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

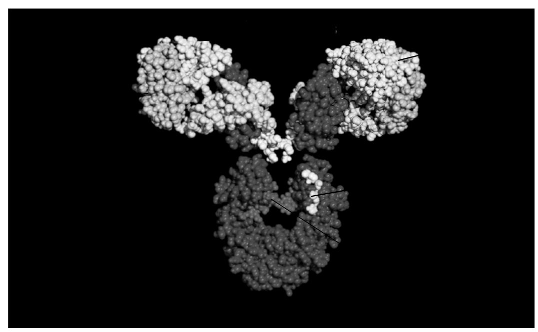
--Albert Szent-György
Nobel prize in Physiology or Medicine, 1937

The Biology of Fc_{γ} Receptors and Complement

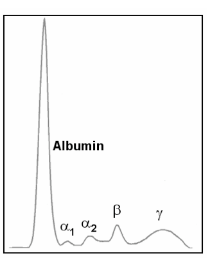
Selected Functions of Ig Isotypes

Antibody isotope	Isotype-specific effector functions
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



Serum Protein Electophoresis (SPEP): the γ-Globulin Peak Contains Multiple Ig Isotypes

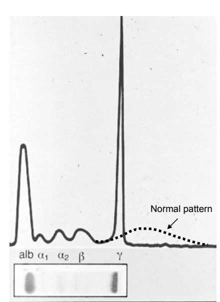


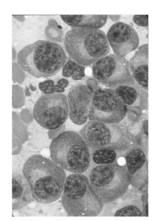
α_1: α_1-antitrypsin
α_2: haptoglobin
β: lipoproteins, transferrin, clotting factors, complement
γ: IgG, IgA, IgM, IgD, IgE

Normal serum total protein: 5.5-9 g/dL
Normal albumin: 3.5-5.5 g/dL

Note that the "$\gamma\alpha\mu\mu\alpha$" in "gammaglobulin" does not refer to the isotype of the antibody (e.g., IgG), but the migration pattern of proteins on SPEP.

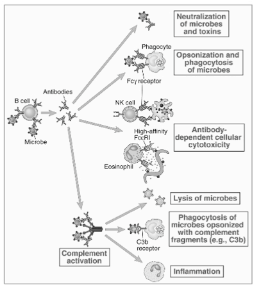
A Monoclonal "Spike" in the SPEP is Seen in Multiple Myeloma, a Plasma Cell Dyscrasia





Bone marrow biopsy from a patient with multiple myeloma

Selected Functions of Fc Receptors



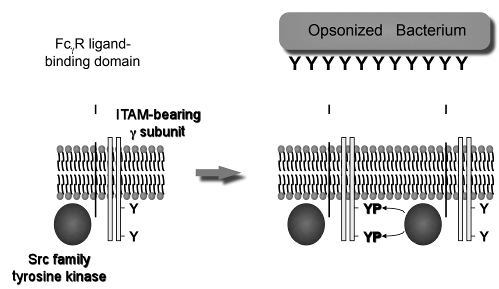
Some Important Receptors for IgG (Fc_γ Receptors)*

FcR	Affinity for immunoglobulin	Cell distribution	Function
FcγRI (CD64)	High ($K_d \sim 10^{-9}$ 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)

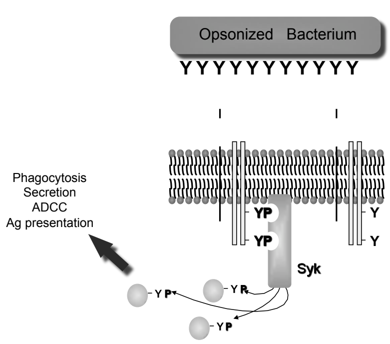
*Do not memorize this list but do learn functions of specific Fc receptors. Of these, all are "activating" receptors, except FcγRIIB, which is an "inhibitory" Fc receptor.

How do Fc_γ Receptors Perform Effector Functions?

Fc_γ Receptor Signaling: Phophorylation of Immunoreceptor Tyrosine-based Activation Motifs (ITAMs)

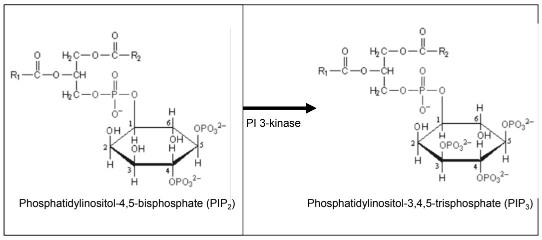


Phosphorylated ITAMs Recruit Another Tyrosine Kinase, Syk, which Phosphorylates Other Substrates



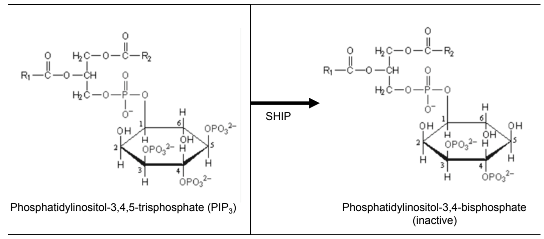
Two Enzymes Worth Knowing

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)

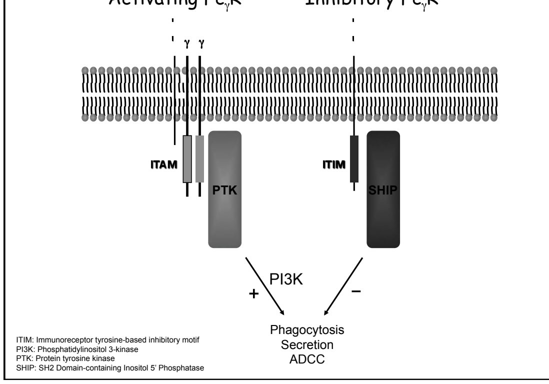
SHIP, an Inositol 5′ Phosphatase



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

Fc_{γ}RIIB: an Inhibitory Fc_{γ} Receptor

Activating $Fc_{\gamma}R$ Inhibitory $Fc_{\gamma}R$



Hypothesis: The balance of activating* and inhibitory Fc_{γ} receptors determines the outcome of IgG-initiated events in health and disease

*Activating: Fc_{γ}RI, Fc_{γ}RIIA, Fc_{γ}RIII
Inhibitory: Fc_{γ}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

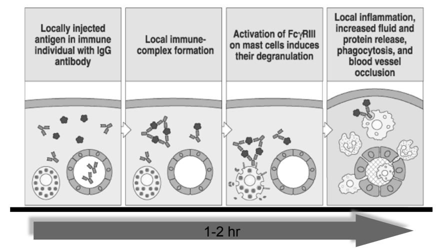
Vasculitis
Kawasaki disease
ANCA-positive systemic vasculitis
Antiphospholipid syndrome
Recurrent spontaneous abortions
Rheumatoid arthritis and Felty's syndrome
Juvenile Rheumatoid Arthritis
SLE

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

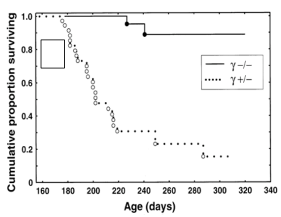
*Other than replacement therapy for hypogammaglobulinemia. Do not memorize this list.
Blue denotes diseases in which IVIg plays a major, established therapeutic role

The "Dark Side" of Fc Receptors: Immune Complex-mediated Injury

The Arthus Reaction: A Model of Type III Hypersensitivity



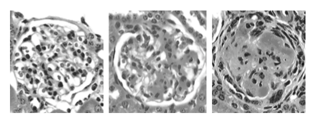
Requirement of Activating Fc_γRs in Immune Complex-mediated Glomerulonephritis



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_γRs 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_γ receptors

1. Ig has multiple isotypes with unique functions

3. 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.

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

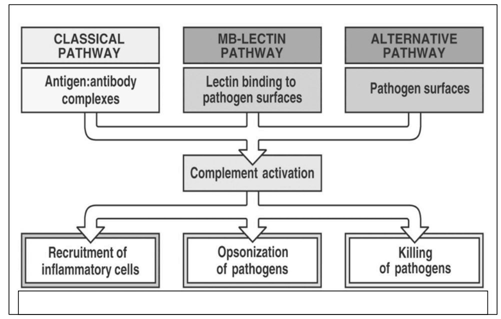
6. Unregulated activation of Fcγ receptors can lead to immune complex disease.

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

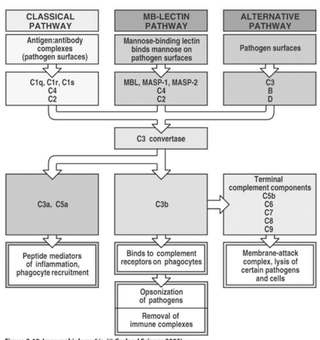
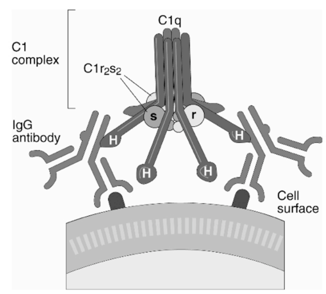
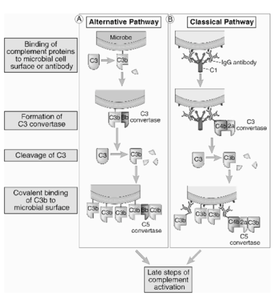


Figure 2-19 Immunobiology, 6/e. (© Garland Science 2005)

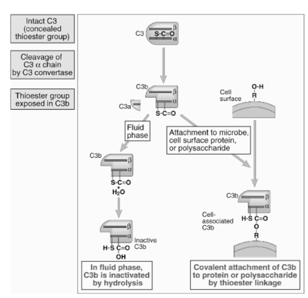
C1q, the Initiator of the Classical Pathway of Complement Activation



Formation of the C3 and C5 Convertases



C3 Contains a Latent, Reactive Thioester Group



The Classical Pathway of Complement Activation

http://www.brown.edu/Courses/Bio_160/Projects1999/ies/how.html

The Mannose-binding Lectin Resembles C1q

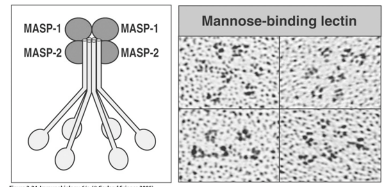
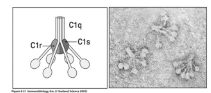


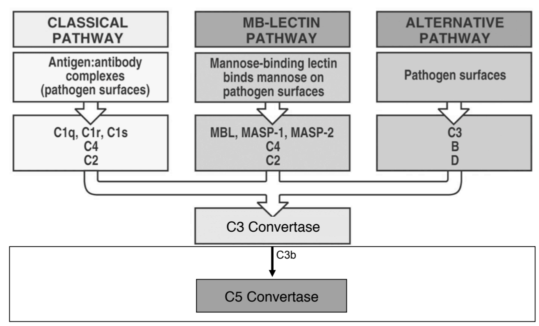
Figure 2-24 Immunobiology, 6/e. (© Garland Science 2005)



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

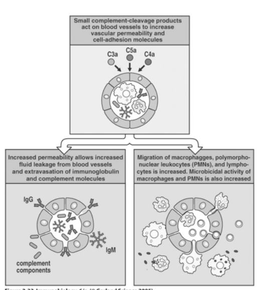
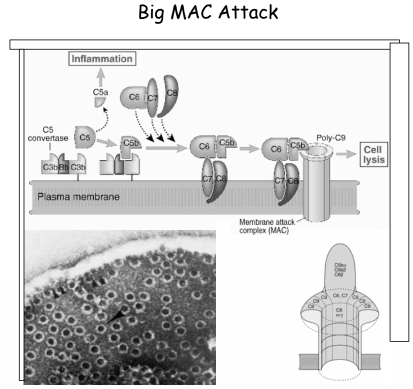
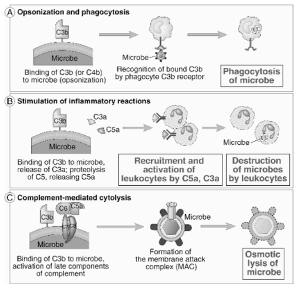


Figure 2-33 Immunobiology, 6/e. (© Garland Science 2005)

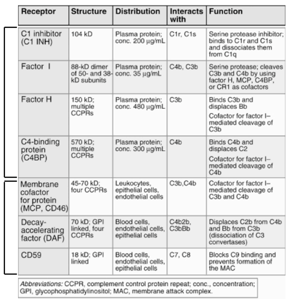
Big MAC Attack



Summary: Three Major Functions of Complement in Host Defense



Complement Regulatory Proteins*

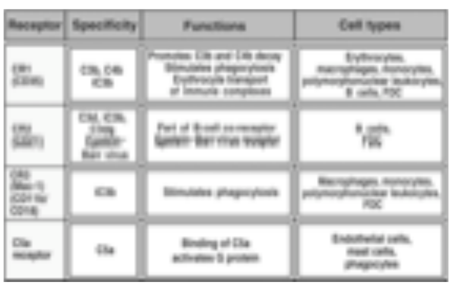


	Receptor	Structure	Distribution	Interacts with	Function
Fluid-phase	C1 inhibitor (C1 INH)	104 kD	Plasma protein; conc. 200 µg/mL	C1r, C1s	Serine protease inhibitor; binds to C1r and C1s and dissociates them from C1q
	Factor I	88-kD dimer of 50- and 38-kD subunits	Plasma protein; conc. 35 µg/mL	C4b, C3b	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 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 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, C3bBb	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, glycophosphatidylinositol; 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
[illegible]	[illegible]	[illegible]	[illegible]
[illegible]	[illegible]	[illegible]	[illegible]
[illegible]	[illegible]	[illegible]	[illegible]
[illegible]	[illegible]	[illegible]	[illegible]

β_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

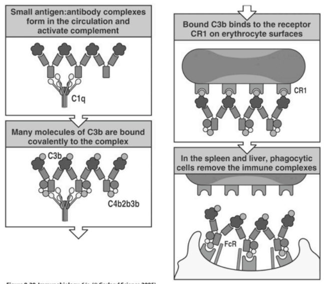


Figure 9-29 Immunobiology, 6/e. (© Garland Science 2005)

Functions of Complement: Disposal of Apoptotic Debris

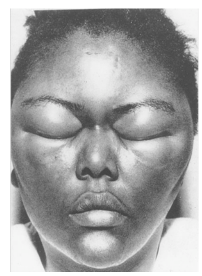
C1q helps removal of apoptotic cell debris (antibody not required)

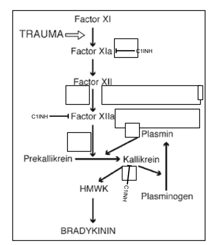
Potential immune consequences of C1q deficiency:

(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

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 autoimmun
Properdin, Factor D, C6, C7, C8, C9	Increased incidence of *Neisseria* infection
CR3, CR4	Increased incidence of pyogenic
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.