"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 Affector Functions</th>
</tr>
</thead>
<tbody>
<tr>
<td>IgG</td>
<td>Prevention of angiogenesis for pathogen influx into tissue sites</td>
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<tr>
<td></td>
<td>Dysregulation of the renal electrolyte balance</td>
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<td></td>
<td>Inflammation of the respiratory tissue, leading to chronic airway pathology</td>
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<td></td>
<td>Neutrophil migration to the parotid and oral mucosa</td>
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<td></td>
<td>Neutrophil stimulation of the cell signaling</td>
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<tr>
<td>IgM</td>
<td>Activation of the classical pathway of B cell recognition of non-self</td>
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<td></td>
<td>IgA-mediated apoptosis of B cells</td>
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<td></td>
<td>Antibody-dependent cell-mediated cytotoxicity involving complement</td>
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<td></td>
<td>Neutrophil degranulation-mediated respiratory cell lysis</td>
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</tbody>
</table>

Functional Sites on the IgG Molecule

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 “αα” 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
Some Important Receptors for IgG (Fc 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 (K&lt;sub&gt;d&lt;/sub&gt; = 10&lt;sup&gt;-4&lt;/sup&gt; M)</td>
<td>Monocytes, macrophages, neutrophils</td>
<td>Phagocytosis, opsonization</td>
</tr>
<tr>
<td>FcγRIIA (CD32)</td>
<td>Low (K&lt;sub&gt;d&lt;/sub&gt; = 10&lt;sup&gt;-7&lt;/sup&gt; M)</td>
<td>Monocytes, macrophages, neutrophils</td>
<td>Phagocytosis, opsonization (macrophags)</td>
</tr>
<tr>
<td>FcγRIIB (CD32)</td>
<td>Low (K&lt;sub&gt;d&lt;/sub&gt; = 10&lt;sup&gt;-7&lt;/sup&gt; M)</td>
<td>Leukocytes</td>
<td>FcγRIIB-mediated regulation of cells</td>
</tr>
<tr>
<td>FcγRI (CD89)</td>
<td>Low (K&lt;sub&gt;d&lt;/sub&gt; = 10&lt;sup&gt;-7&lt;/sup&gt; M)</td>
<td>Leukocytes</td>
<td>ADCC (in T cells)</td>
</tr>
<tr>
<td>FcγRIIB (CD16)</td>
<td>Low (K&lt;sub&gt;d&lt;/sub&gt; = 10&lt;sup&gt;-7&lt;/sup&gt; M)</td>
<td>Monocytes, neutrophils</td>
<td>Neutrophil chemotaxis, chemokinesis</td>
</tr>
<tr>
<td>FcγRIIB</td>
<td>High (K&lt;sub&gt;d&lt;/sub&gt; = 10&lt;sup&gt;-6&lt;/sup&gt; M)</td>
<td>Lymphatic endothelial cells</td>
<td>Neutrophil chemotaxis, chemokinesis</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.

Selected Functions of Fc Receptors

How do Fc, Receptors Perform Effector Functions?

Phosphorylated ITAMs Recruit Another Tyrosine Kinase, Syk, which Phosphorylates Other Substrates

Two Enzymes Worth Knowing
Phosphatidylinositol-4,5-bisphosphate (PIP$_2$)

Phosphatidylinositol-3,4,5-trisphosphate (PIP$_3$)

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$_γ$RIIB: an Inhibitory Fc$_γ$ Receptor

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

Therapeutic Uses of Intravenous Immunoglobulin (IVIg)*

*Activating: Fc$_γ$RI, Fc$_γ$RIIA, Fc$_γ$RIII

Inhibitory: Fc$_γ$RIIB

*Other than replacement therapy for hypogammaglobulinemia. Do not memorize this list. Blue denotes diseases in which IVIg plays a major, established therapeutic role.
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.

Summary: Fc, 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

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)
- MASP1s 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.

C5a Increases Vascular Permeability and is a Potent Chemoattractant

All Roads Lead to Rome

C3 Convertase

C5 Convertase

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

- Activation
- Membrane attack complex
- Mediation of inflammatory reactions

Complement Regulatory Proteins*

*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>Names</th>
<th>CD</th>
<th>Ligands</th>
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<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

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>Deficiency</th>
<th>Description</th>
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<tbody>
<tr>
<td>C1q, C1r, C1s, C2, C4</td>
<td>Markedly increased incidence of autoimmune disease</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 Neisseria infection</td>
</tr>
</tbody>
</table>

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.
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.