B Cells and Antibodies

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Lecture outline

• Functions of antibodies

• B cell activation; the role of helper T cells in antibody production

• Therapeutic targeting of B cells
The Importance of Antibodies

- Humoral immunity is the defense mechanism against extracellular microbes
  - Most current vaccines work by stimulating effective antibody responses

- Antibodies are mediators of many immune/inflammatory diseases

- Antibodies are used as therapeutic agents

Principles of Humoral Immunity

- Antibodies are produced only by B lymphocytes.
- Humoral immune responses are initiated by binding of antigen to membrane bound antibody on B cells.
- Antibody responses are specialized and fine tuned by signals from helper T cells.
- Activated B cells secrete soluble antibodies of the same specificity as the membrane receptors.
Diverse immunoglobulin (Ig) molecules are generated by recombination of gene segments and variations introduced at sites of recombination.

B cells express Ig receptors for antigens and secrete the same Ig after activation.
The effector functions of antibodies

Leukocyte Fc receptors

- Activating Fc receptors on phagocytes (macrophages, neutrophils) ingest opsonized microbes for destruction: FcγRI

- Fc receptor on NK cells binds to opsonized cells and kill the cells: FcγRIII

- Fc receptors with other functions: FcγRII, neonatal Fc receptor (FcRn)
Inhibitory Fc receptors

• One class of Fc receptor on B cells (also macrophages and DCs) delivers inhibitory signals: FcγRII

• Function and clinical significance:
  - Terminates B cell responses after antibodies are produced (Ab engages inhibitory FcR): antibody feedback
  - Intravenous IgG (IVIg) is used to treat inflammatory diseases; may work by engaging inhibitory FcR
  - Mutations in FcγRIIb gene associated with lupus-like disease in mice; humans? (uncontrolled B cell activation)

IgG recycling by FcRn

Blood (physiological pH)

Serum protein

FcγRn

IgG

Endocytic vesicle

Acidified endosome

Sorting of FcRn-IgG complexes

Non-receptor bound proteins are degraded in the lysosome

Lysosome

Monocyte or endothelial cell

Derry C. Roopenian & Shreeram Akilesh
**T-dependent and T-independent antibody responses**

- B cells can recognize a wide variety of chemical structures (proteins, polysaccharides, lipids) and make antibodies against these
  - T cells recognize only MHC-associated peptides

- Helper T cells help B cells and stimulate isotype switching, affinity maturation, and generation of long-lived plasma cells and memory cells
  - T-dependent responses can occur only against proteins (the antigens for T cells)
  - These are the most varied and effective antibody responses

**Take home messages**

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**Subsets of B cells**

- Spinal cord, other lymphoid organs
- Follicular B cells
  - Protein antigen + helper T cell
  - Germinal center reaction
  - Isotype-switched, high-affinity antibodies; long-lived plasma cells
  - Mainly IgG: short-lived plasma cells

- Marginal zone B cells
  - Polysaccharides, lipids, etc.
  - Mainly IgM: short-lived plasma cells

- Mucosal tissues, peritoneal cavity
  - B-1 B cells
  - Polysaccharides, lipids, etc.
Helper T cell-dependent B cell responses

Sequence of T-dependent B cell activation

- Initial T-B interaction occurs at the edge of lymphoid follicles
  - Early antibody response, generates small numbers of short-lived plasma cells

- Some activated B and T cells migrate back into the follicle and form the germinal center (GC)
  - Tremendous B cell proliferation in GC
  - Role of follicular helper T cells
  - Induction of enzyme activation-induced deaminase (AID) in B cells, which stimulates isotype switching and somatic mutation

Take home messages
Molecular basis of T-B cell interaction: how do these cells communicate?

• B cells bind native protein antigen, internalize it, process it, and present peptides on MHC-II to helper T cells
  - B cells see native protein, T cells see peptides derived from the protein

• Helper T cells are activated to express CD40L and secrete cytokines, which stimulate B cell responses

Mechanisms of helper T cell-mediated activation of B lymphocytes
**Actions of helper T cells**

- Helper T cells stimulate B cells to produce large amounts of antibodies, undergo isotype switching and affinity maturation, and generate long-lived plasma cells and memory B cells
  - Mostly in germinal centers
  - Role of follicular helper T cells (IL-21?)
  - Many of the reactions are dependent on induction of the enzyme AID in B cells

**Take home messages**

Heavy chain isotype (class) switching:
B cells make different antibodies with the same specificity as the original antibody

<table>
<thead>
<tr>
<th>V</th>
<th>Sµ</th>
<th>Cµ</th>
<th>Sγ</th>
<th>Cγ</th>
<th>IgM</th>
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<tbody>
<tr>
<td>Naïve B cells</td>
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Deletion of Cµ, joining of V and Cγ

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<td>Activated (isotype switched) B cells</td>
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T cell help: CD40L --> AID
Cytokines target different C regions

V = variable region
C = constant region
S = "switch" segment
Immunoglobulin (Ig) heavy chain isotype (class) switching

**Affinity maturation of antibodies**

- In responses to protein antigens, the affinity of the antibody increases with repeated stimulation
- Caused by somatic mutations in Ig V genes, followed by selection of high-affinity B cells
- Stimulated by helper T cells in GCs (Tfh cells) activating AID in B cells, which induces somatic mutations in Ig genes
Somatic mutations in Ig V genes --> Selection of high-affinity B cells

Early antibody response

Response to repeated stimulation with protein antigens

Mutations

Affinity maturation of antibodies

Low-affinity antibody

High-affinity antibody

Selection of high-affinity B cells in germinal centers

B cells with somatically mutated Ig V genes and Ig with varying affinities for antigen

B cells with high-affinity membrane Ig bind antigen on follicular dendritic cell and present antigen to helper T cells

B cells that recognize antigen on follicular dendritic cells or interact with helper T cells are selected to survive; other B cells die
Plasma cells and memory B cells

- Plasma cells generated during GC reaction migrate to bone marrow and survive for years, producing antibody.
  - Much of circulating IgG is produced by long-lived plasma cells, provides initial protection.

- Some activated B cells develop into memory cells, which recirculate and do not secrete antibody but can be rapidly reactivated to become plasma cells.
  - Choice of plasma cells and memory cells is determined by expression of different transcription factors in the activated B cells.

Take home messages

Therapeutic strategies targeting B cells and antibodies

- Plasmapheresis (in severe cases of autoimmunity)

- B cell depletion: anti-CD20 antibody

- IVIg (does it act on B cells or on FcRN?)

- BAFF antagonists; other approaches
B cell depletion therapy

- Rituximab is an anti-CD20 mAb approved for treatment of RA, and in clinical trials for several other autoimmune diseases.
- Rituximab appears to be effective in RA, SLE, and surprisingly MS.
- CD20 is expressed on most mature B cells, but not plasma cells.
- Rituximab treatment results in long term, profound depletion of circulating B cells, although circulating memory B cells and tissue B cells are not as fully depleted, and plasma cells are not reduced.

Why does B cell depletion therapy work?

- Reduced levels of pathogenic autoantibodies
  - Does not always correlate with clinical response
- Role of B cells in presenting antigens to T cells
- Activated B cells can secrete cytokines
- Activated B cells can organize tertiary lymphoid tissue