“Discovery consists of seeing what everybody has seen, and thinking what nobody has thought”

--Albert Szent-György
Nobel prize in Physiology or Medicine, 1937

The Biology of Fcγ Receptors and Complement
Selected Functions of Ig Isotypes

<table>
<thead>
<tr>
<th>Antibody isotope</th>
<th>Isotype-specific effector functions</th>
</tr>
</thead>
<tbody>
<tr>
<td>IgG</td>
<td>Opsonization of antigens for phagocytosis by macrophages and neutrophils</td>
</tr>
<tr>
<td></td>
<td>Activation of the classical pathway of complement</td>
</tr>
<tr>
<td></td>
<td>Antibody-dependent cell-mediated cytotoxicity mediated by natural killer cells and macrophages</td>
</tr>
<tr>
<td></td>
<td>Neonatal immunity; transfer of maternal antibody across the placenta and gut</td>
</tr>
<tr>
<td></td>
<td>Feedback inhibition of B cell activation</td>
</tr>
<tr>
<td>IgM</td>
<td>Activation of the classical pathway of complement</td>
</tr>
<tr>
<td></td>
<td>Antigen receptor of naive B lymphocytes</td>
</tr>
<tr>
<td>IgA</td>
<td>Mucosal immunity; secretion of IgA into the lumens of the gastrointestinal and respiratory tracts</td>
</tr>
<tr>
<td>IgE</td>
<td>Antibody-dependent cell-mediated cytotoxicity involving eosinophils</td>
</tr>
<tr>
<td></td>
<td>Mast cell degranulation (immediate hypersensitivity reactions)</td>
</tr>
</tbody>
</table>

Functional Sites on the IgG Molecule

- V<sub>L</sub>
- V<sub>H</sub>
- C1q binding site
- FcγR binding site
- Glycosylation site
Serum Protein Electrophoresis (SPEP): the γ-Globulin Peak Contains Multiple Ig Isotypes

- α₁: α₁-antitrypsin
- α₂: haptoglobin
- β: lipoproteins, transferrin, clotting factors, complement
- γ: IgG, IgA, IgM, IgD, IgE

Normal serum total protein: 5.5-9 g/dL
Normal albumin: 3.5-5.5 g/dL

Note that the "γμµα" in "gammaglobulin" does not refer to the isotype of the antibody (e.g., IgG), but the migration pattern of proteins on SPEP.

A Monoclonal "Spike" in the SPEP is Seen in Multiple Myeloma, a Plasma Cell Dyscrasia

Bone marrow biopsy from a patient with multiple myeloma
Selected Functions of Fc Receptors

Some Important Receptors for IgG (Fcγ Receptors)*

<table>
<thead>
<tr>
<th>FcR</th>
<th>Affinity for Immunoglobulin</th>
<th>Cell Distribution</th>
<th>Function</th>
</tr>
</thead>
<tbody>
<tr>
<td>FcγRI (CD64)</td>
<td>High (Kd = 10^{-9} M); binds IgG1 and IgG3, can bind monomeric IgG</td>
<td>Macrophages, neutrophils, eosinophils, also eosinophils</td>
<td>Phagocytosis; activation of phagocytes</td>
</tr>
<tr>
<td>FcγRIIA (CD32)</td>
<td>Low (Kd &gt; 10^{-7} M)</td>
<td>Macrophages, neutrophils, eosinophils, platelets</td>
<td>Phagocytosis; cell activation (inefficient)</td>
</tr>
<tr>
<td>FcγRIIB (CD32)</td>
<td>Low (Kd &gt; 10^{-7} M)</td>
<td>Leukocytes</td>
<td>Feedback inhibition of B cells</td>
</tr>
<tr>
<td>FcγRIIA (CD16)</td>
<td>Low (Kd &gt; 10^{-6} M)</td>
<td>Leukocytes</td>
<td>Phagocytosis (inefficient)</td>
</tr>
<tr>
<td>FcγRIIB (CD16)</td>
<td>Low (Kd &gt; 10^{-6} M); GPI-linked protein</td>
<td>Leukocytes</td>
<td>ADCC in NK cells</td>
</tr>
<tr>
<td>FcγRI</td>
<td>High (Kd &gt; 10^{-10} M); binds monomeric IgE</td>
<td>Mast cells, basophils, eosinophils</td>
<td>Cell activation (degranulation)</td>
</tr>
</tbody>
</table>

*Do not memorize this list but do learn functions of specific Fc receptors. Of these, all are “activating” receptors, except FcγRIIB, which is an “inhibitory” Fc receptor.
How do Fcγ Receptors Perform Effector Functions?

Fcγ Receptor Signaling: Phosphorylation of Immunoreceptor Tyrosine-based Activation Motifs (ITAMs)

FcγR ligand-binding domain

ITAM-bearing γ subunit

Opsonized Bacterium

Src family tyrosine kinase
Phosphorylated ITAMs Recruit Another Tyrosine Kinase, Syk, which Phosphorylates Other Substrates

Two Enzymes Worth Knowing
Phosphatidylinositol 3-kinase (PI 3-kinase)

Lipid products of PI 3-kinase (i.e., PIP₃) bind and activate other proteins (e.g., Bruton’s tyrosine kinase)

SHIP, an Inositol 5’ Phosphatase

SHIP counteracts positive signals generated by PI 3-kinase (by catalyzing the hydrolysis of its lipid product, PIP₃)
FcγRIIB: an Inhibitory Fcγ Receptor

Activating FcγR

Inhibitory FcγR

ITIM: Immunoreceptor tyrosine-based inhibitory motif
PI3K: Phosphatidylinositol 3-kinase
PTK: Protein tyrosine kinase
SHIP: SH2 Domain-containing Inositol 5' Phosphatase

Phagocytosis
Secretion
ADCC

+ PI3K

-
Hypothesis: The balance of activating* and inhibitory Fcγ receptors determines the outcome of IgG-initiated events in health and disease

*Activating: FcγRI, FcγRIIA, FcγRIII
Inhibitory: FcγRIIB

Therapeutic Uses of Intravenous Immunoglobulin (IVIg)*

**Autoimmune Cytopenias**
- Idiopathic thrombocytopenic purpura (ITP)
- Acquired immune thrombocytopenias
- Autoimmune neutropenia
- Autoimmune hemolytic anemia
- Autoimmune erythroblastopenia
- Parvovirus B19-associated red cell aplasia
- Anti-factor VIII autoimmune disease
- Acquired von Willebrand’s disease

**Neurological diseases**
- Guillain-Barré syndrome
- Chronic inflammatory demyelinating polyneuropathy
- Myasthenia gravis
- Multifocal neuropathy
- Polymyositis
- Dermatomyositis

**Vasculitis**
- Kawasaki disease
- ANCA-positive systemic vasculitis
- Antiphospholipid syndrome
- Recurrent spontaneous abortions
- Rheumatoid arthritis and Felty’s syndrome
- Juvenile Rheumatoid Arthritis
- SLE
- Thyroid ophthalmopathy
- Birdshot retinochoroidopathy
- Graft versus host disease
- Multiple sclerosis
- Insulin-dependent Diabetes mellitus
- Steroid-dependent asthma
- Steroid-dependent atopic dermatitis
- Crohn’s disease

*Other than replacement therapy for hypogammaglobulinemia. Do not memorize this list. Blue denotes diseases in which IVIg plays a major, established therapeutic role
The “Dark Side” of Fc Receptors: Immune Complex-mediated Injury

The Arthus Reaction: A Model of Type III Hypersensitivity

- Locally injected antigen in immune individual with IgG antibody
- Local immune-complex formation
- Activation of FeγRII on mast cells induces their degranulation
- Local inflammation, increased fluid and protein release, pleocytosis, and blood vessel occlusion

1-2 hr
Absence of the γ subunit of Fc receptors leads to enhanced survival in the F1 generation of NZB/NZW (lupus-prone) mice, a model for autoimmune, immune complex-mediated glomerulonephritis.


Glomerulonephritis is blocked in γ chain-deficient NZB/NZW (lupus-prone) mice. Pathological features include mesangial thickening and hypercellularity evolving into end-stage sclerotic and crescentic changes.

Summary: Fcγ receptors

1. Ig has multiple isotypes with unique functions

3. Receptors for the Fc portion of IgG (Fcγ receptors) come in two basic types: ITAM-containing activating receptors that bind PTKs and an ITIM-containing inhibitory receptor that antagonizes the PI 3-kinase pathway. Their relative expression determines the outcome of a given engagement of IgG ligand.

5. Fcγ receptors mediate a variety of immune functions: phagocytosis, secretion of pro-inflammatory mediators, and ADCC.

6. Unregulated activation of Fcγ receptors can lead to immune complex disease.

Biology of Complement
Recognized Functions of Complement

1. Host defense
2. Clearance of immune complexes
3. Disposal of apoptotic debris
4. Regulation of the immune response

Complement Activation in Host Defense

- Classical Pathway
  - Antigen:antibody complexes
- MB-Lectin Pathway
  - Lectin binding to pathogen surfaces
- Alternative Pathway
  - Pathogen surfaces

Complement activation leads to:
- Recruitment of inflammatory cells
- Opsonization of pathogens
- Killing of pathogens
Components of Complement

C1q, the Initiator of the Classical Pathway of Complement Activation
Formation of the C3 and C5 Convertases

C3 Contains a Latent, Reactive Thioester Group
The Classical Pathway of Complement Activation


The Mannose-binding Lectin Resembles C1q

The Lectin Pathway and Other Activators of Complement in the Absence of Antibodies

- A lectin is a molecule that binds to carbohydrate structures

- A collectin (like C1q or Mannose Binding Lectin) is a lectin with collagen-like features

- MBL first binds to mannose on bacterial cell walls. It then binds serine proteases MASP-1, -2 or -3 (Mannose binding lectin Associated Serine Protease)

- MASP can then activate C4 and C2, thus creating a C3 convertase without involving antibodies

- Deficiency in MBL is associated with increased susceptibility to bacterial infections

- It is simplistic to think of each “pathway” as acting in isolation. Thus, once the classical pathway has produced some C3b, these C3b molecules produce more C3b using the alternative pathway

- C-reactive protein (CRP) – An “acute phase” protein produced by the liver, binds to bacterial cell wall lipopolysaccharides. C1q then binds to CRP and thus activates complement without involving antibodies.

All Roads Lead to Rome

[Diagram showing classical, lectin, and alternative pathways leading to C3 and C5 convertases]
**C5a Increases Vascular Permeability and is a Potent Chemoattractant**

- Small complement cleavage products act on tissue vessels to increase vascular permeability and cell adherence molecules.
- Increased permeability allows increased entry of immune cells and extravasation of immune cells and complement molecules.
- Migration of macrophages, polymorphonuclear leukocytes (PMNs), and lymphocytes is increased.
- Microbicidal activity of macrophages and PMNs is also increased.

![Diagram](image.png)

**Big MAC Attack**

- Inflammation
- C5a
- C9
- Cell lysis
- Plasma membrane
- Membrane attack complex (MAC)

![Diagram](image.png)
Summary: Three Major Functions of Complement in Host Defense

- Opsonization and phagocytosis
  - Binding of C3b or C4b to microbe (opsonization)
  - Recognition of bound C3b by macrophage C3b receptor
  - Phagocytosis of microbe

- Stimulation of inflammatory reactions
  - Binding of C5a to mast cell, release of cytokines, proteolysis of C5, releasing C5a
  - Recruitment of neutrophils by C5a
  - Destruction of microbes by C5a

- Complement-mediated lysis
  - Binding of C3b to microbe, activation of key components of complement
  - Formation of the membrane attack complex (MAC)
  - Membrane lysis

Complement Regulatory Proteins*

<table>
<thead>
<tr>
<th>Receptor</th>
<th>Structure</th>
<th>Distribution</th>
<th>Interacts with</th>
<th>Function</th>
</tr>
</thead>
<tbody>
<tr>
<td>C1 inhibitor</td>
<td>60 kDa</td>
<td>Plasma, serum, 200 μg/mL</td>
<td>C1s, C1r, C2, C4</td>
<td>Inhibits C1 activation</td>
</tr>
<tr>
<td>Factor I</td>
<td>98 kDa</td>
<td>Plasma, serum, 20 μg/mL</td>
<td>C1r, C1s</td>
<td>Inhibits C1 activation</td>
</tr>
<tr>
<td>Factor H</td>
<td>100 kDa</td>
<td>Plasma, 80 μg/mL</td>
<td>C3b, C5a, C5b</td>
<td>Inhibits C3 activation</td>
</tr>
<tr>
<td>C4-binding protein (C4bp)</td>
<td>575 kDa</td>
<td>Plasma, serum, 330 μg/mL</td>
<td>C4b</td>
<td>Inhibits C4 activation</td>
</tr>
<tr>
<td>Membrane co-factor (MCP)</td>
<td>45 kDa</td>
<td>Membrane, 500 μg/mL</td>
<td>C3b, C5b</td>
<td>Inhibits C3 activation</td>
</tr>
<tr>
<td>Decay accelerating factor (DAF)</td>
<td>45 kDa</td>
<td>Blood cells, endothelial cells</td>
<td>C3b, C4b</td>
<td>Inhibits C3 activation</td>
</tr>
<tr>
<td>C3b and C4b</td>
<td>45 kDa</td>
<td>Blood cells, endothelial cells</td>
<td>C3b, C4b</td>
<td>Inhibits C3 activation</td>
</tr>
</tbody>
</table>

*Do not memorize this list but do learn that complement regulatory proteins are either present in soluble form or membrane-bound. Collectively, they interfere with multiple stages of complement activation.
## Complement Receptors Worth Knowing

<table>
<thead>
<tr>
<th>Receptor</th>
<th>Specificity</th>
<th>Functions</th>
<th>Cell types</th>
</tr>
</thead>
<tbody>
<tr>
<td>CR1 (CD35)</td>
<td>C3b, C4b, iC3b</td>
<td>Promotes C3b and C4b decay</td>
<td>Erythrocytes, macrophages, monocytes, polymorphonuclear leukocytes, B cells, FDC</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Stimulates phagocytosis</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Erythrocyte transport of immune complexes</td>
<td></td>
</tr>
<tr>
<td>CR2 (CD11)</td>
<td>C3d, iC3b, C2dg</td>
<td>Part of B-cell co-receptor</td>
<td>B cells, FDC</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Epstein–Barr virus receptor</td>
<td></td>
</tr>
<tr>
<td>CR3 (Mac-1)</td>
<td>IC3b</td>
<td>Stimulates phagocytosis</td>
<td>Macrophages, monocytes, polymorphonuclear leukocytes, FDC</td>
</tr>
<tr>
<td>(CD11b/CD18)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>C5a receptor</td>
<td>C5a</td>
<td>Binding of C5a activates G protein</td>
<td>Endothelial cells, mast cells, phagocytes</td>
</tr>
</tbody>
</table>

## \( \beta_2 \) (Leukocyte) Integrins

<table>
<thead>
<tr>
<th>Names</th>
<th>CD</th>
<th>Ligands</th>
</tr>
</thead>
<tbody>
<tr>
<td>LFA-1</td>
<td>CD11a/CD18</td>
<td>ICAMs</td>
</tr>
<tr>
<td>CR3 (Mac-1)</td>
<td>CD11b/CD18</td>
<td>iC3b, ICAMs, many others</td>
</tr>
<tr>
<td>CR4 (p150, 95)</td>
<td>CD11c/CD18</td>
<td>C3b, iC3b</td>
</tr>
</tbody>
</table>
Leukocyte Adhesion Deficiency (LAD)

Absence of CD18
Decreased to absent surface expression of LFA-1, CR3, CR4
Phagocytosis impaired
Diapedesis impaired
Patients susceptible to bacterial infections

Recognized Functions of Complement

1. Host defense
2. Clearance of immune complexes
3. Disposal of apoptotic debris
4. Regulation of the immune response
Clearance of Immune Complexes by Complement Bound to CR1 on Red Blood Cells

Small antigen-antibody complexes form in the circulation and activate complement. Bound C3b binds to the receptor CR1 on erythrocyte surfaces. In the spleen and liver, phagocytic cells remove the immune complexes.

Functions of Complement: Disposal of Apoptotic Debris

C1q helps removal of apoptotic cell debris (antibody not required)

Potential immune consequences of C1q deficiency:
(1) Increased deposition of debris in kidney
(2) Possible stimulation of autoantibody production
Disorders of the Complement System

Hereditary Angioneurotic Edema is Due to Deficiency in C1INH*

*Angioneurotic edema can also be acquired in the course of certain diseases. It is due to a lack of sufficient C1INH, a serine protease inhibitor. C1INH has a dual function: it inhibits activation of the classical pathway of complement activation (via C1q). C1INH also inhibits pathways leading to bradykinin formation, which is why patients with this disease develop edema.
**Paroxysmal Nocturnal Hemoglobinuria**

- Defect in enzymes that synthesize GPI-linked proteins (such as DAF and CD59)
- Red cells and platelets cannot repair damage caused by unregulated complement
- Patients suffer hemolysis and thrombosis

**Inherited Complement Deficiencies**

<table>
<thead>
<tr>
<th>Component</th>
<th>Effect</th>
</tr>
</thead>
<tbody>
<tr>
<td>C1q, C1r, C1s, C2, C4</td>
<td>Markedly increased incidence of autoimmune disease</td>
</tr>
<tr>
<td></td>
<td>Moderate increased incidence of pyogenic infections</td>
</tr>
<tr>
<td>H, I, C3</td>
<td>Increased incidence of pyogenic infections. Moderately increased incidence of autoimmune disease</td>
</tr>
<tr>
<td>Properdin, Factor D, C6, C7, C8, C9</td>
<td>Increased incidence of <em>Neisseria</em> infection</td>
</tr>
<tr>
<td>CR3, CR4</td>
<td>Increased incidence of pyogenic infections</td>
</tr>
<tr>
<td>C1INH</td>
<td>Hereditary angioedema</td>
</tr>
<tr>
<td>DAF, CD59</td>
<td>Paroxysmal nocturnal hemoglobinuria</td>
</tr>
</tbody>
</table>
### How is Complement Activity Measured?

**Method:** Incubate antibody-coated erythrocytes with serial dilutions of serum

**Results:**

<table>
<thead>
<tr>
<th>Serum Dilutions</th>
<th>Hemolysis</th>
</tr>
</thead>
<tbody>
<tr>
<td>1/50</td>
<td>100%</td>
</tr>
<tr>
<td>1/100</td>
<td>100%</td>
</tr>
<tr>
<td>1/150</td>
<td>50%</td>
</tr>
<tr>
<td>1/200</td>
<td>20%</td>
</tr>
</tbody>
</table>

The more you are able to dilute the serum to obtain a given degree of hemolysis, the more functional complement is present in the serum. In this case, the CH$_{50}$ = 150 (Reciprocal of 1/150).

CH$_{50}$ tends to fall in some autoimmune diseases due to complement consumption

### Summary: Complement

1. Complement is an ancient system of host defense that has well-defined functions in host defense: it opsonizes microbes (C3b, C3bi), stimulates inflammation (C3a, C4a, C5a), and mediates lysis of pathogens by the membrane attack complex (C5-9).

2. Additional functions of complement include clearance of immune complexes and apoptotic debris. These functions have major implications for the emergence of autoimmunity.

3. Among the known inherited complement deficiencies include Leukocyte Adhesion Deficiency (LAD) and complement component deficiencies; these are associated with frequent infections and, in the latter case, autoimmunity.