COMPLEMENT

Date: 4/11/11
Reading Assignment: **Janeway's Immunobiology,** 7th Edition, pp. 54-55, 61-82, 406-409, 514-515.
Figures: (Unless otherwise noted) **Janeway's Immunobiology,** 7th Edition, Murphy *et al.*, Garland Publishing.

KEY CONCEPTS AND LEARNING OBJECTIVES

You will be able to describe the mechanism and consequences of the activation of the complement system.

To attain the goals for these lectures you will be able to:

a. List the components of the complement system.
b. Describe the three activation pathways for complement.
c. Explain the consequences of complement activation.
d. Describe the consequence of complement deficiency.

CONTENT SUMMARY

Introduction

The complement system is a group of more than 30 plasma and membrane proteins that play a critical role in host defense. When activated, complement components interact in a highly regulated fashion to generate products that:

Recruit inflammatory cells (promoting inflammation).

Opsonize microbial pathogens and immune complexes (facilitating antigen clearance).

Kill microbial pathogens (via a lytic mechanism known as the membrane attack complex).

Generate an inflammatory response.

Complement activation takes place on antigenic surfaces. However, the activation of complement generates several soluble fragments that have important biologic activity.

There are three distinct pathways of activation of complement: the Classical, the MB-lectin, and the Alternative Pathways. See **Figure 1**.

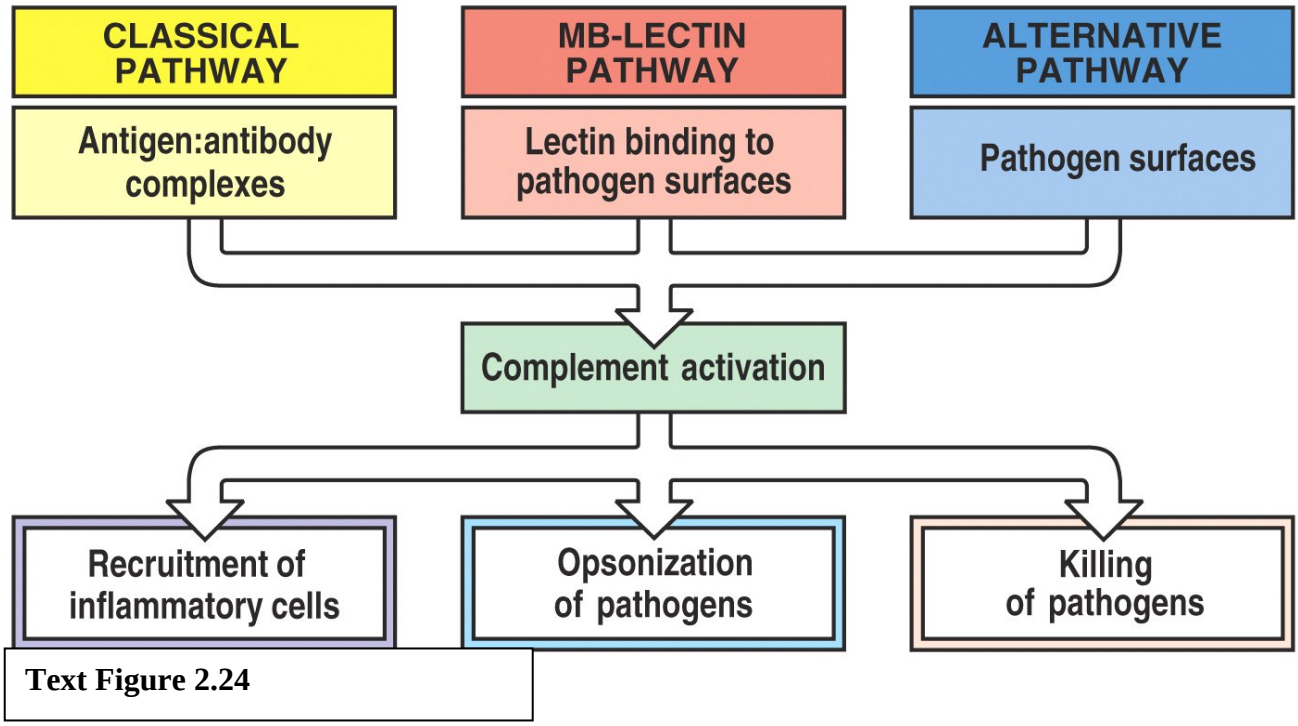


Figure 1. The three pathways of complement activation.

Nomenclature

The components of the classical pathway are designated by the letter C followed by a simple number designation, e.g. C3.

Many complement components are proteases that become active following proteolytic cleavage.

When the components are cleaved during activation, the resulting fragments are given lower case letter designations, such as C3a and C3b.

Components of the alternative pathway are named by capital letters, such as factor B and factor D.

For the MB-lectin pathway, components are designated by acronyms, such as MASP-1 (**M**annan binding lectin-**A**ssociated **S**erine **P**rotease-1)

The lower case "i" is added to indicate that a component is inactive, e.g. iC3b.

Activation of Complement

Activation of the **CLASSICAL PATHWAY:**

Classical pathway activation is initiated after immune complex formation.

Complement component C1 recognizes the antigen-antibody complex.

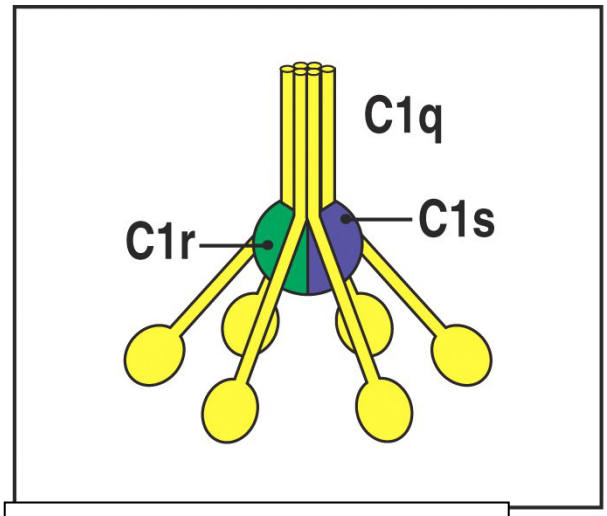
The binding of antibody to antigen induces a conformational change in the antibody constant region. This exposes a site on the Fc portion that can be bound by the first complement component of the classical pathway, C1.

C1 is a macromolecule that consists of C1q (comprised of 6 globular heads and extended tails) in complex with C1r and C1s (the C1qrs complex). See **Figure 2**.

Activation of the C1qrs complex occurs when at least two of the C1q globular heads are simultaneously bound to antibody. See **Figure 2**.

For this to occur, two Fc portions need to be in within close molecular proximity of each other on the antigenic surface.

In contrast to IgG, the pentameric nature of IgM allows a single molecule of antigen bound IgM to activate C1.



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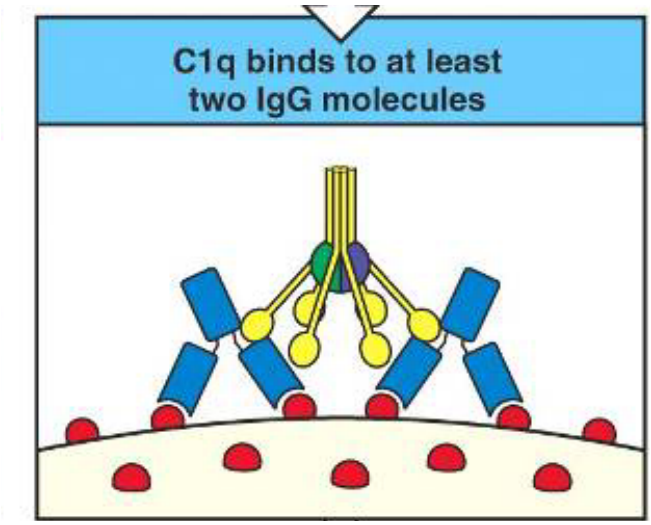

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Figure 2. Complement component C1.

Once C1q is bound to antibody, C1r undergoes a conformational change and becomes enzymatically active.

C1r then cleaves C1s, which after cleavage is enzymatically active as well.

Activation of the **MB-LECTIN PATHWAY:**

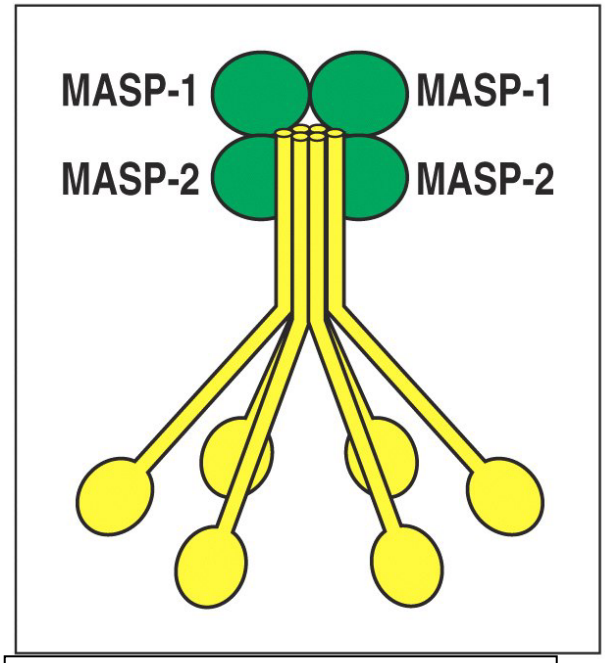
Activation of the mannan binding lectin pathway is similar to the classical pathway.

Except that the MB-lectin pathway is initiated by a protein, Mannan Binding Lectin (MBL), which is homologous to C1q.

MBL binds to mannose and certain other complex carbohydrates that are found on the surface of many microbial pathogens. See **Figure 3**.

MBL is physically associated with two serine proteases, MASP-1 and MASP-2 (mannan binding lectin-associated serine protease-1) that are similar to C1r and C1s.

When MBL binds to the pathogen, MASP-1 and MASP-2 become activated.



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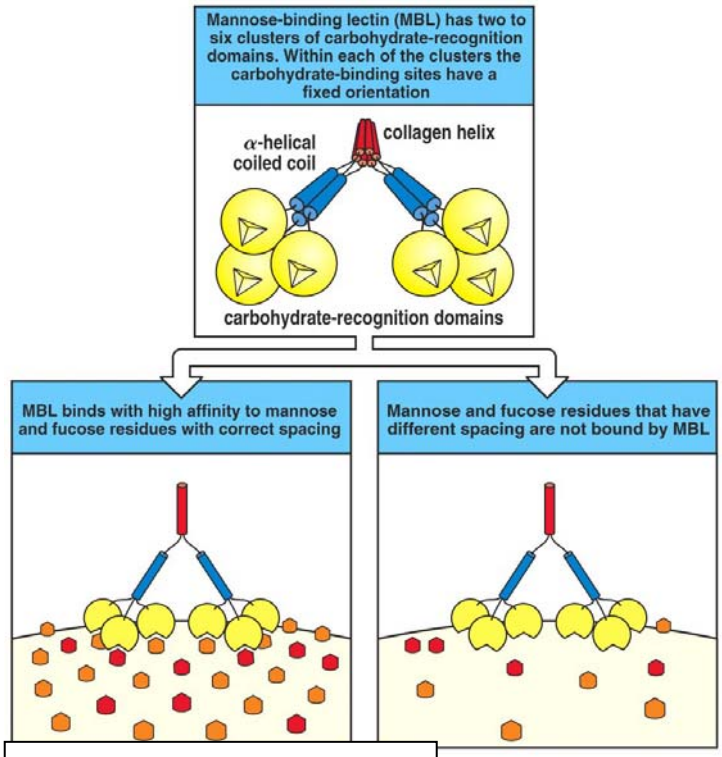

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Figure 3. Mannan binding lectin (MBL).

Further complement sequence progression.

Activated C1qrs (or separately MBL activated MASP-1 and MASP-2) cleaves C4, and the C4b fragment becomes bound to a cell surface (e.g. microorganism). Bound C4b fragment binds C2. See **Figure 4**.

Once bound to C4b, C2 is also cleaved by C1s, forming the C4b2a complex, which remains bound to the cell surface. (For MB-Lectin Pathway activation, MBL can be substituted for C1q, substitute MASP-1 and MASP-2 for C1r and C1s in **Figure 4**).

C4b2a is a C3 convertase, capable of cleaving C3 into C3b and C3a. This is a major point of amplification of the pathways, since one C3 convertase can cleave up to 1000 molecules of C3.

C3b, bound to the antigenic surface, acts as a powerful opsonin and enhances the uptake of antigenic particle by phagocytes.

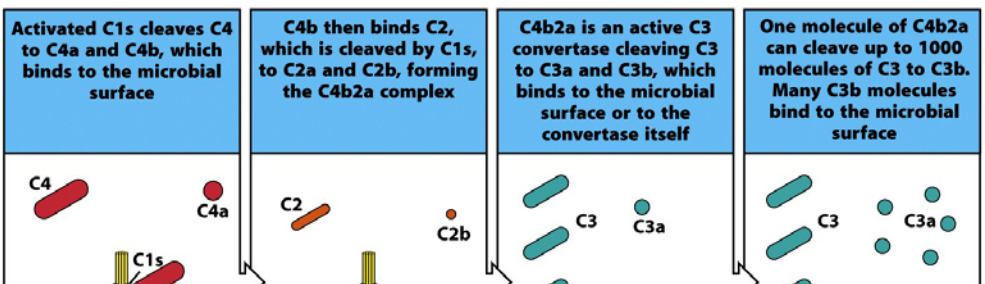

Figure 4. C3 convertase and C5 convertase generation.

C4b2a3b complex, also called C5 convertase, cleaves C5 into C5a, which is a soluble inflammatory mediator, and C5b, which is capable of complexing with additional complement components. The generation of C5b initiates the final phase of complement activation, which is the formation of the **M**embrane **A**ttack **C**omplex (MAC). See **Figure 5.**

The MAC is identical for all pathways of complement activation.

C3a and C5a remain soluble and produce local inflammatory effects.

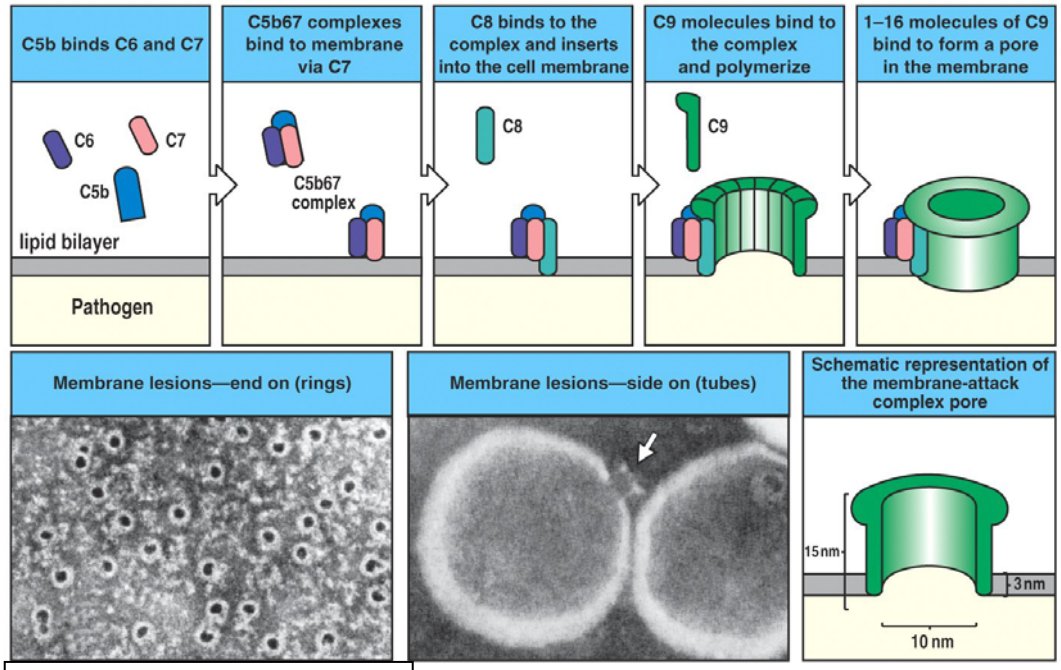

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Figure 5. Membrane attack complex, C5-C9.

Activation of the **ALTERNATIVE COMPLEMENT PATHWAY:**

The alternative pathway depends upon the slow hydrolysis of C3, which spontaneously occurs in plasma.

Hydrolyzed C3 can bind and cleave Factor B, and the resulting C3 (H_2O) Bb complex is a C3 convertase that generates additional molecules of C3b. See **Figure 6.**

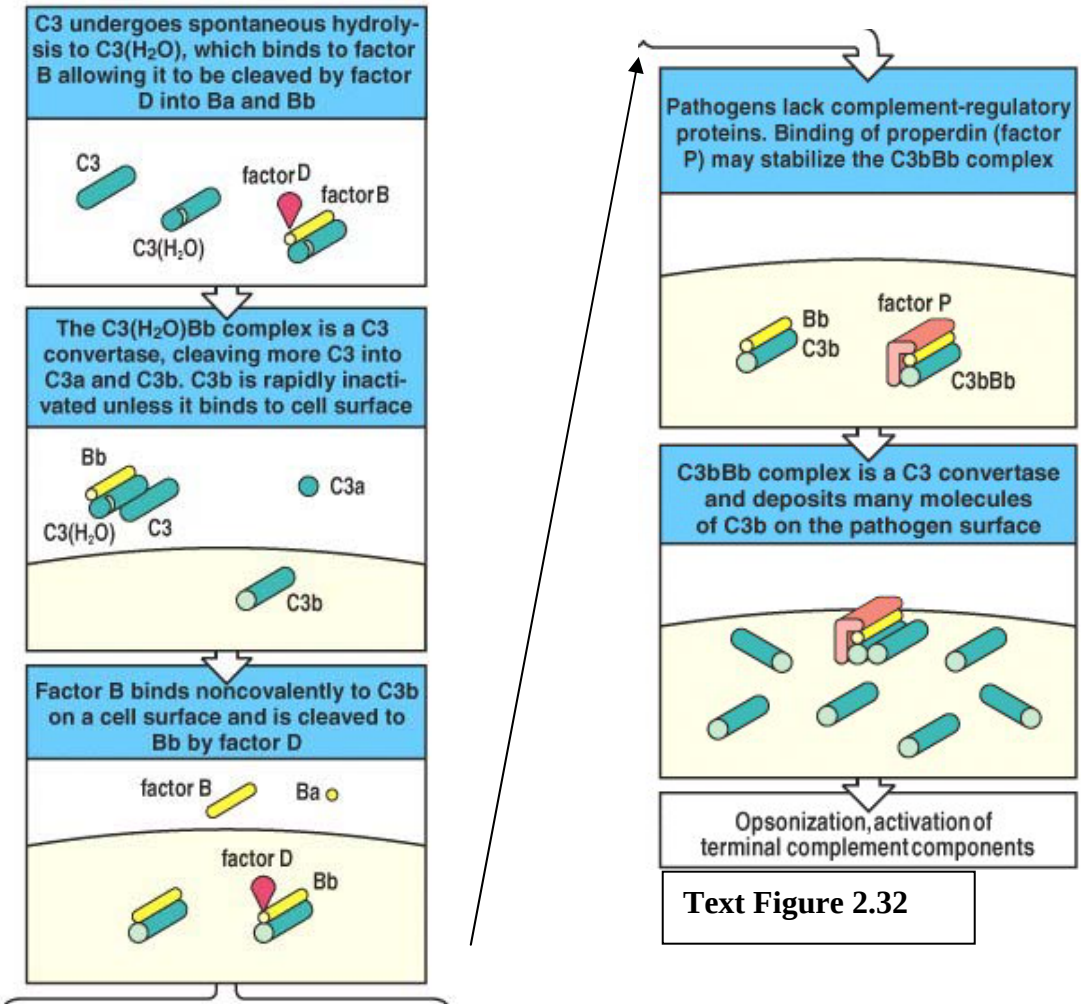


Figure 6. Alternative complement pathway activation.

C3b is rapidly degraded, unless stabilized by attachment to certain favorable pathogenic surfaces.

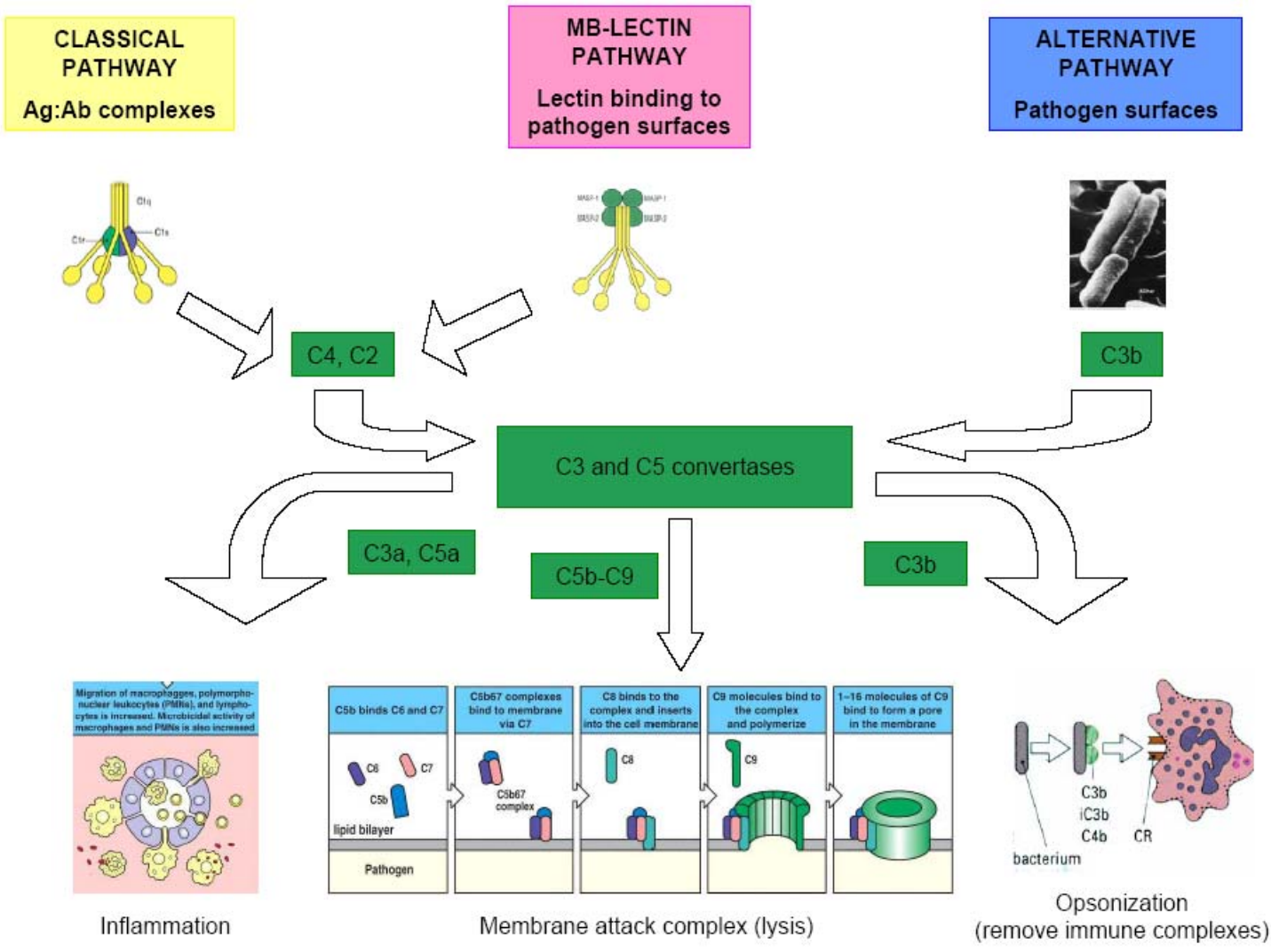
Once attached, C3b binds Factor B, and Factor B is cleaved by Factor D, forming bound C3bBb.

When stabilized by Factor P (properdin), the C3bBb complex acts as a C3 convertase, analogous to C4b2a of the classical pathway.

When another molecule of C3b associates with C3bBb (forming C3bBbC3b), a C5 convertase is formed.

This C5 convertase is analogous to C4b2a3b of the classical pathway. From this point (the cleavage of C5), the alternative and classical pathways converge, leading to the formation of a MAC complex as in **Figure 5**.

The three pathways of complement activation are summarized in **Figure 7**.



Animation: MAC (double click) movie showing complement activation

Figure 7. Integration of the three pathways of complement activation.

Biological Consequence of Complement Activation

Cell lysis and viral neutralization. The MAC complex (C5b→9) creates a pore in the cell membrane, and disrupts cell homeostasis, by cellular lysis (e.g. Gram-negative bacteria). Certain viruses with a membrane coat can also be lysed in this manner. See **Figure 8**.

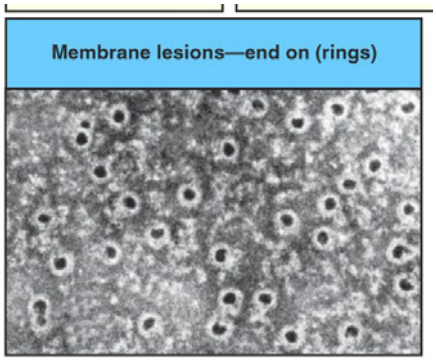


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Figure 8. Pore in a cell membrane as a consequence of MAC.

Opsonization. Phagocytic leukocytes, including neutrophils and macrophages, carry receptors for C3b (CR1). When an antigenic particle is coated with C3b, C3b (also C4b) assists in the adherence and ultimate ingestion of the particle by the phagocytic cell. The C5a fragment also enhances phagocytosis by stimulating phagocytic cells to ingest C3b coated antigens. See **Figure 9**.

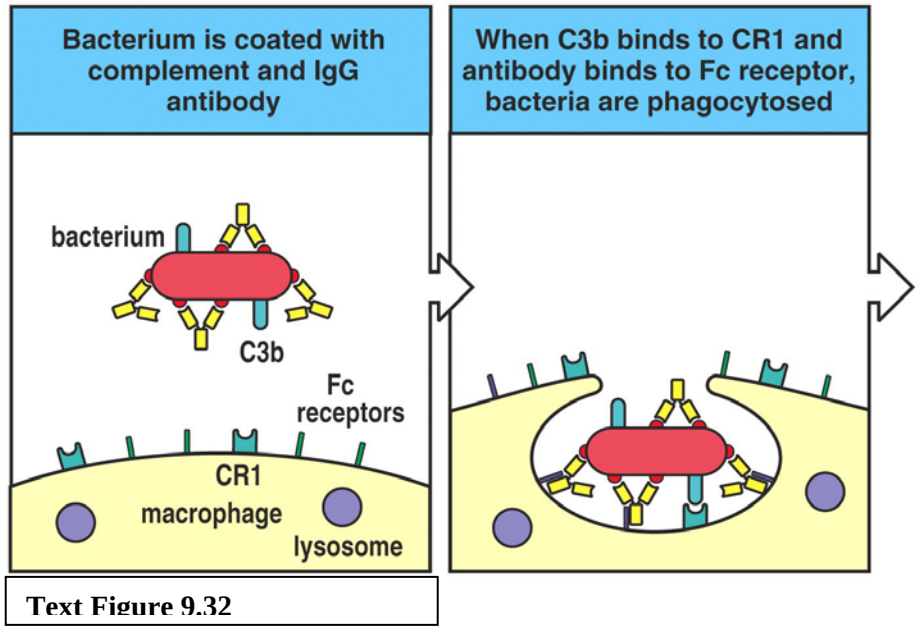

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Figure 9. Opsonization and phagocytosis via C3b and CR1.

Clearance of Immune Complexes. The removal of antigen-antibody complexes from the circulation depends upon C3b. Via C3b, antigen-antibody complexes bind to complement receptors on circulating red blood cells. As the RBCs pass through the spleen and liver, the coated complexes are stripped off of the RBCs by resident phagocytes. See **Figure 10**.

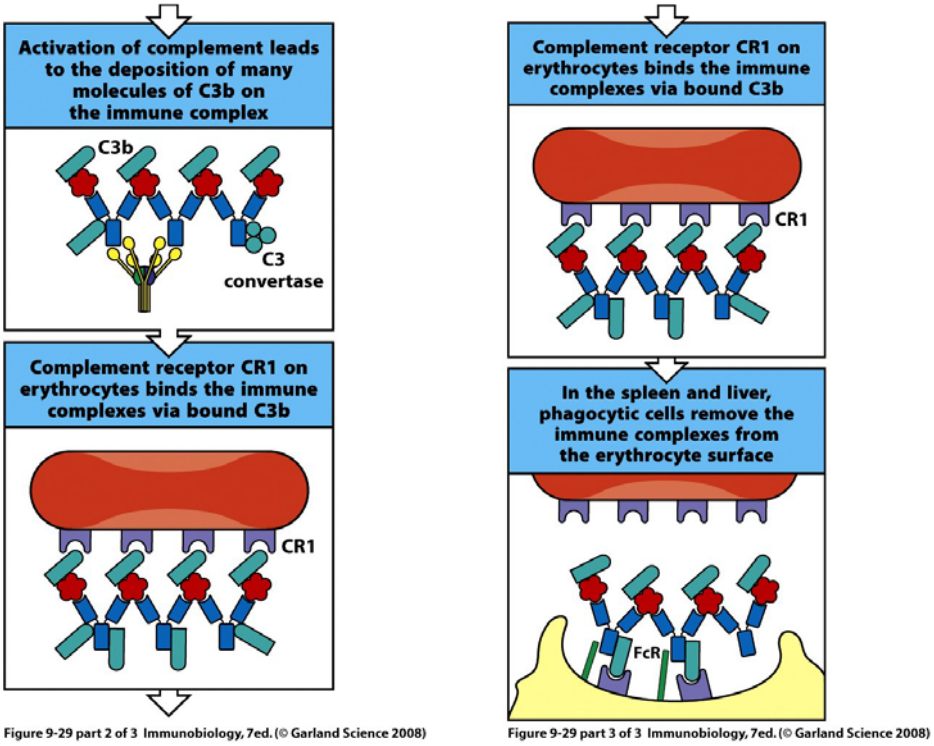


Figure 10. Clearance of immune complexes.

Inflammation. The soluble fragments that are produced during complement activation play several roles in inflammation. See **Figure 11.**

Chemotaxis. C5a is an important chemoattractant for neutrophils, eosinophils, basophils, and monocytes. The development of a C5a gradient at sites of complement activation assists in the recruitment of leukocytes to the area of antigenic challenge.

Vascular Changes. The fragments C3a, C4a, and C5a are capable of binding to specific receptors on mast cells and basophils, triggering granule release by these cells. The release of histamine leads to vascular changes, including increased vascular permeability. Because of this property, C3a, C4a and C5a are called anaphylatoxins.

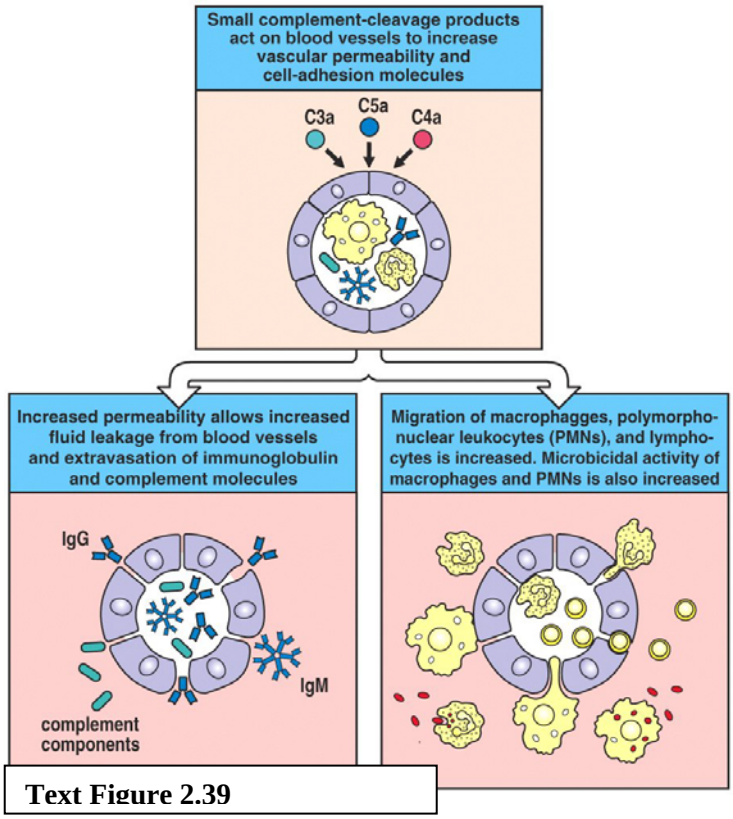


Figure 11. Inflammation mediated by complement.

Regulation of Complement Activation

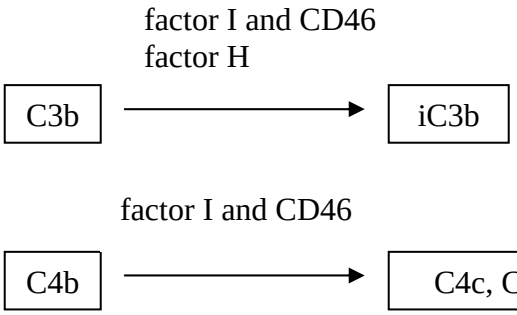
A series of proteins serve to protect host cells from accidental damage by acting at various different stages of complement activation and dissociating complexes or catalyzing enzymatic degradation of covalently bound complement proteins. If not regulated, activated complement can cause excessive inflammation and tissue damage. See below with summaries in **Figures 12, 13 and 14**.

Regulation by protease inhibition. See Figure 12.

C1 inhibitor (C1INH)

C1r:C1s ⟶ C1r, C1s

Regulation by catalytic cleavage. See Figure 12.



Factor I: catalyzes the cleavage of surface bound C3b or C4b.

CD46: binds either C3b or C4b and promoting inactivation by Factor I. CD46 therefore functions to limit C3 and C5 convertase activity, and provides regulation for the classical, MBL and alternative pathways.

Factor H: binds C3b and serves as a cofactor for the cleavage of surface bound C3b by Factor I.

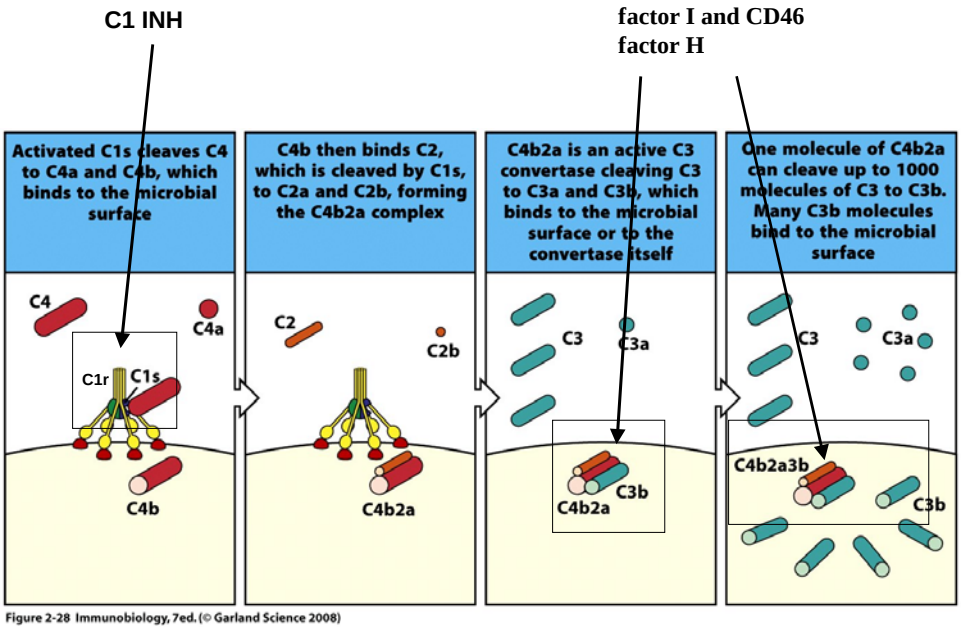
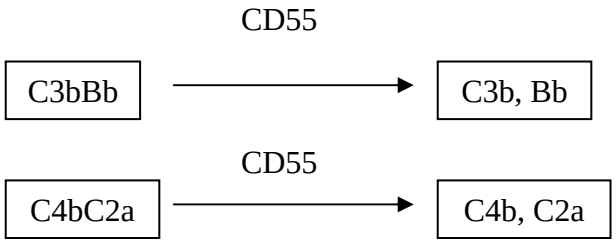


Figure 12. Complement regulation.

Regulation by decay acceleration. See Figure 13.



CD55: a membrane protein that serves to disengage C2a from C4b in the classical and MBL pathways and Bb from C3b for the alternative pathway. In both cases, CD55 inhibits convertase activity.

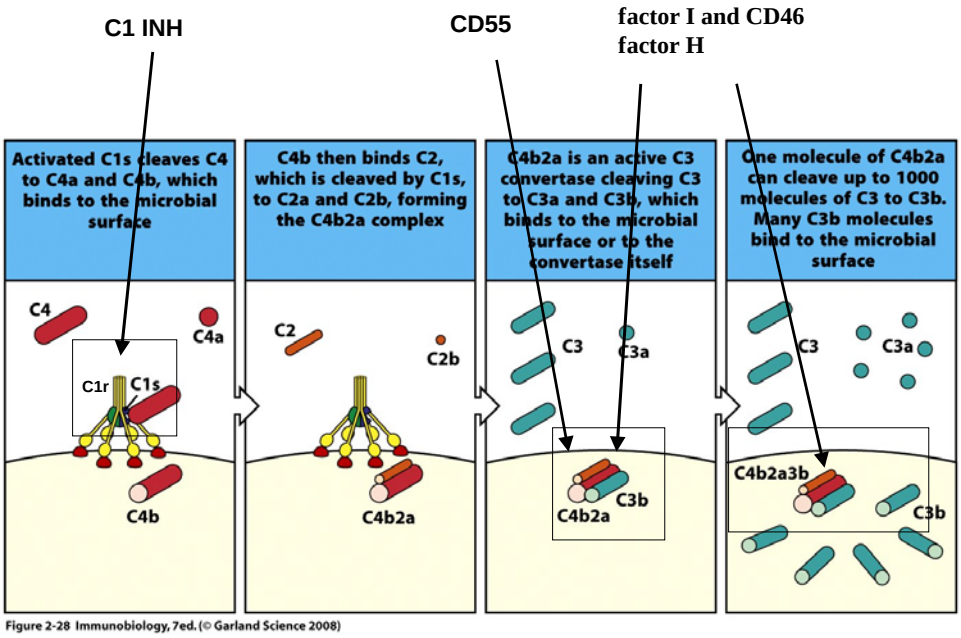
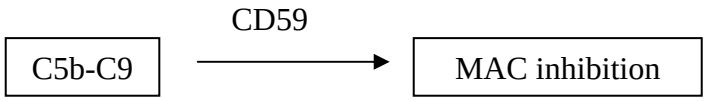


Figure 13. Complement regulation.

Regulation by inhibition of lysis. See Figure 14.



CD59: prevents the assembly of C5b-9 at the final C8/C9 stage.

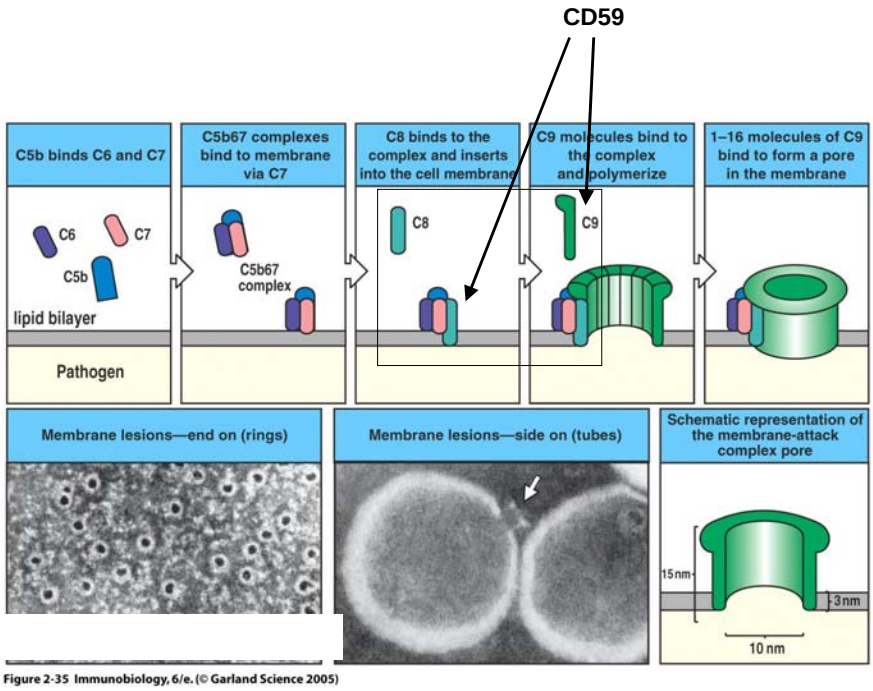


Figure 14. Complement regulation.

Human Complement Component Deficiencies

Deficiencies of components. Deficiencies of complement components are very rare. Defects in the early components of the classical pathway do not lead to overwhelming infection, as the MBL and alternative pathways can bypass this defect. See **Figure 13**.

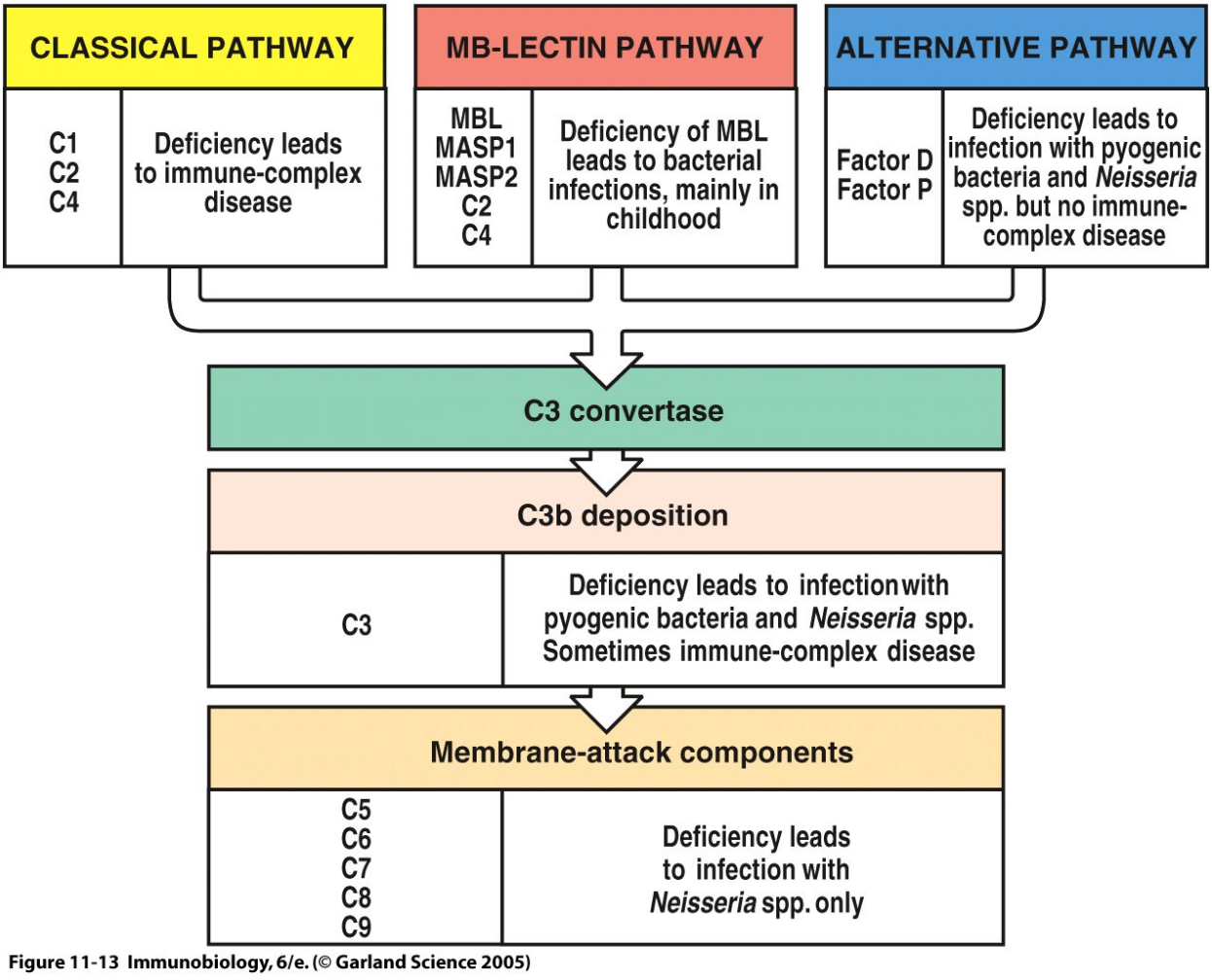


Figure 15. Complement deficiency consequences.

Complement Deficiencies and Associated Disease

Hereditary Angioneurotic Edema – **C1 INH** deficiency – failure to regulate C1 resulting in fluid accumulation, epiglottal swelling.

Paroxysmal Nocturnal Hemoglobinurea – **CD55** and **CD59** failure to function – lack of complement regulation leads to RBC lysis.

A deficiency of the early components of complement results in poor clearance of immune complexes resulting in increased immune complex disease.

STUDY QUESTIONS

1. Compare and contrast the Classical, MB-Lectin, and Alternative pathways of complement activation.
2. Match individual complement components with their specific biological activities.
3. Identify complement regulatory proteins and their effect on specific complement components.
4. Describe the significance of individual complement component deficiencies.

EXAMPLE OF TEST QUESTION

A deficiency of complement component C9 results in susceptibility to:

A. Pyogenic bacteria.

B. *Neisseria* spp. only.

C. Immune complex disease.

D. Reduced immune complex clearance.

E. No phenotype.

CORRECT ANSWER TO ABOVE QUESTION: B

COMPLEMENT

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Introduction

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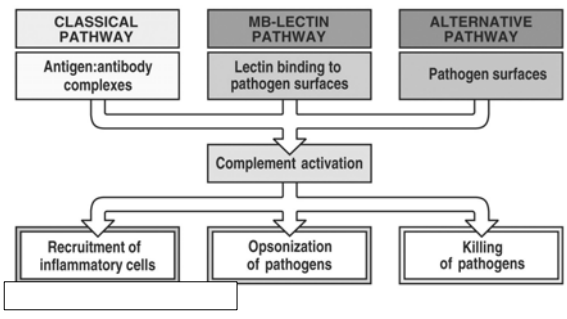
The complement system



Nomenclature

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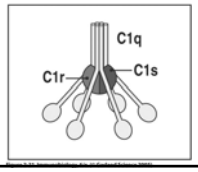

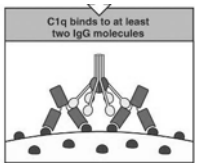

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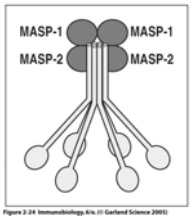
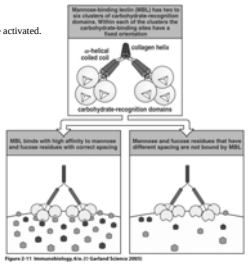
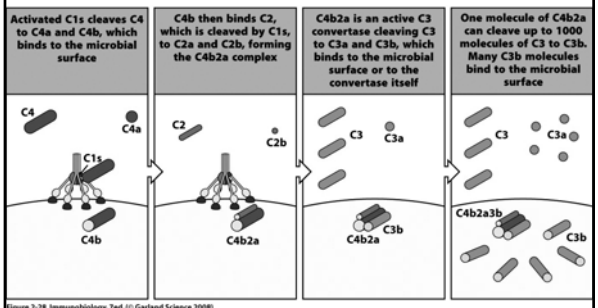
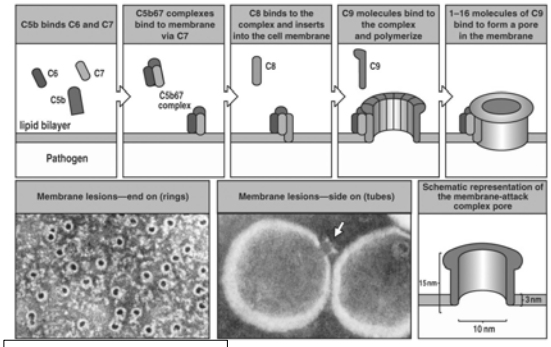




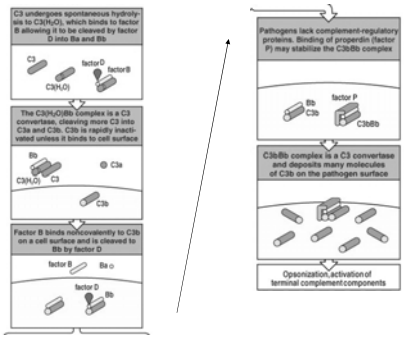
Further complement sequence progression.

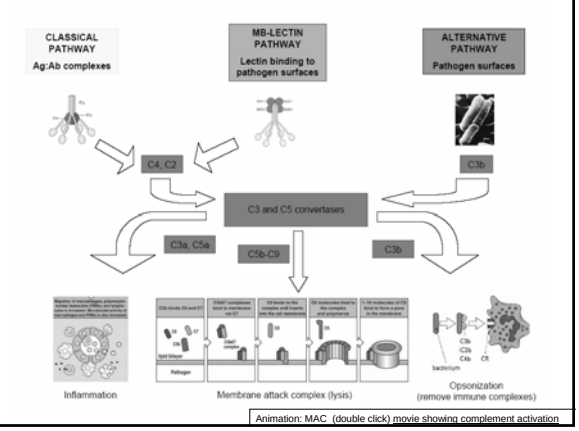


Membrane Attack Complex (MAC)



Activation of the **ALTERNATIVE COMPLEMENT PATHWAY:**

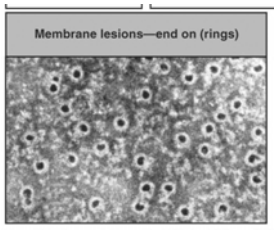




Animation: MAC (double click) movie showing complement activation

Biological Consequence of Complement Activation

Cell lysis and viral neutralization. The MAC complex (C5b→9) creates a pore in the cell membrane, and disrupts cell homeostasis, by cellular lysis (e.g. Gram-negative bacteria). Certain viruses with a membrane coat can also be lysed in this manner.



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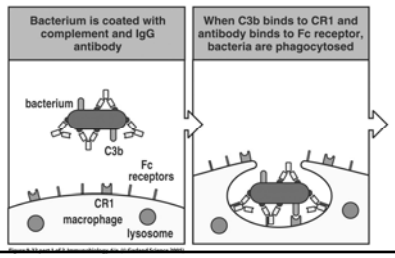
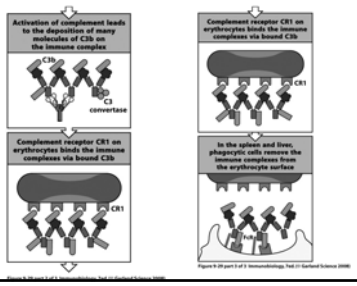


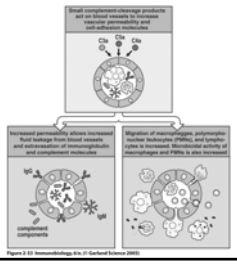
Clearance of Immune Complexes. The removal of antigen-antibody complexes from the circulation depends upon C3b. Via C3b, antigen-antibody complexes bind to complement receptors on circulating red blood cells. As the RBCs pass through the spleen and liver, the coated complexes are stripped off of the RBCs by resident phagocytes.



Inflammation. The soluble fragments that are produced during complement activation play several roles in inflammation.

Chemotaxis. C5a is an important chemoattractant for neutrophils, eosinophils, basophils, and monocytes. The development of a C5a gradient at sites of complement activation assists in the recruitment of leukocytes to the area of antigenic challenge.

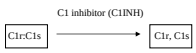
Vascular Changes. The fragments C3a, C4a, and C5a are capable of binding to specific receptors on mast cells and basophils, triggering granule release by these cells. The release of histamine leads to vascular changes, including increased vascular permeability. Because of this property, C3a, C4a and C5a are called anaphylatoxins.



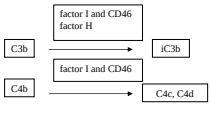
Regulation of Complement Activation

A series of proteins serve to protect host cells from accidental damage by acting at various different stages of complement activation and dissociating complexes or catalyzing enzymatic degradation of covalently bound complement proteins. If not regulated, activated complement can cause excessive inflammation and tissue damage.

Regulation by protease inhibition.



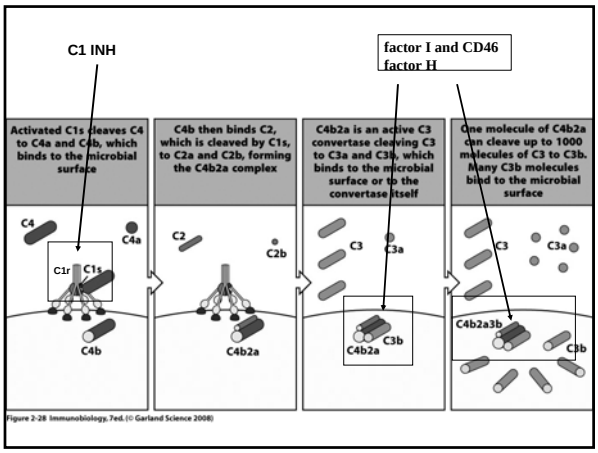
Regulation by catalytic cleavage.



Factor I: catalyzes the cleavage of surface bound C3b or C4b.

CD46: binds either C3b or C4b and promoting inactivation by Factor I. CD46 therefore functions to limit C3 and C5 convertase activity, and provides regulation for the classical, MBL and alternative pathways.

Factor H: binds C3b and serves as a cofactor for the cleavage of surface bound C3b by Factor I.



Regulation by decay acceleration.

CD55
C3bBb → C3b, Bb

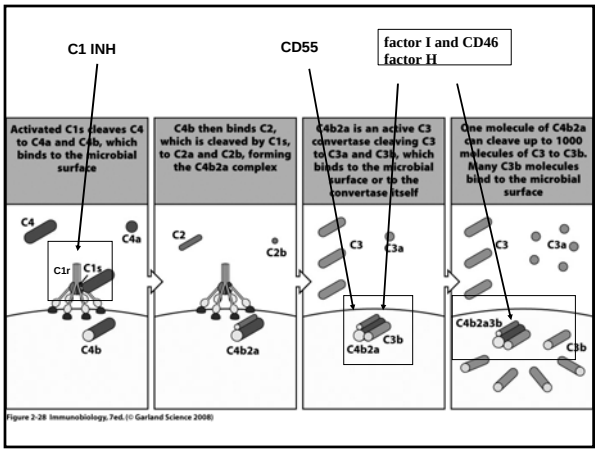
CD55
C4bC2a → C4b, C2a

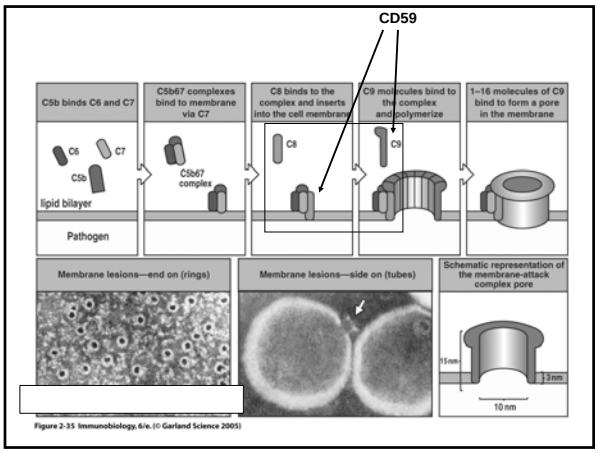
CD55: a membrane protein that serves to disengage C2a from C4b in the classical pathway and Bb from C3b for the alternative pathway. In both cases, CD55 inhibits convertase activity.

Regulation by inhibition of lysis.

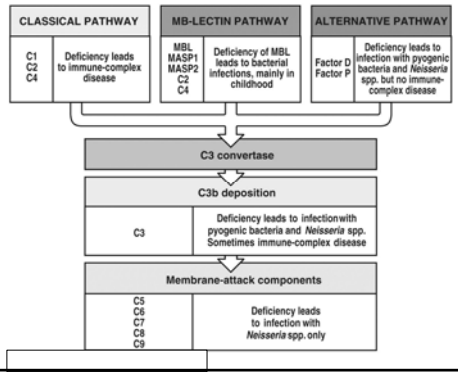
CD59
C5b-C9 → MAC inhibition

CD59: prevents the assembly of C5b-9 at the final C8/C9 stage.



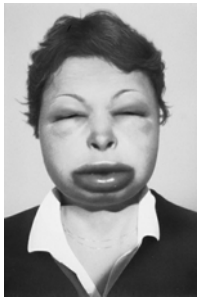


Human Complement Component Deficiencies



Hereditary deficiency of C1 inhibitor (C1 INH)

- Congenital deficiency of functional C1 INH
- Clinically manifests as hereditary angioedema
- C1-INH is central to the regulation of the **complement**
- **Clinical symptoms:**
  swelling,
  abdominal attacks,
  edema of the upper airway
- **Treatment:**
  acute attacks - C1-INH concentrate;



Complement Deficiencies and Associated Disease

- Hereditary Angioneurotic Edema – **C1 INH** deficiency – failure to regulate C1 resulting in fluid accumulation, epiglottal swelling.
- Paroxysmal Nocturnal Hemoglobinurea – **CD55** and **CD59** failure to function – lack of complement regulation leads to RBC lysis.

STUDY ACTIVITIES

1. Compare and contrast the Classical, MB-Lectin, and Alternative pathways of complement activation.
2. Match individual complement components with their specific biological activities.
3. Identify complement regulatory proteins and their effect on specific complement components.
4. Describe the significance of individual complement component deficiencies.

HOST DEFENSE

SMALL GROUP PROBLEM SOLVING SESSION

HOW GENE PROFILING AND NANOMEDICINE ARE GOING TO CHANGE THE WAY YOU UNDERSTAND MEDICINE AND TREAT PATIENTS

Small Group Classrooms

LEARNING GOALS

- Understand how gene profiling by array technology is fueling fundamental changes in the way scientists and physicians think about genes, diseases, and therapeutics

- Understand how a synergistic fusion of biologic concepts, physico-chemical engineering and therapeutic advances is going to produce a quantum leap forward in patient care

BACKGROUND READING: Many of the immunologic concepts have been covered in previous lectures. **The critical material is posted on the Host Defense web site.**

DEVELOPED BY

John A. Robinson, M.D.

I. Introduction

I know that you have already had lectures and small groups elsewhere on array techniques but it very important that you understand how critical this technology and its offspring are to the practice of medicine in the 21st Century. Another way of gaining perspective on their importance is to realize that the use of molecular immunology and pathology technology (DNA and tissue arrays are just the beginning) are going to displace almost all traditional biochemical and histological study of disease. It is not fanciful to suggest that medical school curricula within a decade will have almost no resemblance to current ones. The new curricula will be rich in, among other things, bioinformatics and artificial intelligence applications, customized gene profiling of patients and their responses to drugs, gene profiling that redefines many diseases entirely, and also predicts their prognosis and response to therapy. All the aforegoing will be taught in the context of how a physician can assemble and interpret vast amounts of data and correlate it with the individual patient. But I digress…this doesn't mean you shouldn't study "classical basic science" for Boards next year!

Close behind the application of array technology to biologic processes and clinical medicine is the rapidly developing field of nanomedicine. More on it later in this small group.

II. MICROARRAYS-the future is now

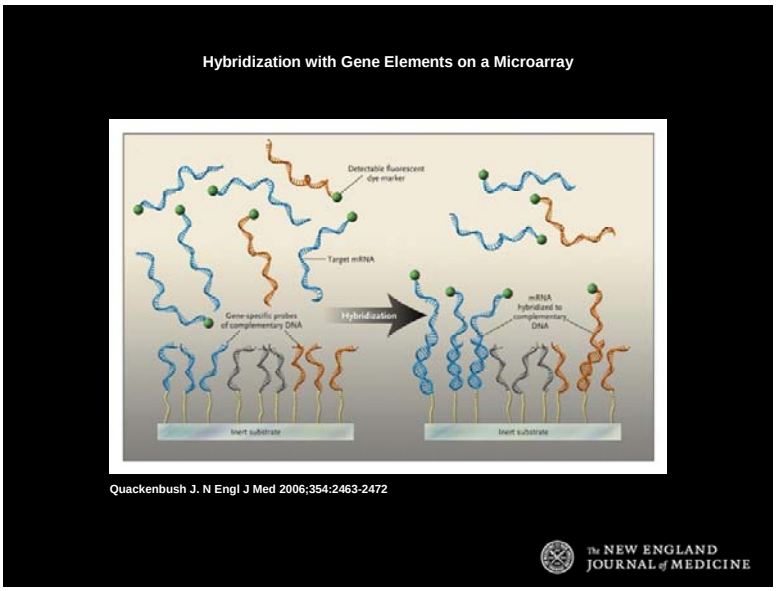
The new science of genomics provides a powerful way to measure the expression of thousands of genes simultaneously in very small amount of tissue, even single cells. This represents a powerful new technology created by a synergistic intersection of biology, computers and automation. Already artificial intelligence techniques are already being used to "mine" the vast amounts of data generated by arrays. Not only gene expression per se can be measured but also, more importantly, **associations between gene** groups can be detected. Although microarrays are not really immunological techniques per se, you need to understand not only how they will rapidly expand the understanding of basic immunologic responses but also revolutionize the diagnosis, prognosis and treatment of many inflammatory and neoplastic diseases. **The applications and implications of the technology are vast and compelling.**

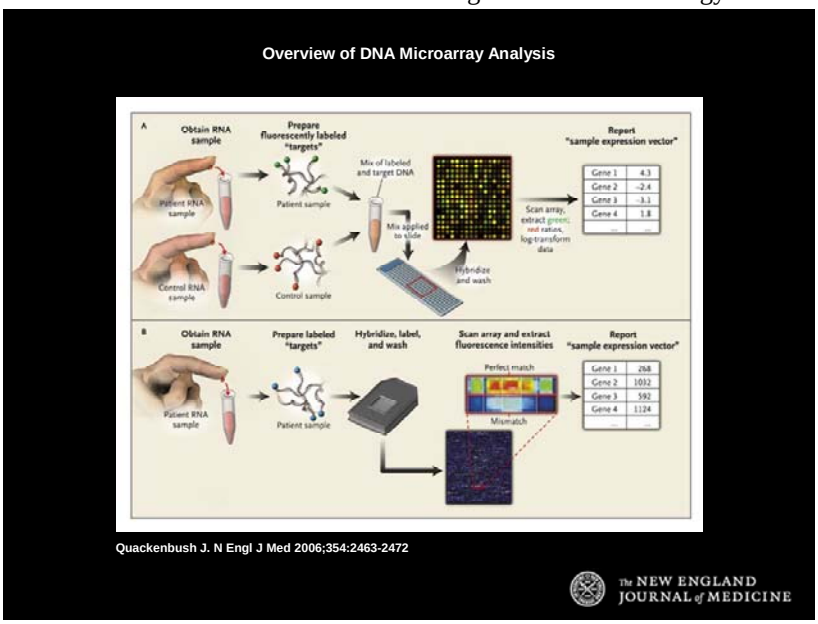
A. Fundamentals of a DNA microarray: (you should know this already)

1. Large numbers of target DNAs are attached to a solid support. In some instances the genetic function of the DNA is known. More often however the DNA is simply a gene fragment or expressed sequence tag developed from cells or tissues.
2. mRNA is extracted from the sample to be studied-for example, a tumor.
3. The mRNA is reverse transcripted.
4. The resultant cDNA is labeled with a fluorescent dye
5. The spotted DNA is probed/hybridized with the labeled DNA
6. All the "spots" on the plate are then scanned to measure the presence or absence of fluorescence-hence the presence or absence of gene expression.

There are many variations of the fundamental technique. For example, cDNA can be spotted and mRNA from a tumor can be labeled and studied.

I. FIRST STEP: Study the 2 diagrams below that demonstrate the stepwise array process used to shed new light on disease causation, best ways to construct vaccines and improve diagnostics. Two journal articles posted on the HD web site for this small group will provide more information if you need it

- 

- 

Students: To prepare for the remainder of this small group, you must go to the Host Defense website and read the posted articles. This is necessary because the arrays will not reproduce well by standard copying techniques.

IMPORTANT-*When you read the articles, do* ***NOT*** *be concerned with the specific methodology and most of the reagents, physical/chemical details and gene terminology- read them for the* ***concepts*** *only*

Article # 1 provides a beautiful example of how gene profiles of dendritic cells confirm that these critical APC detect specific pathogen molecular patterns and then tailor the appropriate innate immune response to a specific pathogen-remember the innate response being driven by a spectrum of TLRs? Cells that first encounter potential pathogens could discriminate between three major pathogen groups (bacteria, fungi and viruses) and then initiate appropriate innate immune reactions to counter the threat. Not only can gene expression be detected but also the sequence of expression can be studied. The combination of detection, sequence of expression and associations of multiple genes provide powerful insights into how the immune system works. The gene profiles also emphasize the extreme redundancy built into the immune system as a safeguard.
The varying gene expression during innate responses could be detected on a gene chip that contained 6800 immune cell genes. This type of fundamental research was impossible before the availability of array technology and guarantees that exponential leaps in understanding immune responses are at hand.

Questions related to Article #1:

1. Do the results in the article confirm that the innate immune response to a virus like influenza is different than to a bacterium like *E. Coli*? Does the difference seem significant and support the notion that the immune system had to invent new ways of dealing with viruses?

2. How does the data in this article support the clinical observations made for over a hundred years by physicians that individual patients vary widely in "how sick" they get when infected with a given organism. For example, if the entire class of 2013 was infected with virus "X", many would have no symptoms, some a mild illness, others would stay home for a few days and maybe one would end up in ICU! Later in the course, you will discover what is behind the heterogeneity of clinical disease expression and how it will show up in an array.

Article #2 & 3 These studies demonstrate how array technology will significantly change the way medicine is practiced **by the time all of you are residents**!

Questions related to Article 2 & 3 and related editorials

1. The lymphoma study posted on the HD site emphasizes that studying cells and subcellular phenomena in isolation can be very misleading. Tumor cells are so interesting that we forget to look at where they live (and don't live). Intuitively one might think that the genetic characteristics of the tumor cells would be the most valuable information to guide care of the patient. How does this study convince you this is not the case?

2. Discuss the clinical implications of how a “single” type of tumor defined by classic histologic methods, when analyzed by DNA microarray, is revealed to be not so “single”.(Hint: read the breast cancer article posted on the HD site)

3. If microarray chips can predict the response to chemotherapy in women with breast cancer and molecular probing of biopsies can predict which ones will become malignant, be ready to discuss the implications for oncologists, pathologists, patients, pharma companies and economists.

The following section is for the sake of knowledge only and will give you the basis for exciting dinner conversations with family, loved ones and non-medical people**. YOU WILL NOT BE TESTED ON THIS SECTION.**

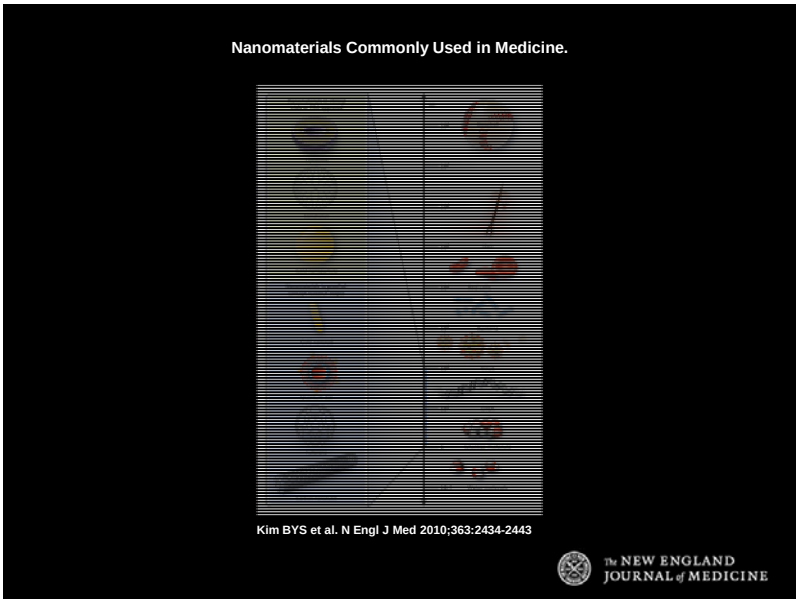
III. **NANOMEDICINE**- the future is almost now!

A. What is it? Need to understand nanotechnology first. Nanomaterials are defined as the “intentional design, characterization, production and application of materials, structures, devices and systems by controlling their size and shape in the nanoscale range- 1-100 nm. This is a similar range within which many molecular and pathologic processes operate.

B. Nanomaterials are being designed for transport, insertion and accumulation of diagnostic and therapeutic agents into tissue and individual cell sites that have been very difficult to access by traditional vehicles. Tumors are a prime example- delivering effective doses of chemotherapy to a tumor currently forces an oncologist to use toxic dosages that limit use in humans.

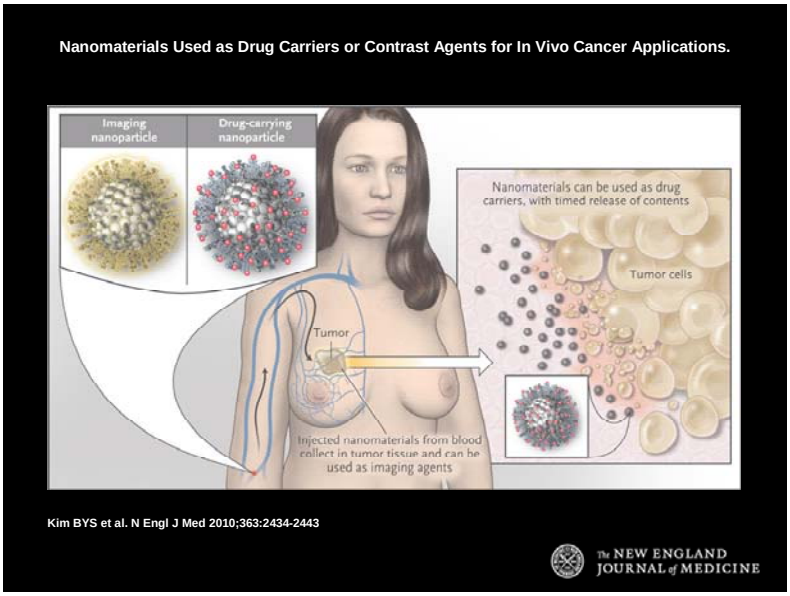
C. A common feature of nanomaterials is a marked increase of surface area relative to their volume and those expanded surfaces can be coated with a high density of molecules of interest. Nanomaterials can be composed of metal ions with magnetic, electronic and optical properties that can be “turned on” once they have gained access to a site of interest to produce local heat that can kill a tumor cell. They can be constructed in a way that allows loading the surface or inner core with potent cytokines or anti-tumor drugs that become active after delivery

D. Each student group, after reading the following, should try and conjure up some exotic way to use nanomedicine in a patient.



D. nanomaterials may also enhance diagnostic imaging, especially MRI, where their magnetic properties can be exploited.

1. An example of diagnostic and/or therapeutic nanotechnology:



These nanobeads can be infused into patient and target a tumor with tumor specific antibody localization, then release chemotherapeutic drugs into the tumor. They can also carry an MRI imaging agent so tumor can be found.

E. In vitro Nanodiagnosis

A Two areas of high interest are using individual cells as diagnostic probes and gold nanoparticles that can replace PCR technology for the highly specific detection of infectious agents.

B. Some examples follow:

1. B lymphocytes have been engineered as pathogen sensors by inserting genes for:
a. bioluminescent jellyfish proteins that emit light when their surface antibody is cross-linked.
b. and using monoclonal antibody technology to produce antibody to the specific pathogen of interest.

2. The sensitivity and speed of the assay is spectacular. Anthrax specific B cells, when exposed to as few as **50 units** of the organism from a nasal swab, emit light that can be easily detected with available sensors **within 5"!**

PATHOGEN SENSING B CELLS

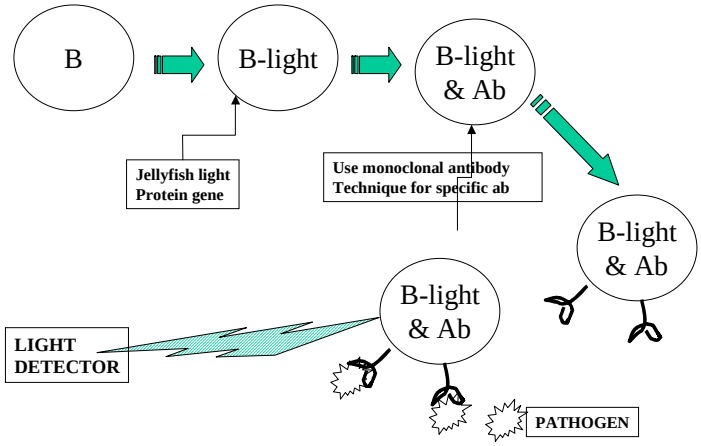
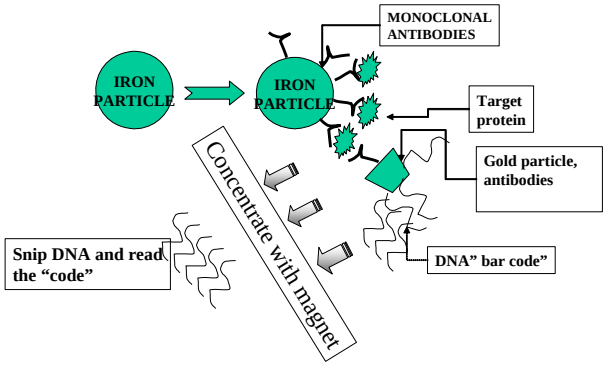


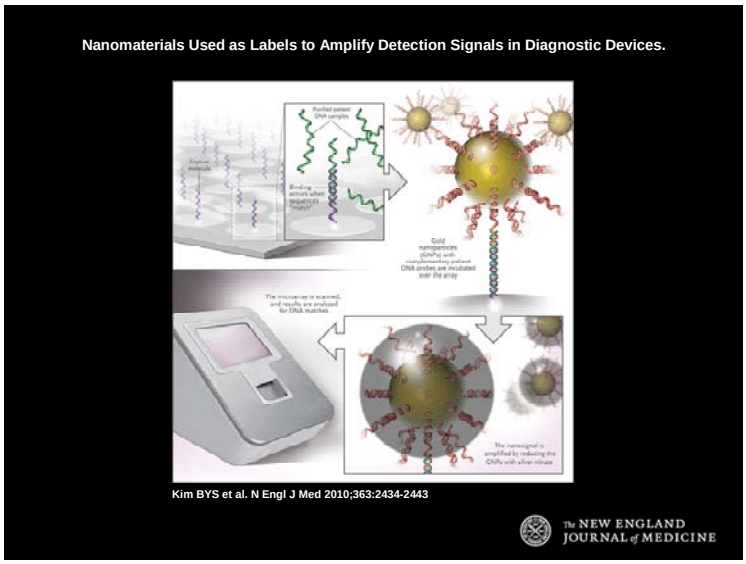
Fig. by J. Robinson

B. Nanotechnology uses minute iron and gold particles that increase the sensitivity **a million fold** over current assays.

1. The technique exploits magnetic properties of iron particles that are encased in plastic coat that has been coated with a monoclonal antibody against a protein of interest.
2. The coated iron particles are reacted with the protein and also with gold nanoparticles coated with a polyclonal antibody to protein (the sandwich technique similar to ELISA assays) and short strands of DNA that act like a "bar code". The iron particles that have migrated to the magnetic field are isolated, the DNA snipped off and the "bar code" read by ultrasensitive DNA techniques.

NANOIMMUNOLOGY




Nanomaterials Used as Labels to Amplify Detection Signals in Diagnostic Devices.
Kim BYS et al. N Engl J Med 2010;363:2434-2443
The NEW ENGLAND JOURNAL of MEDICINE