Stage I: Receptor gene Rearrangement

Stage II: Elimination of self-reactive cells

Stage III: Responders

Stage IV: Effectors
1. pre-BCR mediated positive selection

Bone Marrow

- Early pro-B (A)
- Intermediate pro-B (B)
- Late pro-B (C)
- Large pre-B (C')
- Small pre-B (D)
- Immature B (E)
- Mature B (F)

- $D_H$ to $J_H$ rearrangement
- $V_H$ to $D_J_H$ rearrangement
- $V_L$ to $J_L$ rearrangement

2. BCR mediated emigration

3. BCR mediated maintenance

Follicle

Spleen
Negative selection
Positive selection I
Pre-B receptor dependent
XLA
Syk KO
μKO
Positive selection II
Negative selection and Receptor editing
PERIPHERY

Pre-pro B → Pre-B1 → Pro-B2/preB → C → C' → D Y → E → F

Large pre-B → Small Pre-B → Immature B → Mature B

V to DJ out of frame
Pre-B receptor expressing cells proliferate and allelically exclude

**Common lymphoid progenitor**

- **IL-7 SDF-1**

- **IL-7**

- **D-J_H**

- **B pro-B**

- **VDJ+ In-frame**
  - **C' Large pre-B**

- **VDJ+ In-frame**
  - **C' Large pre-B**

- **VDJ+ In-frame**
  - **C' Large pre-B**

- **VDJ- VDJ-**
  - **C' Large pre-B**
Commitment

Positive selection I
Allelic exclusion

Positive selection II
Negative selection

T

B
Transcriptional regulation of early lymphoid development

- CLP
- ProB
- PreB
- B
- DN
- DP
- SP
- Tcf-1−/
- Gata-3−/
- Ebf−/
- Ikaros−/
- PU.1−/
- E2a−/
- Sox-4−/
- Pax-5−/
Entry versus commitment

- Commitment implies irreversibility and in wild type B cells has occurred when Ig H-chain gene rearrangement is initiated.
- Certain transcription factors such as EBF and E2A are required to turn on genes required early in B cell development.
- In the absence of Pax-5 cells “enter” the B lineage but remain highly plastic.
Pre-B receptor

B cell receptor

Pre-B receptor

B cell receptor

Syk

Blk/Lyn/Fyn

(Src family kinases)

Syk

Blk/Lyn/Fyn/Fgr

(Src family kinases)

Igα

Igβ

ITAM

Igα

Igβ

ITAM

Btk (Bruton's tyrosine kinase)
Defective in X-linked agammaglobulinemia

Other signaling pathways

Other signaling pathways
1) Survival
2) Proliferation
3) Allelic exclusion
4) Induction of κ rearrangement
5) Shut off of surrogate light chain expression
B cell tolerance

Multivalent OR Paucivalent

ANERGY
Chronic crosslinking model
Ca++ influx seen but not sustained
NFAT and ERK activated normally
NFκB and JNK NOT activated

RECEPTOR EDITING
Rag gene reexpression
Deletion of old V κ-Jκ rearrangement
New κ or λ lightchain

CLONAL DELETION
BCR signals induce caspase activation
Apoptotic death

Multivalent OR Paucivalent

ANERGY
Chronic crosslinking model
Ca++ influx seen but not sustained
NFAT and ERK activated normally
NFκB and JNK NOT activated
Red Pulp

White pulp

marginal zone and marginal sinus
Poly A sites

\[ \delta \]

Unspliced IgD message

mRNA for IgD heavy chain

mRNA for IgM heavy chain

L VDJ

\[ \mu \]

Cap site

ATG
NO SIGNAL

monosaccharide

antigen receptor

SIGNAL TRANSDUCTION

polysaccharide
The B-1/CD5 B "lineage"
THREE DISTINCT TYPES OF PERIPHERAL B LYMPHOCYTES

MARGINAL ZONE B CELLS

FOLLICULAR B CELLS

SPLEEN

PERITONEUM / MUCOSAL SITES

B-1 B CELLS
MZ and Follicular B cells

Are only “chosen” B cells selected by endogenous antigens?

OR

Do all B cells get tickled via the antigen receptor?
<table>
<thead>
<tr>
<th>Mutation</th>
<th>Signal Strength</th>
<th>MZ</th>
<th>FO</th>
<th>B-1</th>
</tr>
</thead>
<tbody>
<tr>
<td>BCR</td>
<td>&quot;No BCR signals&quot;</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Btk</td>
<td>&quot;Weak BCR signals&quot;</td>
<td>+</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>PLCγ2</td>
<td>&quot;Weak BCR signals&quot;</td>
<td>+*</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>PKCβ</td>
<td>&quot;Weak and Intermediate BCR signals&quot;</td>
<td>+</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>None</td>
<td>&quot;Weak, Intermediate, and Strong BCR signals&quot;</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
</tbody>
</table>
LONG-LIVED PERIPHERAL B CELL POPULATIONS

Strong BCR Signals .......B-1 cells

Intermediate strength BCR signals .......Follicular B cells

Relatively weak BCR signals .....MZ B cells
**BONE MARROW**

- Early pro-B (A)
- Intermediate pro-B (B)
- Late pro-B (C)
- Large pre-B (C')
- Small pre-B (D)
- Immature B (E)
- Mature B (F)

**SPLEEN**

- Newly formed B cells
- MZ B CELLS
- FOLLICLE

**Pre-BCR MEDIATED POSITIVE SELECTION**

**BCR MEDIATED EMIGRATION**

**D_H to J_H rearrangement**

**V_H to D_J_H rearrangement**

**V_L to J_L rearrangement**
Y

MHC class II (loaded)

TCR

B

BCR

CD4

gp 39/CD40L

CD40

activated

T
1. Dendritic cells (interdigitating) in T cell zones
2. Follicular dendritic cells in B cell areas
3. Macrophages everywhere
low affinity
Follicular Dendritic cell

high affinity
T cell

somatic mutation
isotype switching
Memory B cells
Plasma cells

Receptor diversification and rescue
Follicular Dendritic cell

APC

T cell

X
Activation of APCs via CD40 to release IL-12 and drive a TH1 type response

CD40

TCR

CD4

gp 39/CD40L

?Signals to T cell

BCR

MHC class II (loaded)

Required for T-dependent immune responses
- proliferation
- class switching
- germinal center formation
- somatic mutation

B

CD40

CD40L mutations lead to X-linked hyper-IgM syndrome
Dark zone

Light zone

PALS (T cell area)

Follicle (B cell area)

Germinal center

Centroblasts

Centrocytes

memory B cells

plasma cells

apoptosis

V gene hypermutation
\[ J_H C_\mu C_\delta C_{\gamma 3} C_{\gamma 1} C_{\alpha 1} C_{\gamma 2} C_{\gamma 4} C_\varepsilon C_{\alpha 2} \]
Loopying out and deletion

Switched to IgA
Switch regions and I-region promoters

\[\text{VDJ} \rightarrow \text{C} \rightarrow \text{LCR} \]

- γ2b, γ2a and ε C regions
- Unspliced pre-MRNA
- Spliced Iμ transcript
Class Switching (Murine)

1. IL-4 promotes switching to IgG1 and IgE
2. TGF-β promotes switching to IgA
3. γ-IFN promotes switching to IgG2a
Somatic mutation-I

1. Point substitutions. Non-templated single base changes in rearranged H- and L-chain V region genes

2. Requires T cell help, occurs in centrocytes

3. $10^{-4}$ to $10^{-3}$ base pairs/generation

4. Bell shaped curve of mutations starts in leader intron and ends about 1.5 kb downstream

5. Hotspot motifs

6. Transitions more common than transversions
SOMATIC MUTATION -II

7. Requires enhancer

8. Mechanism: AID DEPENDENT DNA DEAMINATION
   a. Cytosines converted to uracils
   b. Replication or error prone repair generates mutations

AID required for both class switching and somatic mutation

• AID is a novel Activation induced cytidine deaminase
• Related to a protein involved in RNA editing
• Required for class switching and also for somatic mutation
• Aid-/- mice have large germinal centers
• Humans lacking AID present with
Immature B
c
Large pre-B
Early pro-B
Intermediate pro-B
Late pro-B
Small pre-B
Immature B
Mature B

1. pre-BCR MEDIATED POSITIVE SELECTION

2. BCR MEDIATED EMIGRATION

3. BCR MEDIATED MAINTENANCE

BONE MARROW

D_H to J_H rearrangement
V_H to D_J_H rearrangement
V_L to J_L rearrangement

pre-BCR

FOLLICLE

SPLEEN