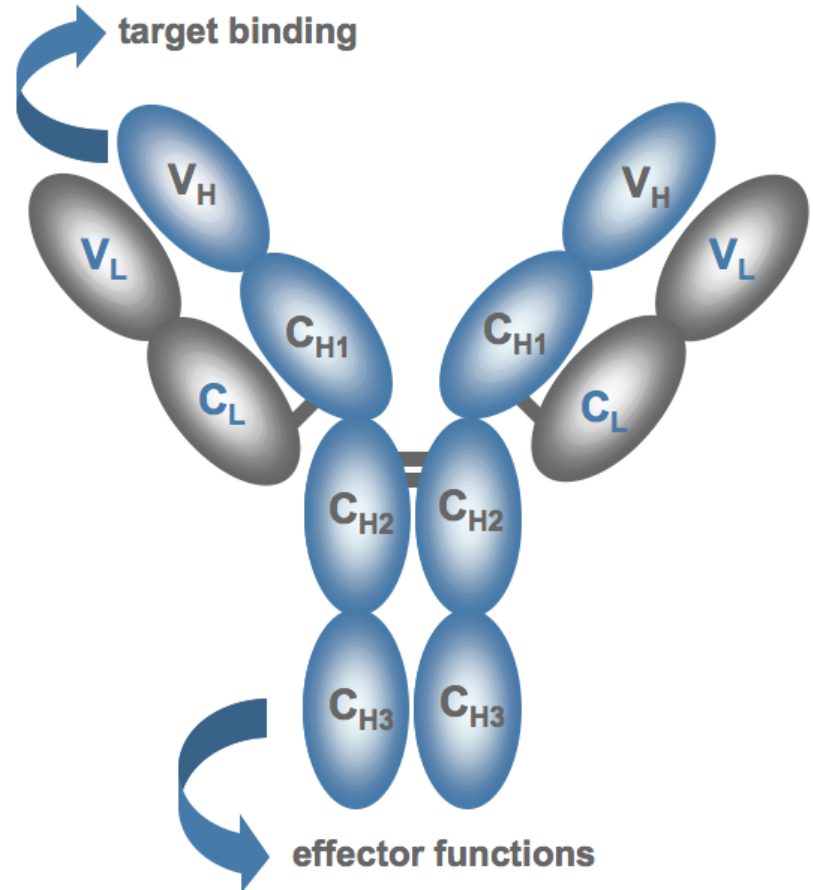
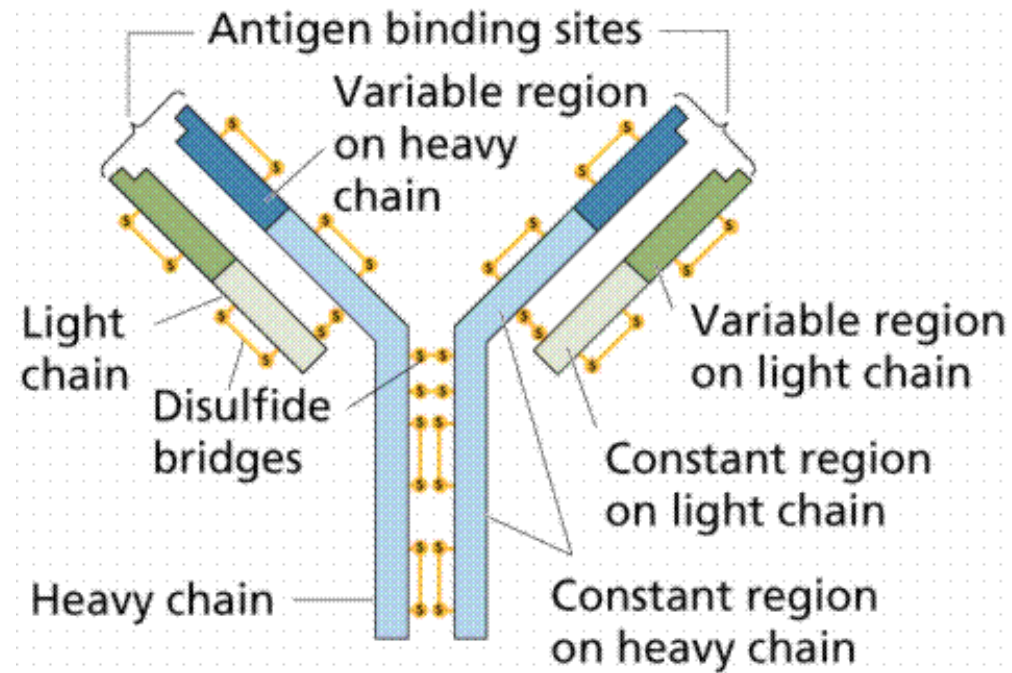
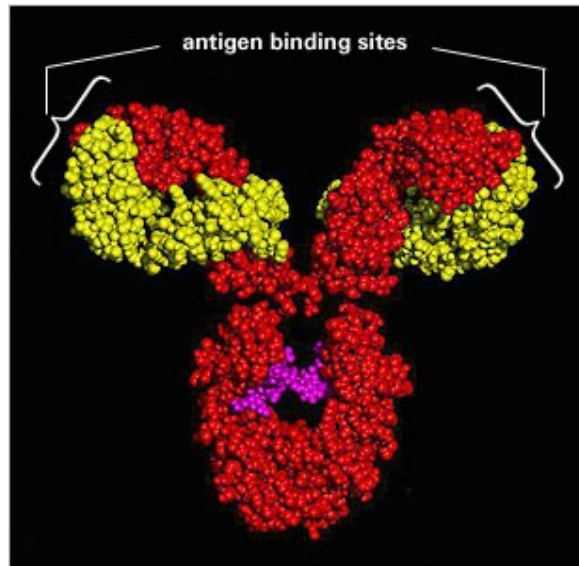
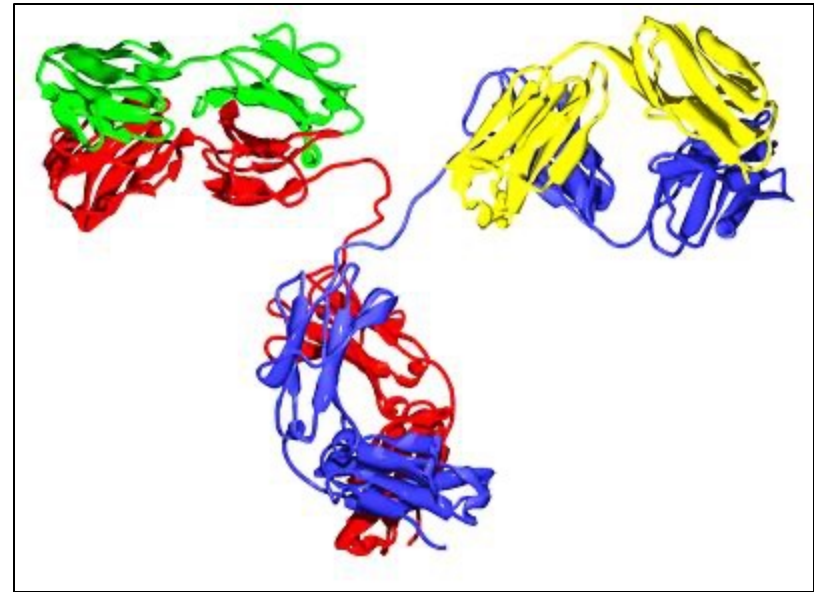
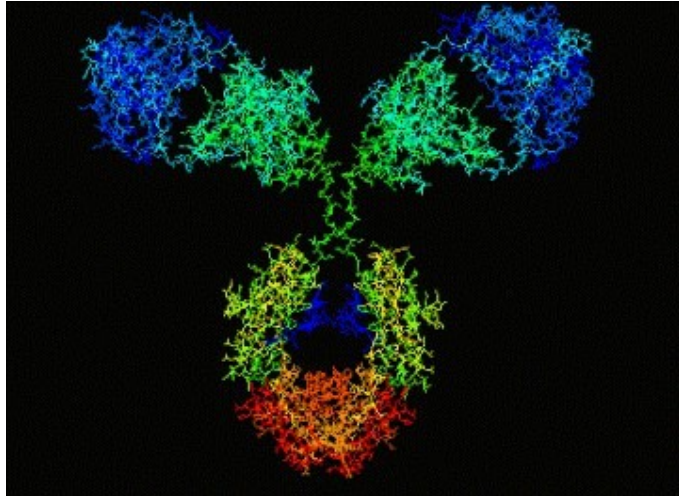


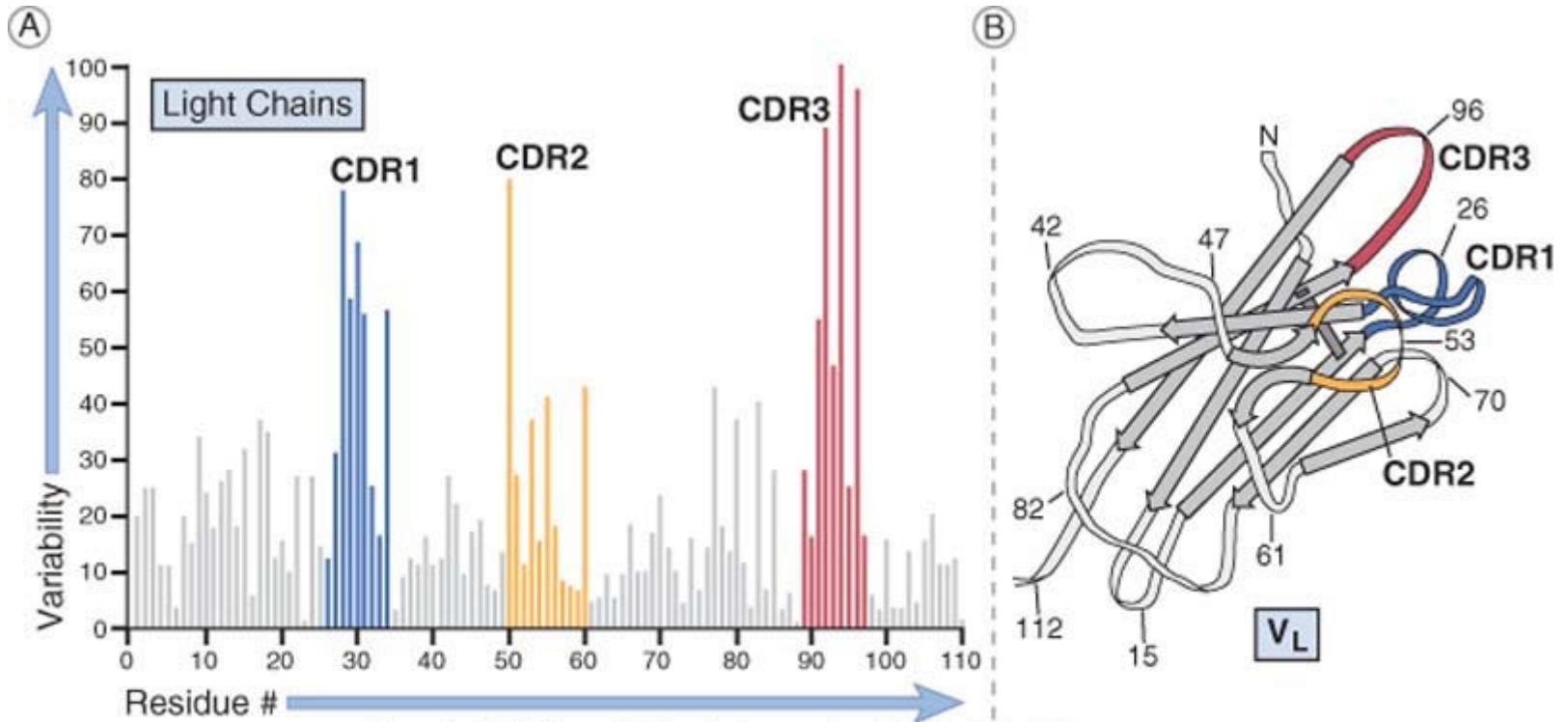
Mechanisms of action of monoclonal antibodies

Antibody





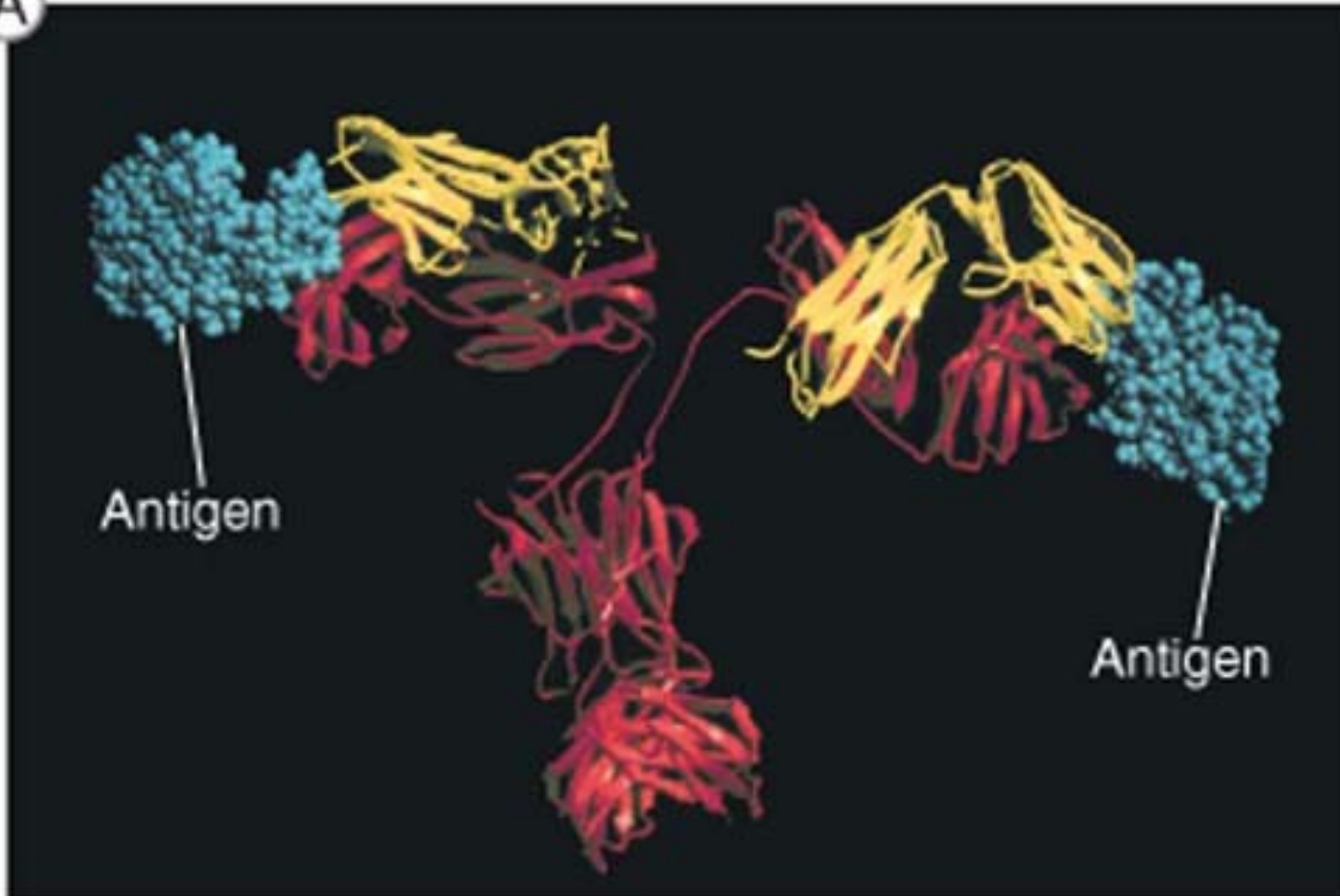
Aminoacid variability in antibody sequence



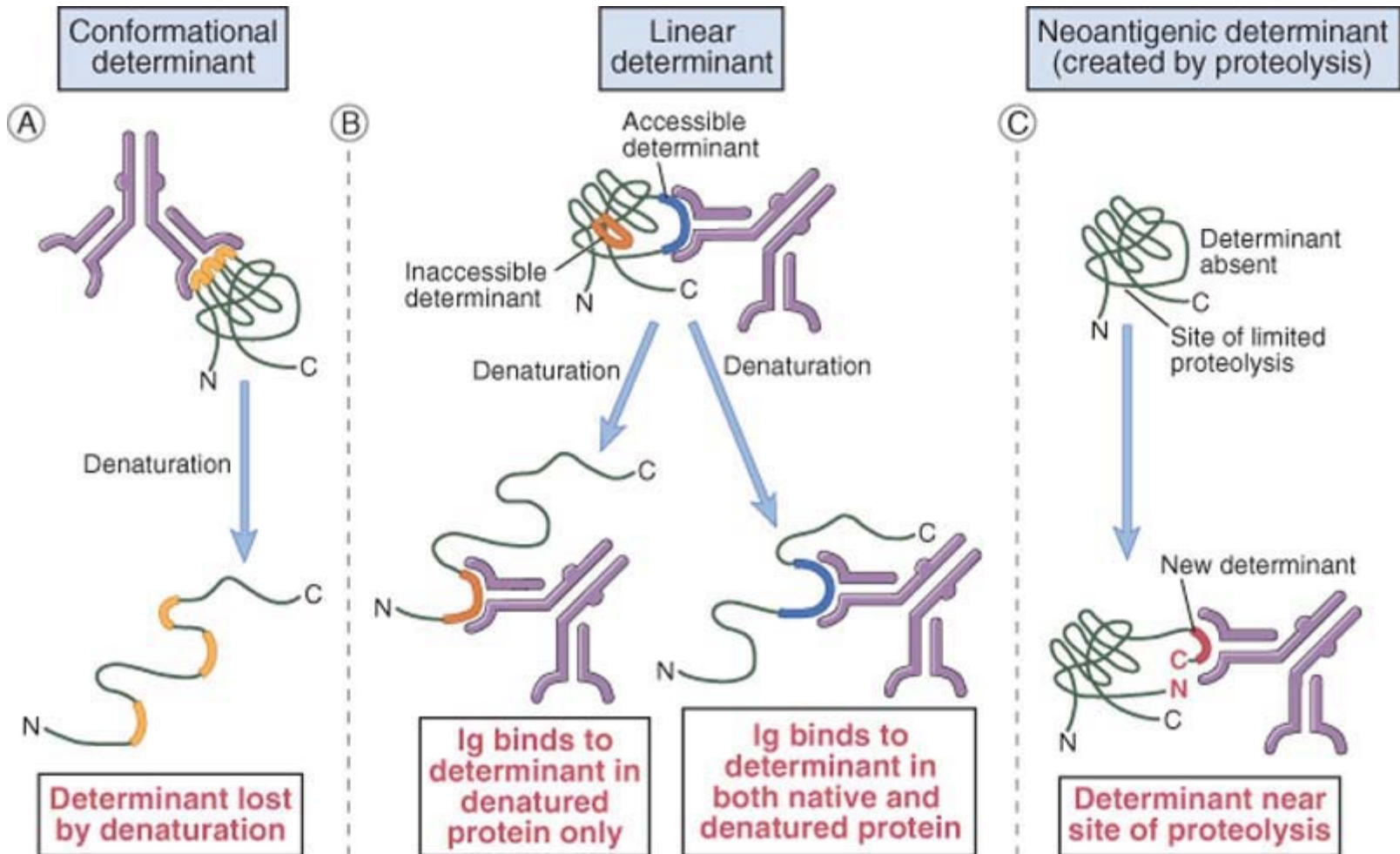
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Antigen/antibody complex

A

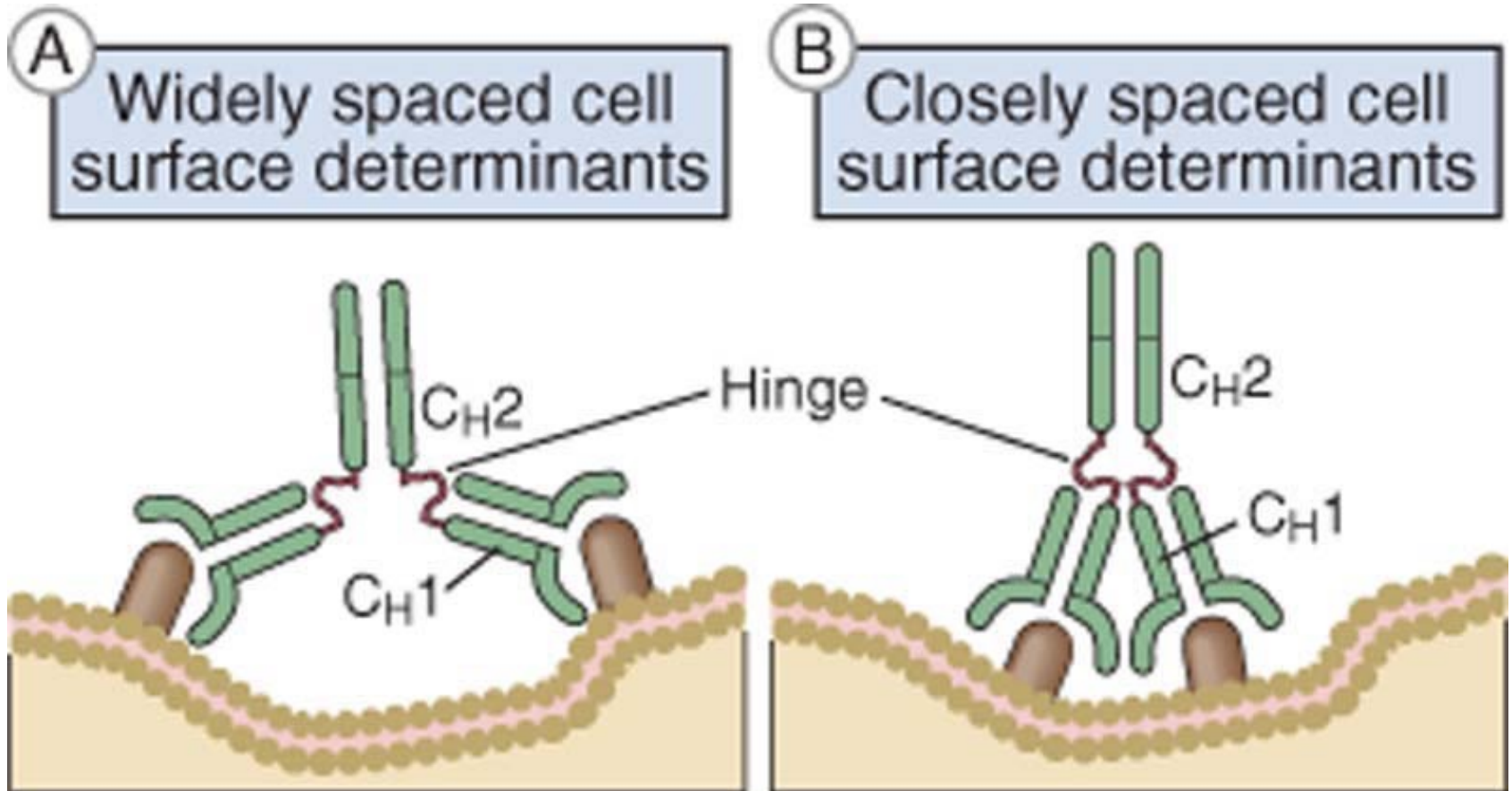


Antigenic determinant (epitope)



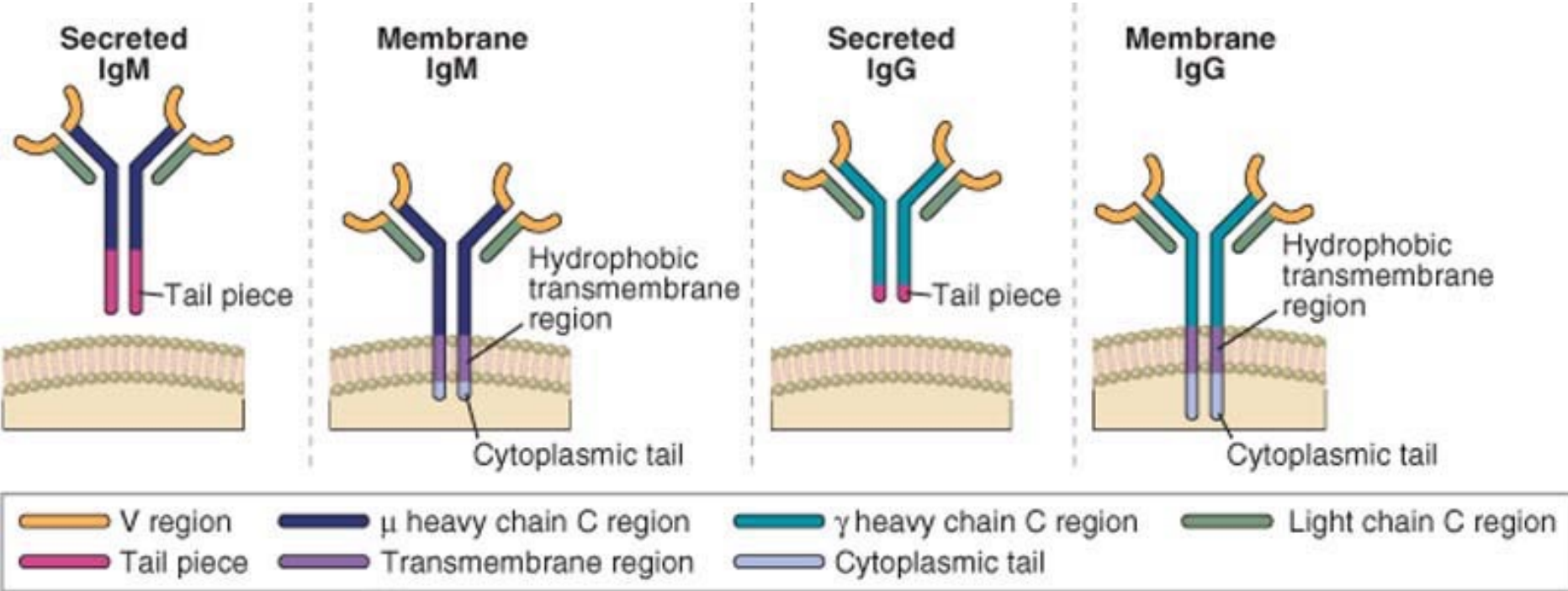
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Antigen/antibody complex on cell membrane



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secreted and membrane antibodies



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class	subclass	plasma concentration (mg/ml)	halflife in plasma (days)	Secreted form
IgA	1,2	3,5	6	<p>IgA (dimer) Monomer, dimer, trimer</p>
IgD	-	-	3	-
IgE	-	0,05	2	<p>IgE Monomer</p>
IgG	1-4	13,5	23	<p>IgG1 Monomer</p>
IgM	-	1,5	5	<p>IgM Pentamers, hexamers</p>

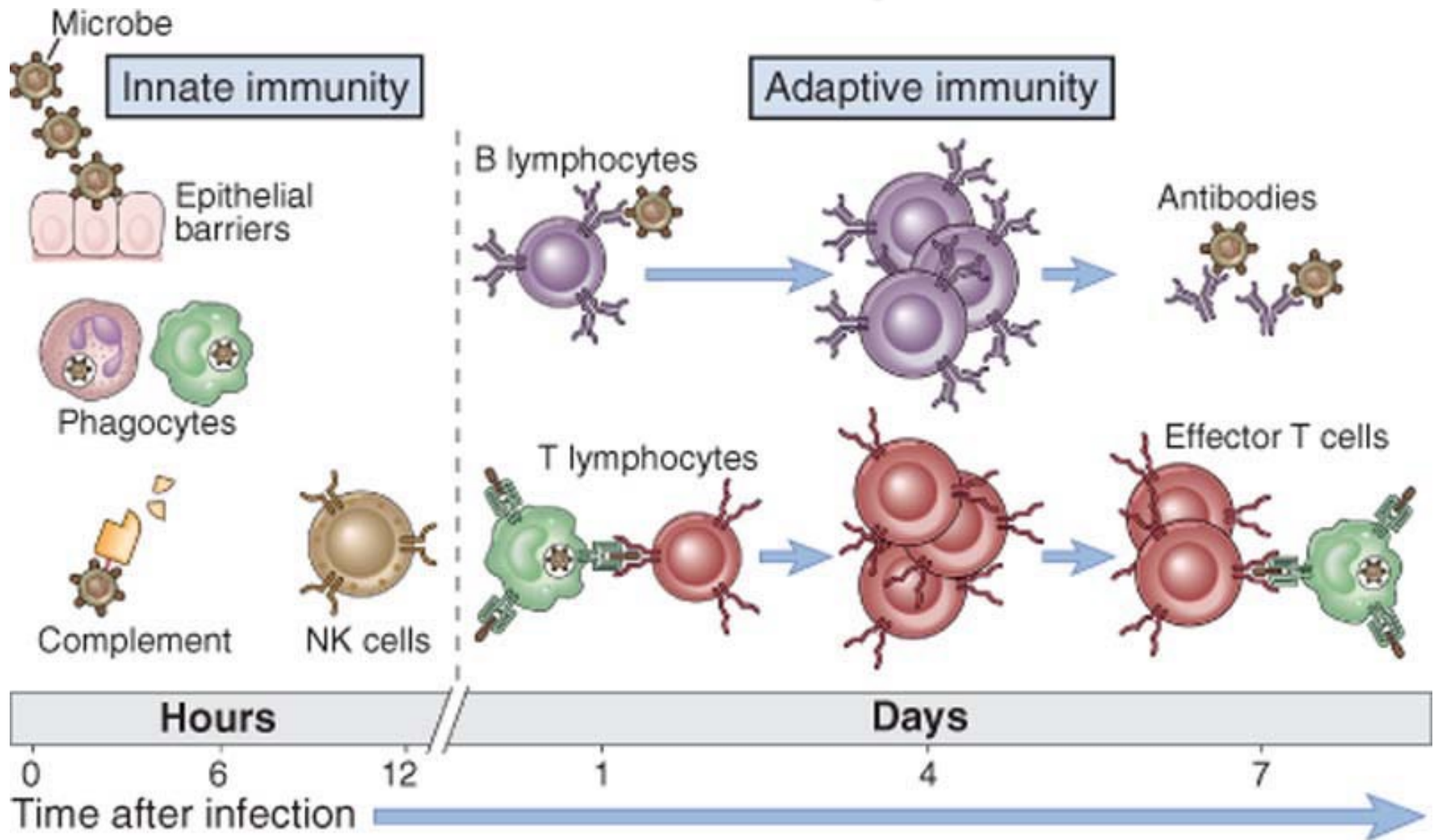
Antibody Isotope	Isotype-specific effector functions
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Characteristics of an antibody

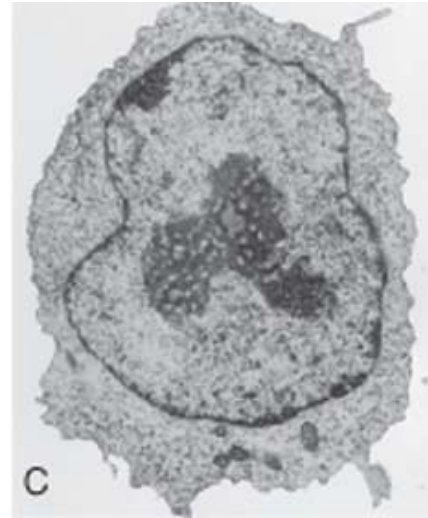
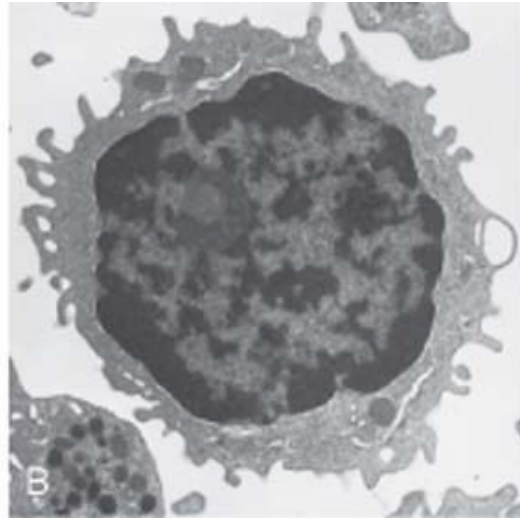
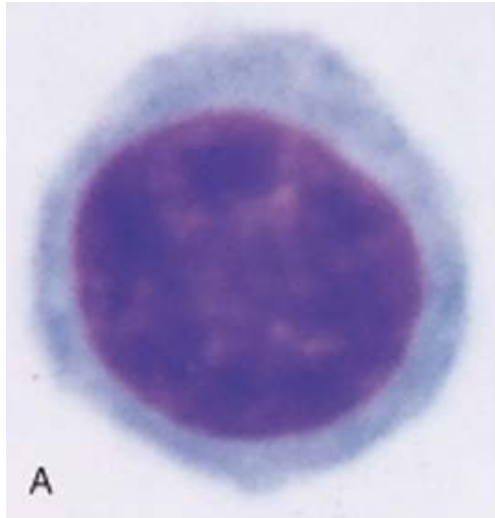
- **Specificity in its action**
- Biodistribution/half-life
- Activation of immune system

Immune response

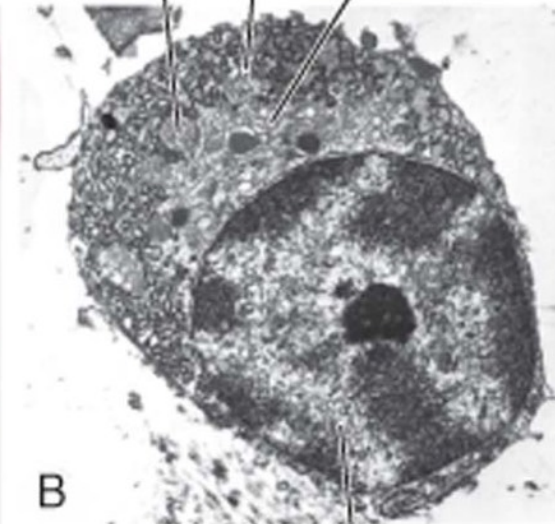
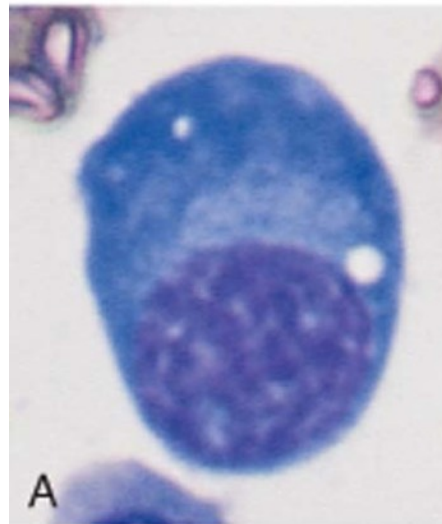


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B lymphocytes and plasma cells



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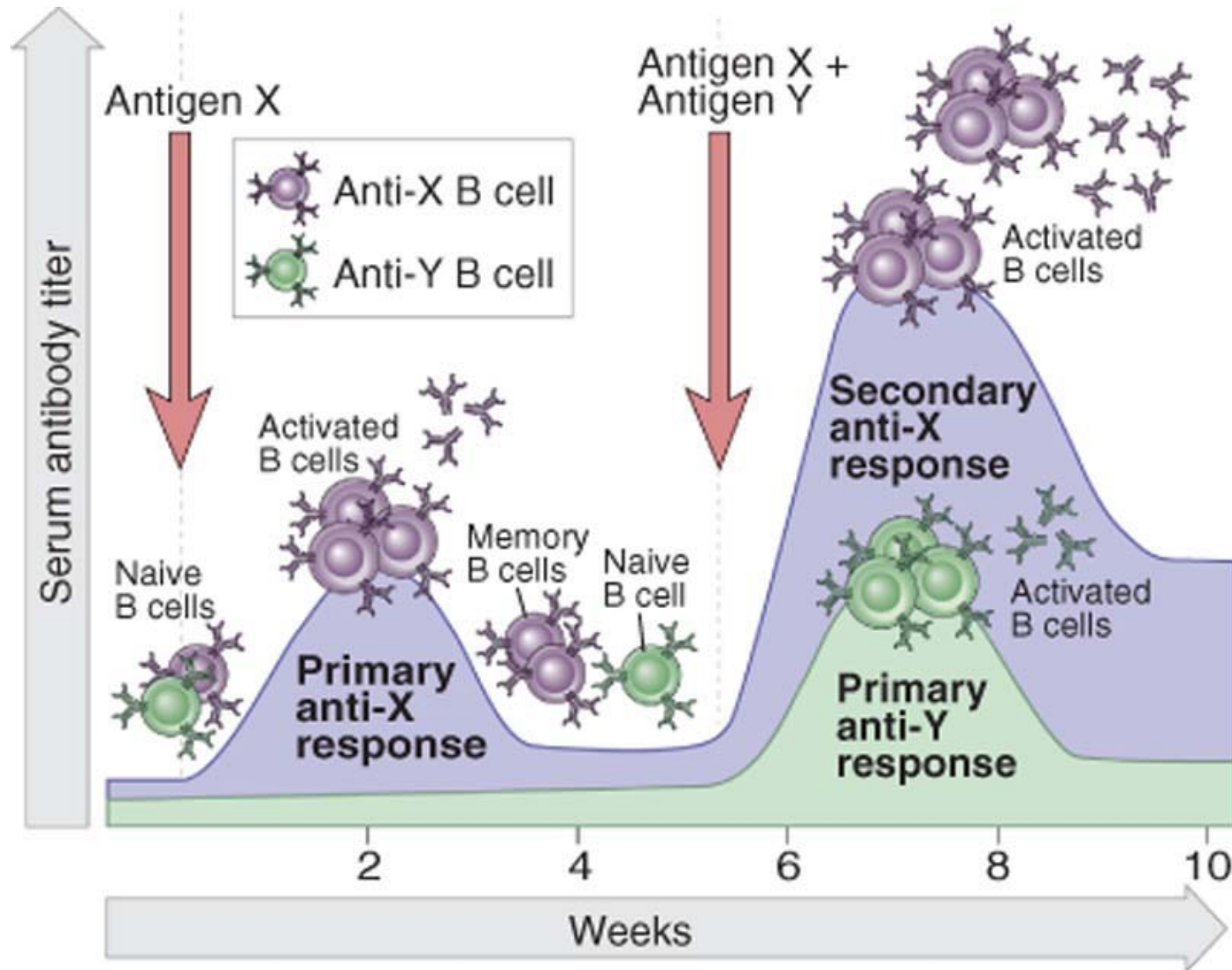


Rough endoplasmic reticulum
Mitochondrion
Golgi complex

Nucleus

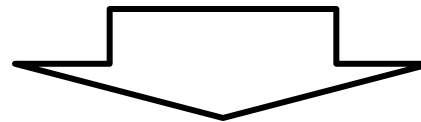
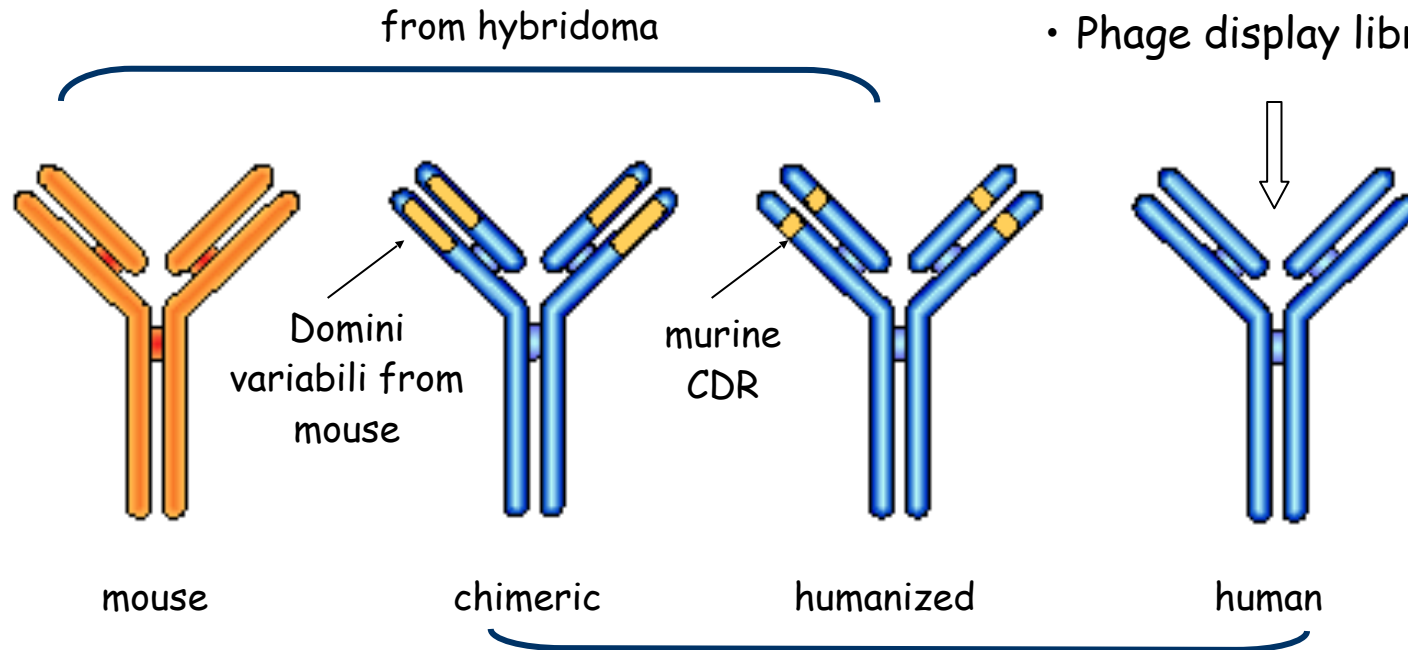
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Timing for antibody production



Antibody engineering

- transgenic mice
- Phage display library



induce immune-reaction;
short half life; murine Fc
poorly activate human
immune functions

less immunogenic;
longer half life; human Fc
activate human immune
functions

Types of antibodies/antibody fragments

1. Antagonist/Blocker
2. Agonist
3. Immuno-activator

1. Blocking antibodies

An antibody able to bind an antigen and neutralize its function

Examples of neutralizing antibodies used in clinic

1. Natalizumab: humanized anti-CD49d (multiple sclerosis)
2. Eculizumab: humanized (IgG4) anti-C5 (PNH, Hemolytic Uremic syndrome, glomerulonephritis)
3. Bevacizumab: humanized anti-VEGF (colon-rectal cancer, lung carcinoma, other solid tumors, macula degeneration)
4. Cetuximab: chimeric anti-EGFR (colon-rectal cancer)
5. Infliximab: chimeric anti-TNF α (Rheumatoid arthritis, Crohn disease and other inflammatory diseases)
6. Adalimumab: human anti-TNF α (Rheumatoid arthritis, Crohn disease and other inflammatory diseases)
7. Pembrolizumab: humanized anti-PD1 (cancer immunotherapy)

2. Agonistic antibodies

Antibodies able to bind and activate its target (usually cell membrane receptors)

These antibodies can be used to induce cell apoptosis (for cancer therapy) or to induce immune cell proliferation (in immune deficiencies)

Examples of agonistic antibodies

1. Trastuzumab: humanized anti-HER2 (breast cancer)
2. Tigatuzumab: humanized anti-TRAIL-R1 (breast cancer)
3. humanized anti-IL2R (infections, immune deficiencies, cancer immunotherapy)

3. Immune-activator antibodies

Antibodies able to bind their target (cell membrane receptors) and activate immune response.

Used in cancer immunotherapy (for the killing of cancer cells) or in autoimmune diseases (to eliminate B lymphocytes and, as a consequence, the production of autoantibodies)

Examples of immune-activators antibodies

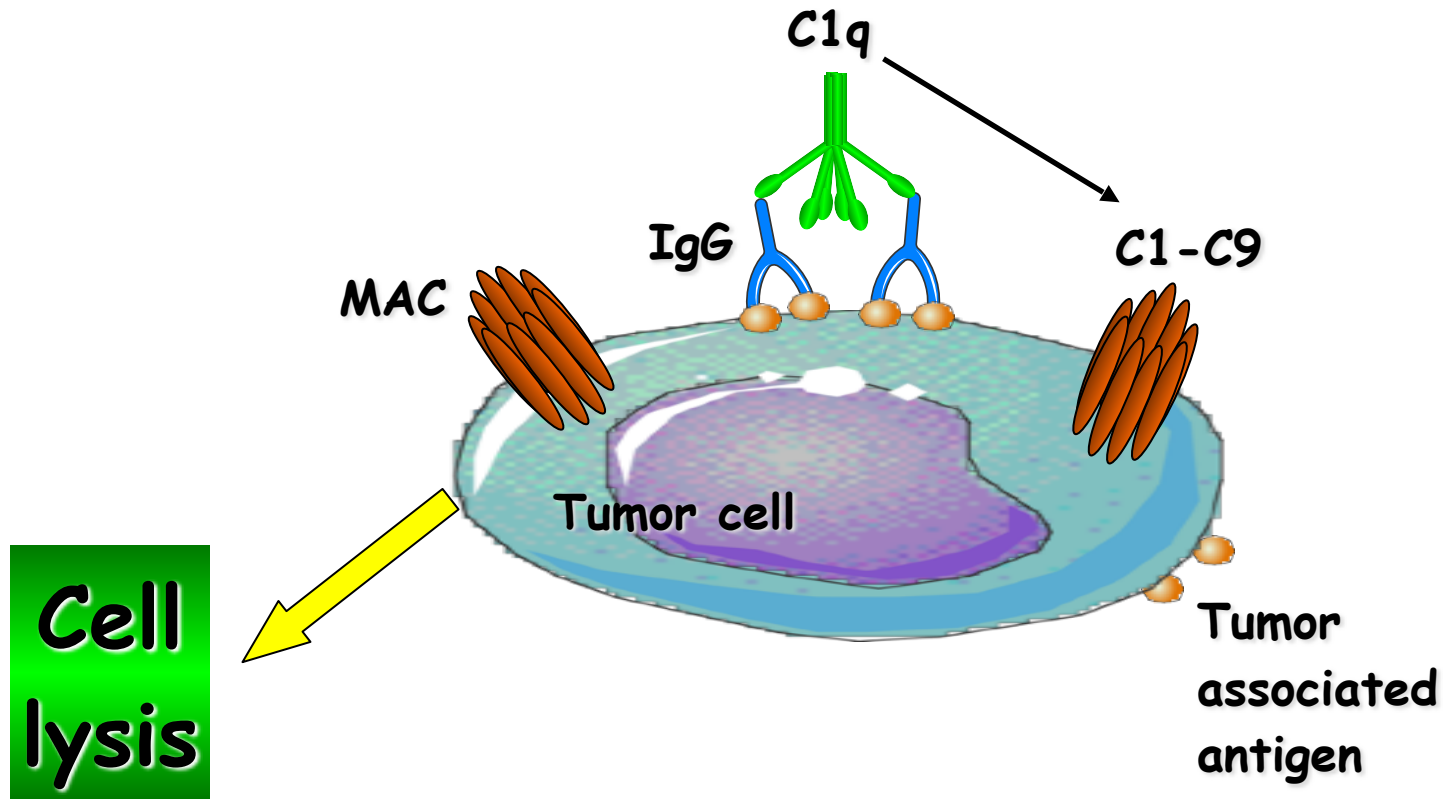
1. Rituximab: chimeric anti-CD20 (B cell lymphoma and leukemia, rheumatoid arthritis)
2. Alemtuzumab: humanized anti-CD52 (B or T cell lymphoma and leukemia)
3. Ofatumumab: human (IgG1) anti-CD20 (B cell lymphoma and leukemia)
4. Trastuzumab: humanized anti-HER2 (breast cancer)
5. cMOV18 e cMOV19: umanizzati anti-folate receptor (alpha isoform) (Ovarian cancer)

class	subclass	plasma concentration (mg/ml)	halflife in plasma (days)	Secreted form
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IgD	-	-	3	-
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IgE	Mast cell degranulation (immediate hypersensitivity reactions)
IgD	Antigen receptor of naive B lymphocytes

Immune-activator monoclonal antibodies:

CDC (Complement-Dependent Cytotoxicity)



ALTERNATIVE PATHWAY

LECTIN PATHWAY

CLASSICAL PATHWAY

Activating surfaces

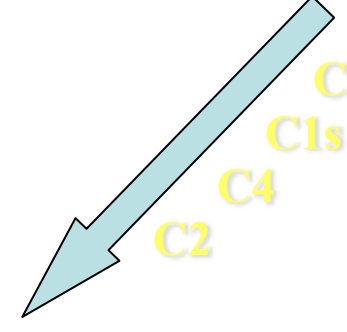
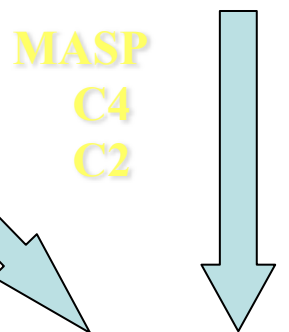
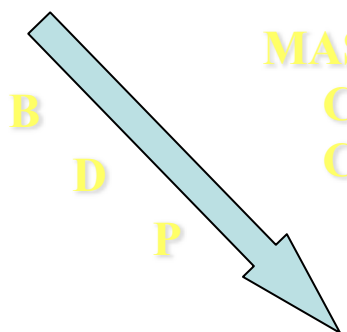
Carbohydrates

Immune complexes

C3b C3H₂O

MBL

C1q



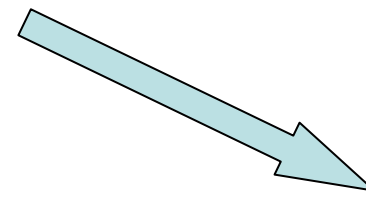
C3

RECOGNITION



C3b

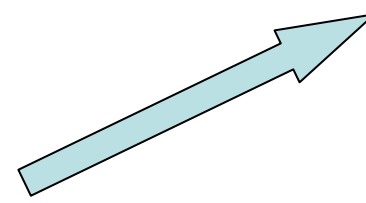
OPSONIZATION



C3a
C5a

INFLAMMATION

C5

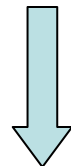


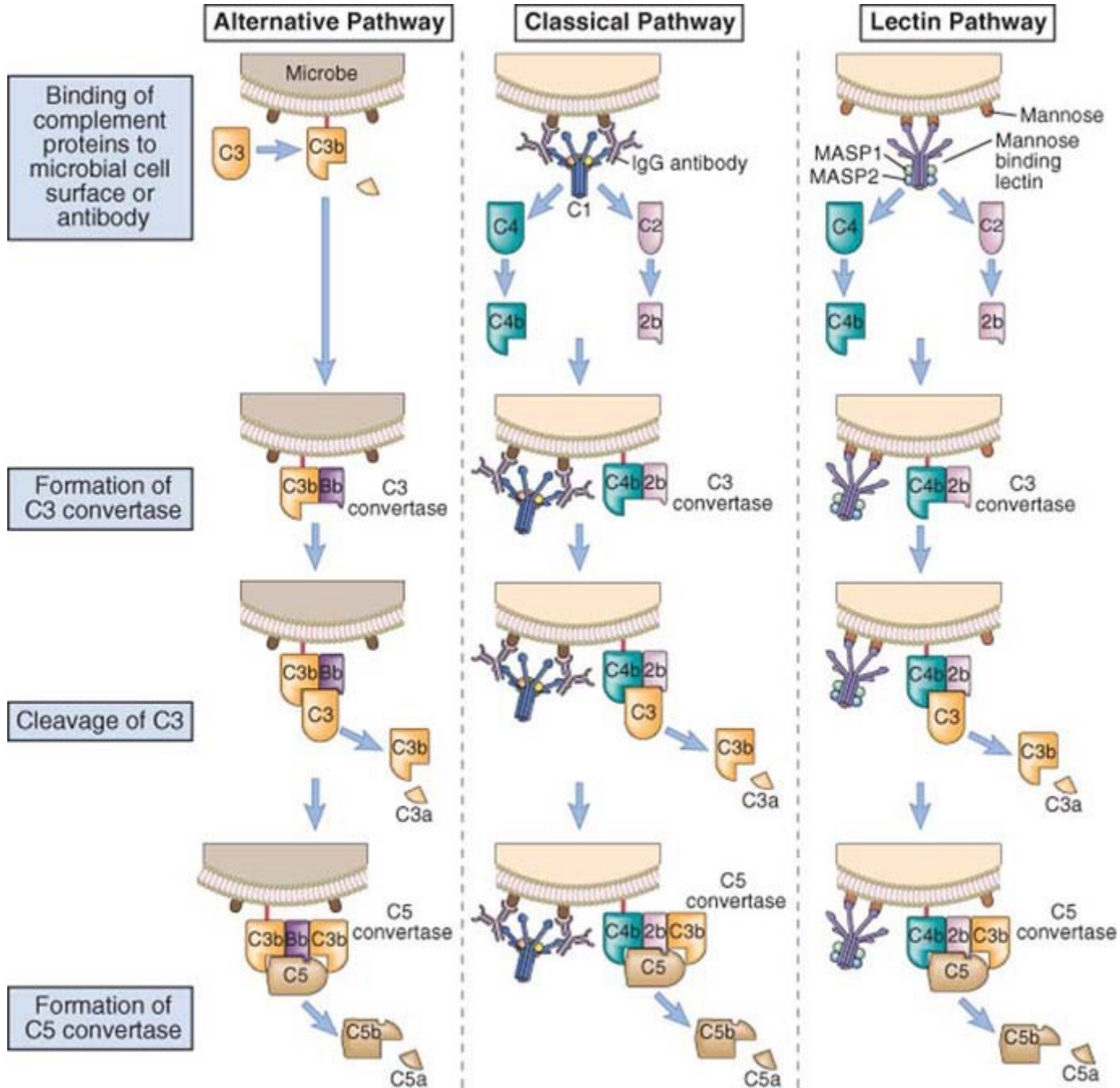
C6C7

C8 C9

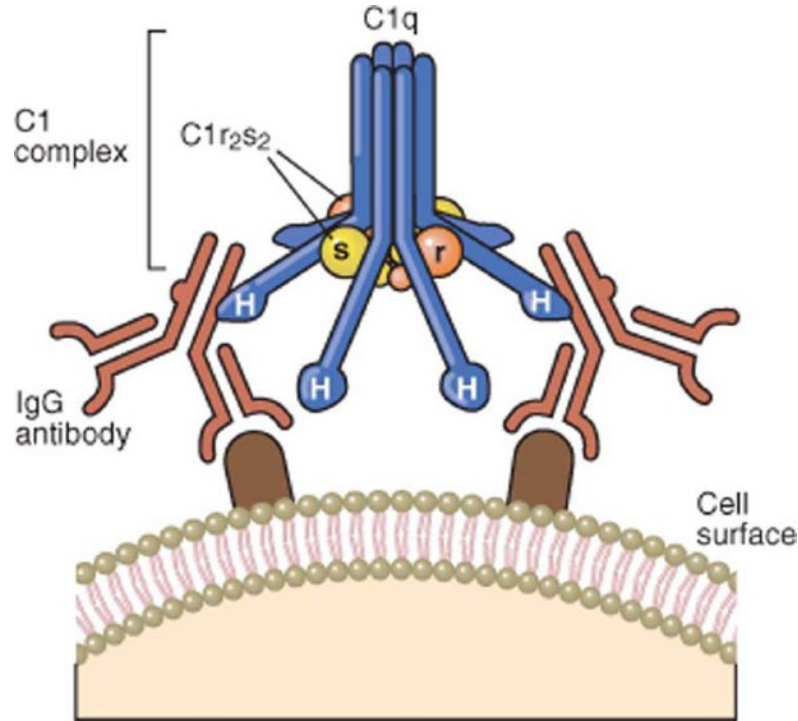
CYTOLYSIS
INFLAMMATION

C5b-9

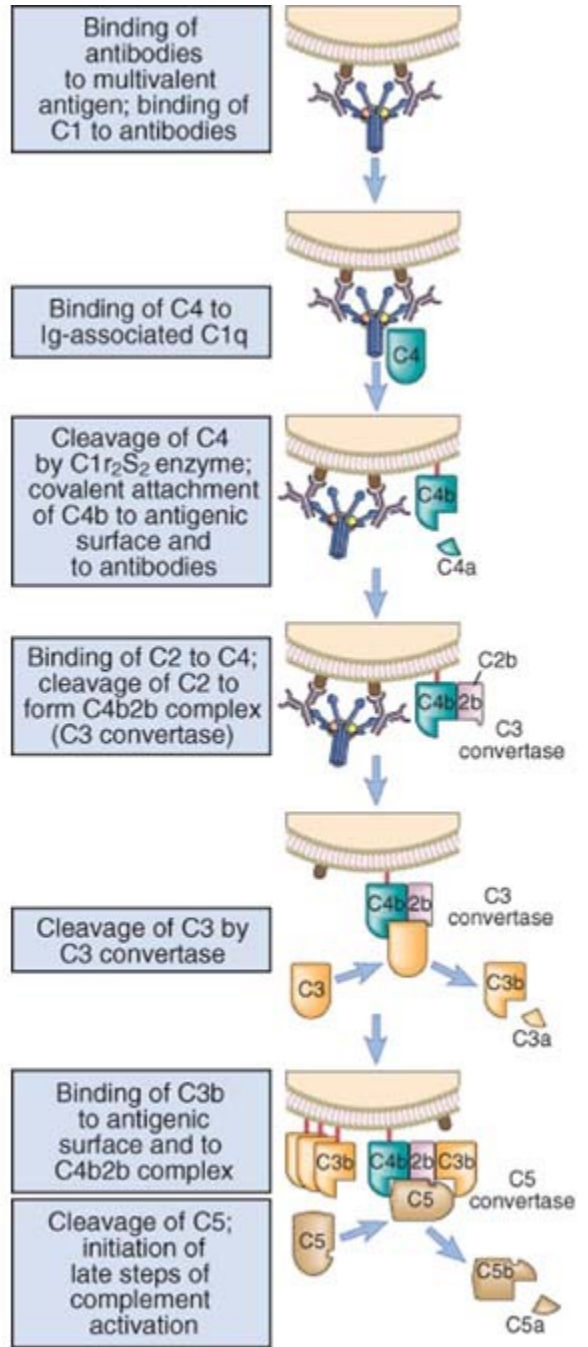




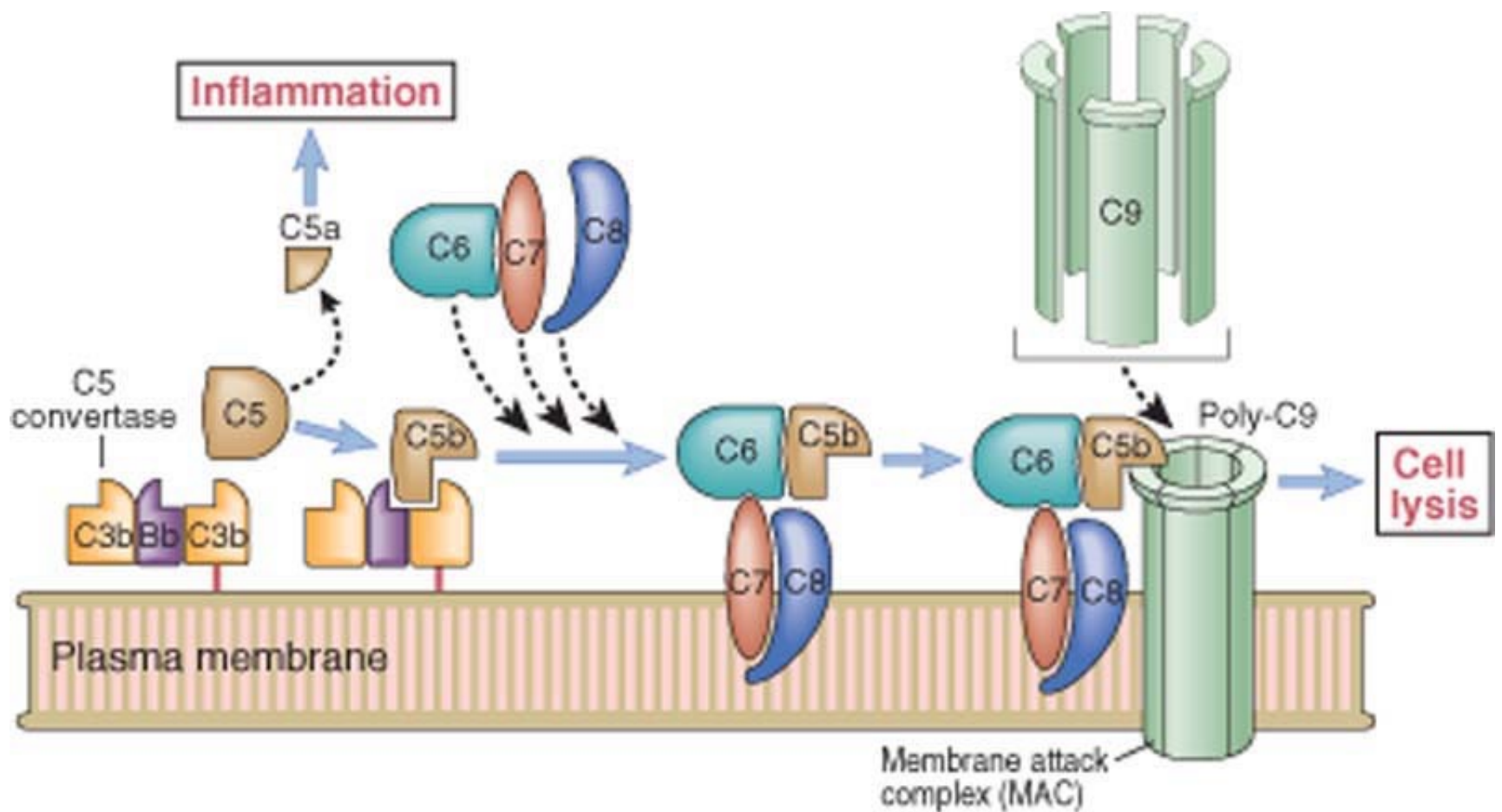
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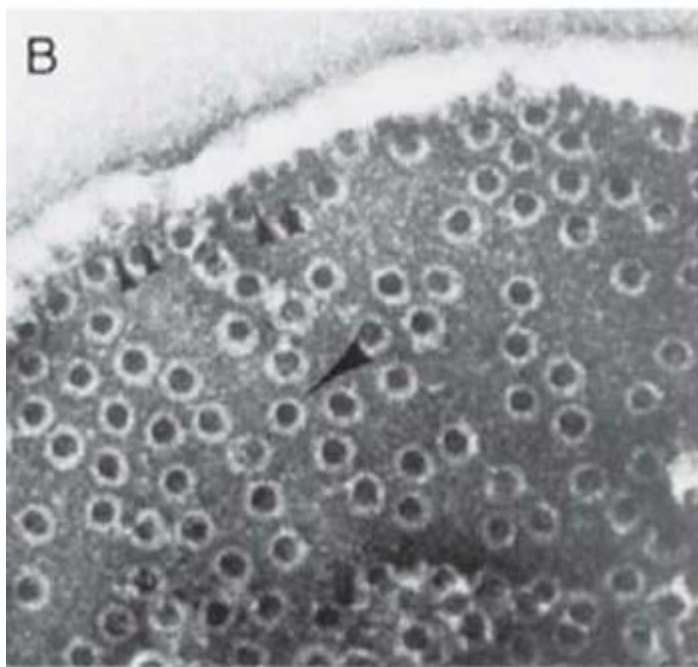
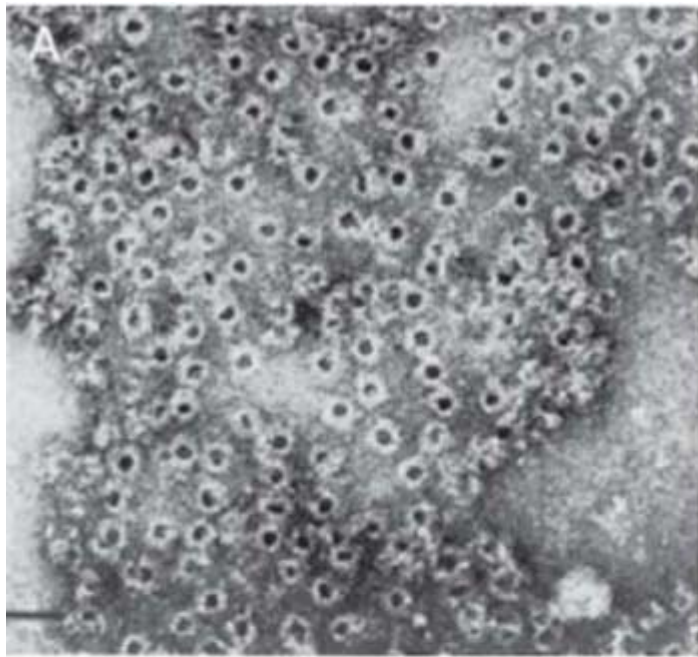
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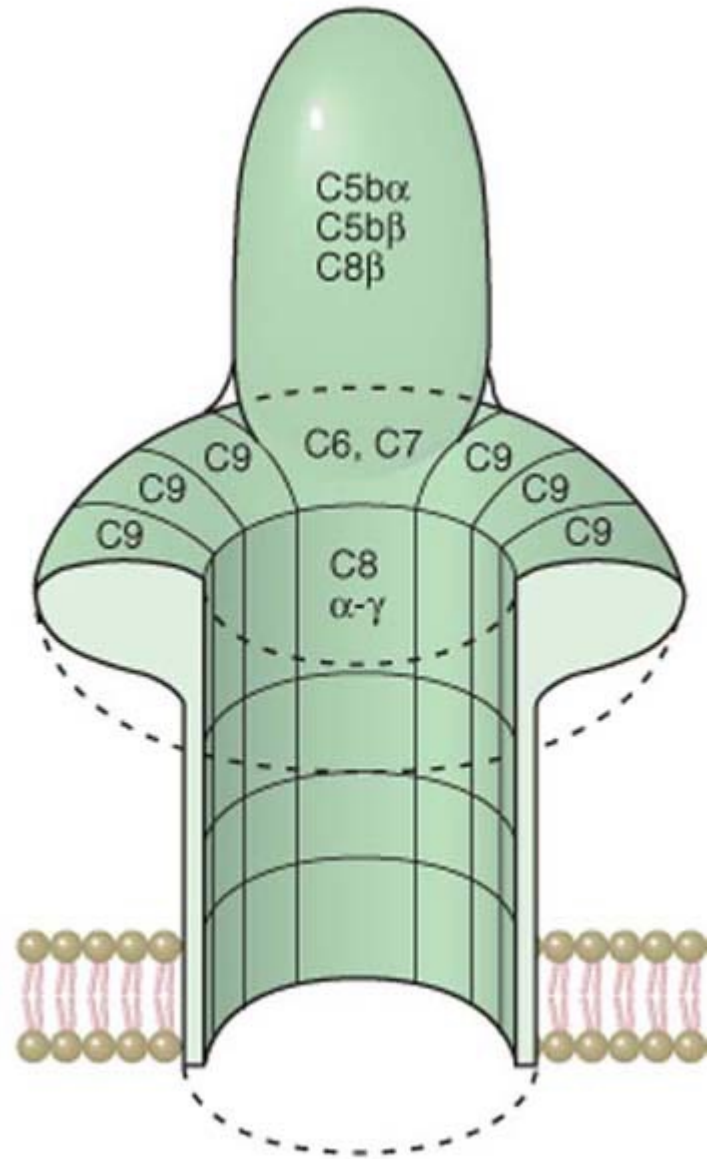
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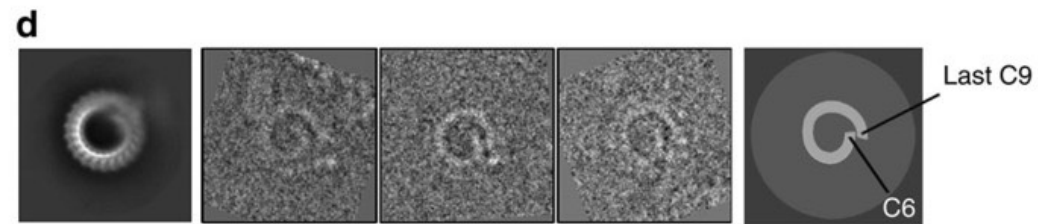
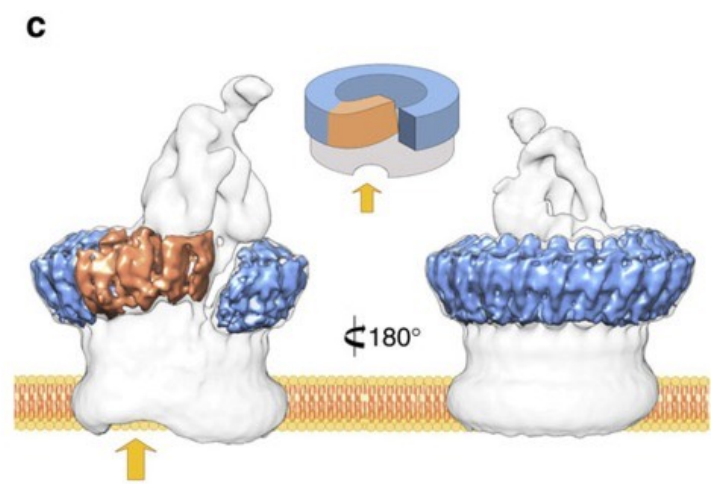
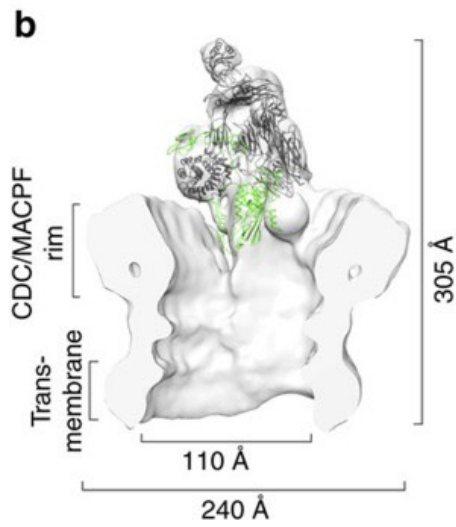
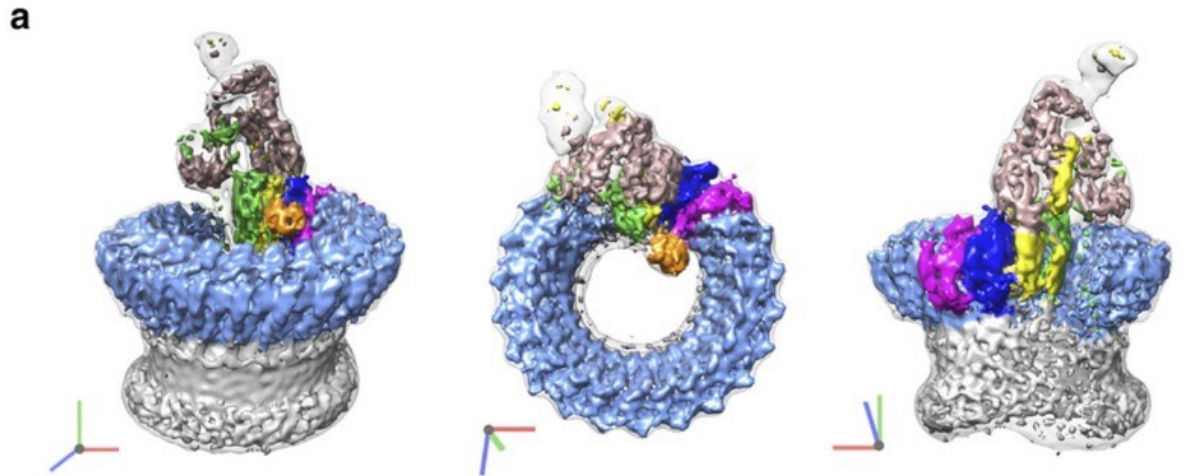


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C



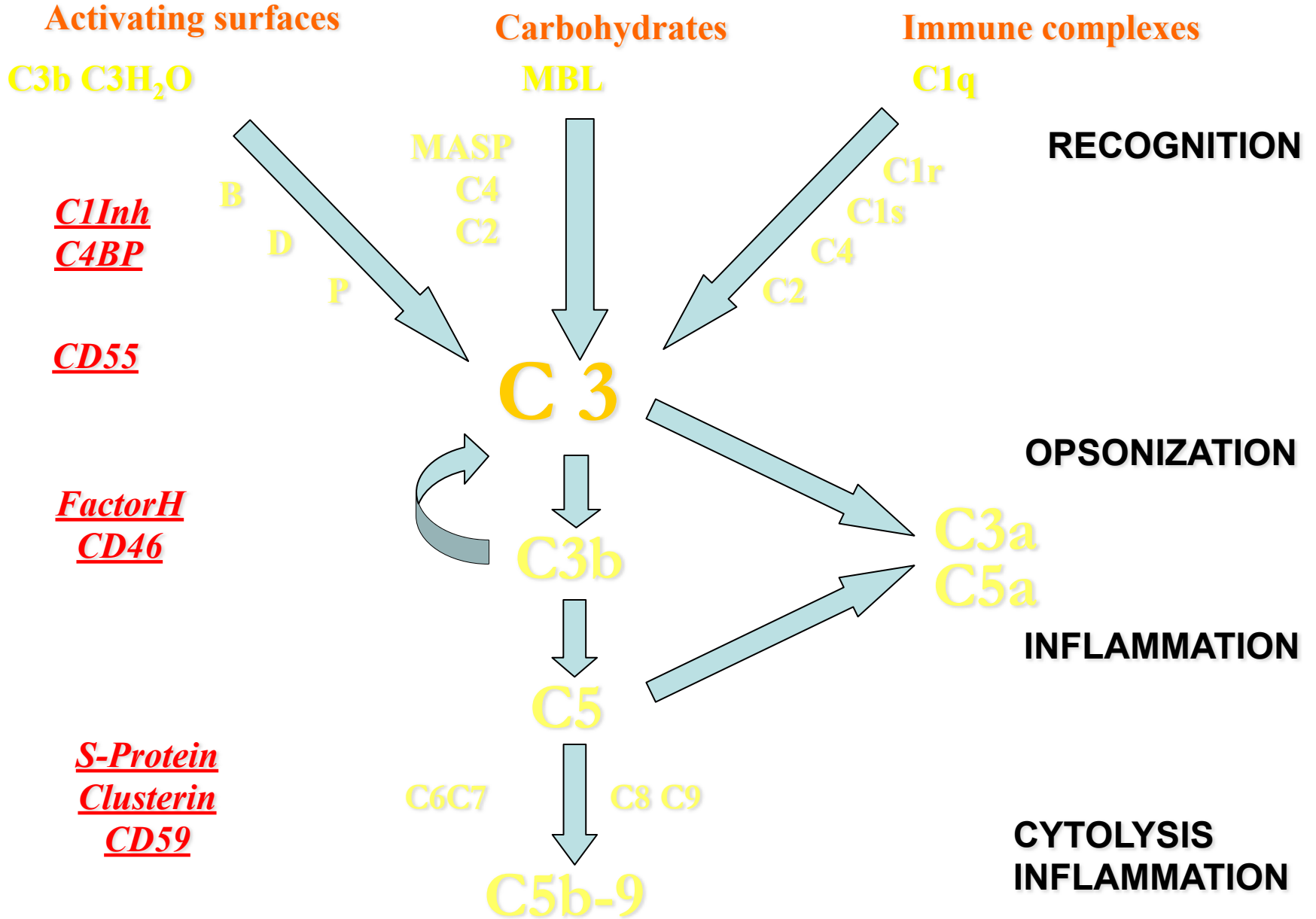


Seva et al,
Nat Comm, 2016

ALTERNATIVE PATHWAY

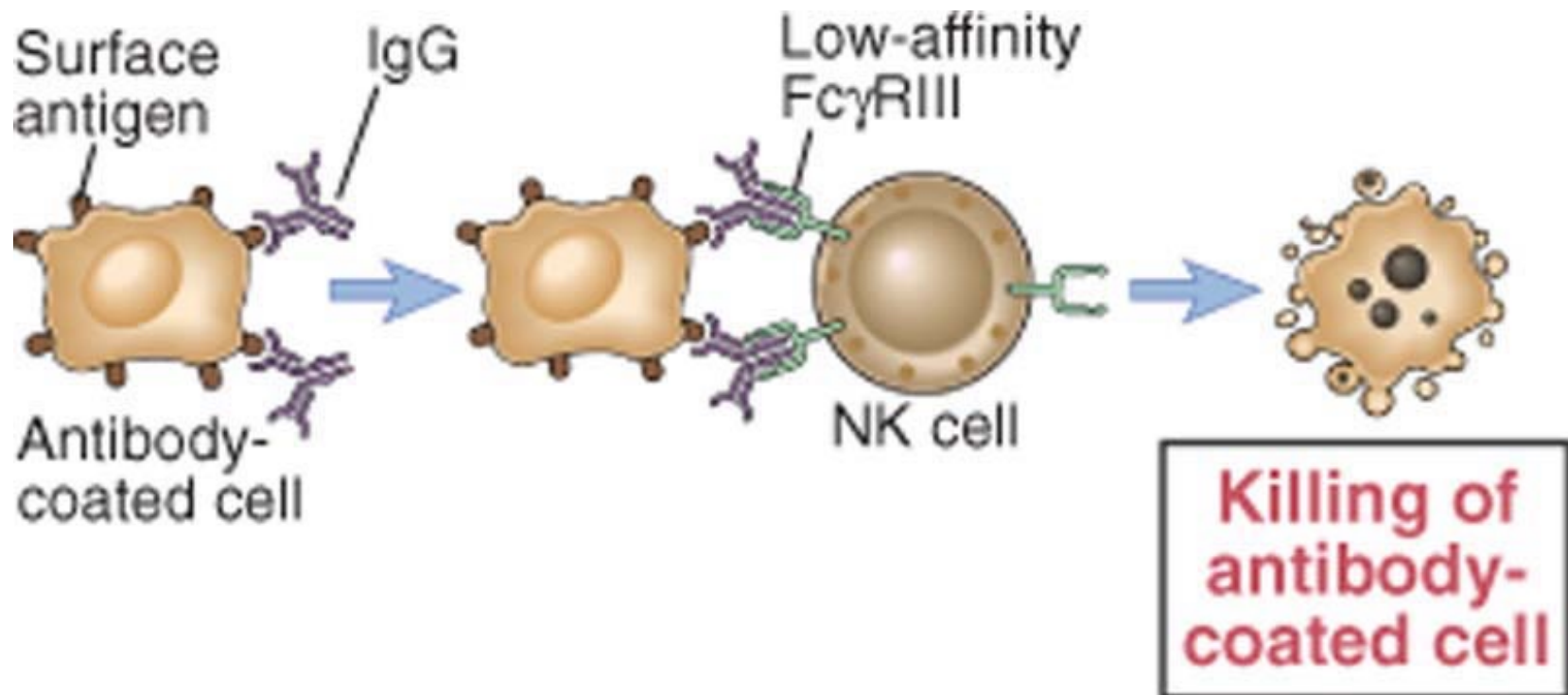
LECTIN PATHWAY

CLASSICAL PATHWAY



FcR	Affinity for immunoglobulin	Cell Distribution	Function
Fc γ RI (CD64)	High (Kd $\sim 10^{-9}$ M) binds IgG1 and IgG3	Macrophages, neutrophils; also eosinophils	Phagocytosis, activation of phagocytes
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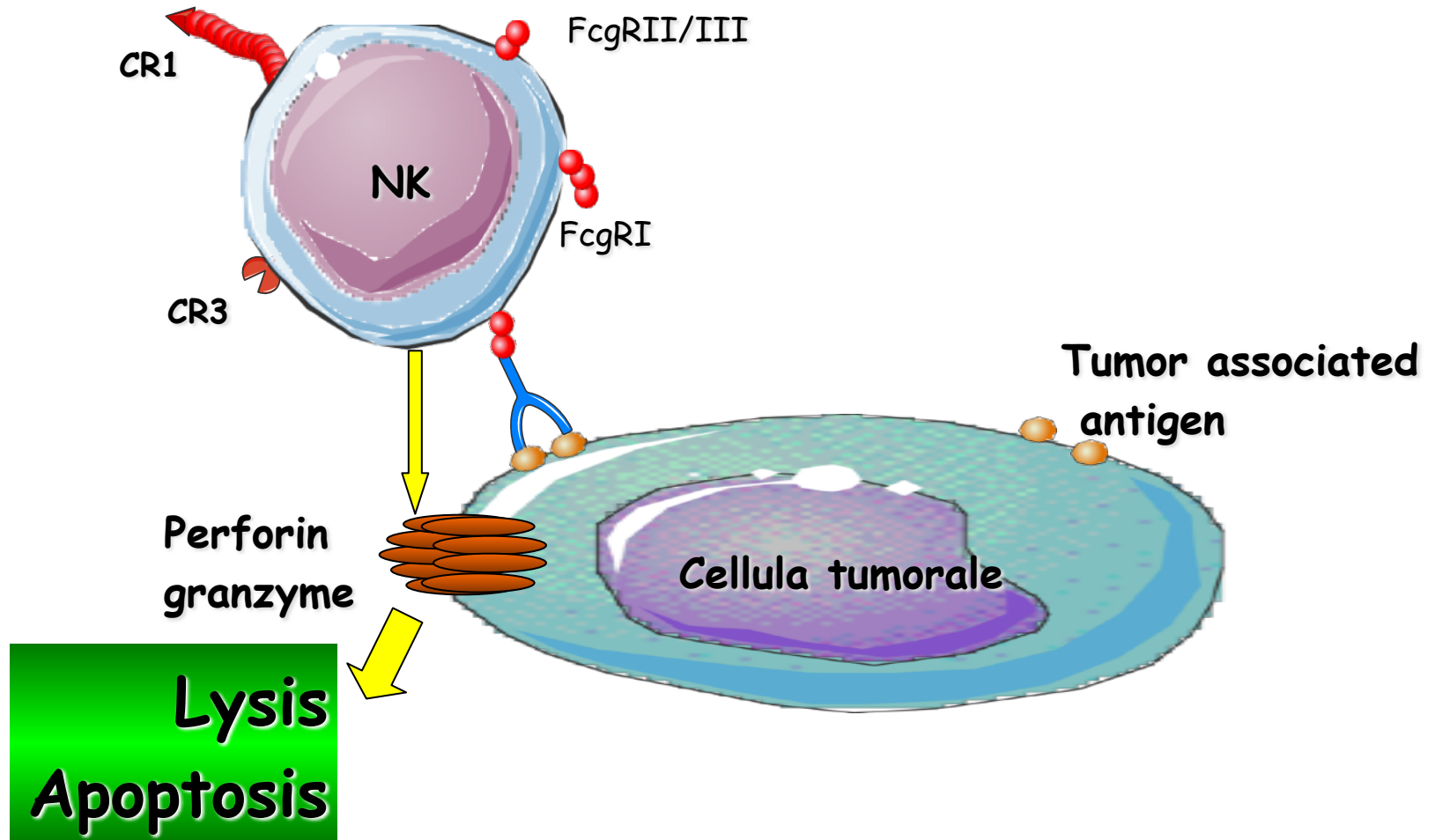
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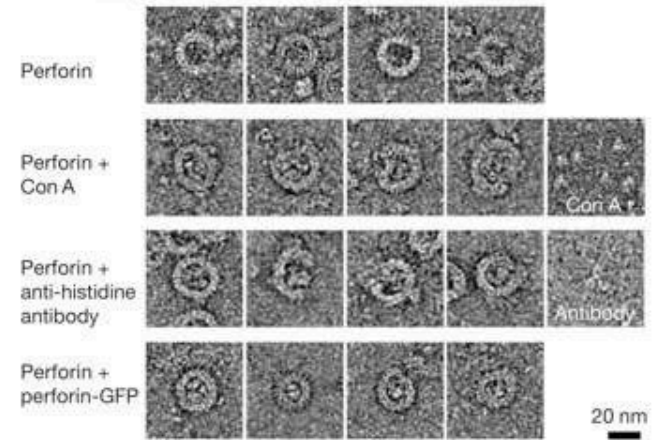
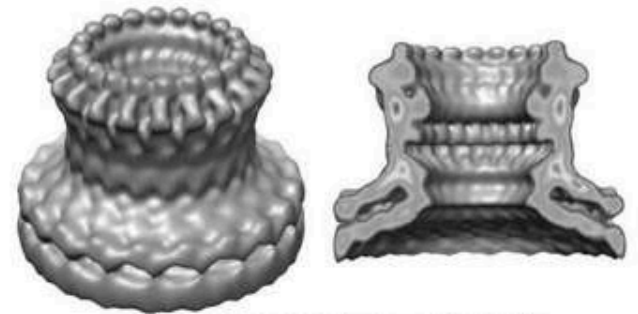
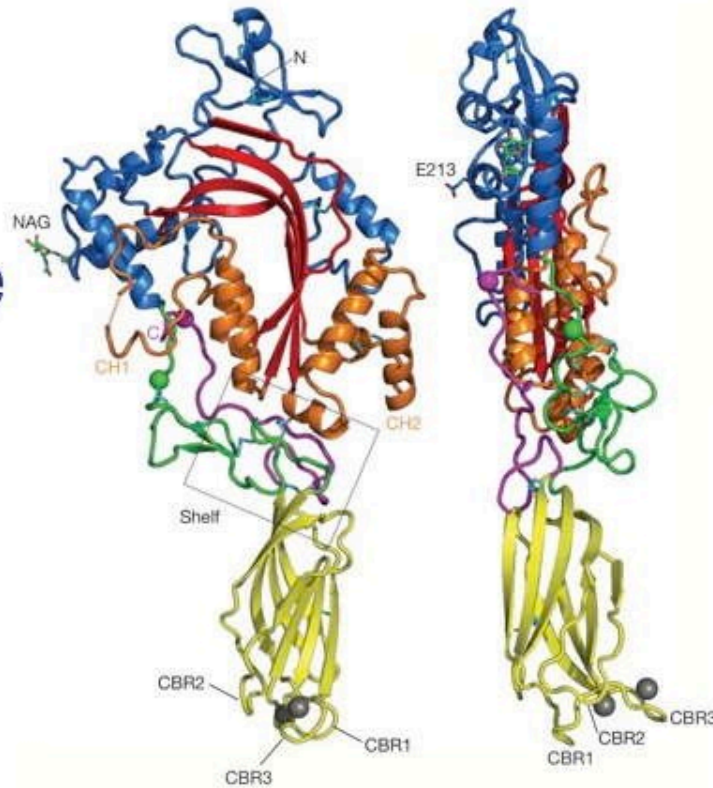
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Immune-activator monoclonal antibodies:

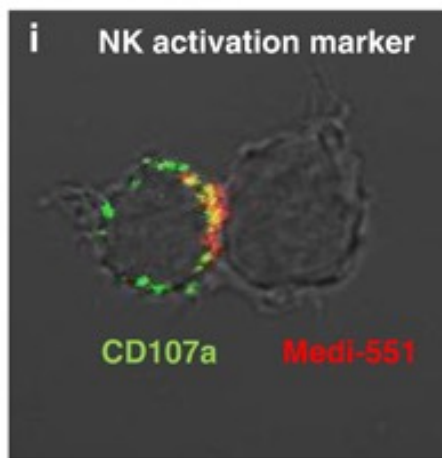
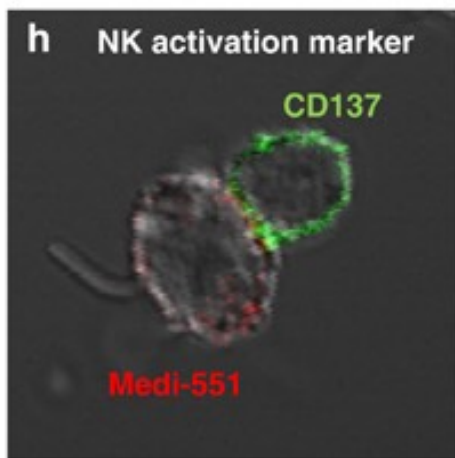
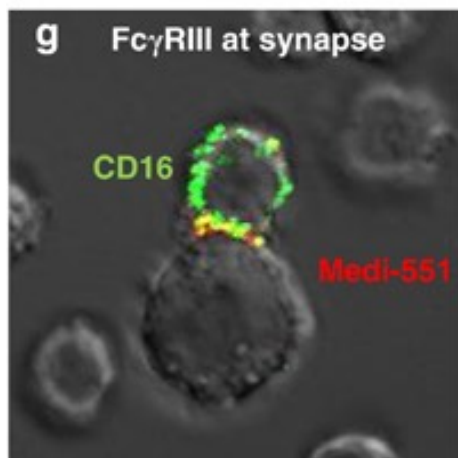
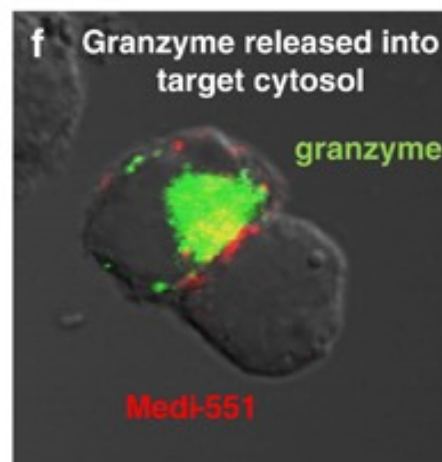
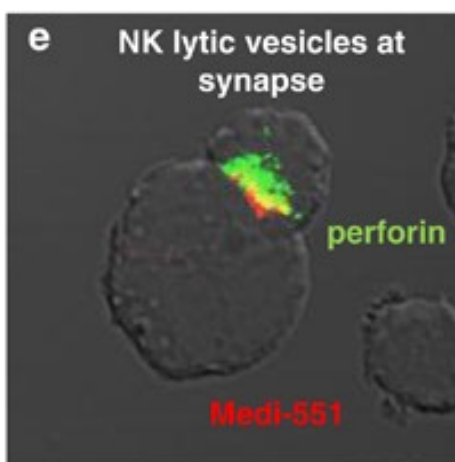
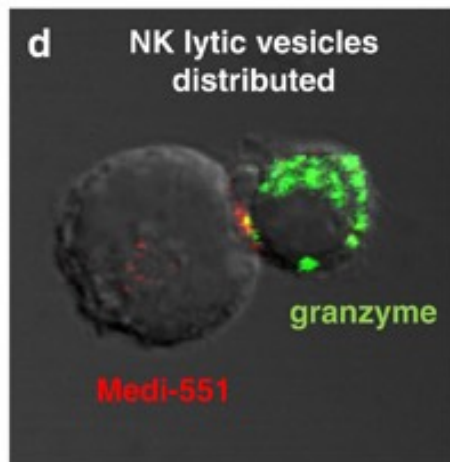
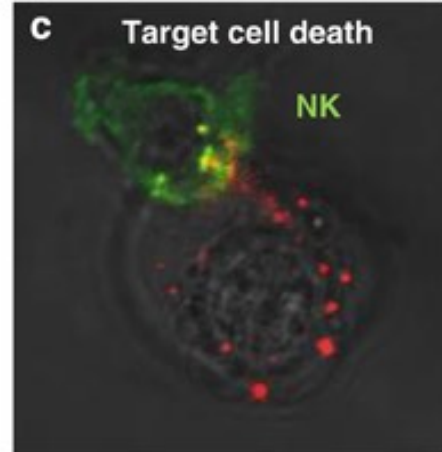
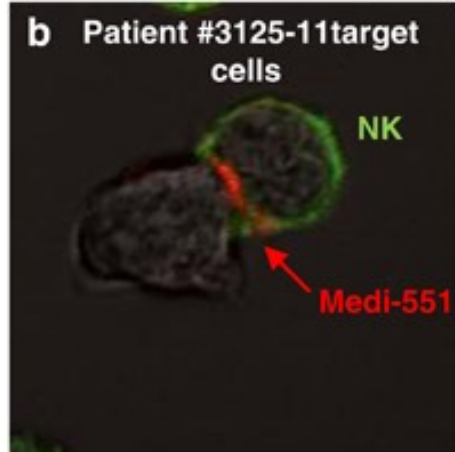
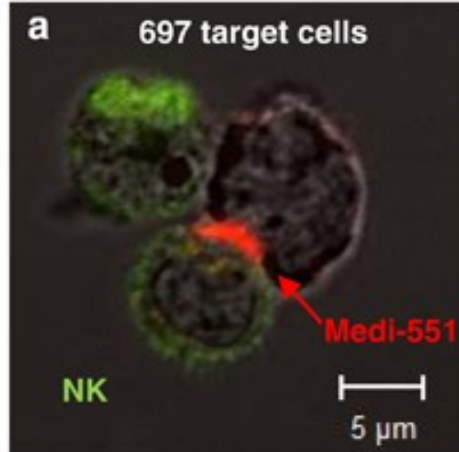
ADCC (Antibody-Dependent Cellular Cytotoxicity)



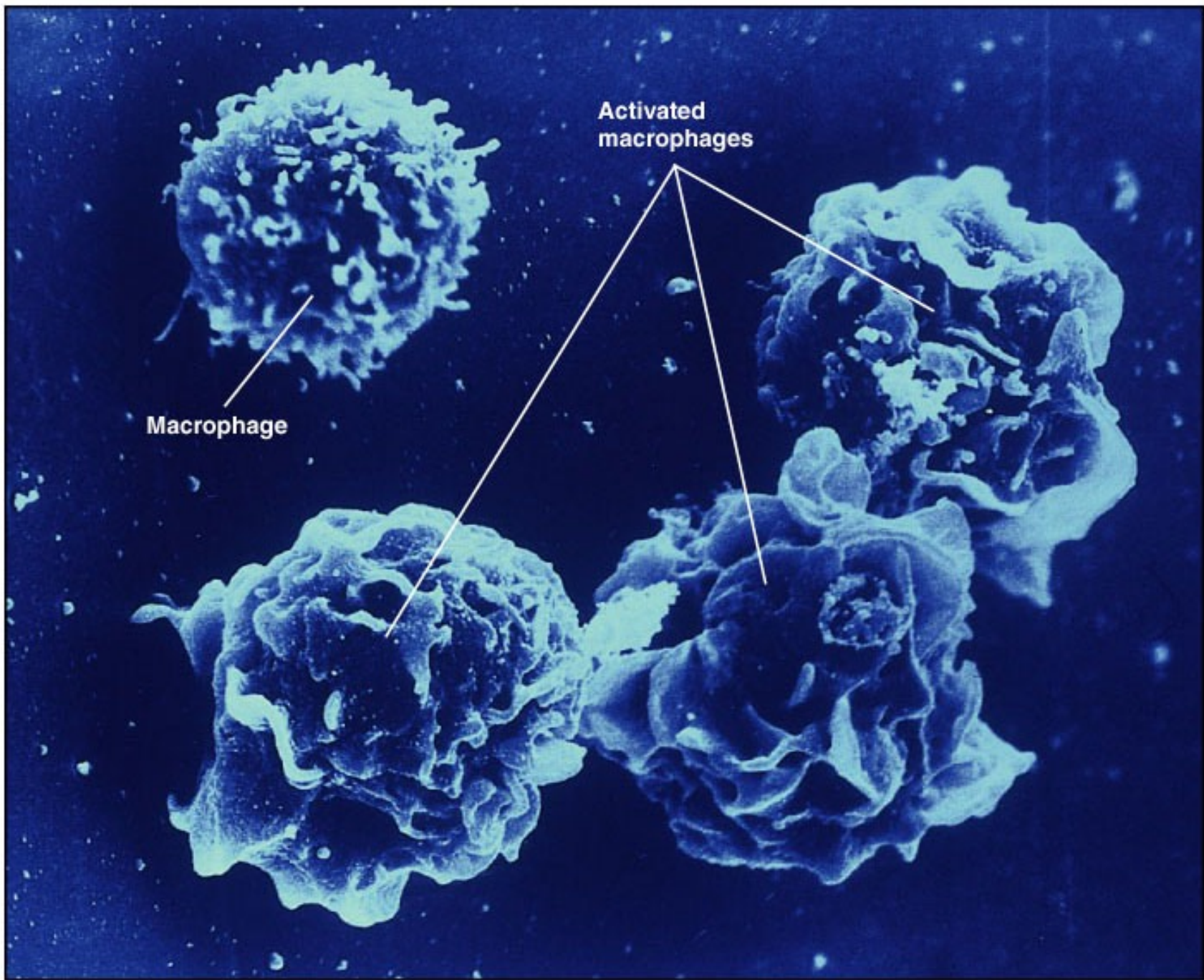
Structures of the lymphocyte perforin monomer and pore network



Law RHP et al Nature 468: 447 (2010)

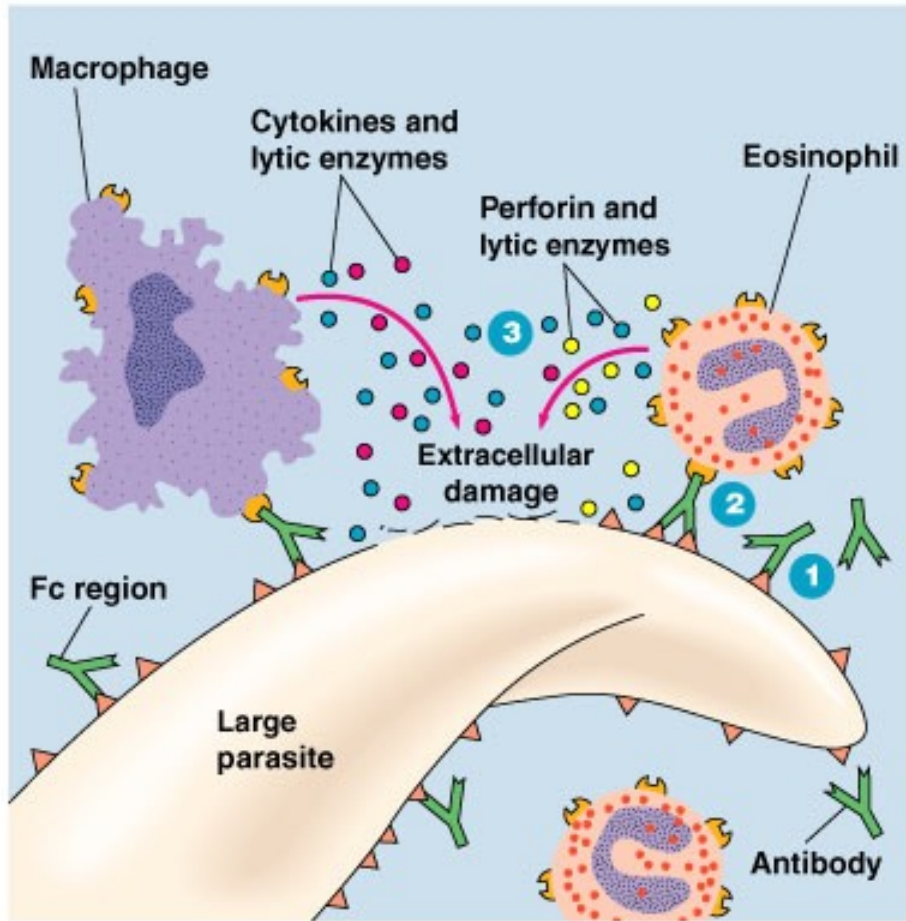


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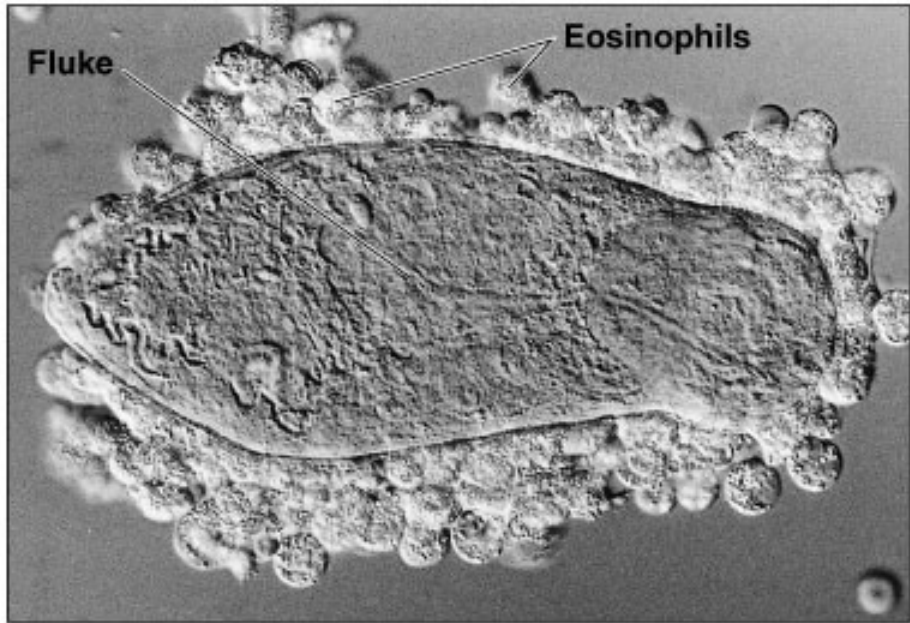
Macrophage

**Activated
macrophages**



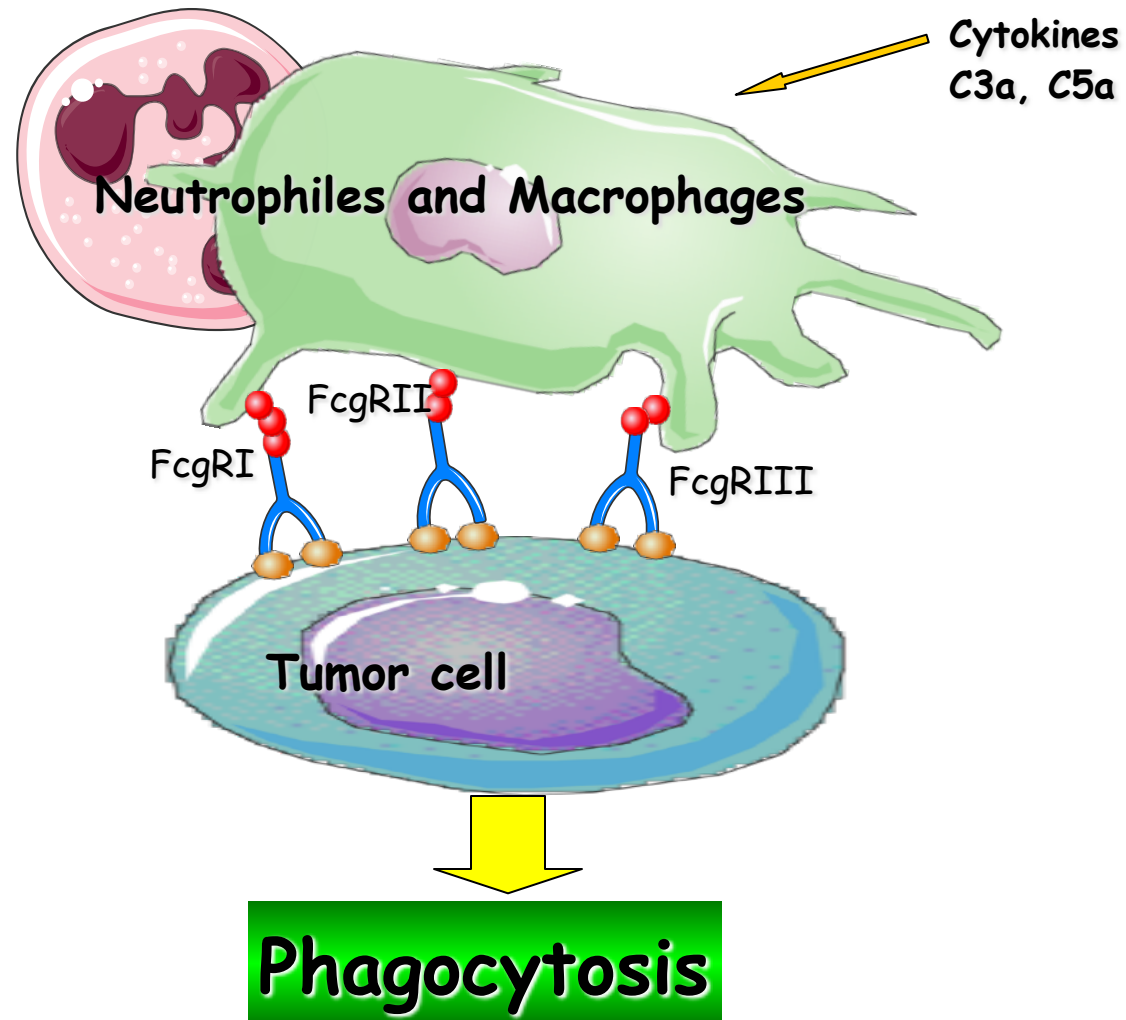
(a)

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(b)

Immune-activator monoclonal antibodies: Phagocytosis



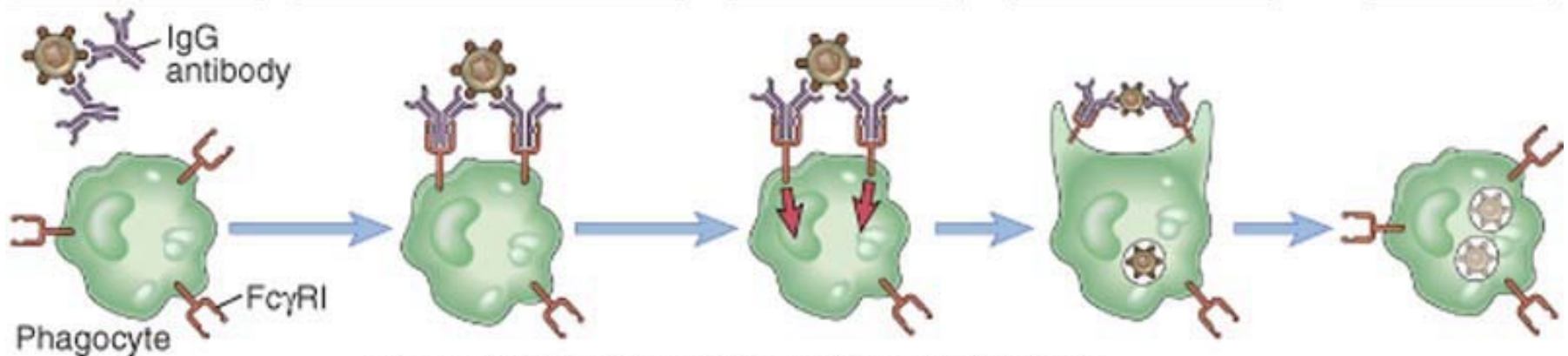
Opsonization of microbe by IgG

Binding of opsonized microbes to phagocyte Fc receptors (Fc γ RI)

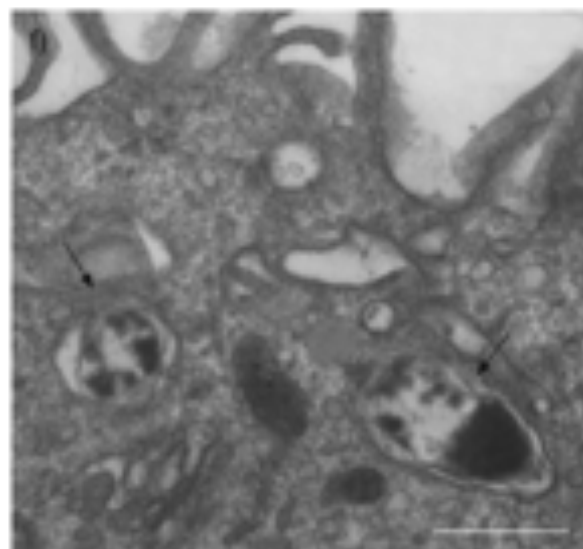
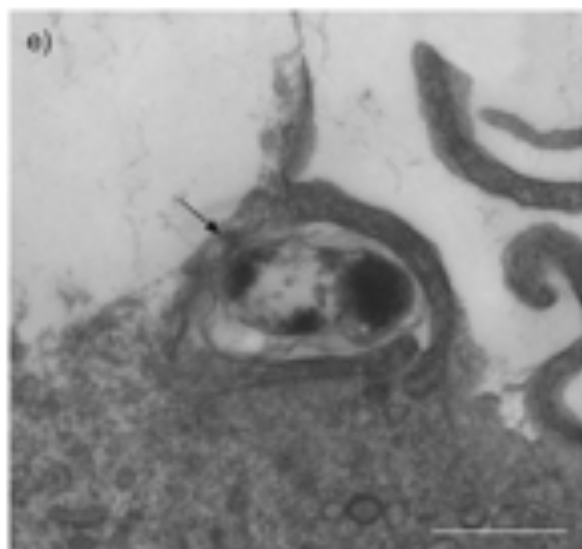
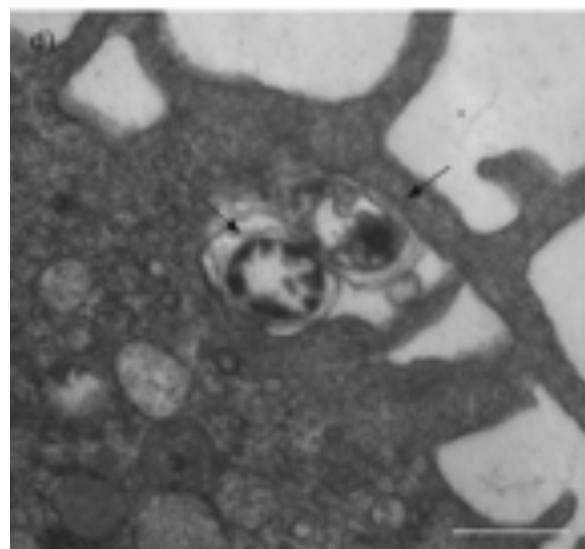
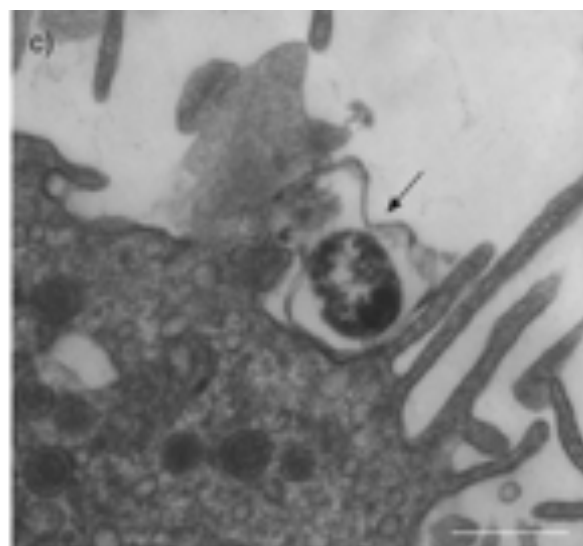
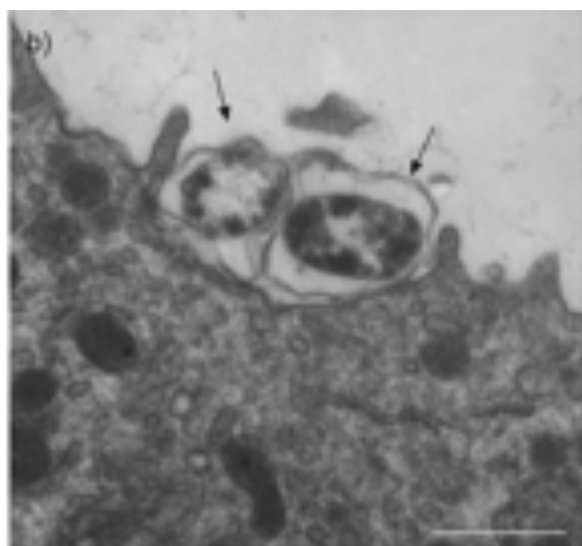
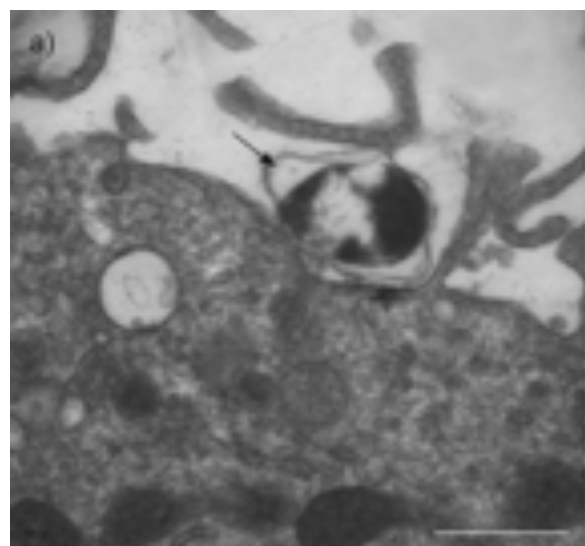
Fc receptor signals activate phagocyte

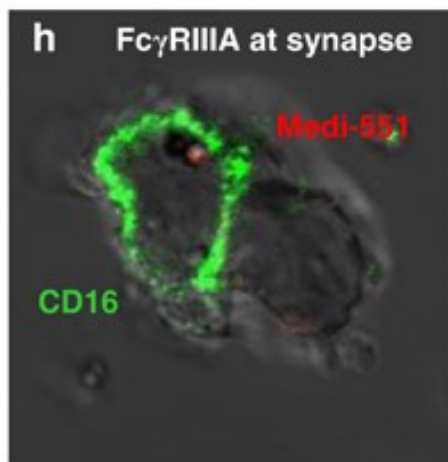
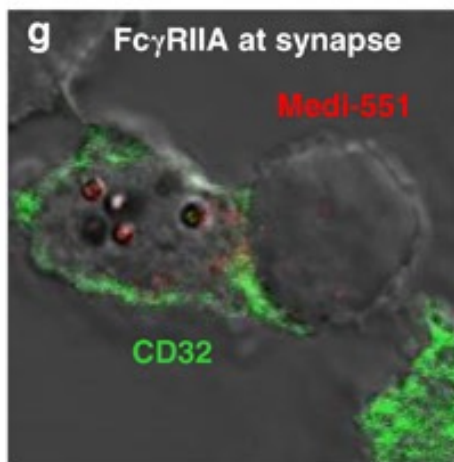
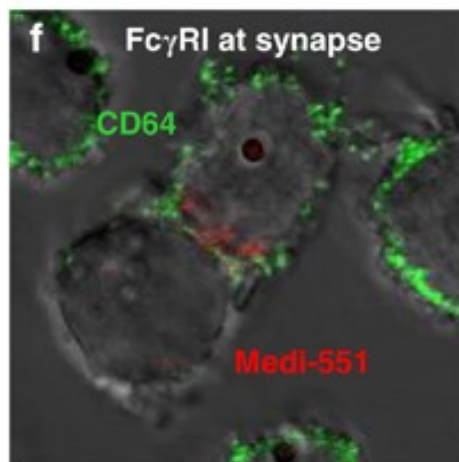
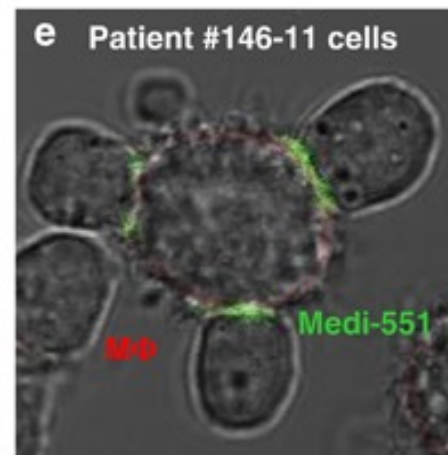
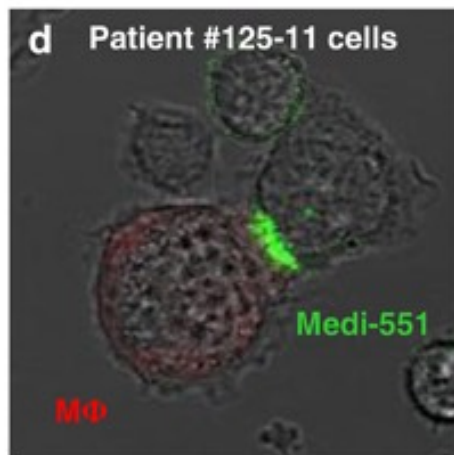
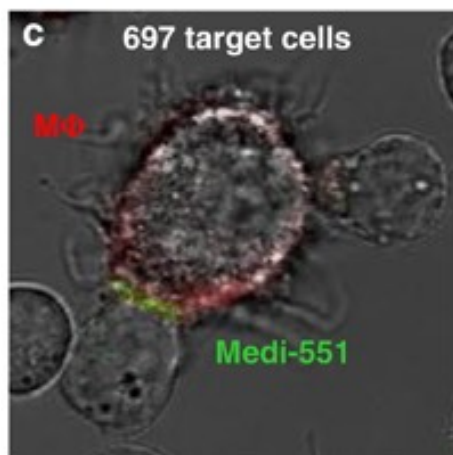
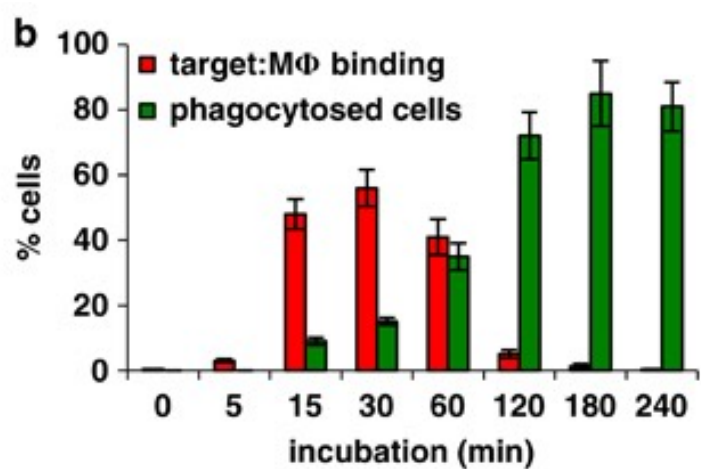
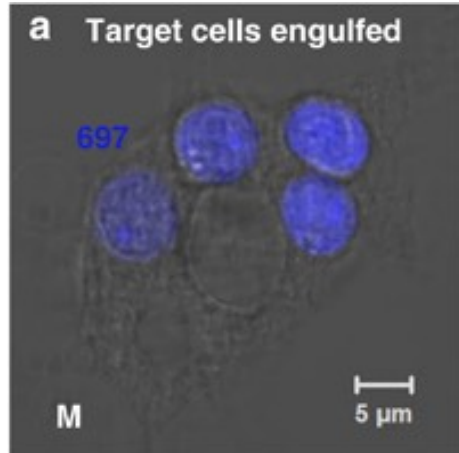
Phagocytosis of microbe

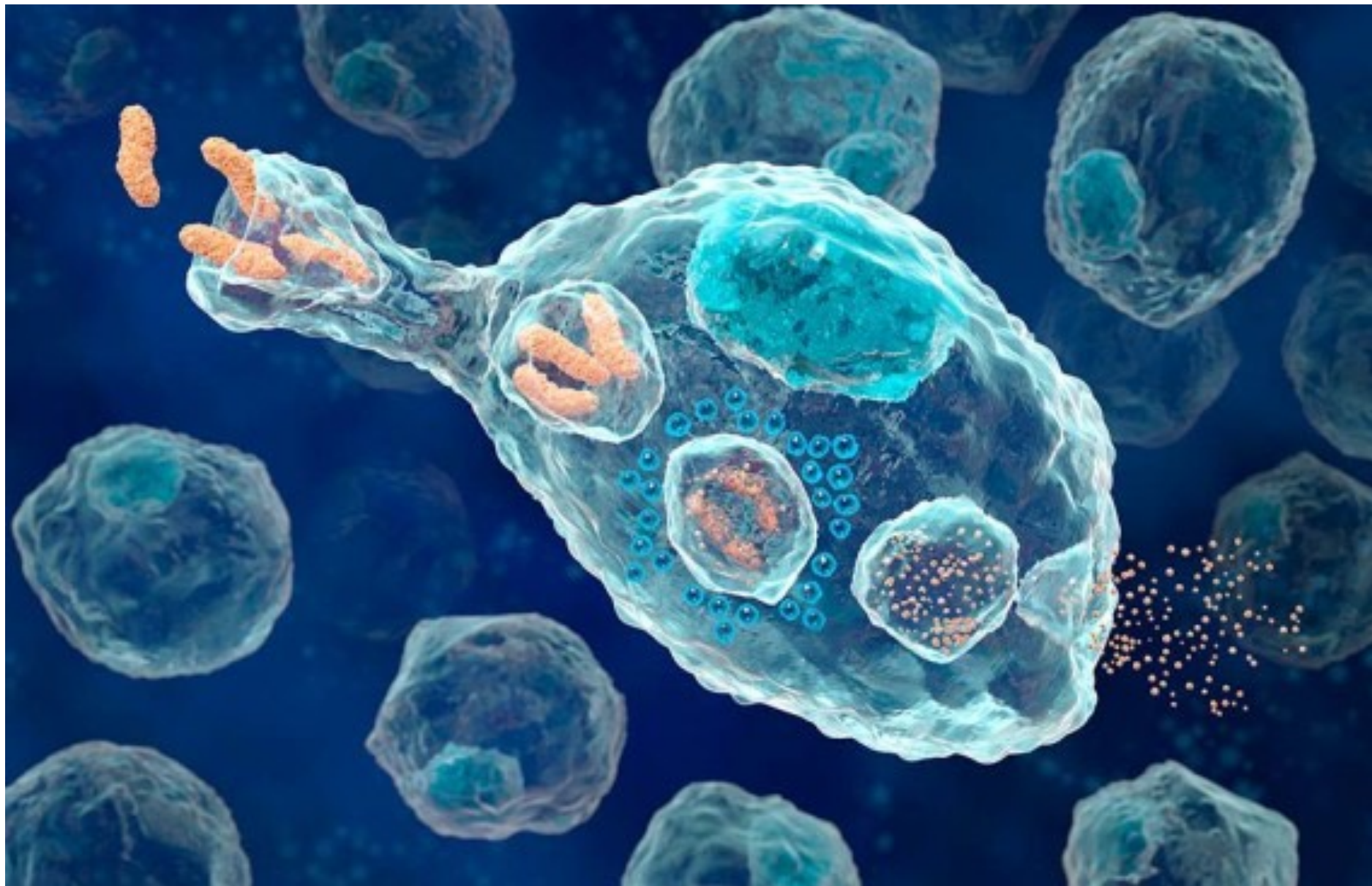
Killing of ingested microbe



Abbas et al: Cellular and Molecular Immunology, Updated 6th Edition.
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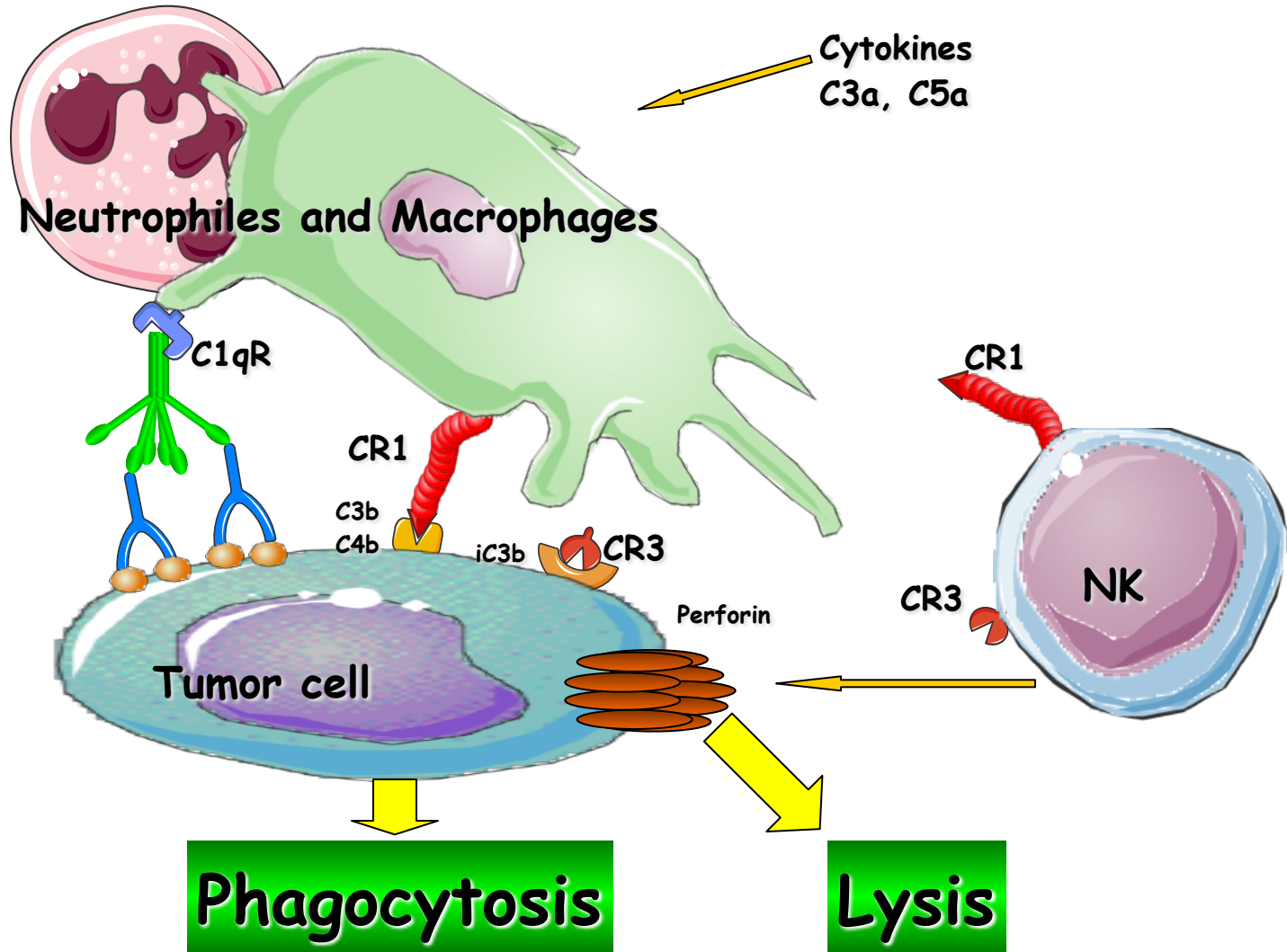






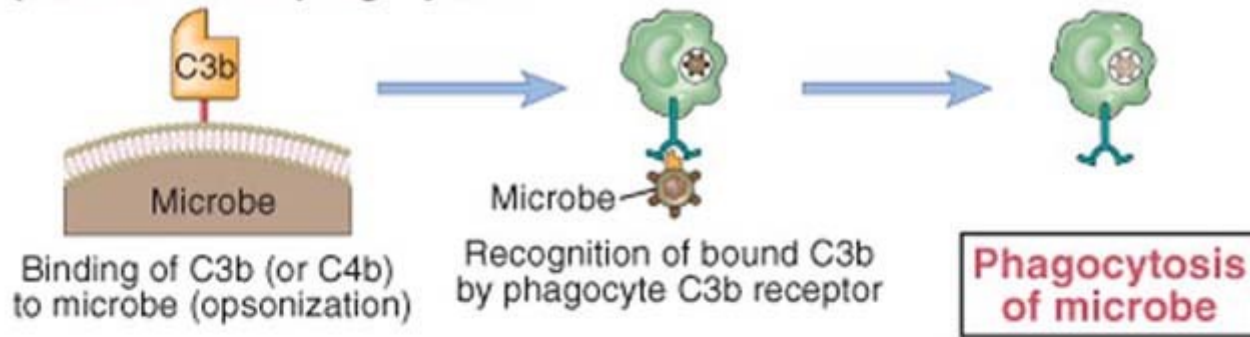
Immune-activator monoclonal antibodies:

CDCC (Complement-Dependent Cellular Cytotoxicity)



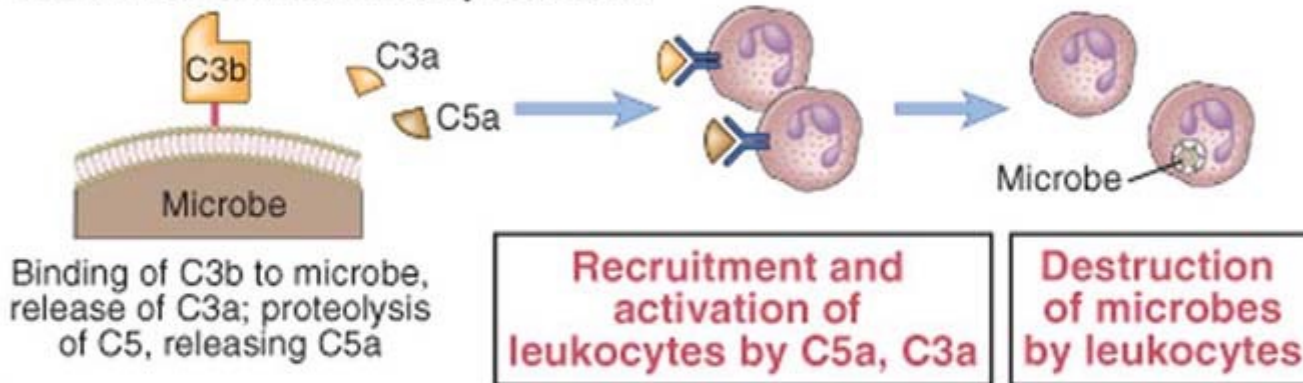
(A)

Opsonization and phagocytosis



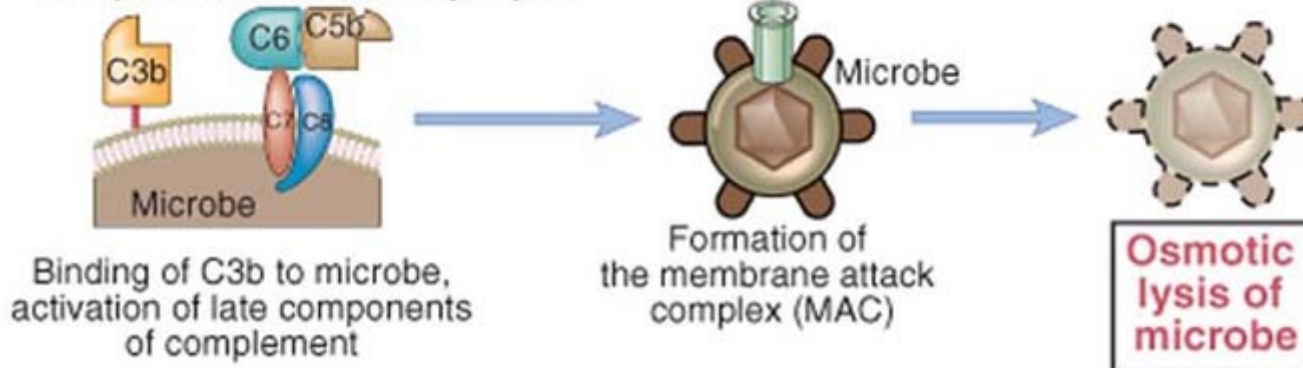
(B)

Stimulation of inflammatory reactions

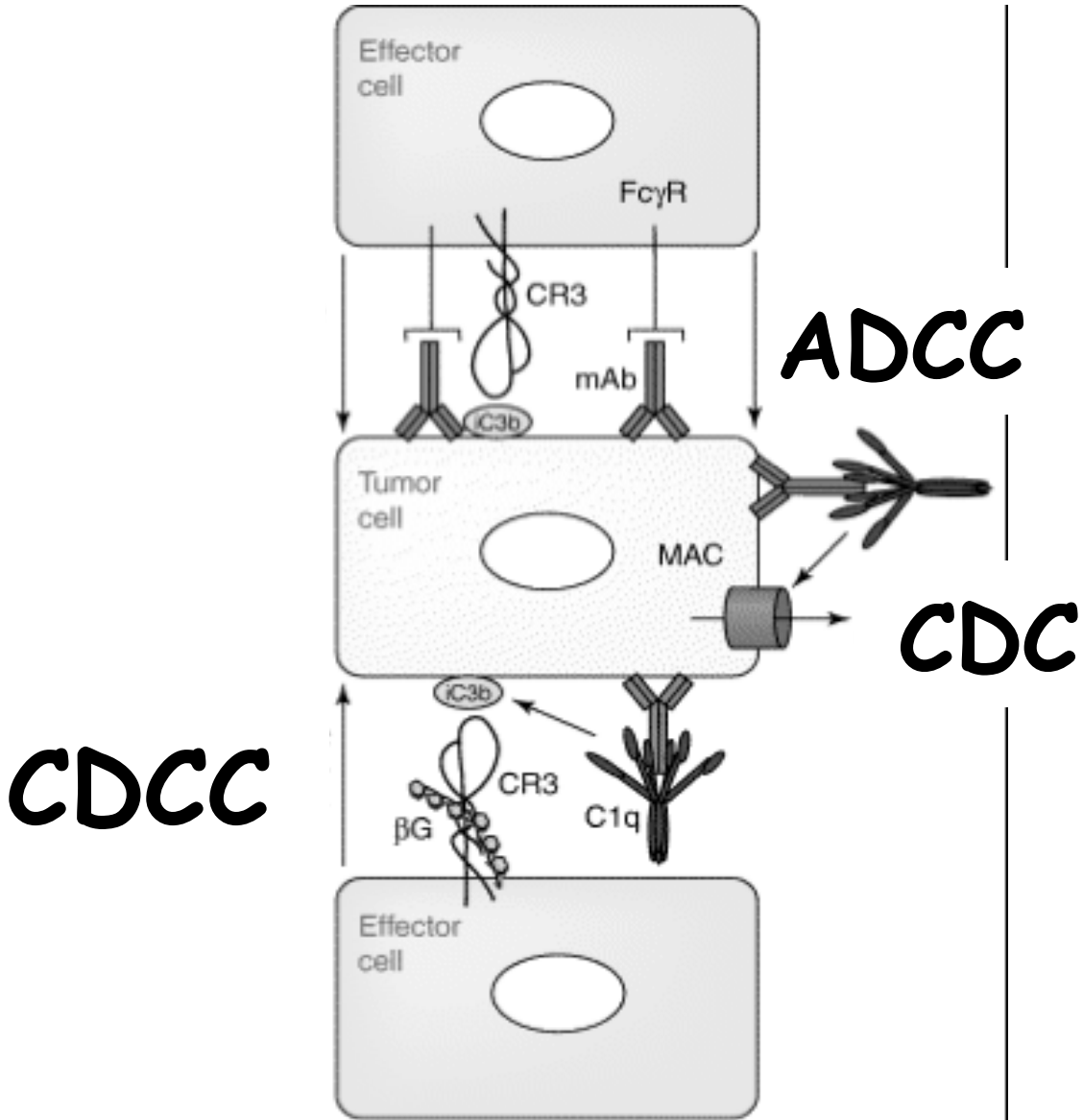


(C)

Complement-mediated cytotoxicity



Effector mechanisms of monoclonal antibodies



Modified from Gelderman et al.
Trends in Immunol, 2004

Mechanisms of action of immune-activator antibodies

CITOTOSSICITA' CELLULARE ANTICORPO DIPENDENTE (ADCC)	Mediata in particolare dalle cellule NK, che tramite il recettore FcγRIII riconosce la porzione Fc dell'anticorpo. Liberazione del contenuto dei granuli citoplasmatici (perforine, granzimi).
OPSONIZZAZIONE E FAGOCITOSI	Gli anticorpi rivestono la cellula tumorale e ne favoriscono l'internalizzazione da parte dei fagociti che riconoscono la porzione Fc mediante i recettori per Fc.
APOPTOSI	Da aggregazione dell'antigene sulla superficie cellulare.
ATTIVAZIONE DELLA VIA CLASSICA DEL COMPLEMENTO	Legame di C1q all'Fc dell'anticorpo; lisi cellulare (CDC); i prodotti generati dall'attivazione del complemento (anafilotossine e opsonine) inducono flogosi e promuovono la fagocitosi.