“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 Isotype</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>
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<tr>
<td></td>
<td>Activation of the classical pathway of complement</td>
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<td></td>
<td>Antibody-dependent cell-mediated cytotoxicity mediated by natural killer cells and macrophages</td>
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<td></td>
<td>Neonatal immunity: transfer of maternal antibody across the placenta and gut</td>
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<tr>
<td></td>
<td>Feedback inhibition of B cell activation</td>
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<tr>
<td>IgM</td>
<td>Activation of the classical pathway of complement</td>
</tr>
<tr>
<td></td>
<td>Antigen receptor of naïve 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>H</sub>**: Heavy chain variable region
- **V<sub>L</sub>**: Light chain variable region
- **C1q binding site**
- **FcγR binding site**
- **Glycosylation site**
Serum Protein Electrophoresis (SPEP): the $\gamma$-Globulin Peak Contains Multiple Ig Isotypes

$\alpha_1$: $\alpha_1$-antitrypsin
$\alpha_2$: haptoglobin
$\beta$: lipoproteins, transferrin, clotting factors, complement
$\gamma$: 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 “" 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>FcγR</th>
<th>Affinity for immunoglobulin</th>
<th>Cell distribution</th>
<th>Function</th>
</tr>
</thead>
<tbody>
<tr>
<td>FcγRI (CD64)</td>
<td>High (Kd = 10^6 M)</td>
<td>Macrophages, neutrophils, eosinophils</td>
<td>Phagocytosis; activation of phagocytes</td>
</tr>
<tr>
<td>FcγRIIA (CD32)</td>
<td>Low (Kd = 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 = 10^7 M)</td>
<td>Leukocytes</td>
<td>Feedback inhibition of B cells</td>
</tr>
<tr>
<td>FcγRIIA (CD16)</td>
<td>Low (Kd = 10^7 M)</td>
<td>Leukocytes</td>
<td>ADCC in NK cells</td>
</tr>
<tr>
<td>FcγRIIB (CD16)</td>
<td>Low (Kd = 10^7 M, GPI-linked protein)</td>
<td>Neutrophils, other cells</td>
<td>Phagocytosis (inefficient)</td>
</tr>
<tr>
<td>FcεRI</td>
<td>High (Kd = 10^6 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:
Phophorylation of Immunoreceptor Tyrosine-based Activation Motifs (ITAMs)

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**
- ITAM: Immunoreceptor tyrosine-based activation motif
- PTK: Protein tyrosine kinase

**Inhibitory FcγR**
- ITIM: Immunoreceptor tyrosine-based inhibitory motif
- SHIP: SH2 Domain-containing Inositol 5' Phosphatase

<table>
<thead>
<tr>
<th>Aktivität</th>
<th>PI3K</th>
<th>Phagocytosis</th>
<th>Secretion</th>
<th>ADCC</th>
</tr>
</thead>
<tbody>
<tr>
<td>+</td>
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<tr>
<td>-</td>
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</tbody>
</table>

**Abbreviations:**
- PI3K: Phosphatidylinositol 3-kinase
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 Fc-RIII on mast cells induces their degranulation
- Local inflammation, increased fluid and protein release, phagocytosis, and blood vessel occlusion

1-2 hr
Absence of the $\gamma$ 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 $\gamma$ chain-deficient NZB/NZW (lupus-prone) mice. Pathological features include mesangial thickening and hypercellularity evolving into end-stage sclerotic and crescentic changes.

Summary: $F_{c\gamma}$ receptors

1. Ig has multiple isotypes with unique functions

2. Receptors for the Fc portion of IgG ($F_{c\gamma}$ 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. $F_{c\gamma}$ receptors mediate a variety of immune functions: phagocytosis, secretion of pro-inflammatory mediators, and ADCC.

4. Unregulated activation of $F_{c\gamma}$ 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
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
C5a Increases Vascular Permeability and is a Potent Chemoattractant

Big MAC Attack
Summary: Three Major Functions of Complement in Host Defense

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

B. Stimulation of inflammatory reactions
- Binding of C3b to microbe, release of C5a, proteolysis of C5a, releasing C5a
- Recruitment and activation of leukocytes by C5a, C3a
- Destruction of microbes by leukocytes

C. Complement-mediated cytosis
- Binding of C3b to microbe, activation of late components of complement
- Formation of the membrane attack complex (MAC)
- Cytolysis 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 (C1INH)</td>
<td>104kD</td>
<td>Plasma protein, conc. 300 mg/dL</td>
<td>C1, C3a</td>
<td>Serine protease inhibitor, binds to C1 and C1s and prevents their activation</td>
</tr>
<tr>
<td>Factor I</td>
<td>67-68 kD dimer of two 34 kD subunits</td>
<td>Plasma protein, conc. 30 mg/dL</td>
<td>C3b, C3a</td>
<td>Serine protease; cleaves C3a and C5a by using factor H, MCP, C4BP, or CRI on cells</td>
</tr>
<tr>
<td>Factor H</td>
<td>150-180 kD and soluble CCP proteins</td>
<td>Plasma protein, conc. 400 mg/dL</td>
<td>C3b</td>
<td>Binds C3b and displaces Bb</td>
</tr>
<tr>
<td>Decay accelerating factor (DAF)</td>
<td>50-70 kD, four CCP proteins</td>
<td>Plasma protein, conc. 300 mg/dL</td>
<td>C3b, C4b</td>
<td>Co-factor for factor I; prevents cleavage of C3b</td>
</tr>
<tr>
<td>C4-binding protein (C4BP)</td>
<td>571 kD, monomeric CCP proteins</td>
<td>Plasma protein, conc. 200 mg/dL</td>
<td>C3b, C4b</td>
<td>Co-factor for factor I; promotes cleavage of C3b and C4b</td>
</tr>
<tr>
<td>Membrane cofactor protein (MCP, CD46)</td>
<td>50-70 kD, four CCP proteins</td>
<td>Leukocytes, endothelial cells, epithelial cells</td>
<td>C3b, C4b</td>
<td>Co-factor for factor I; promotes cleavage of C3b and C4b</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, Stimulates phagocytosis, Erythrocyte transport of immune complexes</td>
<td>Erythrocytes, macrophages, monocytes, polymorphonuclear leukocytes, B cells, FDC</td>
</tr>
<tr>
<td>CR2 (CD21)</td>
<td>C3d, iC3b, C3dg, Epstein-Barr virus</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>C5b, 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>

**β₂ (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

Inherited Complement Deficiencies

C1q, C1r, C1s, C2, C4 Markedly increased incidence of autoimmune disease
Moderate increased incidence of pyogenic infections

H, I, C3 Increased incidence of pyogenic infections. Moderately increased incidence of autoimmune disease

Properdin, Factor D, Properdin, Factor D, C6, C7, C8, C9 Increased incidence of Neisseria infection

CR3, CR4 Increased incidence of pyogenic infection

C1INH Hereditary angioedema

DAF, CD59 Paroxysmal nocturnal hemoglobinuria
How is Complement Activity Measured?

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

Results:
Serum Dilutions: 1/50 1/100 1/150 1/200
Hemolysis: 100% 100% 50% 20%

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.