Stage I: Receptor gene Rearrangement

Stage II: Elimination of self-reactive cells

Stage III: Responders

Stage IV: Effectors
1. pre-BCR MEDIATED POSITIVE SELECTION

2. BCR MEDIATED EMIGRATION

3. BCR MEDIATED MAINTENANCE

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
Negative selection
and Receptor editing
Positive selection I
Pre-B receptor dependent
XLA
Syk KO
μKO
Pre-pro B  →  Pro-B I  →  Pro-B2/preB  →  Large pre-B →  Small Pre-B →  Immature B →  Mature B

Positive selection II
Syk KO  μKO
PERIPHERY

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

Pre-B receptor expressing cells proliferation and allelic exclusion.

- Common lymphoid progenitor
  - IL-7
  - SDF-1
  - IL-7
  - D-J<sub>H</sub>
  - B pro-B
  - IL-7
  - VDJ+ in-frame
  - C'<sup>+</sup> Large pre-B
  - VDJ+ in-frame
  - C'<sup>+</sup> Large pre-B
  - VDJ+ in-frame
  - C'<sup>+</sup> Large pre-B
  - VDJ- VDJ-
  - C'<sup>+</sup> Large pre-B
Commitment
Positive selection I
Allelic exclusion
Positive selection II
Negative selection

T

B
Transcriptional regulation of early lymphoid development

CLP

DN

DP

SP

ProB

PreB

Tcf-1^-/

Gata-3^-/

Ikaros^-/

E2a^-/

PU.1^-/

Ebf^-/

Sox-4^-/

Pax-5^-/

Tcf-1^-/

Ikaros^-/

Gata-3^-/

E2a^-/

PU.1^-/

Ebf^-/

Sox-4^-/

Pax-5^-/

Tcf-1^-/

Ikaros^-/

Gata-3^-/

E2a^-/

PU.1^-/

Ebf^-/

Sox-4^-/

Pax-5^-/

Tcf-1^-/

Ikaros^-/

Gata-3^-/

E2a^-/

PU.1^-/

Ebf^-/

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)

Other signaling pathways

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

Blk/Lyn/Fyn/Fgr
(Src family kinases)

Other signaling pathways

Btk

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

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

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

Unspliced IgD message

mRNA for IgD heavy chain

mRNA for IgM heavy chain

Cap site

ATG
The B-1/CD5 B "lineage"

Fetal Liver HSC

HSC

pro-B/pre-B

No TdT
Limited diversity

B cells with bias towards multivalent TI-1 antigens

Microbial antigens
Bacterial LPS

? Microbial antigens

IgM

IgD

B-1 cells are self-renewing and express CD5

B-1

B-1

B-1

B-1

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

MARGINAL ZONE B CELLS

FOLLICULAR B CELLS

PERITONEUM / MUCOSAL SITES

B-1 B CELLS

IgM

CD21

CD1

IgM

IgD

CD21

CD5
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
Immature B
Small pre-B
Large pre-B
Early pro-B
Intermediate pro-B
Late pro-B
Large pre-B
Small pre-B
Immature B
Mature B

CD1
CD21
MZ B CELLS

SPLEEN
FOLLICLE

Newly formed B cells

pre-BCR MEDIATED
POSITIVE SELECTION

pre-BCR MEDIATED
EMIGRATION

BCR MEDIATED
EMIGRATION

BONE MARROW

Early pro-B
Intermediate pro-B
Late pro-B
Large pre-B
Small pre-B
Immature B
Mature B

D<sub>H</sub> to J<sub>H</sub> rearrangement
V<sub>H</sub> to DJ<sub>H</sub> rearrangement
V<sub>L</sub> to J<sub>L</sub> rearrangement
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

T cell

APC

Receptor diversification and rescue

Follicular Dendritic cell
Activation of APCs via CD40 to release IL-12 and drive a TH1 type response.

- gp 39/CD40L
- Signals to T cell

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

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

Light zone

Follicle (B cell area)

PALS (T cell area)

Germinal center

Centroblasts

Centrocytes

V gene hypermutation

memory B cells

plasma cells

apoptosis
Looping out and deletion

Switched to IgA
Switch regions and I-region promoters

unspliced pre-MRNA

spliced $\mu$ transcript

\(\mu\) S\(\mu\) unspliced pre-MRNA

\(\mu\) S\(\mu\) spliced $\mu$ transcript

\(\gamma_2b, \gamma_2a \text{ and } \epsilon \) C regions

LCR
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
Small pre-B
Large pre-B
Early pro-B
Intermediate pro-B
Late pro-B
Large pre-B
Small pre-B
Immature B
Mature B

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

pre-BCR

pre-BCR

FOLLICLE

SPLEEN

IgM
IgD