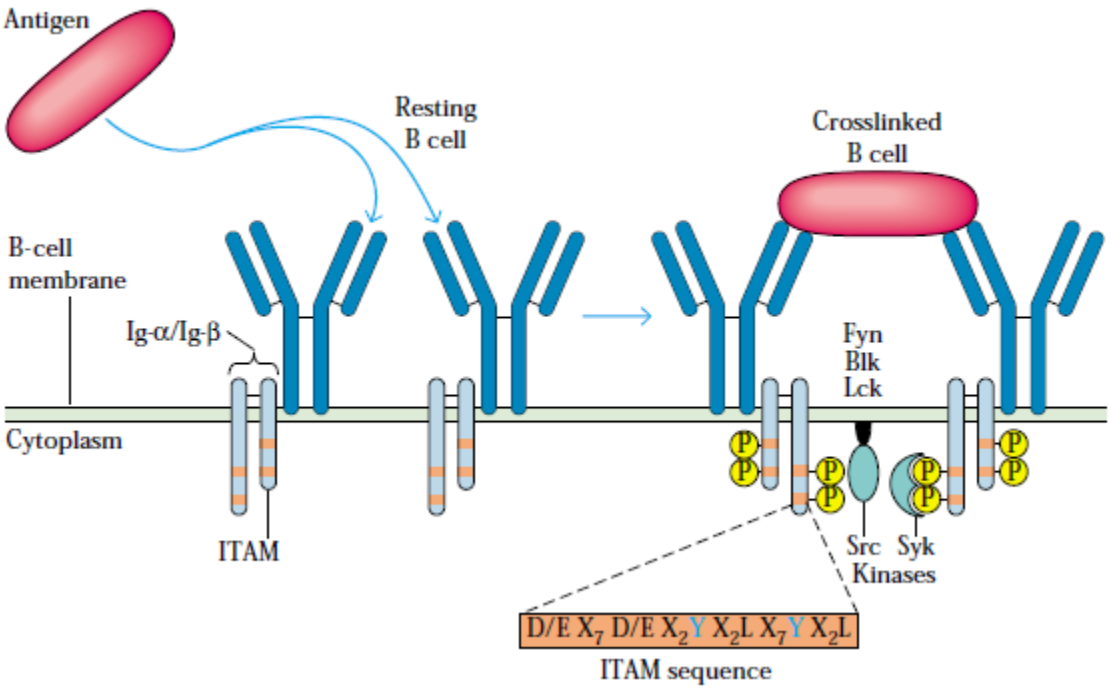

Figure 2: The initial stages of signal transduction by an activated B-cell receptor (BCR). The BCR comprises an antigen-binding mIg and one signal-transducing Igα/Ig β heterodimer. Following antigen crosslinkage of the BCR, the immunoreceptor tyrosine-based activation motifs (ITAMs) interact with several members of the Src family of tyrosine kinases (Fyn, Blk,

and Lck), activating the kinases. The activated enzymes phosphorylate tyrosine residues on the cytoplasmic tails of the Igα/Ig β heterodimer, creating docking sites for Syk kinase, which is then also activated. The highly conserved sequence motif of ITAMs is shown with the tyrosines (Y) in blue. D/E indicates that an aspartate or a glutamate can appear at the indicated position, and X indicates that the position can be occupied by any amino acid.

Figure 3 shows sequence of events in B-cell activation,these include:

- *Compartmentalization of function within receptor subunits:* The antigen-binding unit confers specificity, but has cytoplasmic tails too short to transduce signals to the cytoplasm of the cell. The signaling unit has long cytoplasmic tails that are the signal transducers of the receptor complex.
- *Activation by membrane-associated Src protein tyrosine kinases:* The receptor-associated PTKs (Lyn, Blk, and Fyn in B cells) catalyze phosphorylations during the early stages of signal transduction that are essential to the formation of a functional receptor signaling complex.
- *Assembly of a large signaling complex with protein tyrosine-kinase activity:* The phosphorylated tyrosines in the ITAMs of the BCR provide docking sites for the molecules that endow these receptors with PTK activity; e.g. Syk in B cells.
- *Recruitment of other signal-transduction pathways:* Signals from the BCR result in the production of the second messengers IP3 and DAG. IP3 causes the release of Ca2+ from intracellular stores, and DAG activates PKC. A third important set of signaling pathways are those governed by the small G proteins Ras and Rac that are also activated by signals received through the TCR or BCR.
- *Changes in gene expression:* One of the important outcomes of signal-transduction processes set in motion with engagement of the BCR is the generation or translocation to the nucleus of active transcription factors that

stimulate or inhibit the transcription of specific genes. Failures in signal transduction can have severe consequences such as X-linked agammaglobulinemia.

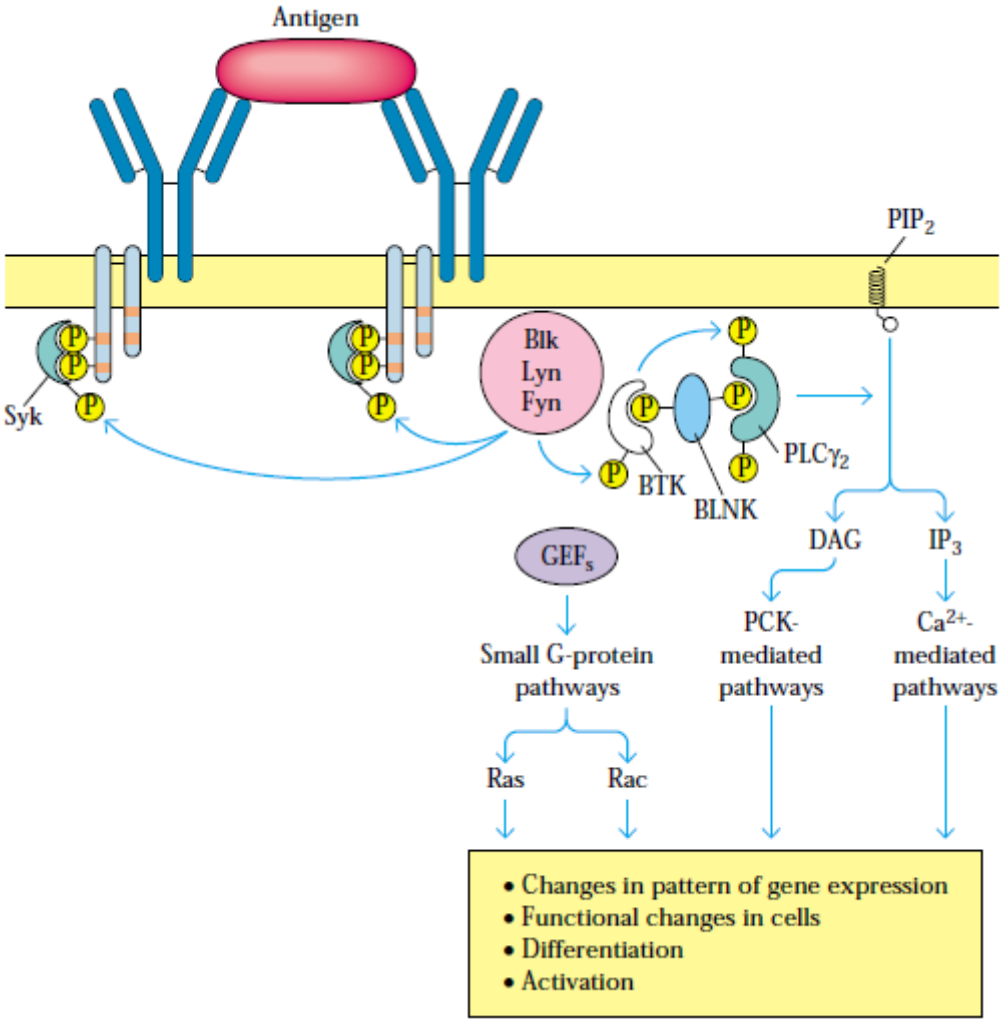

Figure 3: Some of the many signal-transduction pathways activated by the BCR. In one pathway, Syk activates PLCγ2 by tyrosine phosphorylation. PLCγ2 then hydrolyzes PIP2, a membrane phospholipid, to produce the second messengers DAG and IP3. DAG and Ca2+ released by the action of IP3 collaboratively activate the PKC, which induces additional signal-transduction pathways. The activated receptor complex also generates signals that activate the Ras pathway. Activated Ras initiates a cascade of phosphorylations that culminates in the activation of transcription factors that up-regulate the expression of many genes.