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

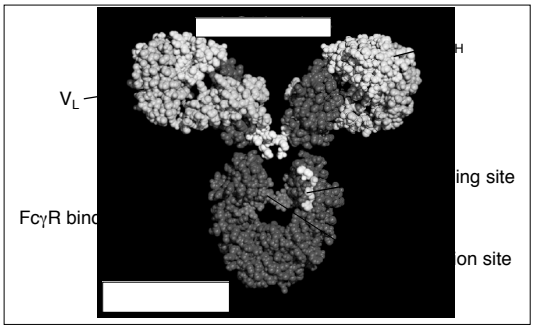
--Albert Szent-György
Nobel prize in Physiology or Medicine, 1937

# The Biology of $Fc_\gamma$ Receptors and Complement

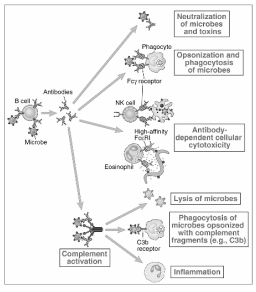
## Selected Functions of Ig Isotypes

| Antibody isotope | Isotype-specific effector functions |
|---|---|
| IgG | Opsonization of antigens for phagocytosis by macrophages and neutrophils<br>Activation of the classical pathway of complement<br>Antibody-dependent cell-mediated cytotoxicity mediated by natural killer cells and macrophages<br>Neonatal immunity: transfer of maternal antibody across the placenta and gut<br>Feedback inhibition of B cell activation |
| IgM | Activation of the classical pathway of complement<br>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<br>Mast cell degranulation (immediate hypersensitivity reactions) |

## Functional Sites on the IgG Molecule




## Selected Functions of Fc Receptors



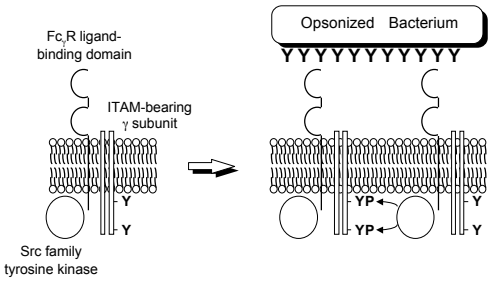

## Some Important Receptors for IgG ( $Fc_\gamma$ Receptors)*

| FcR | Affinity for immunoglobulin | Cell distribution | Function |
|---|---|---|---|
| FcγRI (CD64) | High ($K_d$ ~ $10^{-9}$ M); binds IgG1 and IgG3, can bind monomeric IgG | Macrophages, neutrophils; also eosinophils | Phagocytosis; activation of phagocytes |
| FcγRIIA (CD32) | Low ($K_d$ > $10^{-7}$ M) | Macrophages, neutrophils; eosinophils, platelets | Phagocytosis; cell activation (inefficient) |
| FcγRIIB (CD32) | Low ($K_d$ > $10^{-7}$ M) | Leukocytes | Feedback inhibition of B cells |
| FcγRIIIA (CD16) | Low ($K_d$ > $10^{-6}$ M) | Leukocytes | ADCC in NK cells |
| FcγRIIIB (CD16) | Low ($K_d$ > $10^{-6}$ M); GPI-linked protein | Neutrophils, other cells | Phagocytosis (inefficient) |
| FcεRI | High ($K_d$ > $10^{-10}$ M); binds monomeric IgE | Mast cells, basophils, eosinophils | Cell activation (degranulation) |

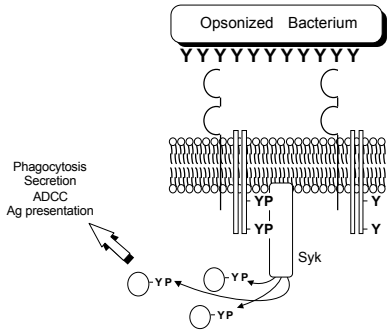
*Do not memorize this list but do learn functions of specific Fc receptors. Of these, all are "activating" receptors, except FcγRIIB, which is an "inhibitory" Fc receptor.

## How do $Fc_\gamma$ Receptors Perform Effector Functions?

## $Fc_\gamma$ Receptor Signaling: Phophorylation of Immunoreceptor Tyrosine-based Activation Motifs (ITAMs)

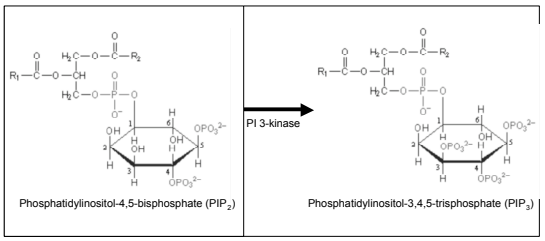



## Phosphorylated ITAMs Recruit Another Tyrosine Kinase, Syk, which Phosphorylates Other Substrates



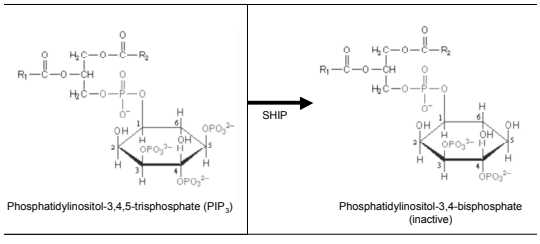

## Two Enzymes Worth Knowing

## Phosphatidylinositol 3-kinase (PI 3-kinase)



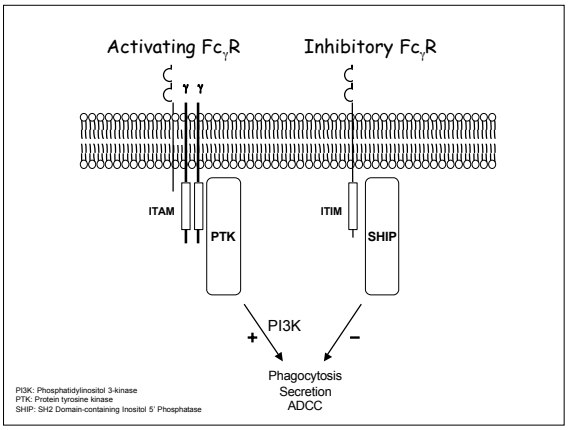

Lipid products of PI 3-kinase (i.e., $PIP_3$) bind and activate other proteins (e.g., Bruton's tyrosine kinase)

## SHIP, an Inositol 5' Phosphatase




SHIP counteracts positive signals generated by PI 3-kinase (by catalyzing the hydrolysis of its lipid product, $PIP_3$)

## $Fc_\gamma RIIB$: an Inhibitory $Fc_\gamma$ Receptor




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

*Activating: $Fc_\gamma RI$, $Fc_\gamma RIIA$, $Fc_\gamma RIII$
Inhibitory: $Fc_\gamma RIIB$

### 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

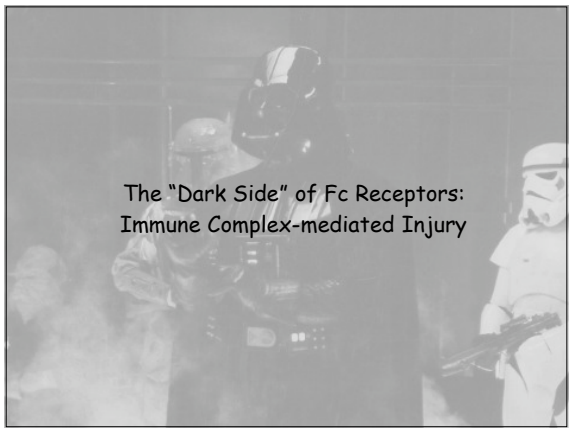
**Neurological diseases**
Guillain-Barré syndrome
Chronic inflammatory demyelinating polyneuropathy
Myasthenia gravis
Multifocal neuropathy

Polymyositis
Dermatomyositis

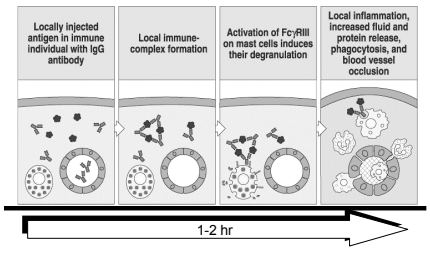
**Vasculitis**
Kawasaki disease
ANCA-positive systemic vasculitis
Antiphospholipid syndrome
Recurrent spontaneous abortions
Rheumatoid arthritis and Felty's syndrome
Juvenile Rheumatoid Arthritis
SLE

Thyroid ophthalmopathy
Birdshot retinochoroidopathy
Graft versus host disease
Multiple sclerosis
Insulin-dependent Diabetes mellitus
Steroid-dependent asthma
Steroid-dependent atopic dermatitis
Crohn's disease

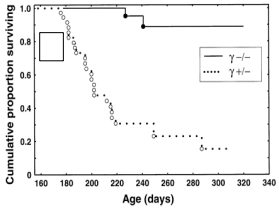
*Other than replacement therapy for hypogammaglobulinemia. Do not memorize this list.
Blue denotes diseases in which IVIg plays a major, established therapeutic role




### The Arthus Reaction: A Model of Type III Hypersensitivity



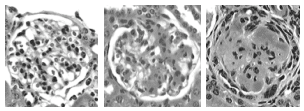
## Requirement of Activating $Fc_\gamma Rs$ in Immune Complex-mediated Glomerulonephritis




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.

From: Clynes et al., *Science* 279:1052, 1998.

## Requirement of Activating $Fc_\gamma Rs$ in Immune Complex-mediated Glomerulonephritis



| Strain: | C57Bl/6 | NZB/NZW | NZB/NZW |
|---|---|---|---|
| γ chain: | -/- | -/- | +/- |

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.

From: Clynes et al., *Science* 279:1052, 1998.

## Summary: $Fc_\gamma$ receptors

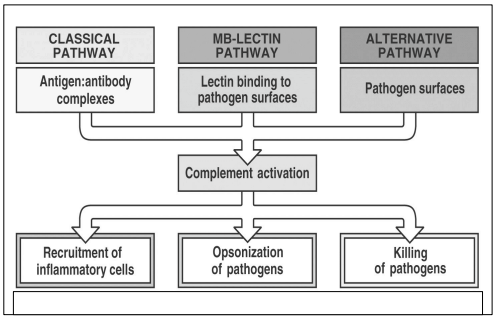
1. Ig has multiple isotypes with unique functions
2. Receptors for the Fc portion of IgG (Fcγ receptors) come in two basic types: ITAM-containing activating receptors that bind PTKs and an ITIM-containing inhibitory receptor that antagonizes the PI 3-kinase pathway. Their relative expression determines 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

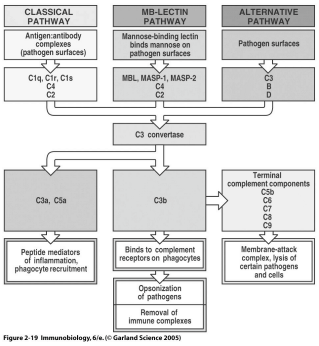
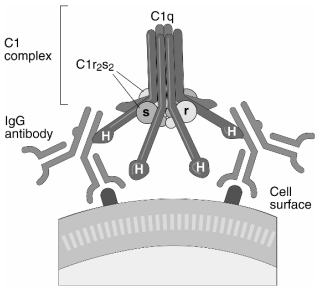
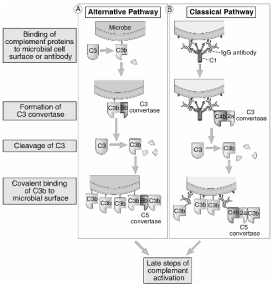



Figure 2-19 Immunobiology, 6/e. (© Garland Science 2005)

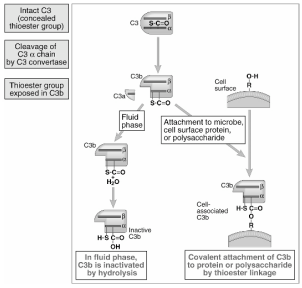
## C1q, the Initiator of the Classical Pathway of Complement Activation




## Formation of the C3 and C5 Convertases




## C3 Contains a Latent, Reactive Thioester Group




## The Mannose-binding Lectin Resembles C1q

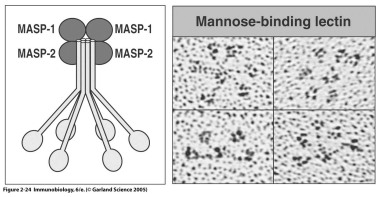
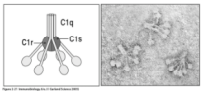


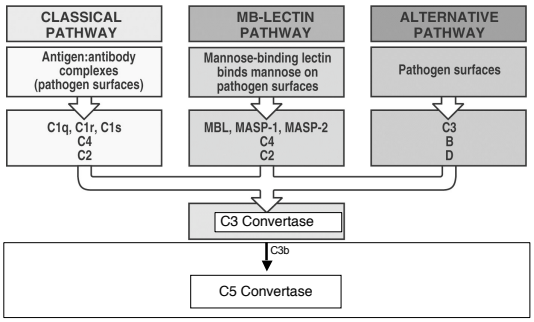

Figure 2-24 Immunobiology, 6/e. (© Garland Science 2005)




## 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)
- 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.

## All Roads Lead to Rome




## C5a Increases Vascular Permeability and is a Potent Chemoattractant

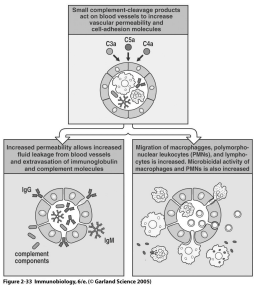
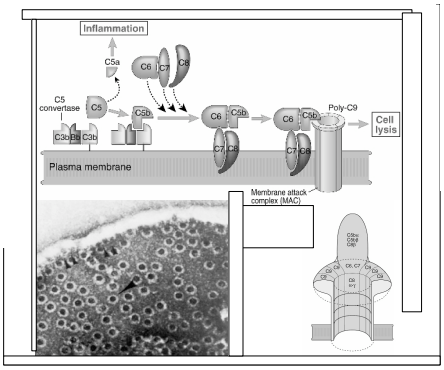
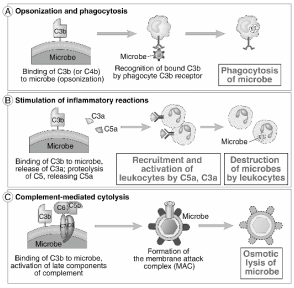



Figure 2-33 Immunobiology, 6/e. (© Garland Science 2005)

## Big MAC Attack




## Summary: Three Major Functions of Complement in Host Defense




## Complement Regulatory Proteins*

| | Receptor | Structure | Distribution | Interacts with | Function |
|---|---|---|---|---|---|
| Fluid-phase | C1 inhibitor (C1 INH) | 104 kD | Plasma protein; conc. 200 μg/mL | C1r, C1s | Serine protease inhibitor; binds to C1r and C1s and dissociates them from C1q |
| | Factor I | 88-kD dimer of 50- and 38-kD subunits | Plasma protein; conc. 35 μg/mL | C4b, C3b | Serine protease; cleaves C3b and C4b by using factor H, MCP, C4BP, or CR1 as cofactors |
| | Factor H | 150 kD; multiple CCPRs | Plasma protein; conc. 480 μg/mL | C3b | Binds C3b and displaces Bb<br>Cofactor for factor I–mediated cleavage of C3b |
| | C4-binding protein (C4BP) | 570 kD; multiple CCPRs | Plasma protein; conc. 300 μg/mL | C4b | Binds C4b and displaces C2<br>Cofactor for factor I–mediated cleavage of C4b |
| Membrane-bound | Membrane cofactor for protein (MCP, CD46) | 45-70 kD; four CCPRs | Leukocytes, epithelial cells, endothelial cells | C3b, C4b | Cofactor for factor I–mediated cleavage of C3b and C4b |
| | Decay-accelerating factor (DAF) | 70 kD; GPI linked; four CCPRs | Blood cells, endothelial cells, epithelial cells | C4b2b, C3b6b | Displaces C2b from C4b and Bb from C3b (dissociation of C3 convertases) |
| | CD59 | 18 kD; GPI linked | Blood cells, endothelial cells, epithelial cells | C7, C8 | Blocks C9 binding and prevents formation of the MAC |

*Abbreviations:* CCPR, complement control protein repeat; conc., concentration; GPI, glycosphosphatidylinositol; MAC, membrane attack complex.

*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

| Receptor | Specificity | Functions | Cell types |
|---|---|---|---|
| CR1 (CD35) | C3b, C4b iC3b | Promotes C3b and C4b decay<br>Stimulates phagocytosis<br>Erythrocyte transport of immune complexes | Erythrocytes, macrophages, monocytes, polymorphonuclear leukocytes, B cells, FDC |
| CR2 (CD21) | C3d, iC3b, C3dg<br>Epstein–Barr virus | Part of B-cell co-receptor<br>Epstein–Barr virus receptor | B cells, FDC |
| CR3 (Mac-1) (CD11b/ CD18) | iC3b | Stimulates phagocytosis | Macrophages, monocytes, polymorphonuclear leukocytes, FDC |
| C5a receptor | C5a | Binding of C5a activates G protein | Endothelial cells, mast cells, phagocytes |

## $\beta_2$ (Leukocyte) Integrins

| Names | CD | Ligands |
|---|---|---|
| LFA -1 | CD11a/CD18 | ICAMs |
| CR3 (Mac-1) | CD11b/CD18 | iC3b, ICAMs, many others |
| CR4 (p150, 95) | CD11c/CD18 | C3b, iC3b |

## 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

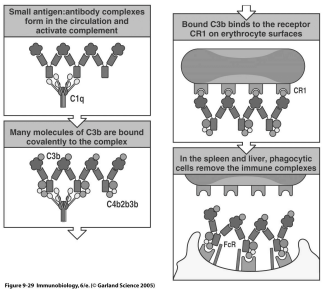


Figure 9-29 Immunobiology, 6/e. (© Garland Science 2005)

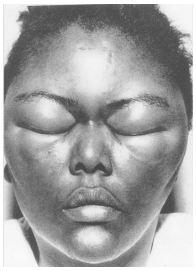
## Functions of Complement: Disposal of Apoptotic Debris

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 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 develp 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

| | |
|---|---|
| C1q, C1r, C1s, C2, C4 | Markedly increased incidence of autoimmune disease<br>Moderate increased incidence of pyogenic infections |
| H, I, C3 | Increased incidence of pyogenic infections. Moderately increased incidence of autoimmune disease |
| 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.