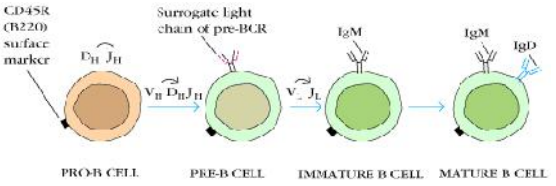
Chapter 11

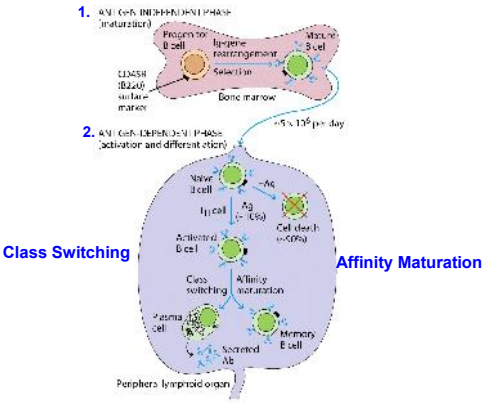
B cell generation, Activation, and Differentiation

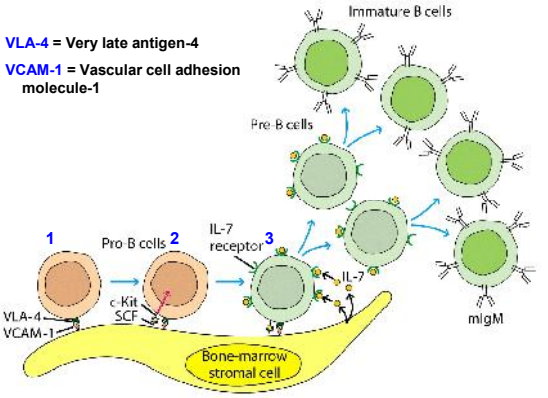


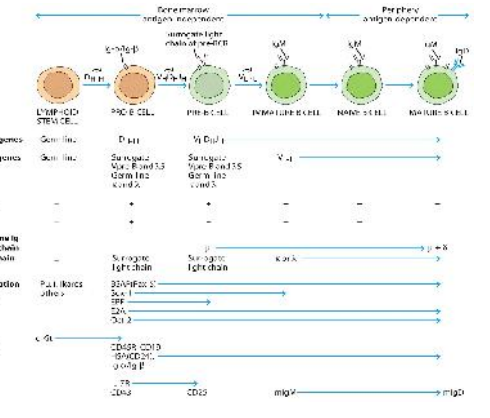
- B cells mature in the bone marrow.

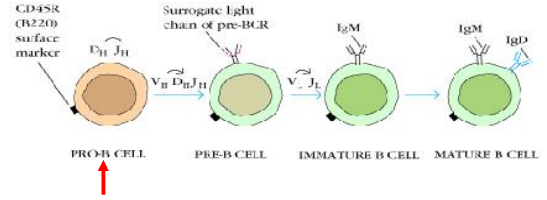
- B cells proceed through a number of distinct maturational stages:

1) Pro-B cell
2) Pre-B cell
3) Immature B cell
4) Mature B cell



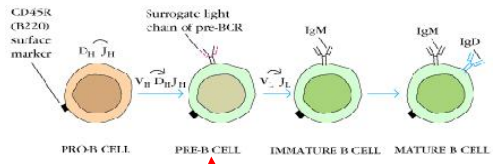






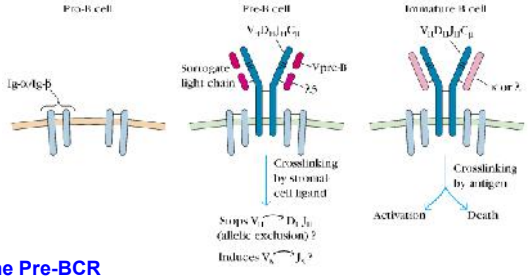
Pro-B cells

- Committed to the B lineage
- Express B220 (CD45R) - a B-lineage specific isoform of CD45,
- Express cKit, CD19
- Express no Ig. They are Igα/Igβ positive
- Are in the process of rearranging the heavy chain genes (D-J).
- Completion of heavy chain rearrangement marks the transition to the pre-B cell stage.



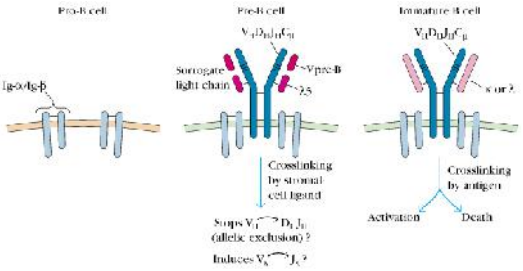
Pre-B cells

- Have successfully rearranged the heavy (H) chain locus but have not yet rearranged a light chain locus.
- **Are Tdt-ve --> so light chain rearrangement does not include incorporation of N-region nucleotides.
- Express the μ heavy chain on their surface in association with the "surrogate light chain" to form the "pre-BCR".
- Are Igα/Igβ positive
- Are also positive for CD25 (IL-2Rα)



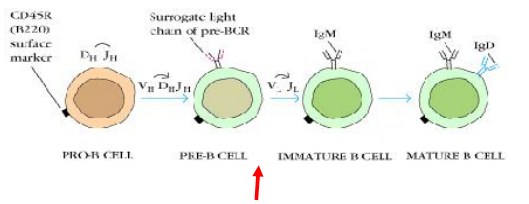
The Pre-BCR

- Associated with Ig-α/Ig-β heterodimers on the surface of pre-B cells
- Surrogate light chain consists of λ5 (constant) and Vpre-B (variable) subunits complexed with heavy chains
- Mediates:
  - Successful heavy chain rearrangement
  - Proliferation of pre-B cells (~256 clones)
  - Initiation of light chain rearrangement



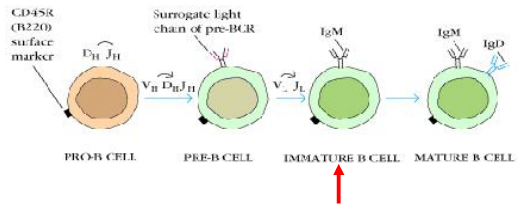
The Pre-BCR (developmental checkpoint):

- Cells that cannot express a complete BCR will not continue maturation.
- Reasons for failure to express a complete pre-BCR:
  - non-productive rearrangement of both heavy chain alleles
  - other? signaling defects?



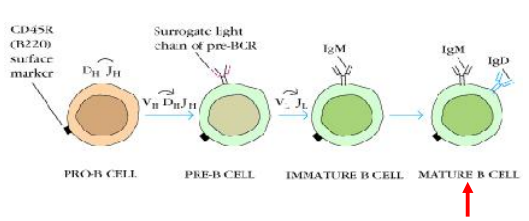
Once a pre-BCR is expressed, then:

- pre-B cells proliferate
  (The vast majority of human acute lymphoblastic leukemias of B cell origin express the surrogate light chain)
- Light chain rearrangement is initiated
- Successful rearrangement of a light chain allele marks transition to the immature B cell stage.



Immature B cells

- Have successfully rearranged both a IgM heavy chain allele and a light chain allele
- Express mIgM (not IgD) on their surface
- No longer express the surrogate light chain, now κ or λ light chains
- Still express RAG-2 and low levels of RAG-1
  - This allows for receptor editing
- Eventually RAG-1 and RAG-2 expression terminates and the cell differentiates into a mature B cell



Mature B cells

- Express both mIgM and mIgD on their surface
- Can exit the bone marrow.

A COMPARISON OF T CELL AND B CELL MATURATION

	T cells	B cells
	Proliferation	Proliferation
Rearrangement of:	α chain	Light chain
If rearrangement is nonproductive:	Death by apoptosis	Death by apoptosis
Expression on surface of:	TCR	BCR
Selection events:	Positive and negative selection *Selection of cells with affinity for self-MHC and elimination of self-reactive cells*	Negative selection only *Elimination of self-reactive cells*
	Loss of CD4/CD8	Expression of surface IgD
Final stage:	Mature, "single-positive" T cell	Mature, IgM+, IgD+ B cell
	Leaves thymus	Leaves bone marrow

B-1 B cells (Remember γ/δ T cells)

- Express CD5 (Ly-1 in mice), which is otherwise found only on T cells.
- Named B-1 B cells, with conventional B cells being referred to as "B-2 B cells" (the term "B cell" also refers to conventional B cells).
- Differ in a number of ways from conventional B cells:
  - Expression of CD5
  - Appear earlier than conventional B cells during fetal development
  - Abundant in peritoneum but scarce in secondary lymphoid tissues
  - Originate in the bone marrow but can proliferate in the periphery in order to maintain their numbers
  - Do not enter germinal centers, do not undergo somatic hypermutation
  - Produce predominantly IgM or IgG3 antibodies
  - Respond mostly to type 2 T-independent antigens (CHO) rather than to T-dependent antigens

Attribute	Conventional B cells (B-2 B cells)	B-1 B cells
Major sites	Secondary lymphoid organs	Peritoneal and pleural cavities
Source of new B cells	From precursors in bone marrow	Self-renewing (division of existing B-1 cells)
V-region diversity*	Highly diverse	Restricted diversity
Somatic hypermutation*	Yes	No
Requirements for T-cell help	Yes	No
Isotypes produced	High levels of IgG	High levels of IgM
Response to carbohydrate antigens	Possibly	Definitely
Response to protein antigens*	Definitely	Possibly
Memory*	Yes	Very little or none
Surface IgD on mature B cells*	Present on naive B cells	Little or none

Function?

- Not well understood
- A first line of defense?
- may have evolved to respond to specific antigens commonly found on microorganisms (type 2 T-independent antigens)

- A B cell lineage analogous to the γδ T cells?

Mature B cells exit the bone marrow and are ready to respond to antigen.

BUT - what prevents them from being activated by self-antigens?

If antibodies are made to self antigens --- autoimmune diseases

1) Antibodies to acetylcholine receptors --> myasthenia gravis

2) Antibodies to TSH receptor on thyroid cells --> Graves' disease

3) Antibodies to red blood cells --> autoimmune hemolytic anemia

SO - presumably some mechanism operates normally to prevent this.

Negative Selection

- Only negative selection
- Self-reactive immature B cells (mIgM) binding to self antigens are deleted in the B.M.
- Only 10% exit the B.M.
- Receptor editing rescues cells that failed negative selection → edits light chain

B cell activation

- B cell activation:
  - 1) Dependent on Th cells
  - 2) Independent of Th cells
- Thymus-dependent (TD) antigens – require direct contact for B cell activation.
- Thymus-independent (TI) antigens- do not require direct contact for B cell activation. Two types:
  - A) TI-type 1= LPS
  - B) TI-type 2= polymers (flagellin, bacterial cell wall components, etc)

Type I T-independent antigens: are mitogens (polyclonal activators) such as lipopolysaccharide (LPS) that activate B cells via nonspecific binding to B cell surface molecules. Any B cell, irrespective of its antigen specificity, can be activated by such molecules.

Type II T-independent antigens: are usually linear polymeric antigens that have a repeating unit structure – such as polysaccharides. The repeating structure allows simultaneous binding to, and cross-linking of, multiple BCRs. This massive BCR cross-linking is thought to provide a sufficient activation signal to over-ride the need for T cell help.

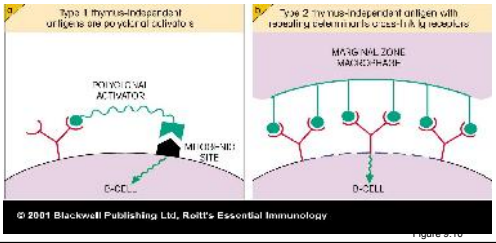
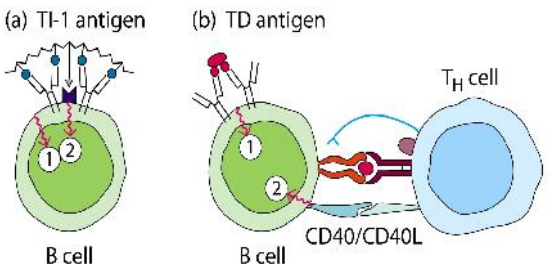


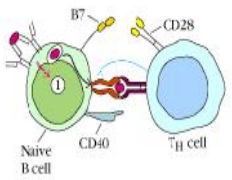
TABLE 11-2 Properties of thymus-dependent and thymus-independent antigens

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
Immunological memory	Yes	No	No
Polyclonal activation	No	Yes (high doses)	No
Produce Abs in nude mice	**No**	**Yes**	

T-Independent and T-dependent antigens



Activation of B cells by T-dependent antigens



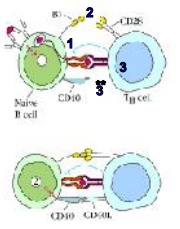
Receptor-mediated endocytosis

Upon binding antigen, B cells can internalize it, degrade it, combine antigenic peptides with class II MHC and present the antigen-MHC on their surface.

1. Activated B cells increase expression of surface MHC-II and also of another cell surface molecule, B7.

If a CD4+ helper T cell recognizes the antigen that is displayed on the B cell surface (i.e. that is being presented on class II MHC by the B cell), the two cells interact, forming a tight T-B cell conjugate.

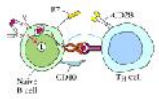
Role of Th cells in humoral immune responses (to T-dependent antigens)

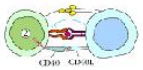


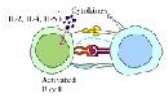
If the Th cell is activated by the antigen, there will be:

- 2) Interaction between the B7- CD28 molecules → **T cells to express CD40L.**
- **3) Now Th cells express CD40L on its surface - which can interact with CD40, which is expressed on the B cell to provide a signal that is essential for B cell activation and proliferation.**
- **B7-CD28 interactions provide co-stimulation for T cell activation.**

Role of T cells in humoral immune responses (to T-dependent antigens)

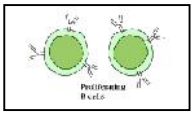




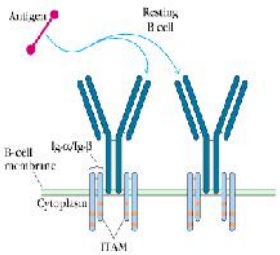


4. The B cell then expresses receptors for cytokines produced by the Th cell, including IL-2, IL-4 and IL-5.

As a result of signals received from cytokines and from the CD40-CD40L interaction, B cell proliferation occurs.



B cell Activation



The mature B cell receptor (BCR)

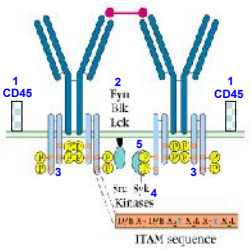
- Ig monomer plus 2 Ig-α/Ig-β heterodimers

- Ig cannot be expressed on the surface without the Ig-α/Ig-β heterodimers.

- The cytoplasmic tails of Ig-α and Ig-β contain ITAMs (immunoreceptor tyrosine-based activation motif)

The ITAM is a recognition site for cellular tyrosine kinases that are involved in B cell activation.

Cross-linked B cell



Syk = ZAP70

ITAM motif contains two tyrosines.

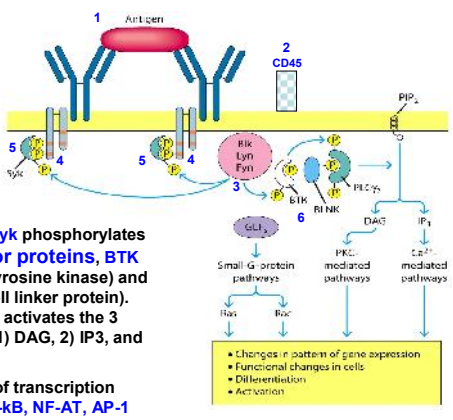
Cross-linking of the BCR by type II T-independent antigen results in recruitment of Src kinases (Blk, Fyn or Lyn) and CD45 tyrosine phosphatase.

1) CD45 activates Src kinases (Blk, Fyn or Lyn) which then phosphorylate tyrosines in ITAMs of Igα/Igβ.

This phosphorylation creates a high affinity binding site for the PTK Syk.

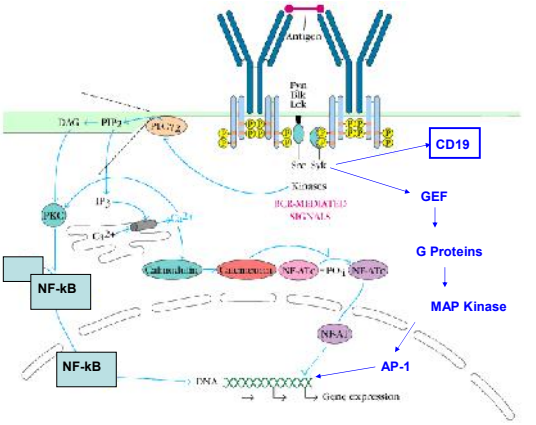
2) Binding of Syk to the ITAM results in its phosphorylation and activation by Blk, Fyn or Lyn.

At least three signal transduction pathways are then activated.



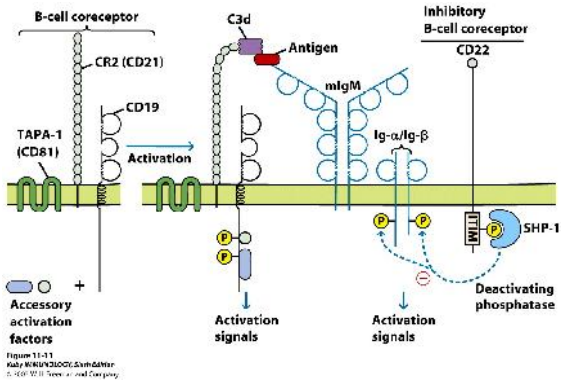
Activated Syk phosphorylates the adaptor proteins, BTK (Bruton's tyrosine kinase) and BLNK (B cell linker protein). BTK in turn activates the 3 pathways: 1) DAG, 2) IP3, and GEF.

Activation of transcription factors: NF-kB, NF-AT, AP-1



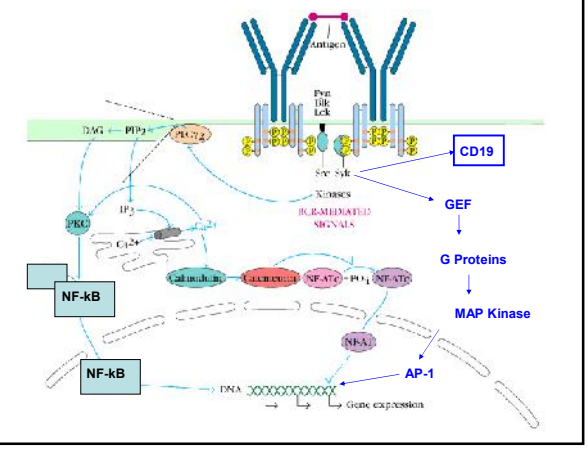
B cell co-receptor

- The B-cell co-receptors provides stimulatory signals
- Three components: CD19, CR2 (CD21) and TAPA-1 (CD81)
- CD19 is member of the Ig superfamily and contains ITAMs in its cytoplasmic tail
- CR2 (CD21) is receptor for a complement degradation product C3d (iC3b).
- CD22 is a negative regulator (SHP-1 – phosphatase)

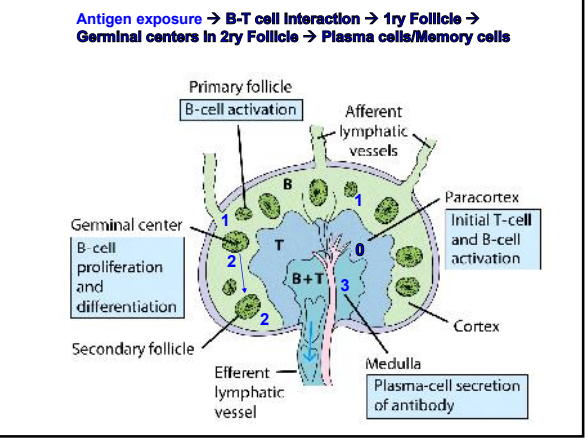


B cell co-receptor

- Antigen coated with C3d is bound by mIg and CR2. This leads to phosphorylation of CD19 by Lyn, Fyn, and others? This provides docking sites for a lipid kinase (PI-3 kinase).
- The PI-3 kinase is activated by Lyn or Fyn.
- This pathway is involved in the GEF pathway and induction of the AP-1 transcription factor
- Co-ligation of the BCR with its co-receptor (CD19/CR2/TAPA-1) increases signaling 100-1000 fold.
- CD22 negative regulator



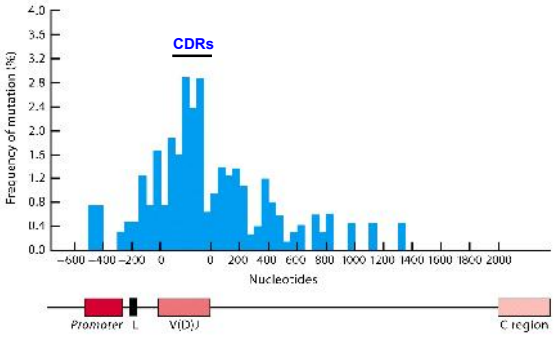
Site for Induction of Humoral Responses



Germinal Centers

- **Affinity maturation-** is the result of somatic hyper-mutation during subsequent exposure to the antigen
  - This is an antigen driven process that generates antibodies with higher affinities and this process and positive selection occurs in the germinal centers
- **Class-switching**- similar recognition sites (specificities) but the effector role of the molecule varies depending on the Ig class.
  - Remember, cytokines can direct class switch from the original IgM.

Frequency of Somatic Hypermutation



GOAL: Any given VH domain to associate with constant region of any isotype

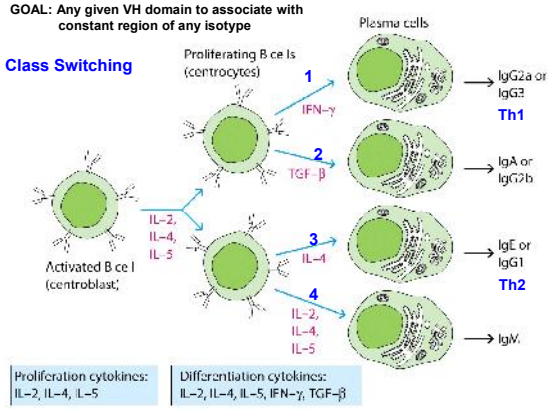


TABLE 11-6 Comparison of naive and memory B cells

Property	Naive B cell	Memory B cell
Membrane markers		
Immunoglobulin	IgM, IgD	IgM, IgD(?), IgG, IgA, IgE
Complement receptor	Low	High
Anatomic location	Spleen	Bone marrow, lymph node, spleen
Life span	Short-lived	May be long-lived
Recirculation	Yes	Yes
Receptor affinity	Lower average affinity	Higher average affinity due to affinity maturation*
Adhesion molecules	Low ICAM-1	High ICAM-1

*Affinity maturation results from somatic mutation during proliferation of centroblasts and subsequent antigen selection of centrocytes bearing high-affinity mIg.

Table 11-6
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THE END

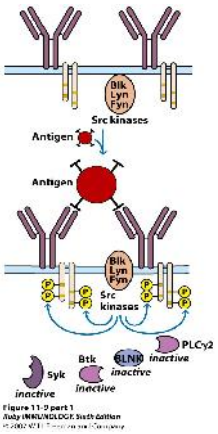

Figure 11-9 part 1
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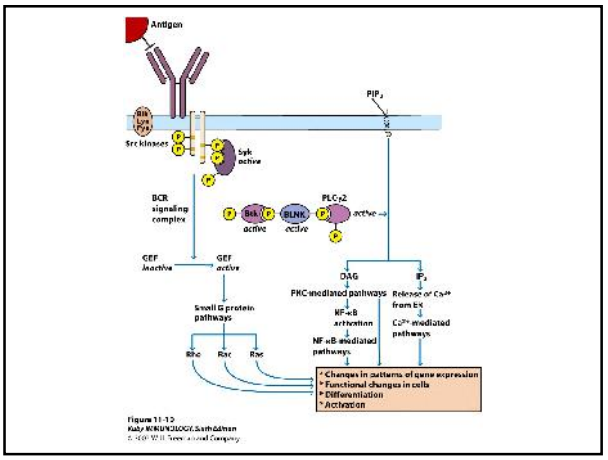

Figure 11-12
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