B Cell Generation, Activation & Differentiation

- **Naïve B cells-**
  - **have not encountered Ag.**
  - **Have IgM and IgD on cell surface : have same binding VDJ regions but different constant region**
  - **leaves bone marrow with single specificity and goes to LN or Spleen**
  - **once activated goes through affinity maturation and class switching**
  - **size of recirculating B cell pool is ~ $2X10^8$ cells**
    - ***short 1/2 life (~3 days to 8 weeks)***
    - ***only small fraction of potential repertoire is displayed at any given time by membrane Ig on circulating B lymphocytes, since potential antibody diversity is ~10^{11}.***

B cell maturation

- **Bone Marrow environment-**
  - **bone marrow stromal cell necessary to supply correct environment for pro-B cell to grow. B cells initially need direct contact with stromal cells (VLA-4 on pro-B cells and VCAM-1 on stromal cells).**
  - **After initial contact a receptor on pro-B, c-Kit, which is a TK interacts with stromal cell molecule called stem-cell factor (SCF), and this activates c-Kit. B cell begins to divide and expresses receptor for IL-7. IL-7 will eventually down-regulate adhesion molecules and B cell is released (still requires IL-7 for growth and maturation.**
- **Gene rearrangements-**
- **$5X10^7$ B cells/day with only $5X10^6$ surviving (10%)**
  - **negative selection & clonal deletion**

B-1 and B-2 β Cells

- B-1 arises before B-2 (B-2 is the major set of cells in humans)
  - B-1 cells appear during fetal life, express surface IgM but little or no IgD
  - B-1 cells arise in BM but renew their population by proliferation outside BM (in spleen and LN)
  - responds poorly to protein Ags and better to CHOs
  - B-1 does NOT undergo much class switch, and therefore antibodies are of low affinity
  - Many chronic leukemias arise from B-1 population

B-2 Population

- B-2 cells major population in humans and mice
  - class switch and get greater affinity for Ag
  - stem cell re-population, not in 2^{o} organs.
  - Have IgM and IgD in naïve state
- B cells undergo negative selection (clonal deletion) to self antigens.
  - 90% die off-- cross linking of IgM without IgD present
  - $5X10^{7}$ B cells made/day, $5X10^{6}$ recruited to periphery

Thymus dependent vs independent B cells

- **Thymus dependent (TD) antigens require direct contact of T_H cells, not just exposure to T_H-derived cytokines**
- **Thymus Independent (TI) antigens can activate B cell in the absence of direct participation of T_H cells**
  - **most TI antigens are polyclonal B cell activators (LPS) and can stimulate as many as 1/3 of all B cells (at low concentration of mitogen only those B cells specific for Ag respond).**
  - **Other TI antigens are highly repetitious like Polysaccharides, Flagellin, Bact cell walls**

TI-1 vs TI-2 Antigens

- **TI-1 antigens are polyclonal mitogens and act by cross-linking IgM**
  - **activate both immature and mature B cells**
    - ***weaker response and NO MEMORY cells made***
- **TI-2 antigens are repeating polysaccharides**
  - **these are not mitogens or polyclonal activators**
  - **activate only mature B cells**
  - **response does not involve direct contact with T_H cells but does require cytokines from them**
    - ***for class switching***
    - ***for proliferation***
    - ***cannot produce Abs in mice that cannot express alpha and beta TCR chains***

Activating Signals

- mIg have cytoplasmic tails
  - tails are too short to activate signal responses
- Membrane Ig is associated with an alpha /beta B cell receptor (BCR)
  - two molecules of BCR associate with one mIg to make ONE BCR
    - *there is a ligand-binding Ig molecule and the signal-transducing molecule (alpha/beta BCR)*
    - *Signaling from ITAM regions of BCR is mediated by tyrosine kinase activity*
    - *B CELL SIGNAL TRANSDUCTION PROCESSES IS SIMILAR TO T CELL SIGNAL TRANSDUCTION*

T-dependent B cell Activation

- After Ag binding to mIg
  - Ag is internalized by receptor-mediated endocytosis and processed into peptides (endocytic pathway as in APC)
  - BRC stimulation upregulates mIg and MHC II as well as B7 (co-stimulatory ligand). B cell functions as APC
  - Ag'ic peptides associate with MHC ii (60 minutes after Ag attaches to mIg)
  - Presents Ag to T_H cell at 100-->10,000 times lower concentration than for macrophages
  - T-B conjugate forms and cytokines made

Signal Transduction in B cells

- **Following Ag cross linking of the BCR the *immunoreceptor tyrosine-based activation motifs (ITAMS)* of BCR α and β chains:**
  - **interact with members of the *src* family of TKs (*Fyn, Blk, Lck*) and activate the kinases**
  - **these activated kinases phosphorylate tyrosine residues on the cytoplasmic tails of the Ig-α and Ig-β chain heterodimers , and this phosphorylation creates "docking" sites for *Syk* kinase which is also activated.**
  - ***Syk* activates phospholipase C (PLC) by tyrosine phosphorylation**
  - **PLC hydolyzes PIP_2 (membrane phospholipid) to produce 2nd messengers DAG and IP_3, which activate PKC (serine/threonine kinase) that activates the nuclear transcription factor CREB**

  - **Ras pathway activated- initiates cascade of phosphorylations known as the MAP Kinase [mitogen-activated protein kinase] cascade, which activates MAPK.**
  - **MAPK then translocates to nucleus where it activates transcription factors and NF-AT [nuclear factor of activated T/B cells], NF-kB, CREB [cyclic-AMP response element/B cell] and MAPK-activated transcription factors act at a number of sites in the genome to induce the expression of various genes.**
  - **2 such genes are Fos and Jun. The expression of these oncogenes forms a heterodimer, fos-jun, which is called an AP-1 complex (activation protein-1) which binds to and activates specific genes**
- **Once activated the B cells express RECEPTORS for IL-2, IL-4, IL-5, and others. These bind to cytokines released by T_H cells**
  - **these induce differentiation and into effector and memory cells, class switching and affinity maturation**

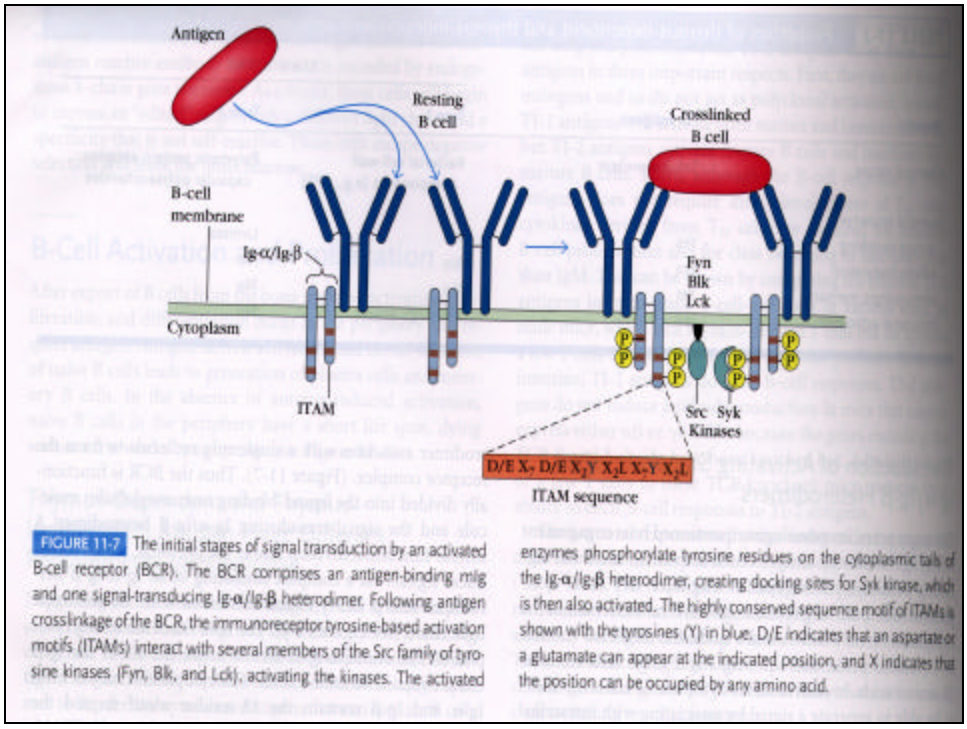

FIGURE 11-7 The initial stages of signal transduction by an activated B-cell receptor (BCR). The BCR comprises an antigen-binding mIg and one signal-transducing Ig-α/Ig-β heterodimer. Following antigen crosslinkage of the BCR, the immunoreceptor tyrosine-based activation motifs (ITAMs) interact with 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 Ig-α/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.

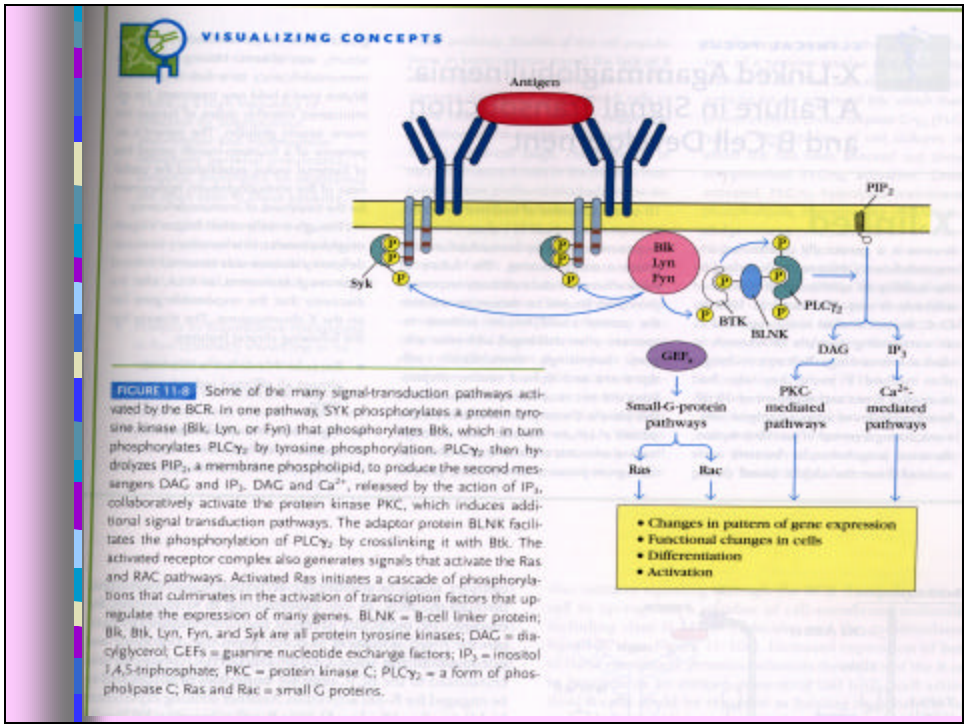

FIGURE 11-8 Some of the many signal-transduction pathways activated by the BCR. In one pathway, SYK phosphorylates a protein tyrosine kinase (Blk, Lyn, or Fyn) that phosphorylates Btk, which in turn phosphorylates PLCγ2 by tyrosine phosphorylation. PLCγ2 then hydrolyzes PIP_2, a membrane phospholipid, to produce the second messengers DAG and IP_3. DAG and Ca^{2+}, released by the action of IP_3, collaboratively activate the protein kinase PKC, which induces additional signal transduction pathways. The adaptor protein BLNK facilitates the phosphorylation of PLCγ2 by crosslinking it with Btk. The activated receptor complex also generates signals that activate the Ras and RAC pathways. Activated Ras initiates a cascade of phosphorylations that culminates in the activation of transcription factors that upregulate the expression of many genes. BLNK = B-cell linker protein; Blk, Btk, Lyn, Fyn, and Syk are all protein tyrosine kinases; DAG = diacylglycerol; GEFs = guanine nucleotide exchange factors; IP_3 = inositol 1,4,5-triphosphate; PKC = protein kinase C; PLCγ2 = a form of phospholipase C; Ras and Rac = small G proteins.

Primary and Secondary B cell Response

- **Lag phase-** after Ag binds responding B cells make clones of themselves
- **Logarithmic increase in serum Abs-** peak plasma and memory cell levels ~ 4-5 days, peak Ab levels ~ 7-10 days. For soluble proteins lag phase is a little longer (~9-14 days)
- **Secondary Response-** shorter lag period and last longer
  - different isotypes of Ig's than IgM in 1° and Ig's have greater affinity
- **Responses occur in LN and spleen-** follicles and germinal centers

Regulation of Response

- When antigen is encountered the response will be either:
  - tolerance
  - active immune response
- Regulation occurs in both humoral and cell-mediated branches- must determine the intensity and duration of response
  - Cytokines play important role in regulation
- **Antigenic Competition-** Injection of one Ag 2 days prior to second Ag suppresses response to 2nd
- **Antibody mediated Suppression-** passively administered Ab competes with specific Ag for binding. Vaccines are not administered to infants before age of 1 because of high presence of maternal antibodies