# Antibody-antigen interactions and complement

# Overview of the immune system

## Innate immunity

- "Natural" immunity
  - Does not "recognize" specific antigens
  - Response does not improve with repeated infections
- Examples include:
  - Anatomical barriers (ie, skin, mucous membranes)
  - Non-specific proteins (ie, complement )
  - Non-specific cells (ie, NK cells)

## Adaptive immunity

- Specificity and memory
  - Cells recognize specific infectious agents
  - Initial exposure leads to increased response if reinfected

### Humoral

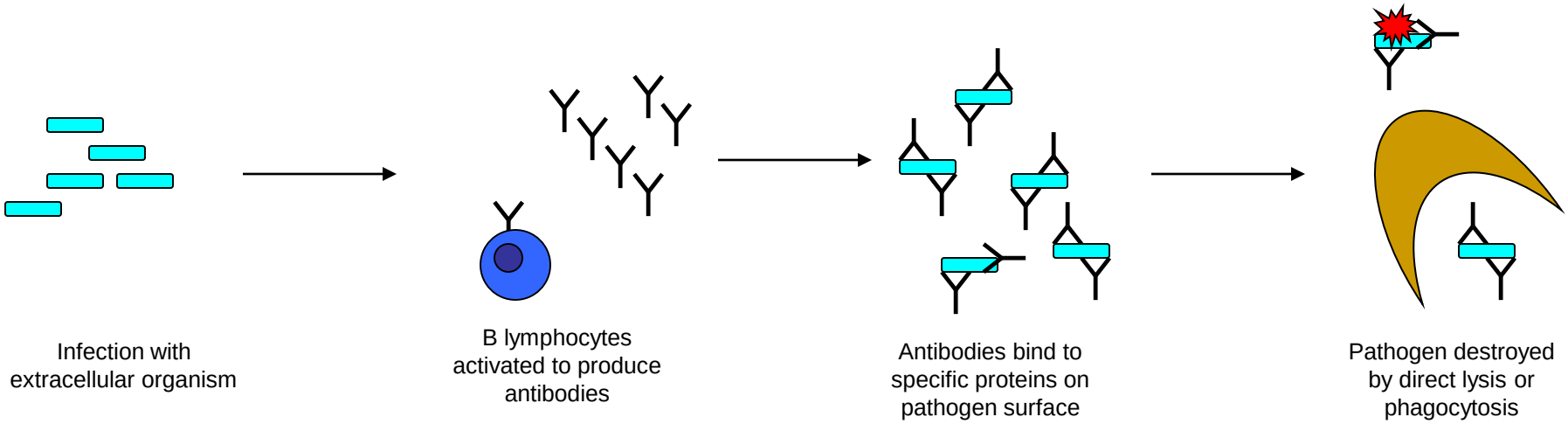
- B cells, antibodies

### Cell-mediated

- T cells

- **Both systems work together in a regulated fashion during an immune response**

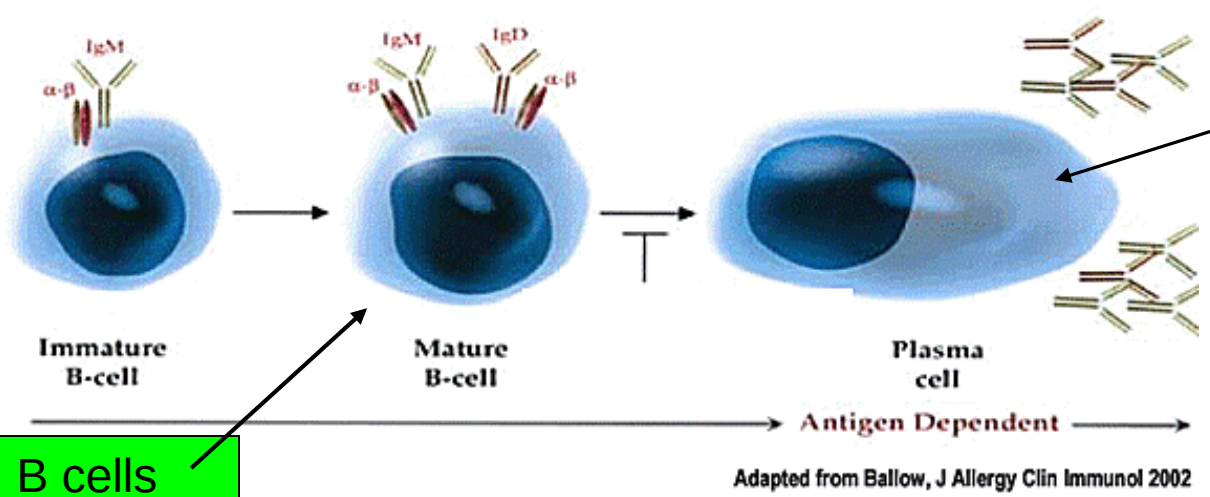
# Overview of humoral immunity




- **The main role of the humoral immune system is to identify <u>extracellular</u> infectious agents and generate antibodies for <u>opsonization</u>, which leads to elimination of the pathogen by either <u>cellular lysis</u> or <u>phagocytosis.</u>**

# B lymphocytes are the primary mediators of humoral immunity

- Circulating B cells express specific immunoglobulins (antibodies) on their cell surface
  - Each B cell expresses antibody specific to a unique antigen
- Exposure of naïve B cells to their antigen leads to clonal expansion of that B cell.
  - This requires help from T cells and cytokines
- Expanded B cell clones can either:
  - Differentiate to plasma cells and secrete antibodies (IgM initially, or others after further differentiation)
  - Differentiate into memory B cells (for future surveillance)




Plasma cells produce antibody

B cells recognize antigen

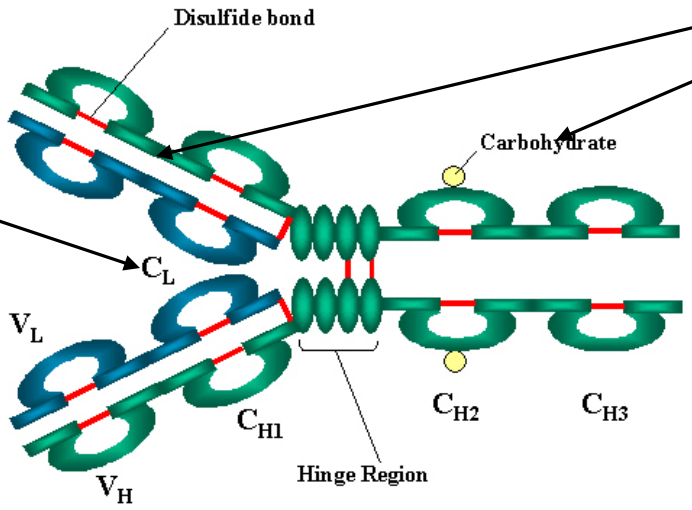
http://www.scielo.br/img/revistas/aabc/v76n4/a07fig1a.gif

# Antibody structure

- A basic, monomeric antibody is made up of two types of chains, each of which consists of two types of regions

Regions:

- Each chain contains variable and constant regions
- Variable sites determine the specificity, or idiotype, of an antibody
  - Every antibody has a unique specificity to a particular antigen

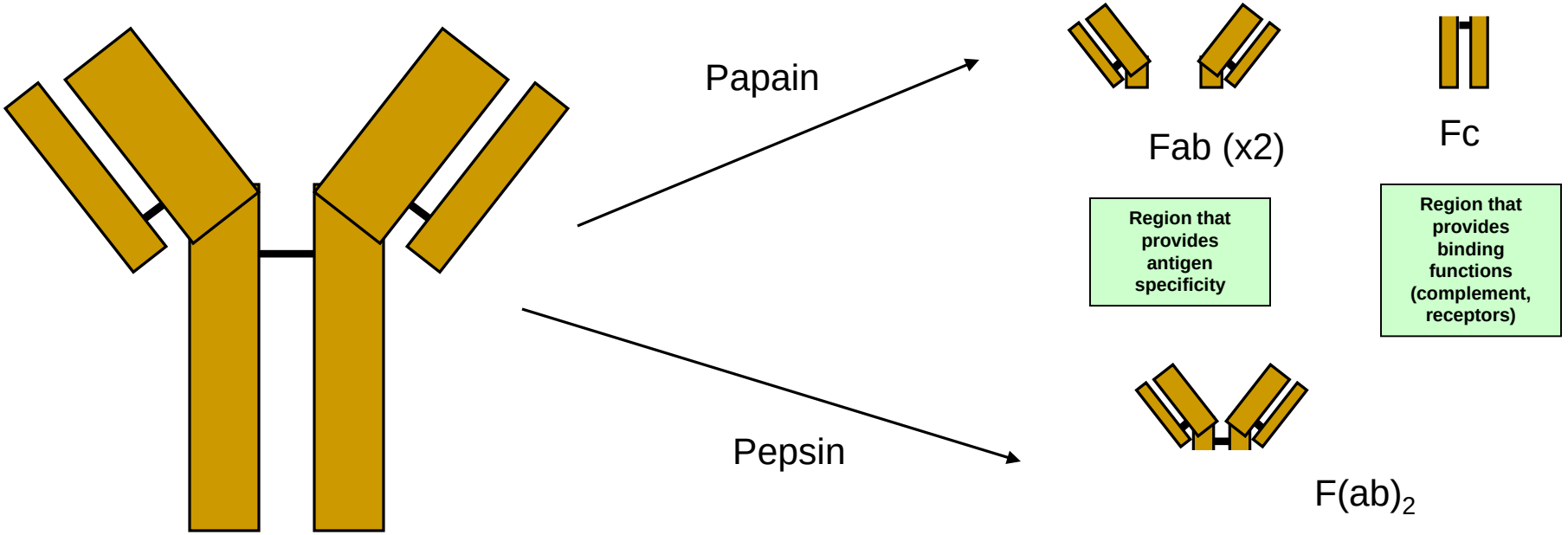



Chains:

- Monomeric antibodies contain 2 heavy and 2 light chains
  - Both copies are identical
- Heavy chain determines isotype of antibody
  - IgG, IgM, IgD, IgA, or IgE
- Light chains can be kappa or lambda

# Enzymatic digestion fragments

- Various enzymes can cut immunoglobulins into functional fragments
  - This does not happen *in vivo* and has no relevance to how the immune response actually works. It's just a convenient way to break the molecule down into functional pieces.

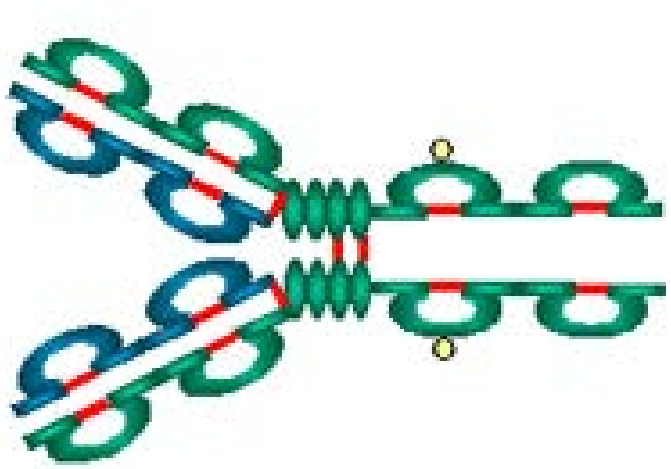


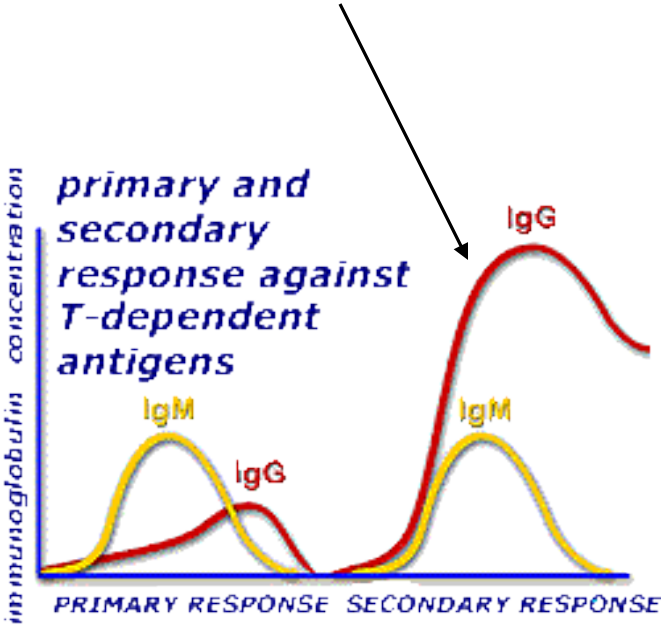
# Different antibody isotypes have different characteristics

| | IgM | IgG | IgA | IgE | IgD |
|---|---|---|---|---|---|
| Serum concentration (mg/dl) | 150 | 1200 | 100 | ND | ND |
| Half-life (days) | 5 | 21 | 5.5 | 2 | NA |
| Circulating form | Pentamer | Monomer | Monomer/Dimer | Monomer | Monomer |
| Molecular W (kD)_ | 900 | 150 | 160 | 200 | 180 |
| Unique structures | J chain | | J chain & secretory piece on mucosal IgA | | |
| Distribution | Intravascular | Intra- and extravascular | Intravascular, Mucosal surfaces | Mast Cells, Basophils | Naïve B cells |
| In breast milk? | | + | +++ | | |
| Cross placenta? | | ++ | | | |
| Activate complement? | +++ | + | | | |

**Adapted from "Essentials of Immunology and Serology". Table 3-1**

# IgG



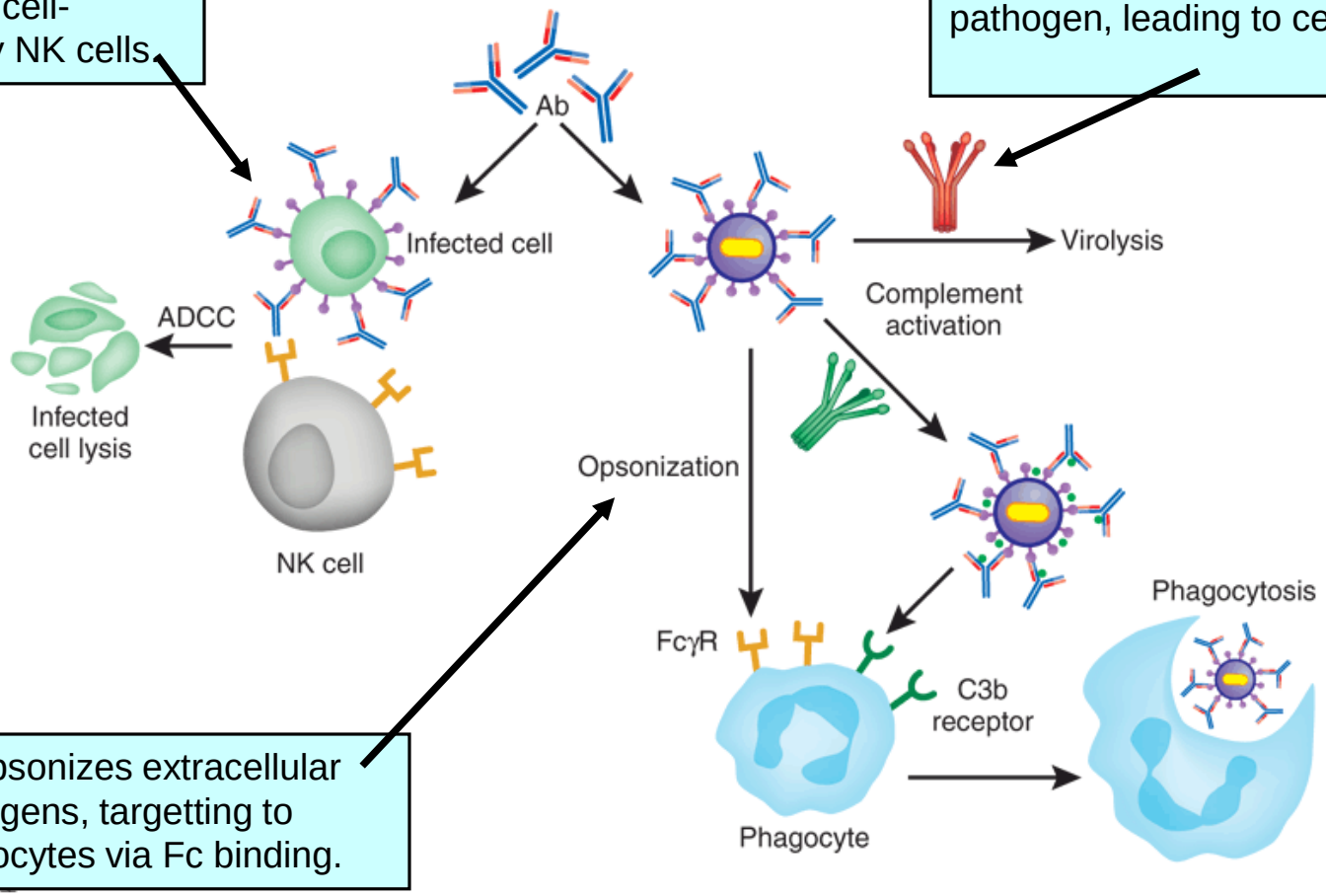



- The major immunoglobulin present in humans, accounting for 70-80% of circulating antibody.
  - 4 subclasses exist (IgG1 > IgG2 >> IgG3, IgG4)
- The most important immunoglobulin for mounting an effective immune response.
  - Can activate complement, opsonize cells for phagocytosis, and target cells for ADCC destruction.
  - Is the main antibody produced in a secondary immune response
- Only class which crosses the placenta, providing fetal immunity.
  - Also supports neonatal immunity for approximately 6 months after birth thanks to long half-life (21 days).

# The action of IgG in the immune response

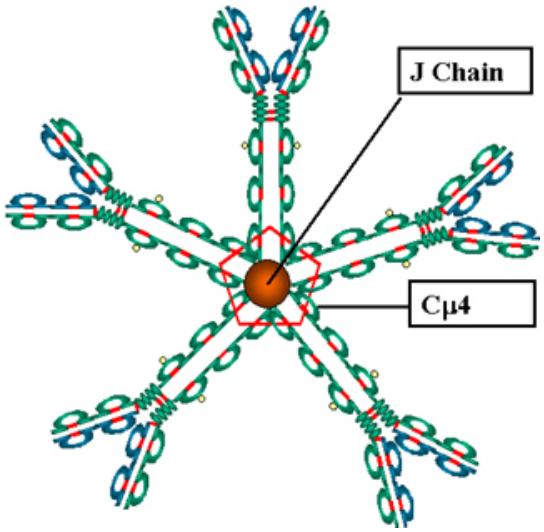
3. Binds to virally infected cells, targeting for cell-mediated killing by NK cells

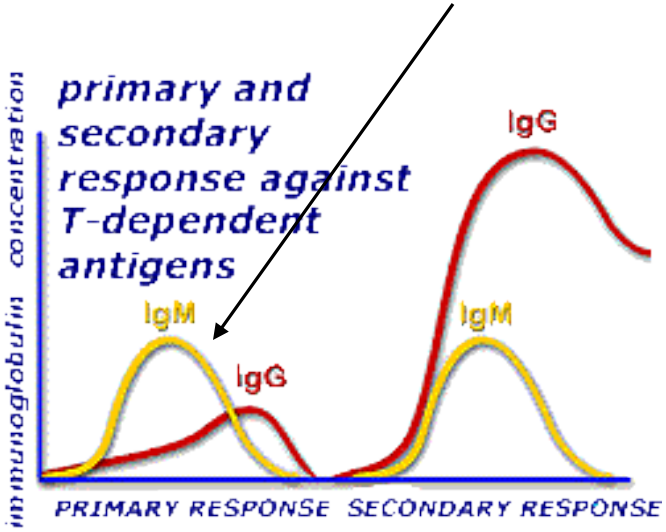
2. Directly recruits complement to opsonized pathogen, leading to cell lysis




1. Opsonizes extracellular pathogens, targetting to phagocytes via Fc binding.

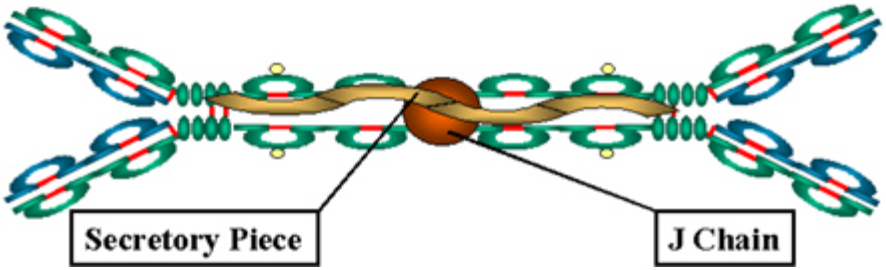
# IgM



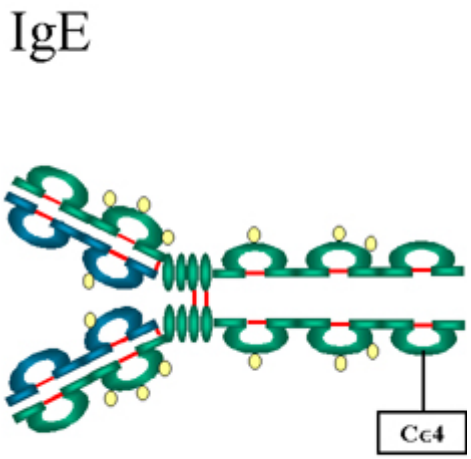

- First antibody to appear in a primary immune response
- Largest isotype, containing 5 immunoglobulin subunits
  - Makes IgM the most efficient complement activator
- Is responsible for naturally-occurring ABO blood group antibodies.

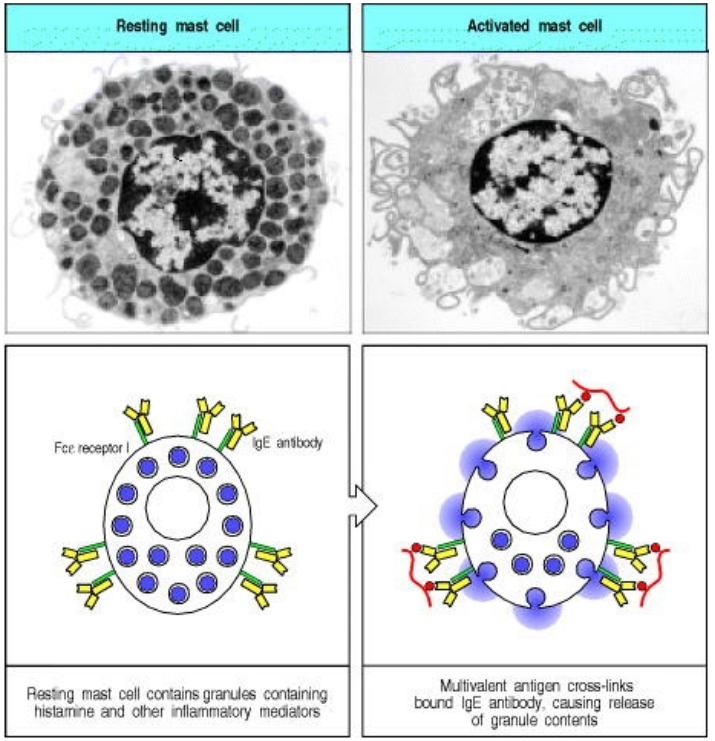
# IgA




- Dimeric immunoglobulin mainly found in mucosal secretions (saliva, tears, breast milk), although some is present in serum.
  - Monomeric forms also exist
- Contains an unusual “secretory piece” which allows IgA to cross from the basolateral to lumenal sides of epithelial cells for secretion.
- IgA deficiency can be a cause of transfusion reactions.

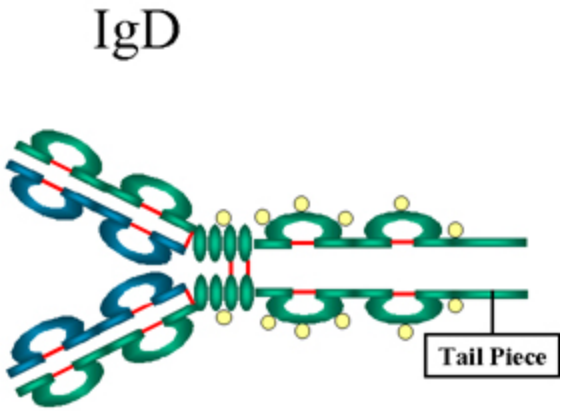
# IgE




- Main isotype involved in allergic reactions. Also plays a role in defending against parasitic infections.
- Circulating levels are normally very low, but majority is bound to tissue cells such as mast cells, eosinophils, and basophils.
- Binding and cross-linking of membrane-bound IgE leads to mast cell degranulation, histamine release, and an anaphylactoid response in sensitized patients.

# IgD




- Isotype found on the surface of naïve B cells (cells which have never been activated by antigen)
- Not secreted from plasma cells, virtually undetectable in circulation.
- Biological relevance is unclear.

# That's it for the easy part

- Any questions?

# Complement – the short version

- Complement is a group of roughly 20 proteins (mostly made by the liver) that when activated by a variety of stimuli help to destroy pathogens by:
  - Generating chemokines (to recruit cells to the site of inflammation)
  - Producing anaphylatoxins (to trigger release of inflammatory mediators)
  - Opsonizing cells (to promote phagocytosis)
  - Permeabilizing cell membranes (to cause cell lysis)

# Complement – the long version

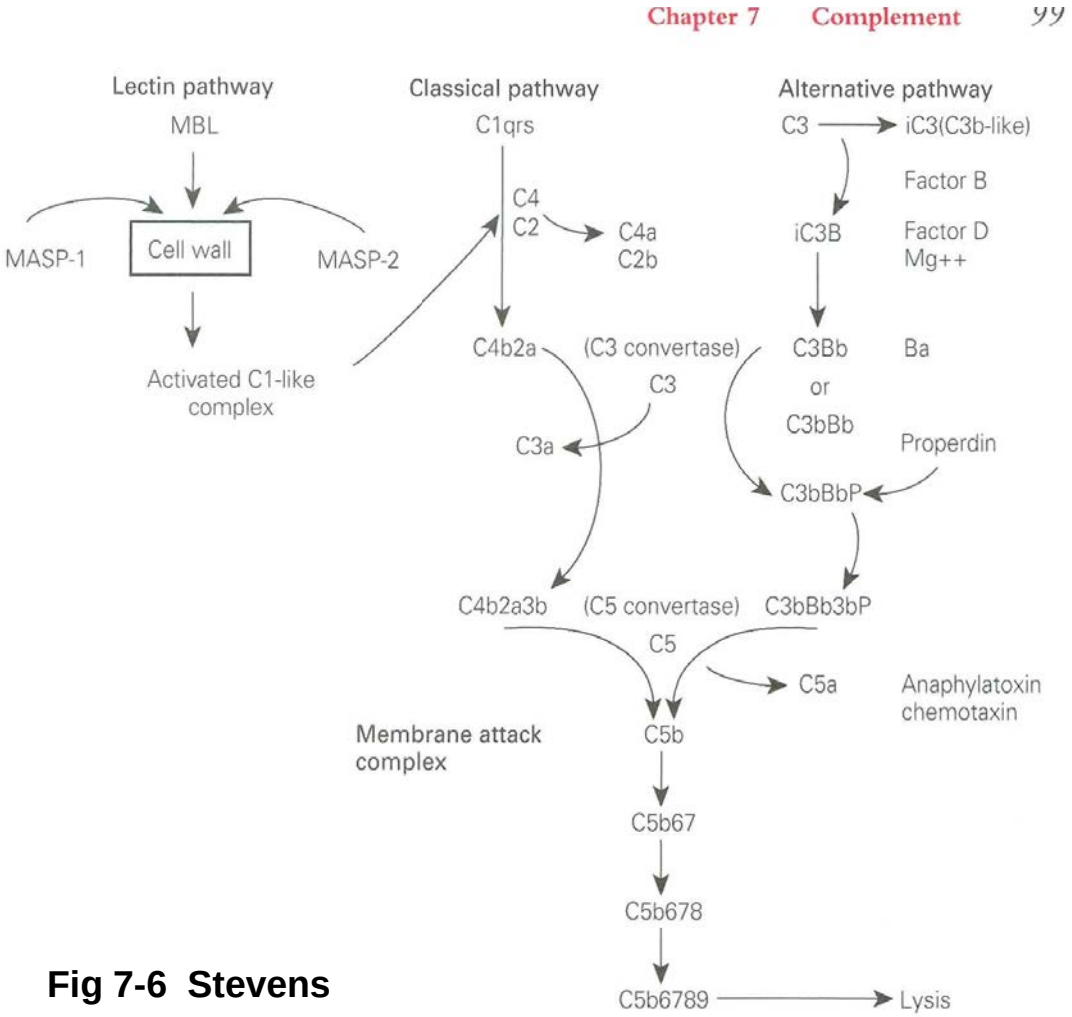



**Fig 7-6 Stevens**

## General rules

- 3 possible activation pathways that converge on a common endpoint
- Cascade of proteins which act to cleave the next one in the series to continue the reaction
- Byproducts given off by the cleavage of these proteins often have effects as well

# Nomenclature rules

- Upper case letters (“B”, “D”) or numbers (C3, C4) indicate the intact protein.
- Lower case letters (usually “a” and “b”) indicates a cleavage fragments of a larger protein.
- The “b” fragment is usually the larger fragment which binds to the cell, while the “a” fragment is usually the smaller fragment that is released.
  - (Except for C2a and C2b, which are reversed)
- Inactive fragments are labeled with an “i”. Active fragments are sometimes indicated by a bar across the top of the protein name.

# The classical pathway

- The classical pathway is the main antibody-mediated way to trigger complement activation
  - IgM and IgG1-3 can use this pathway
- Activation requires binding of 2 IgG molecules near each other to start the cascade
  - 1 IgM molecule will also work, because of its pentameric nature
- Activation proceeds through three stages
  - The recognition unit (C1)
  - The activation unit (C4, C2, and C3)
  - The membrane attack unit (C5 – C9)

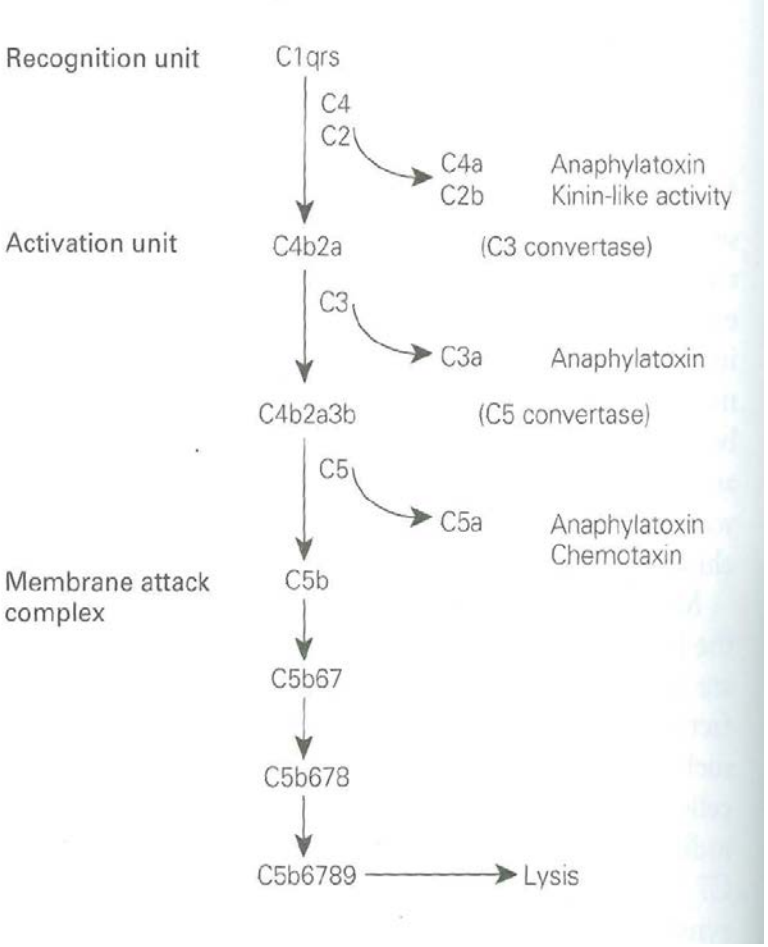



**Fig 7-1 Stevens**

# The recognition unit (C1qrs)

START: C1 complex binds to Fc regions on 2 adjacent IgG antibodies (or 1 IgM)

C1q binding activates C1r

Activated C1r cleaves and activates C1s

END: C1s is activated, and ready to cleave next 2 proteins in cascade (C4 and C2)

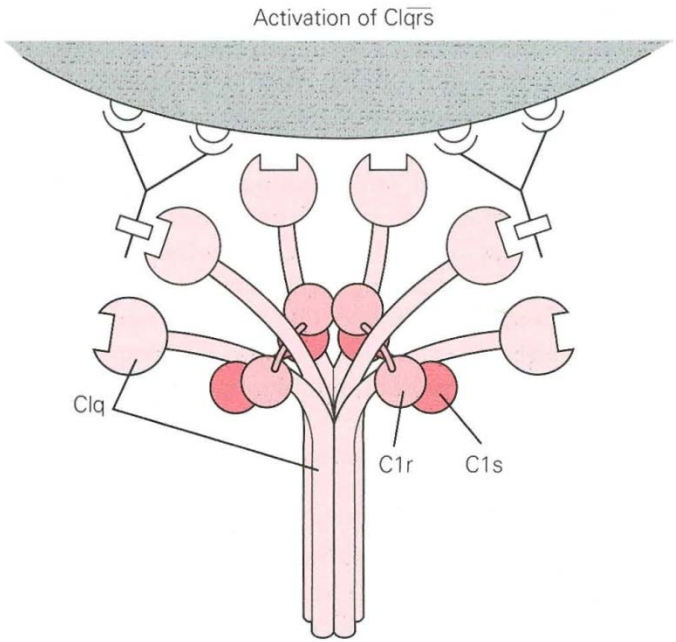



**Fig 7-2 Stevens**

# The activation unit (C4b2a3b)

START: Activated C1qrs on surface of cell

↓

C1qrs cleaves C4 and C2

↓

C4b and C2a form a complex on cell surface. This complex can now cleave lots of C3 (200x)

↓

Lots of C3b deposits on cell surface. C3b alone acts as opsonin. C3b near C4b2a becomes "C5 convertase"

↓

END:

1) "C5 convertase" (C4b2a3b) on surface of cell ready to cleave C5 and start forming membrane attack complex
2) C4a, C2b, and C3a released to stimulate various effects

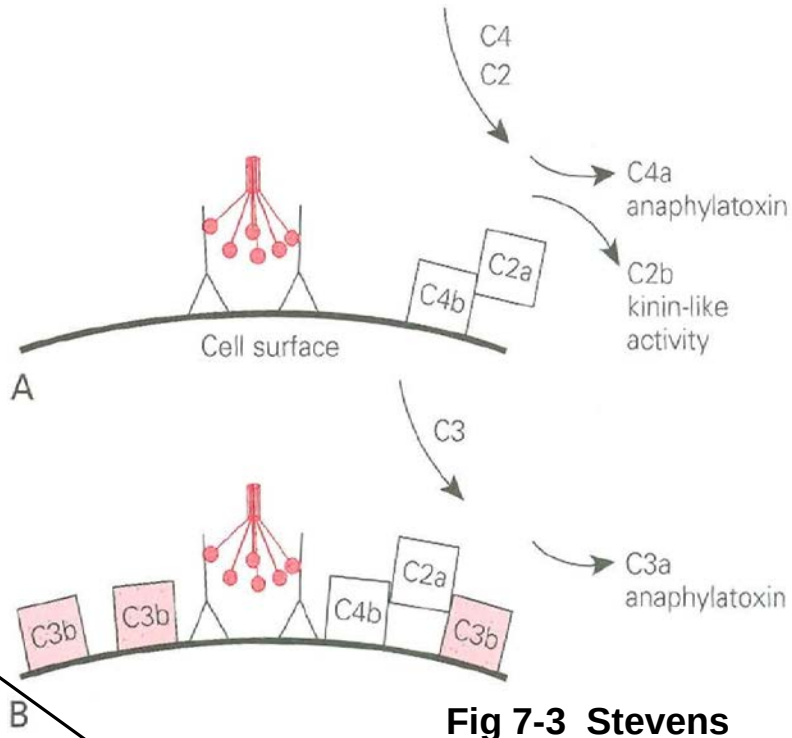



**Fig 7-3 Stevens**

C3 is the most abundant complement protein, and is the key intermediary in all of the cascades

# The membrane attack unit (C5b6789)

START: Activated C4b2a3b on cell surface

↓

C4b2a3b cleaves C5

↓

C5b binds to cell membrane, and recruit C6, C7 and C8

↓

C9 molecules polymerize and forms a transmembrane pore

↓

END: C5b6789 forms the MAC, which leads to cell lysis

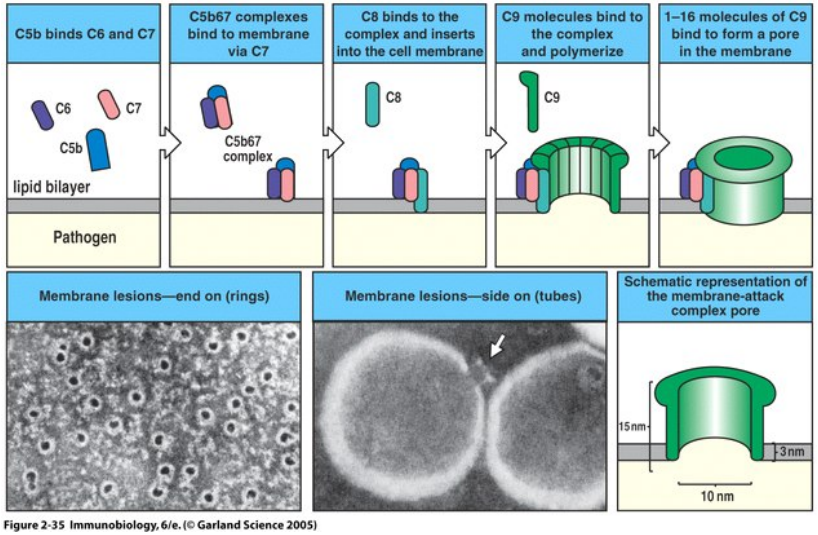


Figure 2-35 Immunobiology, 6/e. (© Garland Science 2005)

**The formation of the MAC and resulting cell lysis is the main point of complement activation (although the release of various cofactors is an added bonus)**

# So what are all those cleavage fragments doing?

- Many of the proteins formed during enzymatic cleavage (aka, “split products”) have pro-inflammatory functions

| Split product | Actions | Definition |
|---|---|---|
| C4a | Weak anaphylatoxin | Increases vascular permeability, releases histamine |
| C2b | Kinin-like activity | Dilate blood vessels, increase vascular permeability |
| C3a | Anaphylatoxin | |
| C5a | Potent anaphylatoxin<br>Chemotaxin | Attracts neutrophils, basophils, monocytes |

# The alternative pathway

- A related cascade that activates complement without using antibodies triggered by :
  - bacterial or fungal cell walls
  - Virally infected cells
  - Some parasites (trypanosomes)

START: "naturally" inactivated C3 in circulation binds factor B

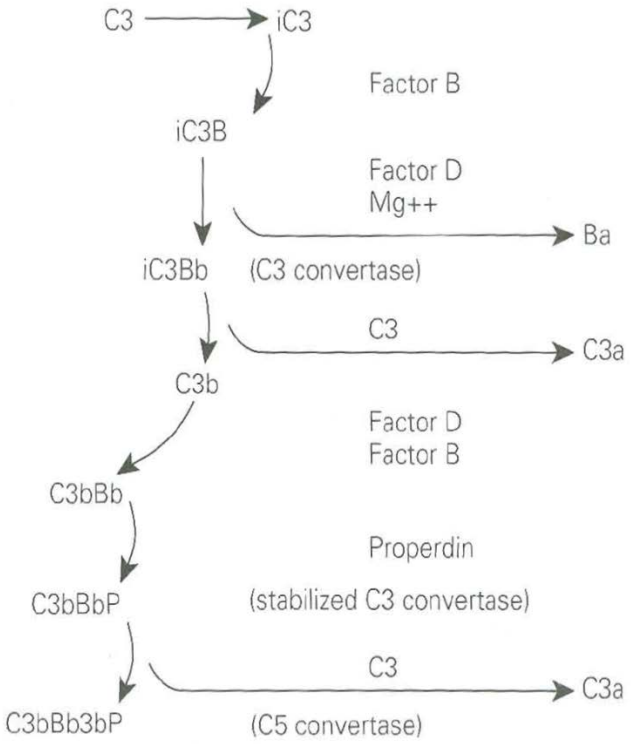
↓

iC3B is cleaved by Factor D to become a C3 convertase (iC3Bb)

↓

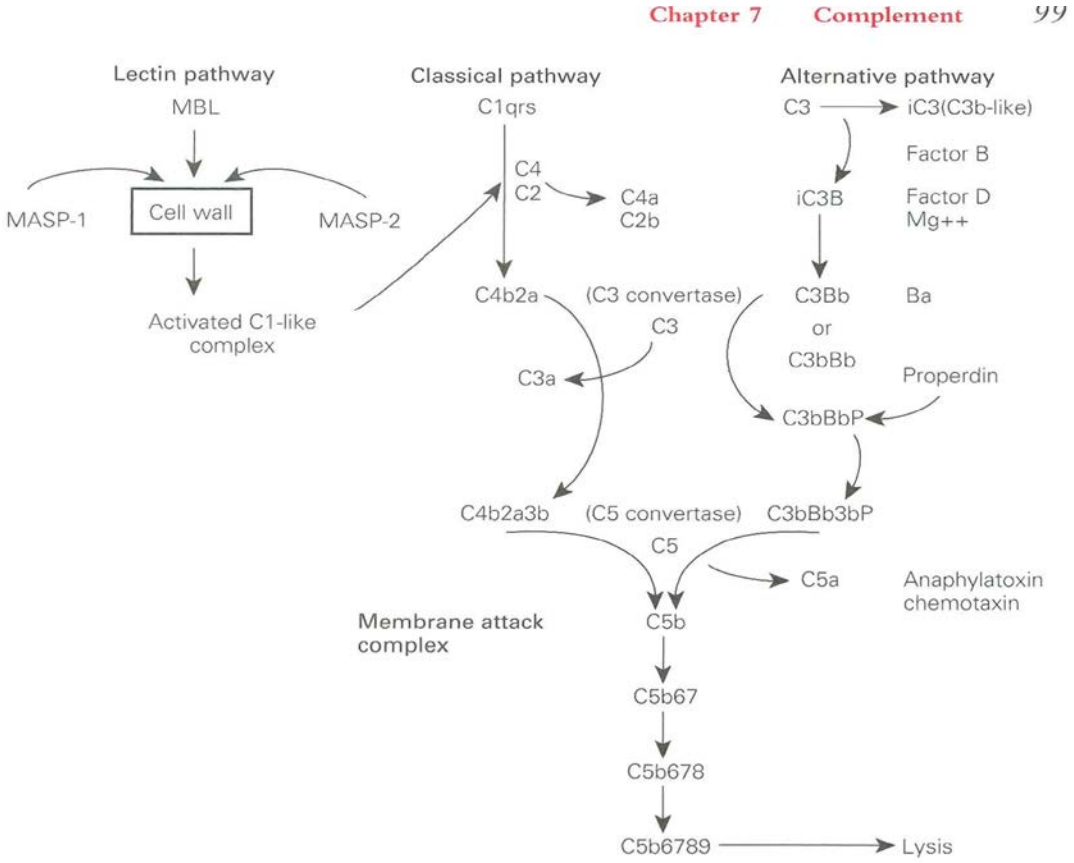
C3bBb complex is stabilized by Properdin to form a C5 convertase (C3bBb3bP)

END: C3bBb3bP complex can cleave C5, to start forming MAC




**The alternative pathway is just a different way to start the process – but the end goal is the same (make an enzyme that will convert C5 -> C5b so you can create the MAC)**

# Convergence of the two pathways

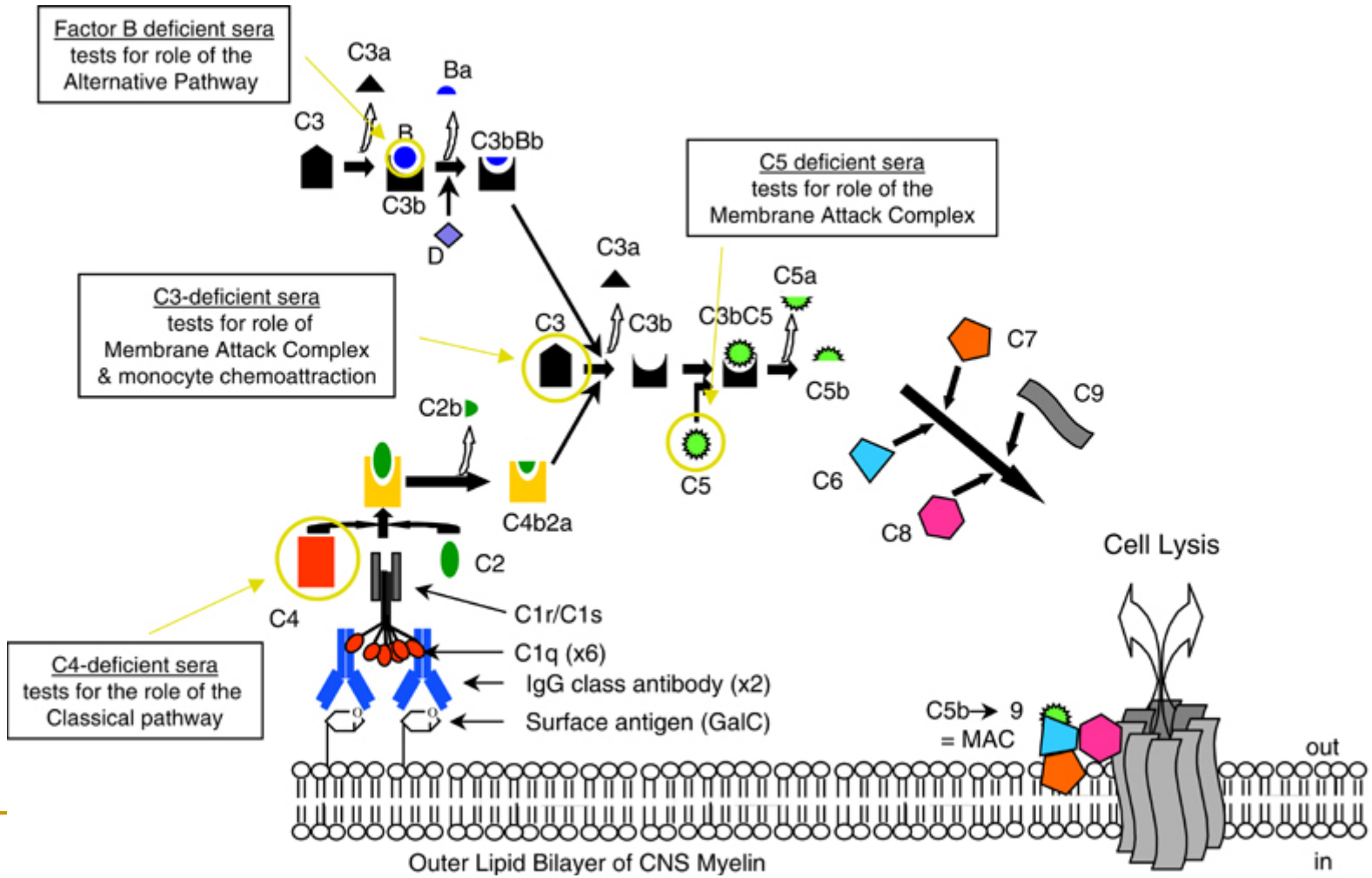


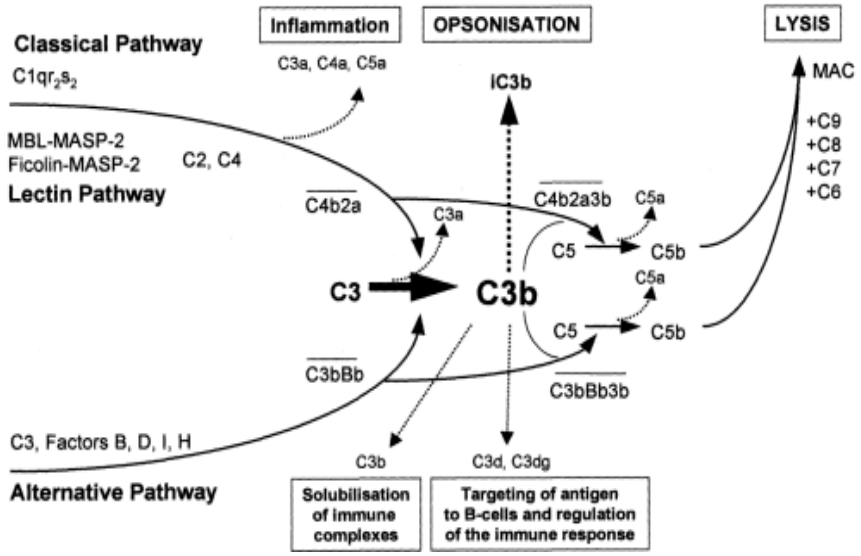

- Both pathways use C3 as the major molecule for amplifying the pathway
- Both ultimately enable the formation of the MAC to lyse pathogens.

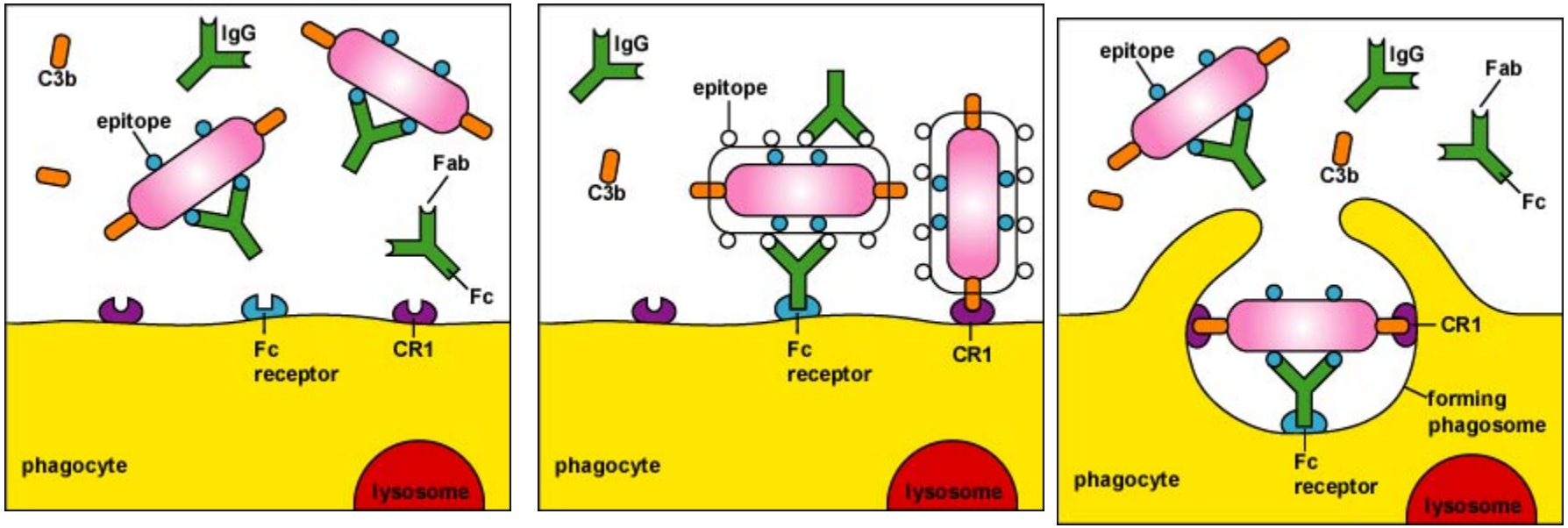
# Conclusions

- Innate and adaptive immune responses work together to eliminate pathogens from the body
- Antibodies play an important role in the adaptive immune response via a number of mechanisms
- Complement acts as a final common factor for both the innate and adaptive systems to promote cell lysis.

# Extra figures


Factor B deficient sera
tests for role of the
Alternative Pathway
C3a
Ba
C3
B
C3bBb
C3b
D
C5 deficient sera
tests for role of the
Membrane Attack Complex
C3a
C5a
C3-deficient sera
tests for role of
Membrane Attack Complex
& monocyte chemoattraction
C3
C3b
C3bC5
C7
C9
C5b
C2b
C5
C6
C8
C4b2a
Cell Lysis
C2
C4
C1r/C1s
C1q (x6)
IgG class antibody (x2)
C4-deficient sera
tests for the role of the
Classical pathway
Surface antigen (GalC)
C5b→ 9
= MAC
out
Outer Lipid Bilayer of CNS Myelin
in


Inflammation
OPSONISATION
LYSIS
Classical Pathway
C1qr2s2
C3a, C4a, C5a
iC3b
MAC
+C9
+C8
+C7
+C6
MBL-MASP-2
Ficolin-MASP-2
C2, C4
Lectin Pathway
C4b2a
C3a
C4b2a3b
C5a
C5
C5b
C3
C3b
C5a
C5
C5b
C3bBb
C3bBb3b
C3, Factors B, D, I, H
Alternative Pathway
C3b
C3d, C3dg
Solubilisation of immune complexes
Targeting of antigen to B-cells and regulation of the immune response


C3b
IgG
epitope
Fab
Fc
Fc receptor
CR1
phagocyte
lysosome
IgG
epitope
C3b
Fc receptor
CR1
phagocyte
lysosome
epitope
IgG
Fab
C3b
Fc
CR1
forming phagosome
Fc receptor
phagocyte
lysosome