B Cell Activation and Differentiation

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Lecture 11   Kuby (Chapter 11)

March 23, 2009
Goals of Lecture

• Distinguish between membrane and secreted form of Ig

• Mechanism of BCR signaling via ITAM sequences

• Distinguish Primary vs Secondary Immune Response

• TI vs TD

• Distinguish between membrane and secreted form of Ig

• T and B cell interactions:
  - Germinal Center Maturation and role of AID.
  - Class Switching (CSR)
  - Somatic Hypermutation (SHM)
  - Memory B cells and Plasma Cells
B Cell Activation

Ab Responses to most Ags require thymus (TD)

Ag must be a protein

Humoral response leads to:
  Affinity maturation
  Isotype Switching
  Memory B cells
TD antigen

B cell

CD40/CD40L

T_H cell
B Cell Activation

Ab Responses to few Ags does not require thymus (TI)
Response is mainly IgM with no memory

TI-1 Ags  Bacterial cell wall components, LPS
act as polyclonal B cell activators or B cell mitogens
LPS can also bind to TLR4 to activate most B cells

TI-2 Ags  Repeating epitopes that induce cross-linking
TI-1 antigen

B cell

Figure 11-7a
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**Figure 11-8**

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**Immunoreceptor tyrosine-based activation motif**

![Diagram showing the structure of the B-cell membrane with Ig-α/Ig-β and ITAM sequences](image-url)
Raft translocation of the BCR by ligand binding.
In resting B cells, the B-cell receptor (BCR) is excluded from lipid rafts, as are most plasma-membrane proteins, including CD45. The rafts concentrate glycosylphosphatidylinositol (GPI)-linked proteins and myristylated proteins, such as LYN and phosphoprotein associated with glycosphingolipid-enriched microdomains (PAG). After antigen engagement, the BCR relocates within rafts. IgH, immunoglobulin heavy chain; IgL, immunoglobulin light chain.
Ag cross links mlg triggers phosphorylation of ITAMs by Src kinases
The activated ITAMS serve as docking site of Syk.

Activation of transcription factors and change in gene expression.

Figure 11-9 part 2
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Figure 11-10

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Antigen cross-links mIg, generating signal ①, which leads to increased expression of class II MHC and co-stimulatory B7. Antigen-antibody complexes are internalized by receptor-mediated endocytosis and degraded to peptides, some of which are bound by class II MHC and presented on the membrane as peptide–MHC complexes.

$T_H$ cell recognizes antigen–class II MHC on B-cell membrane. This plus costimulatory signal activates $T_H$ cell.

1. $T_H$ cell begins to express CD40L.
2. Interaction of CD40 and CD40L provides signal ②.
3. B7-CD28 interactions provide costimulation to the $T_H$ cell.

Figure 11-12 part 1
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1. Tₜₜ cell begins to express CD40L.
2. Interaction of CD40 and CD40L provides signal ②.
3. B7-CD28 interactions provide costimulation to the Tₜₜ cell.

1. B cell begins to express receptors for various cytokines.
2. Binding of cytokines released from Tₜₜ cell in a directed fashion sends signals that support the progression of the B cell to DNA synthesis and to differentiation.

Figure 11-12 part 2
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Figure 11-16
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<table>
<thead>
<tr>
<th>Property</th>
<th>Primary response</th>
<th>Secondary response</th>
</tr>
</thead>
<tbody>
<tr>
<td>Responding B cell</td>
<td>Naive B cell</td>
<td>Memory B cell</td>
</tr>
<tr>
<td>Lag period following antigen administration</td>
<td>Generally 4–7 days</td>
<td>Generally 1–3 days</td>
</tr>
<tr>
<td>Time of peak response</td>
<td>7–10 days</td>
<td>3–5 days</td>
</tr>
<tr>
<td>Magnitude of peak antibody response</td>
<td>Varies depending on antigen</td>
<td>Generally 100–1000 times higher than primary response</td>
</tr>
<tr>
<td>Isotype produced</td>
<td>IgM predominates early in the response</td>
<td>IgG predominates</td>
</tr>
<tr>
<td>Antigens</td>
<td>Thymus dependent and thymus independent</td>
<td>Thymus dependent</td>
</tr>
<tr>
<td>Antibody affinity</td>
<td>Lower</td>
<td>Higher</td>
</tr>
</tbody>
</table>

Table 11-4
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T cells recognize different epitope from B cell epitope: Artificial system hapten (B cell epitope: DNP), carrier (T cell epitope BSA or BGG). Need T help to get secondary Ab response.
T cells recognizing carrier required --BUT not T Cells recognizing B cell epitope (next slide)
$1^\circ$ DNP-BSA + Anti-Thy-1 + complement

$2^\circ$ DNP-BGG

Spleen cells

X-irradiated syngeneic mouse

Secondary anti-DNP response:
• Combinatorial and Junctional diversity creates repertoire of naïve, mature B cells and T cells

• **B cells** undergo further diversification after antigen stimulation
  – Class switching of constant regions
  – Affinity maturation of variable regions (somatic hypermutation)
Mechanisms of Somatic Hypermutation and Isotype Switching

• Both require active transcription of genes
• Both require AID (Activation Induced Cytidine Deaminase)
• Affinity maturation by somatic hypermutation increases antibody affinity from $10^{-7}$ - $10^{-9}$ nM to $10^{-9}$ - $10^{-11}$ nM (100-fold increase in affinity)
Somatic hypermutation (SHM)

- SHM targets immunoglobulin genes (but not T cell receptor genes)
- SHM requires active transcription
- SHM involves DNA single-strand breaks
Model for somatic hypermutation

- **Activation-induced deaminase (AID)**
  - Expressed only in activated B cells
  - Converts C to U in single-stranded DNA
- **Other proteins insert mutations**
  - Uracil DNA glycosylase converts U to an apurinic site
  - AP endonuclease nicks the DNA adjacent to the AP site
  - Exonuclease removes the AP ribose
  - An error-prone polymerase fills in the gap
Phase 1A SHM
C:G → T:A

AID

NH₂
Mutations in variable-region mRNA

AID (+/-)

CDR1

CDR2

AID (-/-)

N terminal

V_H messenger RNA
Isotype Switching involves recombination at **switch signals**

Figure 4-21 part 1 of 2 Immunobiology, 6/e. (© Garland Science 2005)
Isotype Switching

- Other (than IgM/IgD) isotypes are produced by class-switch recombination (CSR), a process that exchanges the constant region of the heavy chain (CH) with a set of downstream constant-region genes.

- This deletional-recombination reaction, which requires the enzyme activation-induced cytidine deaminase (AID), involves the generation of DNA breaks at switch (S) regions, which precede the constant-region genes, followed by the repair of DNA.

- This leads to a rearranged CH locus and deletion of the intervening sequence as an episomal circle.

- Cytokines stimulate transcription through the CH gene and determine the immunoglobulin isotype that the B cell will switch to.
**Isotype Switching**

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Closely spaced nicks (*) on opposite strands converted into double-stranded breaks

Synapsis (possibly inducing H2AX, 53BP1, ATM, LRI, MLH1, DNA-PKcs) and end joining (possibly by NHEJ)

Productive CSR
Proliferating B cells (centrocytes) are induced to differentiate into plasma cells by cytokines such as IFN-γ and TGF-β. Plasma cells secrete specific immunoglobulins:

- IgG2a or IgG3
- IgA or IgG2b
- IgE or IgG1
- IgM

Activated B cell (centroblast) proliferation is induced by IL-2, IL-4, IL-5, and IL-6. Differentiation into plasma cells is influenced by IL-2, IL-4, IL-5, IFN-γ, and TGF-β.

**Proliferation cytokines:** IL-2, IL-4, IL-5

**Differentiation cytokines:** IL-2, IL-4, IL-5, IFN-γ < TGF-β
Cellular interactions important for IgE class-switch recombination.

- Uptake of allergens by dendritic cells allows for the presentation of antigenic determinants to T cells.

- Stimulation of specific CD4+ T cells leads to the production of interleukin-4 (IL-4) and the upregulation of expression of CD40 ligand (CD40L) by T cells.

- CD40 stimulation of allergen-specific B cells upregulates the expression of the co-stimulatory molecules CD80 and CD86, which allows for more efficient T-cell expression of CD40L and enhanced stimulation of B cells through the induction of IL-4.

- CD40-mediated stimulation of B cells also synergizes with IL-4-receptor (IL-4R) signals to enhance the transcription of C germline transcripts (C GLTs) and activation-induced cytidine deaminase (AID), rearrangement of the IgE genomic locus and production of IgE antibodies.
Figure 11-20

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Secondary follicle in a lymph node

From Abbas, Lichtman, & Pober: Cellular and Molecular Immunology. W.B. Saunders, 1999, Fig. 9-14b
<table>
<thead>
<tr>
<th>Property</th>
<th>Naive B cell</th>
<th>Memory B cell</th>
</tr>
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<tbody>
<tr>
<td>Membrane markers</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Immunoglobulin</td>
<td>IgM, IgD</td>
<td>IgM, IgD(?) , IgG, IgA, IgE</td>
</tr>
<tr>
<td>Complement receptor</td>
<td>Low</td>
<td>High</td>
</tr>
<tr>
<td>Anatomic location</td>
<td>Spleen</td>
<td>Bone marrow, lymph node, spleen</td>
</tr>
<tr>
<td>Life span</td>
<td>Short-lived</td>
<td>May be long-lived</td>
</tr>
<tr>
<td>Recirculation</td>
<td>Yes</td>
<td>Yes</td>
</tr>
<tr>
<td>Receptor affinity</td>
<td>Lower average affinity</td>
<td>Higher average affinity due to affinity maturation *</td>
</tr>
<tr>
<td>Adhesion molecules</td>
<td>Low ICAM-1</td>
<td>High ICAM-1</td>
</tr>
</tbody>
</table>

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

Table 11-6
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Fc receptor regulation of B cell activation

Ig cross-linking by polyvalent antigen without Fc receptor coligation

Polyvalent antigen

Antibody-antigen complex

Phosphatase

Fc receptor

Fc receptor–associated phosphatase removes phosphates in B cell receptor complex

B cell receptor signaling

Block in B cell receptor signaling

From Abbas, Lichtman, & Pober: Cellular and Molecular Immunology. W.B. Saunders, 1999, Fig. 9-17