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.
- Activated B cells secrete soluble antibodies of the same specificity as the membrane receptors.
- Antibody responses are specialized and enhanced by signals from helper T cells.
Diverse immunoglobulin (Ig) molecules with different specificities are generated by recombination of gene segments and variations introduced at sites of recombination.
B cell activation and antibody production

- **Antigen recognition**
  - Naive IgM⁺, IgD⁺ B cell

- **Activation of B lymphocytes**
  - Microbe
  - Activated B cell
  - Helper T cells, other stimuli

- **Proliferation**
  - IgG-expressing B cell

- **Differentiation**
  - High-affinity IgG-expressing B cell
  - Memory B cell

**Effector functions**

- **Effector cells:** antibody secreting plasma cells
  - IgM
  - IgG
  - High-affinity IgG
  - Memory B cell

**Isotype switching**

**Affinity maturation**
The effector functions of antibodies

- Neutralization of microbes and toxins
- Opsonization and phagocytosis of microbes
- Antibody-dependent cellular cytotoxicity
- Lysis of microbes
- Phagocytosis of microbes opsonized with complement fragments (e.g., C3b)
- Complement activation
- Inflammation
Leukocyte Fc receptors

- Activating Fc receptors on phagocytes (macrophages, neutrophils) ingest opsonized microbes for destruction: Fc$_\gamma$RI

- Fc receptor on NK cells binds to opsonized cells and kill the cells (ADCC): Fc$_\gamma$RIII

- Fc receptors with other functions: Fc$_\gamma$RII, neonatal Fc receptor (FcRn)

Take home messages
IgG recycling by “neonatal” FcR (FcRn)
Antibody production: activation of B cells

- Naive B cell
- Activated B cell
- Proliferation
- Activated B cells differentiate into antibody-secreting plasma cells

Helper T cells, other stimuli

Microbe

IgG
**T-dependent and T-independent antibody responses**

**T-independent (TI)**

- Clonal expansion; differentiation

**T-cell dependent (TD)**

- 'Activation' signal but no clonal expansion
- Clonal expansion; differentiation

- **T-independent** antigens are multivalent (e.g. bacterial polysaccharides or repeating determinants on the surface of viruses)
  - Responses are fast (within 1-2 days) and predominantly IgM
  - Weak in infants and young children

- **T-dependent** antigens must contain a protein component (true of most antigens) so that T cell help can be received
  - Responses slower (several days), produce all Ig isotypes (IgM, IgG, IgA, IgE)
  - Stronger and can lead to antibody affinity maturation and memory
T-dependent and T-independent antibody responses

**T-dependent**
- Protein antigen
- Helper T cell
- Follicular B cells
- IgM
- Isotype-switched, high-affinity antibodies; memory B cells, long-lived plasma cells

**T-independent**
- Polysaccharide antigen
- B-1 cells, marginal zone B cells
- IgM
- Mainly IgM, low-affinity antibodies; short-lived plasma cells

*Other signals (e.g. complement protein)*
Steps in T-dependent B cell activation

Initial T-B interaction

Dendritic cell  Antigen  Helper T cell  T cell zone  Initial T-B interaction  B cell zone (primary follicle)
Steps in T-dependent B cell activation

### Initial T-B interaction
- **Dendritic cell**
- Antigen
- **Helper T cell**
- **B cell**
- **T cell zone**
- **B cell zone (primary follicle)**

### B cell activation
- **Extrafollicular focus**
- **Extrafollicular helper T cell**
- **Follicular dendritic cell**
- **Follicular helper T (Tfh) cell**
- **Germinal center B cells**
- **Long-lived plasma cells**
- **Germinal center reaction**
Antigen presentation by B lymphocytes to helper T cells

1. B cell recognition of native protein antigen
2. Receptor-mediated endocytosis of antigen
3. Antigen processing and presentation
4. T cell recognition of antigen
Mechanisms of helper T cell-mediated activation of B lymphocytes

Activated helper T cell expresses CD40L, secretes cytokines

B cells are activated by CD40 engagement, cytokines

B cell proliferation and differentiation
The germinal center reaction

- Some B cells that are activated outside follicles migrate back to form germinal centers, where they undergo isotype switching and affinity maturation, and generate long-lived plasma cells and memory B cells
  - Driven by T cell help (follicular helper T cells)
  - Many of the reactions are dependent on induction of the enzyme AID in B cells
TFH cells: a unique helper T cell subset

Naïve CD4 T cell

- IL-12
- IFN-γ

Th1
- Tbet
- STAT1
- STAT4
- IFN-γ
- IL-4
- IL-5
- IL-13

Th2
- GATA3
- STAT6
- IL-4

Th17
- RORγt
- STAT3
- IL-17

IL-21
- ICOS
- IL-1
- IL-6
- IL-23

Follicular helper T (Tfh) cells
- BCL-6
- IFN-γ
- IL-4
- IL-13?
Follicular helper T cells (Tfh)

- Some effector T cells express the chemokine receptor CXCR5, migrate to lymphoid follicles, and help B cells (isotype switching, affinity maturation)

- Characteristics of Tfh:
  - Surface CXCR5, ICOS
  - Transcription factor: BCL-6
  - Cytokines secreted: IL-21 + IL-4 or IFN$\gamma$
    (or IL-17?)
Immunoglobulin (Ig) heavy chain isotype (class) switching

Isotype switching

B cell

Helper T cells: CD40L, cytokines

Various

IL-4

Cytokines produced in mucosal tissues, e.g. TGF-β, BAFF, others

IgM

IgG subclasses (IgG1, IgG3)

IgE

IgA

Principal effector functions

Complement activation

Fc receptor-dependent phagocyte responses; complement activation; neonatal immunity (placental transfer)

Immunity against helminths

Mast cell degranulation (immediate hypersensitivity)

Mucosal immunity (transport of IgA through epithelia)
Ig Heavy chain class (isotype) switching

IgM+ naive B cell

IgG+ memory cell

IgG secreting plasma cell

T cell help (cytokines, CD40L)

AID = Activation Induced Deaminase

VDJ

55 kb

μ

γ

ε

α

variable

constant

AID = Activation Induced Deaminase

T cell help (cytokines, CD40L)
Activation-induced deaminase (AID)

- Enzyme induced in B cells by Tfh signals (mainly via CD40)

- Role in isotype switching: switch regions are rich in palindromic AGCT sequences, sites of double-stranded DNA breaks; repair leads to recombination of different switch regions
Role of T-cell cytokines in Ig isotype switching

• Different cytokines induce switching to different Ig isotypes
  - Cytokines largely produced by Tfh cells (same as Th1/Th2 cytokines)
  - IL-4 $\rightarrow$ IgE
  - In mice, IFN$_\gamma$ $\rightarrow$ IgG; cytokine(s) involved in switching to IgG in humans still not clearly established

• Old statement that Th1 = cell-mediated immunity and Th2 = humoral immunity is incorrect
Affinity maturation of antibodies

Response to repeated stimulation with protein antigens

Early antibody response

Somatic mutations in Ig V genes ⇒ Selection of high-affinity B cells

Low-affinity antibody

High-affinity antibody

Mutations
Affinity maturation of antibodies

### Point mutation

**Heavy chain V regions**

- **Day 7 primary**
  - CDR1
  - CDR2
  - CDR3

- **Day 14 primary**
  - CDR1
  - CDR2
  - CDR3

- **Secondary**
  - CDR1
  - CDR2
  - CDR3

- **Tertiary**
  - CDR1
  - CDR2
  - CDR3

**Light chain V regions**

- **Day 7 primary**
  - CDR1
  - CDR2
  - CDR3

- **Day 14 primary**
  - CDR1
  - CDR2
  - CDR3

- **Secondary**
  - CDR1
  - CDR2
  - CDR3

- **Tertiary**
  - CDR1
  - CDR2
  - CDR3

**K_d (10^-7 M)**

- **Day 7 primary**
  - 3.6
  - 4.0
  - 6.0

- **Day 14 primary**
  - 0.4
  - 0.1
  - 0.2

- **Secondary**
  - 0.9
  - 0.02
  - 1.1

- **Tertiary**
  - 0.03
  - 0.03
  - 0.03
Activation-induced deaminase (AID)

- Enzyme induced in B cells by Tfh signals (mainly via CD40)

- Role in affinity maturation: V region sequences are hotspots for AID-induced mutations; selection increases the frequency of CDR mutations that result in high affinity
Selection of high-affinity B cells in germinal centers

Naive B cell

B cell activation by protein antigen and helper T cells

Migration into germinal center

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

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

B cells that recognize antigen on FDCs or interact with helper T cells are selected to survive; other B cells die

High-affinity B cell
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 vs memory cells is determined by expression of different transcription factors in the activated B cells

*Take home messages*
The germinal center reaction

• Site of development of sophisticated antibody responses
  • Isotype switching, affinity maturation, long-lived plasma cells, memory B cells
  • Driven by follicular helper T cells (assays for blood Tfh cells in humans?)

• Need to maximize the reaction for development of effective vaccines

• Does dysregulation of the GC reaction contribute to autoimmune diseases?
  • Strong autoantibody responses
  • Generation of self-reactive B cells?
Therapeutic strategies targeting antibody producing cells

- IVIg (does it act on B cells?)
- B cell depletion: anti-CD20 antibody
- BAFF antagonists
- Anti-CD40, CD40L (trials)
- Depletion of plasma cells: bortezomib (proteasome inhibitor)?
- Plasmapheresis (in severe cases of autoimmunity)