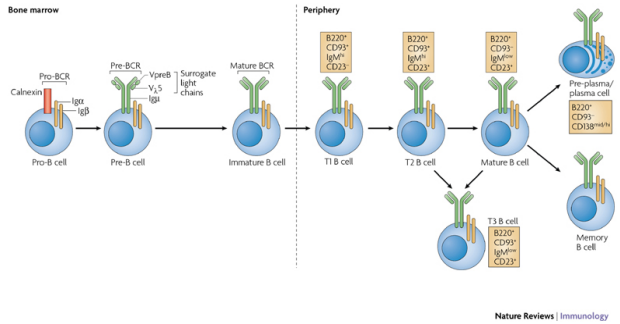
## The Development of B lymphocytes: Chap. 4 and 6




## Stages in development B cells

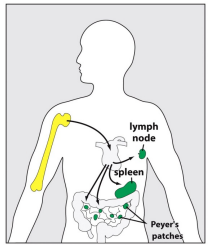
| Phase 1 | Phase 2 | Phase 3 |
|---|---|---|
| Generation of diverse and clonally expressed B-cell receptors in the bone marrow | Alteration, elimination or inactivation of B-cell receptors that bind to components of the human body | Promotion of a fraction of immature B cells to become mature B cells in the secondary lymphoid tissues |
| Repertoire assembly | Negative selection | Positive selection |

BM

Figure 6.1 The Immune System, 4th ed. (© Garland Science 2015)

| Phase 4 | Phase 5 | Phase 6 |
|---|---|---|
| Recirculation of mature B cells between lymph, blood, and secondary lymphoid tissues | Activation and clonal expansion of B cells by pathogen-derived antigens in secondary lymphoid tissues | Differentiation to antibody-secreting plasma cells and memory B cells in secondary lymphoid tissue |
| Searching for infection | Finding infection | Attacking infection |

SLN

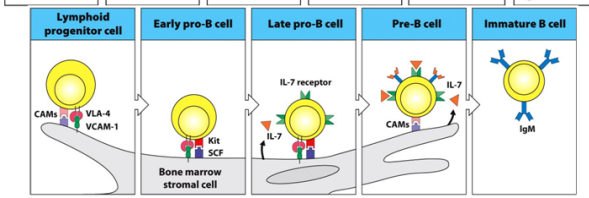



## B cell development: Today's subjects

- Phase I: Productive vs. non-productive rearrangement of immunoglobulin gene segments
- Phase II & III : Tolerance induction (= no self reactivity)
  - Receptor editing
  - Clonal deletion

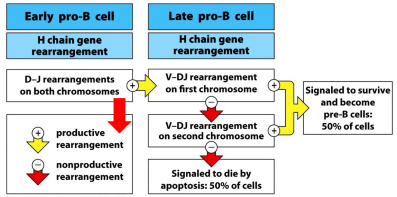
## Stages of BCR development: Phase I

| | Stem cell | Early pro-B cell | Late pro-B cell | Large pre-B cell | Small pre-B cell | Immature B cell |
|---|---|---|---|---|---|---|
| H-chain genes | Germline | D–J rearrangement | V–DJ rearrangement | VDJ rearranged | VDJ rearranged | VDJ rearranged |
| L-chain genes | Germline | Germline | Germline | Germline | V–J rearranging | VJ rearranged |
| Ig status | None | None | None | μ heavy chain is made | μ chain in endoplasmic reticulum | μ heavy chain. λ or κ light chain. IgM on surface. |



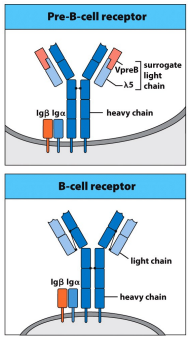
## Heavy chain rearrangement inefficient process

- In every Ig rearrangement there is 2/3 chance that you generate an out-of-the-frame sequence
- Many B cells die because they can not make a functional Ig
- Most loses are during heavy chain development
- Light chain: more trials!




## Quality check pre-B cell receptor

- Is rearranged heavy chain capable to combine with light chain?
  - ➢Use a templeate: surrogate light chain consisting of VpreB and λ5
- IgB shuts down further rearrangement




## Pre-B cells and allelic exclusion

- Eliminates B cells that do not make a functional heavy chain
- Prevents B cells from making more than one heavy chain
  - ✓Successful rearrangement and expression of heavy chain
    - ➢Stop transcription of RAG
    - ➢Signal degradation of RAG
    - ➢Chromatin is reorganized into a state that resists gene rearrangement

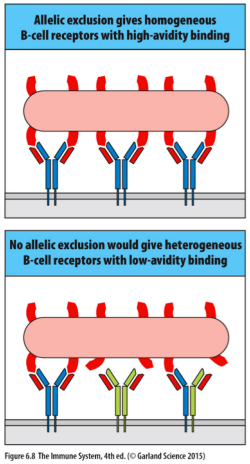
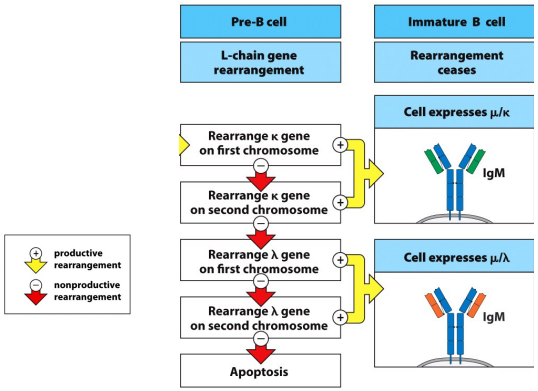


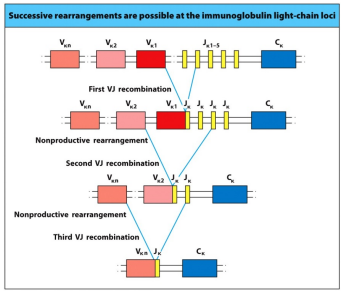

Figure 6.8 The Immune System, 4th ed. (© Garland Science 2015)

## Pre-B cell to Immature B cell

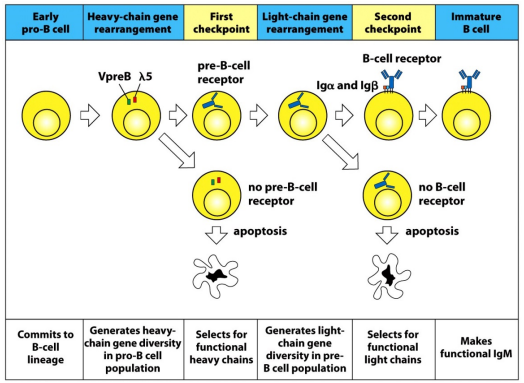


## Light chain rearrangement is more efficient

- Large pre-B cell with rearranged heavy chain divides yielding approx. 100 cells per B cell clone (i.e. with identical VDJ)
- Kappa light chain rearranges first, if unsuccessful followed by lambda
- Only V and J need to recombine, therefore higher success rate then with additional D
- If first recombination fails to give correct fusion, then other V region in upstream position will recombine with downstream J
- If again non-productive, then recombination of kappa locus on other chromosome
- If kappa fails, then lambda locus rearranges
- 85% of pre-B cells yield productive light chain rearrangement




## Checkpoints during B cell development in bone marrow




## Several proteins play a role in B cell development

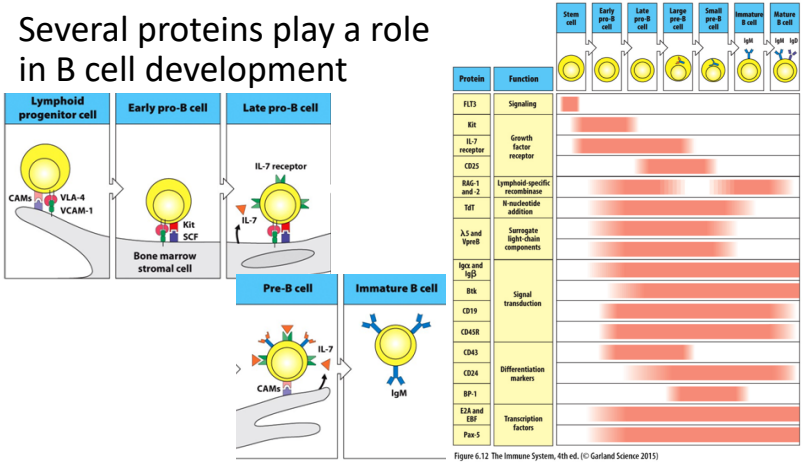



Figure 6.12 The Immune System, 4th ed. (© Garland Science 2015)

## Stages in development B cells

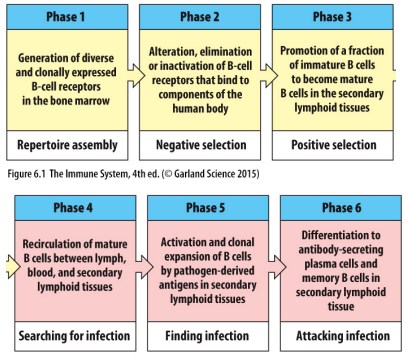
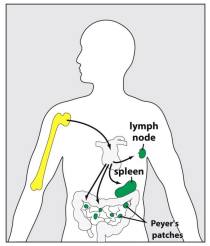



Figure 6.1 The Immune System, 4th ed. (© Garland Science 2015)

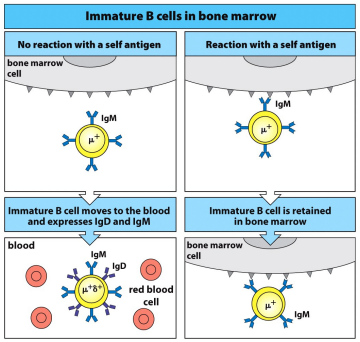
BM

SLN

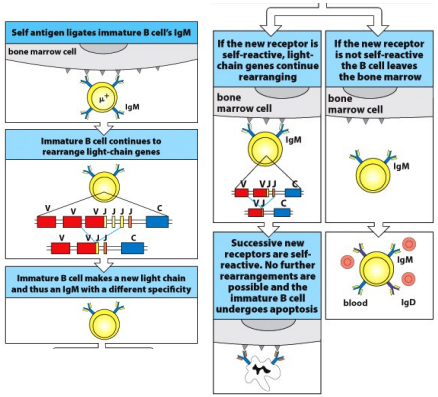


# Phase II in B cell development: Central Tolerance induction (Negative selection)

- Negative selection starts in bone marrow on self antigens presented by stromal and hematopoietic cells
- Potential *multivalent* self antigens are glycoproteins, proteoglycans and glycolipids on cells
- Immature B cells with self reactivity are maintained within bone marrow, others will go into circulation
- Immature B cells in the bone marrow that are self-reactive (up to 75%) need to dealt with:
  - clonal depletion
  - Receptor editing (give them another chance)

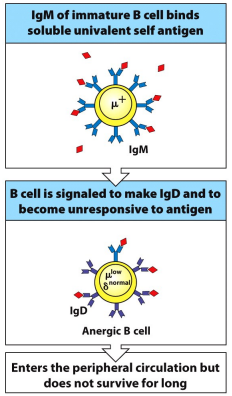



# Receptor editing to change self reactivity (multivalent antigens)




# Clonal activation of self reactive B cells (monovalent antigen): anergy

- Monovalent antigens are usually soluble proteins
- B cell becomes non-responsive:
  - Expresses IgD but hardly any IgM
  - Half-life: 1-5 days




# Genes that are involved in B cell tolerance

Table 1 | **Genes and proteins implicated in B cell central tolerance and receptor editing**

| Gene | Model | Comment |
|---|---|---|
| *ADA* | Human | Deficiency hinders tolerance |
| *AICDA* | Human and mouse | Deficiency hinders tolerance |
| *Bcl2* | Mouse | Enforced expression hinders tolerance |
| *Bim* (also known as *Bcl2l11*) | Mouse | Deficiency hinders tolerance |
| *Blnk* | Mouse | Deficiency hinders editing |
| *BTK* | Human and mouse | Deficiency hinders tolerance |
| *Cbl* and *Cblb* | Mouse | Deficiency promotes autoimmunity |
| *CD19* | Human and mouse | Deficiency hinders tolerance |
| *Cd45* | Mouse | Deficiency hinders tolerance |
| *CIN85* (also known as *SH3KBP1*) | Human cell line | Knockdown promotes B cell survival |
| *Fkbp11* | Mouse | Overexpression hinders tolerance |
| *Gadd45a* | Mouse | Deficiency hinders editing |
| *Hcls1* (encodes HS1) | Mouse cell line | Deficiency hinders apoptosis |
| *IRAK4* | Human | Deficiency hinders tolerance |
| *Irf4* | Mouse | Deficiency hinders editing |
| *Itpkb* | Mouse | Deficiency hinders tolerance |
| *mir-148a* | Mouse | Overexpression hinders deletion |
| *miR-17–92* | Mouse | Overexpression hinders deletion |
| *Myc* | Mouse | Knockdown hinders positive selection |
| *MYD88* | Human | Deficiency hinders tolerance |
| *Nfkb* (also known as *Rela*) | Mouse | Inhibition hinders tolerance |
| *Nras* | Mouse | Constitutive activity drives positive selection |
| *Pik3cd* (encodes p110δ) | Mouse | Deficiency hinders positive selection |
| *Pik3ca* (encodes p110α) | Mouse | Constitutive activity drives positive selection |
| *Pik3r1* (encodes p85α) | Mouse | Deficiency hinders positive selection |
| *Plcg2* | Mouse | Deficiency hinders editing |
| *Prkcd* (encodes PKCδ) | Mouse | Deficiency hinders deletion |
| *Pten* | Mouse | Deficiency hinders tolerance |
| *PTPN22* T | Human | Polymorphism hinders tolerance |
| *Raf1* | Mouse | Constitutive activity suppresses editing |
| *RAG1* | Human | Partial deficiency hinders tolerance |
| *Rasgrp1* | Mouse | Deficiency hinders tolerance |
| *Rasgrp1* or *Rasgrp3* | Mouse | Deficiency hinders apoptosis |
| *Syk* | Mouse | Deficiency hinders positive selection |
| *Syk* and *Zap70* | Mouse | Double deficiency hinders positive selection |
| *TACI* (also known as *TNFRSF13B*) | Human | Mutation hinders tolerance |
| *UNC93B* | Human | Deficiency hinders tolerance |

ADA, adenosine deaminase; AICDA, activation-induced cytidine deaminase; *Bcl2*, B cell leukaemia/lymphoma 2; Blnk, BTK, Bruton's tyrosine kinase; CIN85, CBL-interacting protein of 85 kDa; Fkbp11, FK506-binding protein 11; Gadd45a, g arrest and DNA-damage-inducible 45 alpha; Hcls1, haematopoietic cell-specific Lyn substrate 1; IRAK4, interleukin-1 r associated kinase 4; Irf4, interferon regulatory factor 4; Itpkb, inositol-1,4,5-trisphosphate 3-kinase B; mir-148a, microR miR-17–92, microRNA cluster 17–92; MYD88, myeloid differentiation primary response 88; Nfkb, nuclear factor kappal Pik3cd, phosphoinositide 3-kinase catalytic delta polypeptide; Pik3ca, phosphoinositide 3-kinase catalytic alpha polyp Pik3r1, phosphoinositide 3-kinase regulatory subunit polypeptide 1; Plcg2, phospholipase C gamma 2; Prkcd, protein k Pten, phosphatase and tensin homologue; PTPN22 T, R620W allele of protein tyrosine phosphatase non-receptor type recombination-activating gene 1; Rasgrp1, RAS guanyl-releasing protein 1; Syk, spleen tyrosine kinase; Zap70, zeta-ch protein of 70 kDa; TACI, transmembrane activator and CAML interactor.

*Nemazee, D, Nat Rev Imm. 2015*

## Maturing B cells

- After leaving bone marrow, B cells are still immature. The final stage occurs when immature B cells enter a secondary lymph node
- BCR stimulation dictate how B cell matures (positive selection)

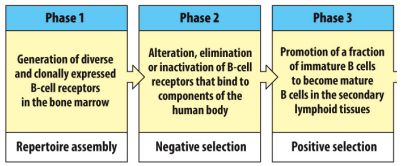
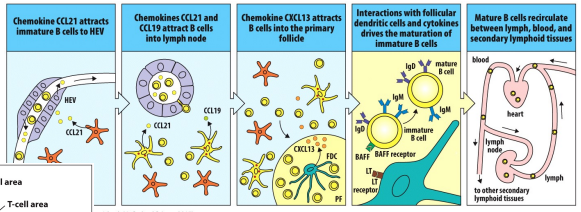
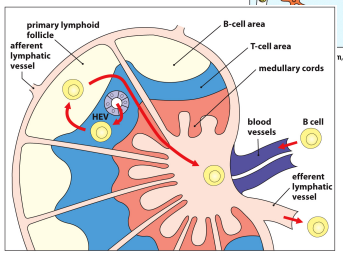



Figure 6.1 The Immune System, 4th ed. (© Garland Science 2015)

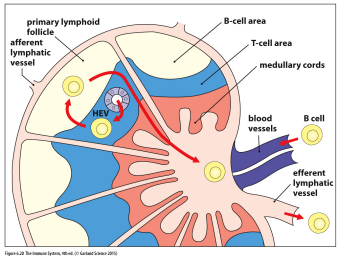
## Maturing B cells



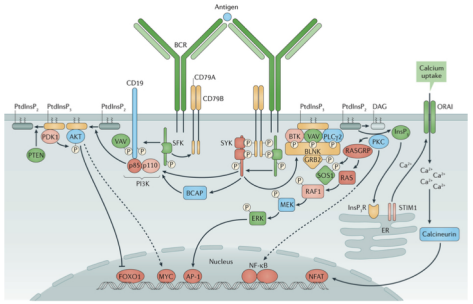




## Mature B cells

- They express both IgM and IgD
- Recirculate between blood and secondary lymphoid organs
- Transition via the lymph node to follicles
  - ➢CXCR5 is the homing receptor
  - ➢On average they remain in the follicles for 16 hours

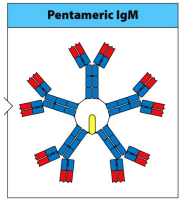



## Encountering antigen initiates many pathways



## Heavy chain isotypes

| | Immunoglobulin class or subclass | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | IgM | IgD | IgG1 | IgG2 | IgG3 | IgG4 | IgA1 | IgA2 | IgE |
| Heavy chain | $\mu$ | $\delta$ | $\gamma_1$ | $\gamma_2$ | $\gamma_3$ | $\gamma_4$ | $\alpha_1$ | $\alpha_2$ | $\varepsilon$ |
| Molecular weight (kDa) | 970 | 184 | 146 | 146 | 165 | 146 | 160 | 160 | 188 |
| Serum level (mean adult mg/ml) | 1.5 | 0.03 | 9 | 3 | 1 | 0.5 | 2.0 | 0.5 | $5 \times 10^{-5}$ |
| Half-life in serum (days) | 10 | 3 | 21 | 20 | 7 | 21 | 6 | 6 | 2 |



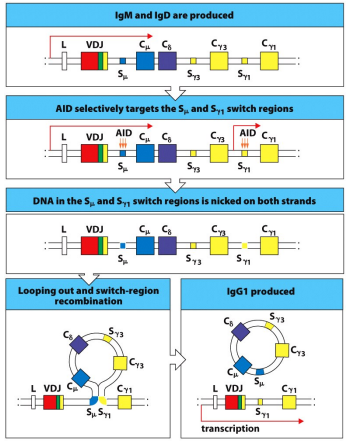

- After successful rearrangement VH and VL low affinity antibody secreted in form of pentameric IgM; high avidity (5x2 binding units) compensates for low affinity of binding unit
- Somatic mutation increases affinity, therefore lower degree of avidity needed: isotype switch from IgM (pentamer) to other types (IgG, IgA or IgE; bivalent)

# Isotype switching

Janeway's immunobiology Isotype switching

## Isotype switching

- Isotype switching ensures fusion of VH to other heavy chain isotype (no change in epitope recognition)
- Isotype light chain not changed
- Initiated by Switch (S) sequences and catalyzed by activation-induced cytidine deaminase (AID)
- Looping out of DNA puts VH in juxtaposition of new isotype




## Functions of isotypes

| Function | IgM | IgD | IgG1 | IgG2 | IgG3 | IgG4 | IgA | IgE |
|---|---|---|---|---|---|---|---|---|
| Neutralization | + | – | +++ | +++ | +++ | +++ | +++ | – |
| Opsonization | – | – | +++ | * | ++ | + | + | – |
| Sensitization for killing by NK cells | – | – | ++ | – | ++ | – | – | – |
| Sensitization of mast cells | – | – | + | – | + | – | – | +++ |
| Activation of complement system | +++ | – | ++ | + | +++ | – | + | – |

- neutralization (e.g. blocking interaction virus with cellular receptor)
- opsonization / activation complement system leading to lysis pathogen and ingestion by phagocytes
- activation NK cells by binding to Fc receptors
- activation mast cells by interaction with IgE receptor

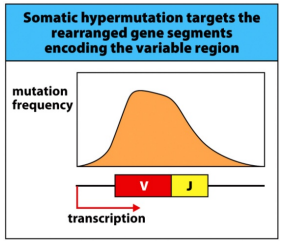
(discussed in Chapter 9)

## Properties of isotypes

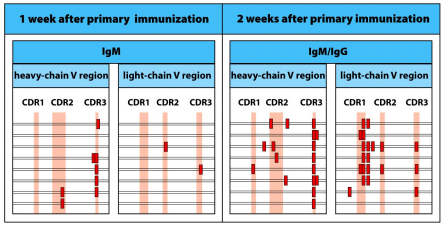
| Property | IgM | IgD | IgG1 | IgG2 | IgG3 | IgG4 | IgA | IgE |
|---|---|---|---|---|---|---|---|---|
| Transport across epithelium | + | – | – | – | – | – | +++ (dimer) | – |
| Transport across placenta | – | – | +++ | + | ++ | ++ | – | – |
| Diffusion into extravascular sites | +/– | – | +++ | +++ | +++ | +++ | ++ (monomer) | + |
| Mean serum level (mg/ml) | 1.5 | 0.03 | 9 | 3 | 1 | 0.5 | 2.5 | $5 \times 10^{-5}$ |

- transport across epithelium: secretion into lumen of gut, milk, saliva, sweat and tears to combat parasites, microbes and viruses outside the body
- transport across placenta to supply fetus with protective antibodies
- diffusion in extravascular sites of damaged or infected tissues (related to size of antibody)

## Affinity maturation by somatic hypermutations



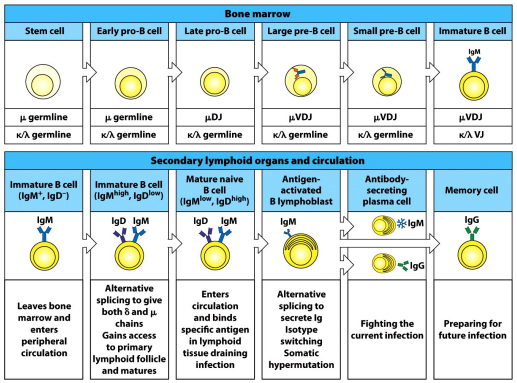

- IgM has low affinity binding
- During B cell development affinity improved by somatic hypermutation
- Random mutations introduced in V gene (left), but those giving better affinity (and therefore targeting CDR loops ) are selected (below)

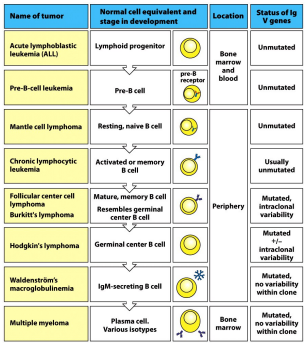



# Affinity Maturation

Movie by Jullian Kirk Elleker

## Summary B cell development



# Various B cell tumors reflect mistakes in different development stages of B cells

- Mistake in rearrangement can lead to activation of oncogenes (controling cell growth, division and differentiation)
- B cell tumors are clonal, i.e. derived from single recombined B cell
- Tumors derived from naive B cells grow in follicles of lymph nodes
  => follicular center cell lymphoma
- Tumors derived from plasma cells grow in bone marrow
  => myeloma