Biology of Fcγ Receptors

Selected Functions of Ig Isotypes

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

Selected Functions of Fc Receptors
Fcγ Receptor Signaling: ITAM Phosphorylation

FcγRIIIA

γ subunit

Src family TK

Opsonized Bacterium

Fcγ Receptor Signaling: Syk Activation

FcγRIIA ligand-binding domain

PTPase

Syk

TK substrates
Some Important 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</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>B lymphocytes</td>
<td>Feedback inhibition of B cells</td>
</tr>
<tr>
<td>FcγRIIA (CD16)</td>
<td>Low (Kd &gt; 10^{-6} M)</td>
<td>NK cells</td>
<td>Antibody-dependent cell-mediated cytotoxicity</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^{-15} 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 ITAM-associated activating receptors, except FcγRIIB, which is an ITIM-associated inhibitory Fc receptor.

Phosphoinositide-metabolizing Enzymes Important in Immunity
Three Important Phospholipid-modifying Enzymes Worth Knowing: PLC-γ

Phosphatidylinositol-4,5-bisphosphate (PIP₂)

Diacylglycerol

Inositol-3,4,5-trisphosphate (IP₃)

Phosphatidylinositol 3-kinase (PI 3-kinase)

Phosphatidylinositol-3,4,5-trisphosphate (PIP₃)
SHIP, an Inositol 5’ Phosphatase

FcγRIIB: an Inhibitory Fcγ Receptor
PTK: Protein tyrosine kinase
SHIP: SH2 Domain-containing Inositol 5' Phosphatase

Activating FcγR

Inhibitory FcγR

Phagocytosis

Clustering of the BCR by AntigenInitiates Signaling
Positive and Negative Regulation of the BCR

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. Do not memorize this list.
Diseases in blue indicate where therapeutic IVIg plays a major, established role.
The “Dark Side” of Fc Receptors: Immune Complex-mediated Injury

Hypersensitivity Diseases

<table>
<thead>
<tr>
<th>Type of hypersensitivity</th>
<th>Pathologic immune mechanisms</th>
<th>Mechanism of tissue injury and disease</th>
</tr>
</thead>
<tbody>
<tr>
<td>Immediate hypersensitivity: Type I</td>
<td>IgE antibody</td>
<td>Mast cells and their mediators (vasoactive amines, lipid mediators, cytokines)</td>
</tr>
<tr>
<td>Antibody mediated: Type II</td>
<td>IgM, IgG antibodies against cell surface or extracellular matrix antigens</td>
<td>Opsonization and phagocytosis of cells</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Complement- and Fc receptor-mediated recruitment and activation of leukocytes (neutrophils, macrophages)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Abnormalities in cellular functions, e.g., hormone receptor signaling</td>
</tr>
<tr>
<td>Immune complex mediated: Type III</td>
<td>Immune complexes of circulating antigen and IgM or IgG antibodies</td>
<td>Complement- and Fc receptor-mediated recruitment and activation of leukocytes</td>
</tr>
<tr>
<td>T cell mediated: Type IV</td>
<td>1. CD4+ T cells (delayed-type hypersensitivity) 2. CD8+ CTLs (T cell-mediated cytolyis)</td>
<td>1. Macrophage activation, cytokine-mediated inflammation 2. Direct target cell killing, cytokine-mediated inflammation</td>
</tr>
</tbody>
</table>
The Arthus Reaction: A Model of Type III Hypersensitivity

Requirement of Activating FcRs in Immune Complex-mediated Glomerulonephritis

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.

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 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 Protein) is a lectin with collagen-like features
- First binds to mannos on bacterial cell walls. It then binds serine proteases MASP-1, -2 or -3 (Mannose binding lectin Associated Serine Protease)
- These 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 test for CRP is frequently ordered in clinical situations where inflammation is suspected

The Mannose-binding Lectin Resembles C1q
The Complement System is Critical for Innate Immunity and is Triggered by Multiple Ligands

C3 Convertase

C5 Convertase

C5a Increases Vascular Permeability and is a Potent Chemoattractant
Summary: Three Major Functions of Complement in Host Defense
**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 kDa</td>
<td>Plasma protein, conc. 200 μg/ml</td>
<td>C1a, C1s</td>
<td>Some protease inhibitors, inhibits C1 and C4 transferase activation</td>
</tr>
<tr>
<td>Factor I</td>
<td>86 kDa dimer of 40- and 46 kDa subunits</td>
<td>Plasma protein, conc. 35 μg/ml</td>
<td>C4b, C2b</td>
<td>Some protease inhibitors, inhibits C1 and C4 transferase activation</td>
</tr>
<tr>
<td>Factor H</td>
<td>134 kDa</td>
<td>Plasma protein, conc. 400 μg/ml</td>
<td>C3b</td>
<td>Binds C3b and prevents C5b-9 formation on ligands</td>
</tr>
<tr>
<td>C4-binding protein (C4BP)</td>
<td>570 kDa</td>
<td>Plasma protein, conc. 200 μg/ml</td>
<td>C4b</td>
<td>Binds C4b and prevents C5b-9 formation on ligands</td>
</tr>
<tr>
<td>Membrane cofactor for protein (MCP, CD46)</td>
<td>47/51 kDa</td>
<td>Leukocytes, epithelial cells, endothelial cells</td>
<td>C2b, C4b, C5b, C9b</td>
<td>Cofactor for factor I-mediated cleavage of C2b, C4b, C5b, C9b</td>
</tr>
<tr>
<td>CD59</td>
<td>18 kDa</td>
<td>Blood cells, epithelial cells, endothelial cells</td>
<td>C5, C7</td>
<td>Displaces C8 from C9b and fills in C9a (inhibition of C9 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, erythrocyte transport of immune complexes</td>
<td>Erythrocytes, macrophages, monocytes, polymorphonuclear leukocytes, B cells, FDC</td>
</tr>
<tr>
<td>CR2 (CD21)</td>
<td>C3d, C3dg, C3d, Epstein-Barr virus</td>
<td>Part of B-cell co-receptor complex, 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

Functions of Complement:
Immune Regulation
Positive and Negative Regulation of the BCR

PI 3-kinase: Generates PIP₃
CR2: Complement Receptor 2; binds C3d

The “Paradox” of Complement Deficiency and Autoimmunity

Despite a positive role for CR2 in setting the “gain” of B cell responsiveness, it has been suggested that deficiencies in CR2 and C3dg deposition pre-dispose to autoimmunity because the amplification afforded by CR2 occurs inappropriately during tolerance induction.
Models for How Complement Deficiency Predisposes to Autoimmunity


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

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

<table>
<thead>
<tr>
<th>Protein(s)</th>
<th>Description</th>
</tr>
</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>

Total Hemolytic Complement Measurement

*Method:* Mix RBC, Anti-RBC, 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<sub>50</sub> = 150 (Reciprocal of 1/150).

CH<sub>50</sub> tends to fall in some autoimmune diseases due to complement consumption.
Summary

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 phagocytosis and, when overactivated by excessive immune complex deposition, tissue injury.

4. Complement is an ancient system of host defense that has well-defined functions in host defense: it opsonizes, stimulates inflammation, and mediates lysis of pathogens by MAC.

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

6. 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.