“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

- FcγR binding site
- C1q binding site
- Glycosylation site

VH
VL
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 IgE</td>
<td>Macrophages, neutrophils; 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>ADCC in NK cells</td>
</tr>
<tr>
<td>FcγRIIB (CD16)</td>
<td>Low (Kd &gt; 10^{-6} M); GPI-linked protein</td>
<td>Neutrophils, other cells</td>
<td>Phagocytosis (inefficient)</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 Fc_{γ} Receptors Perform Effector Functions?

Fc_{γ} Receptor Signaling:
Phosphorylation of Immunoreceptor Tyrosine-based Activation Motifs (ITAMs)

Fc R ligand-binding domain

ITAM-bearing γ subunit

Opsonized Bacterium

Src family tyrosine kinase

YP

YP
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., PIP$_{3}$) 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, PIP$_{3}$)
**Fc,RIIB: an Inhibitory Fc, Receptor**

Activating Fc, R  
Inhibitory Fc, R

PI3K: Phosphatidylinositol 3-kinase  
PTK: Protein tyrosine kinase  
SHIP: SH2 Domain-containing Inositol 5' Phosphatase

PI3K  
Phagocytosis  
Secretion  
ADCC
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

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
2. 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.
3. Fcγ receptors mediate a variety of immune functions: phagocytosis, secretion of pro-inflammatory mediators, and ADCC.
4. 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

CLASSICAL PATHWAY
Antigen-antibody complexes (pathogen surfaces)

MB-LECTIN PATHWAY
Mannose-binding lectin binds mannose on pathogen surfaces

ALTERNATIVE PATHWAY
Pathogen surfaces

C1q, C1r, C1s
C4
C2

C3 convertase

C3a, C5a

Peptide mediators of inflammation, phagocyte recruitment

C3b

Binds to complement receptors on phagocytes

Opsonization of pathogens

Removal of immune complexes

Figure 2.19 Immunobiology, 4th ed. Garland Science (2003)

C1q, the Initiator of the Classical Pathway of Complement Activation

C1 complex

C1q

C1r2S2

IgG antibody

Cell surface
Formation of the C3 and C5 Convertases

C3 Contains a Latent, Reactive Thioester Group
The Mannose-binding Lectin Resembles C1q

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)

MASPs 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.

The Lectin Pathway and Other Activators of Complement in the Absence of Antibodies
The Complement System is Critical for Innate Immunity and is Triggered by Multiple Ligands

All Roads Lead to Rome

**CLASSICAL PATHWAY**
Antigen:antibody complexes (pathogen surfaces)
C1q, C1r, C1s C4 C2

**MB-LECTIN PATHWAY**
Mannose-binding lectin binds mannose on pathogen surfaces
MBL, MASP-1, MASP-2 C4 C2

**ALTERNATIVE PATHWAY**
Pathogen surfaces
C3 B D

C3 Convertase

C3b

C5 Convertase

C5a Increases Vascular Permeability and is a Potent Chemoattractant

Small complement-cleavage products act on blood vessels to increase vascular permeability and anti-adhesion molecules

Increased permeability allows increased fluid leakage from blood vessels and extravasation of immunoglobulins and complement molecules

Migration of macrophages, polymorphonuclear leukocytes (PMNs), and lymphocytes is increased. Monocyte activity of macrophages and PMNs is also increased

Figure 3.23 from week19.png in Garfield:Science 2001
Summary: Three Major Functions of Complement in Host Defense

1. Opsonization and phagocytosis
   - Binding of C3b to microbe
   - Phagocytosis of microbe

2. Stimulation of inflammatory reactions
   - Binding of C3b to microbe, release of C3a, recruitment of neutrophils
   - Destruction of microbes by leukocytes

3. Complement-mediated lysis
   - Binding of C3b to microbe, activation of late components of complement
   - Complement lysis of microbe
**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 (C1 INH)</td>
<td>184 kD</td>
<td>Plasma protein, conc. &gt; 200 μg/ml</td>
<td>C1r, C1s</td>
<td>Some protease inhibitor, neutralizes C1 and C4a and cleaves them from C1</td>
</tr>
<tr>
<td>Factor I</td>
<td>94 kD dimer</td>
<td>Plasma protein, conc. &gt; 200 μg/ml</td>
<td>C4b, C2b</td>
<td>Some protease destroys C4b and C2b, inactivating B cell opsonization</td>
</tr>
<tr>
<td>Factor H</td>
<td>150 kD</td>
<td>Plasma protein, conc. &gt; 400 μg/ml</td>
<td>C3b</td>
<td>Binds C3b and prevents its complement deposition of C3b</td>
</tr>
<tr>
<td>C4-binding protein (C4BP)</td>
<td>78 kD</td>
<td>Plasma protein, conc. &gt; 200 μg/ml</td>
<td>C4b</td>
<td>Binds C4b and stabilizes C3</td>
</tr>
<tr>
<td>Membrane cofactor protein (MCP, CD46)</td>
<td>45 kD</td>
<td>Leukocytes, epithelial cells, endothelial cells</td>
<td>C3b, C4b</td>
<td>Cofactor for factor I- mediated cleavage of C3b and C4b</td>
</tr>
<tr>
<td>Decay accelerating factor (DAF)</td>
<td>18 kD</td>
<td>Blood cells, epithelial cells, endothelial cells</td>
<td>C3b, C8</td>
<td>Displaces C3b from DAF and inhibits its complement 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.

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**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; Stimulates phagocytosis of immune complexes</td>
<td>Erythrocytes, macrophages, monocytes, polymorphonuclear leukocytes, B cells, FDC</td>
</tr>
<tr>
<td>CR2 (CD21)</td>
<td>C3d, iC3b, C3dg</td>
<td>Part of B-cell co-receptor; Epstein-Barr virus receptor</td>
<td>B cells, FDC</td>
</tr>
<tr>
<td>CR3 (Mac-1) (CD11b/CD18)</td>
<td>iC3b</td>
<td>Stimulates phagocytosis</td>
<td>Macrophages, monocytes, polymorphonuclear leukocytes, FDC</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
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
Complement Deficiencies

<table>
<thead>
<tr>
<th>Complement Components</th>
<th>Effects</th>
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</thead>
<tbody>
<tr>
<td>C1q, C1r, C1s, C2, C4</td>
<td>Markedly increased incidence of autoimmune disease, 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 infection</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 $C_{H50} = 150$ (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.