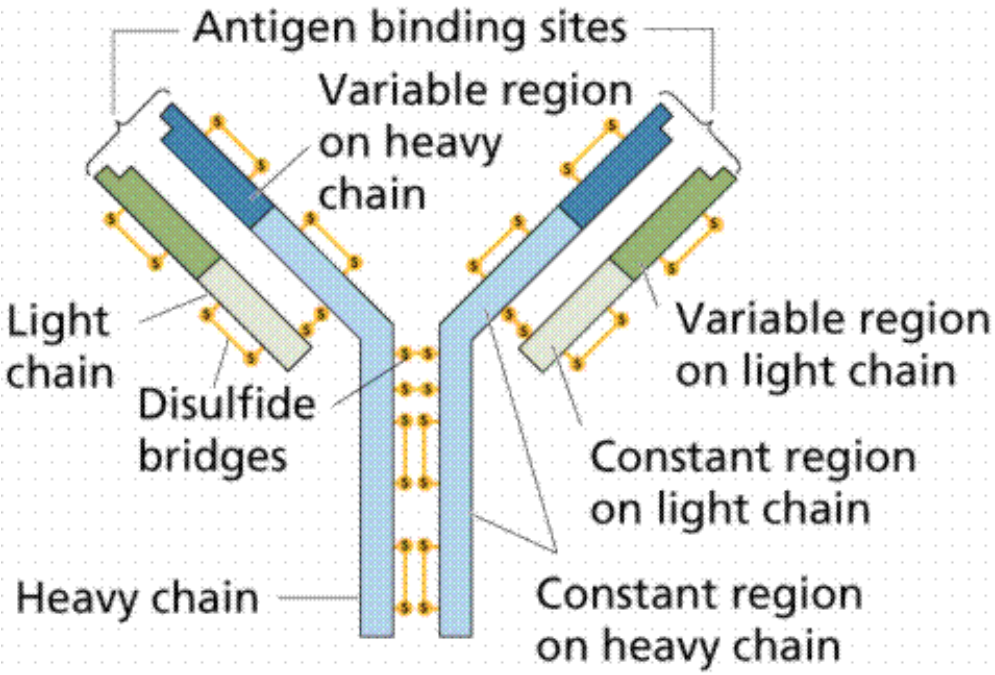
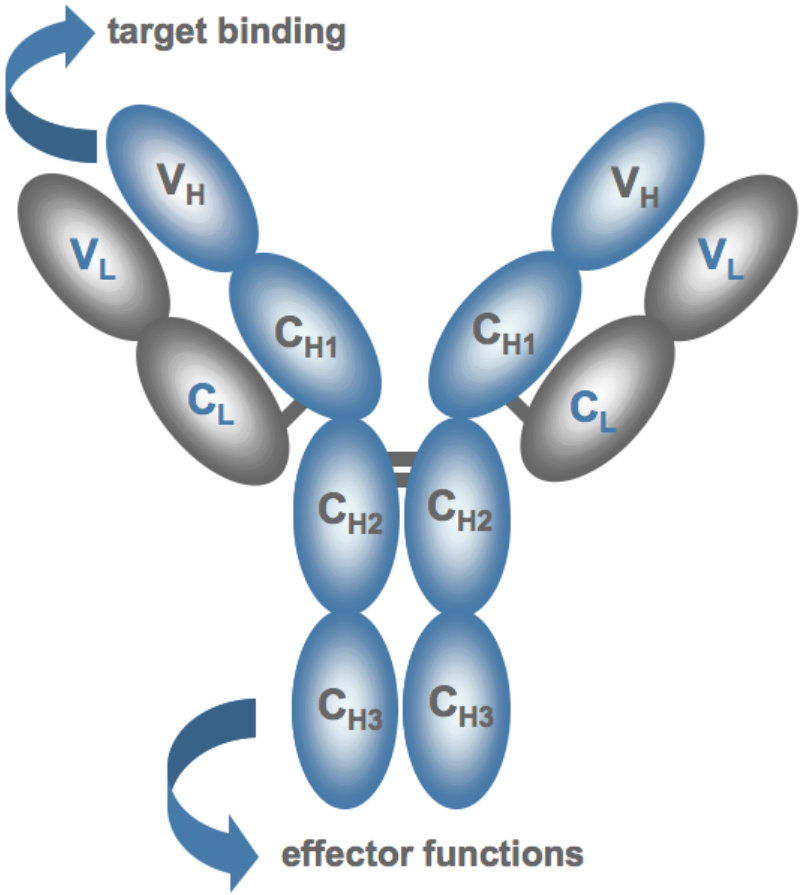
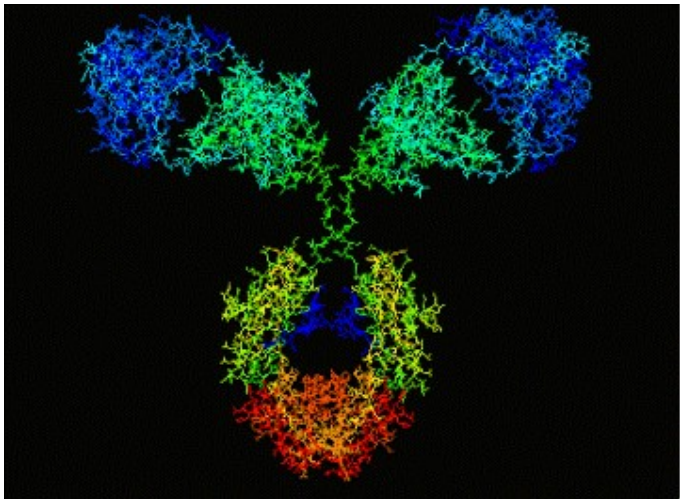
Mechanisms of action of monoclonal antibodies

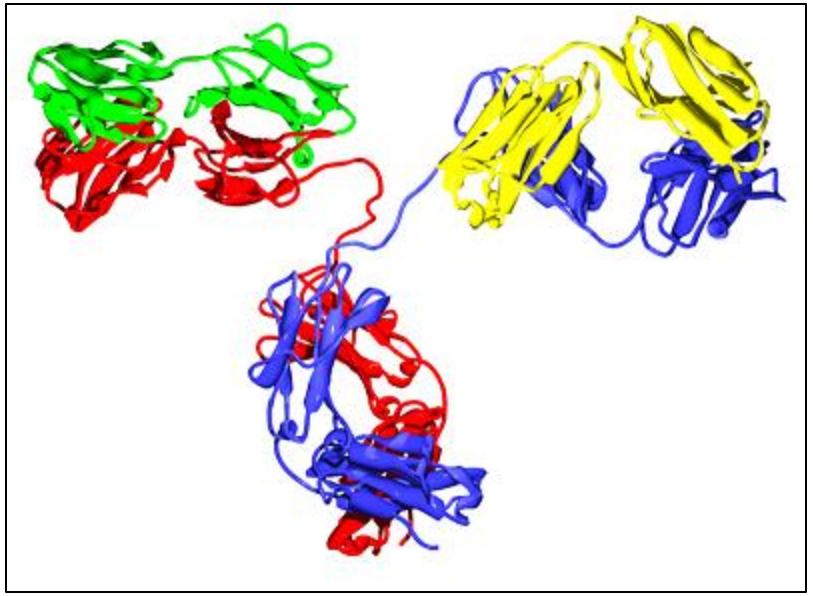
Antibody

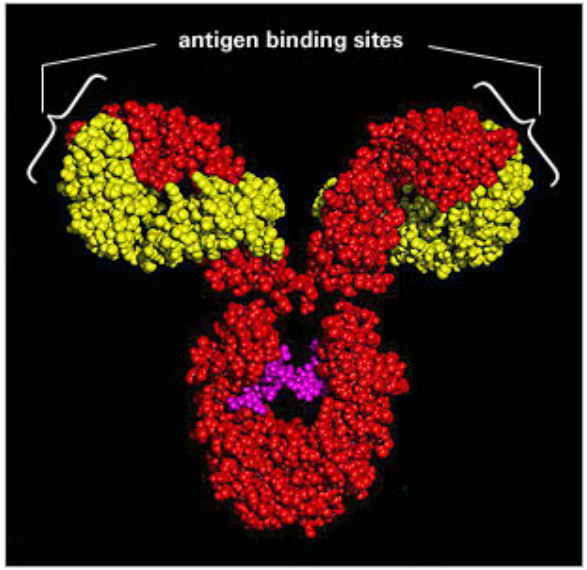




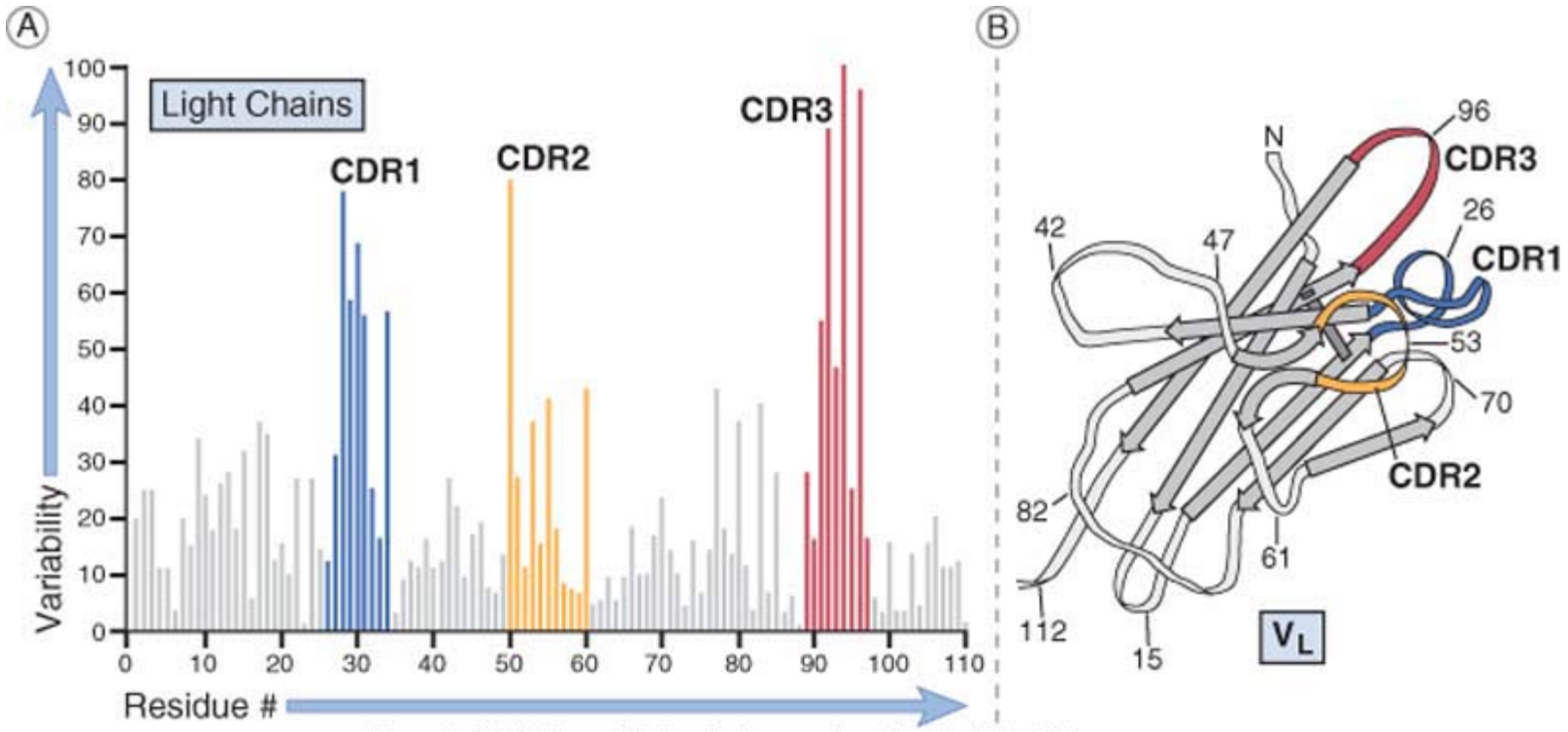
Google Immagini



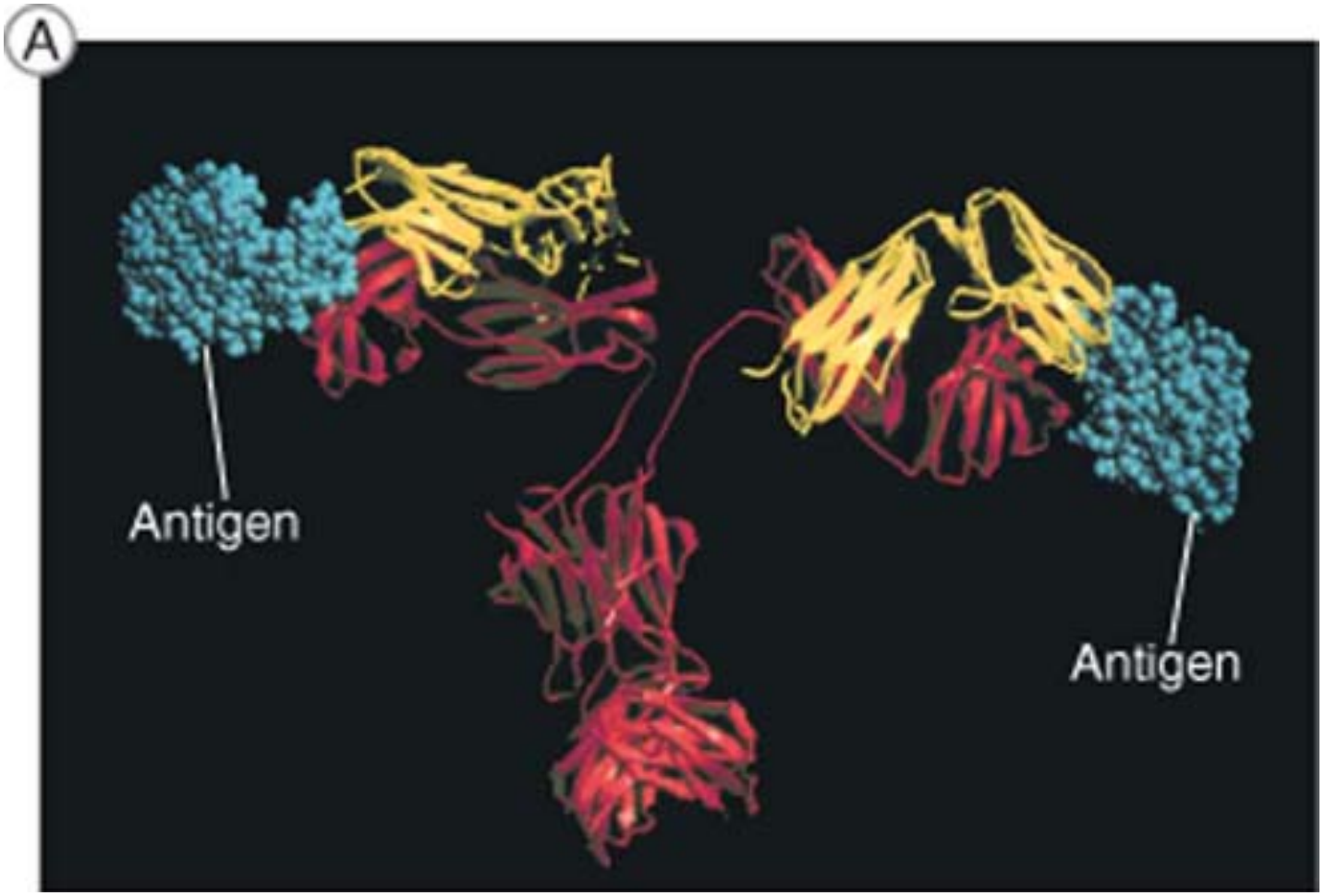



antigen binding sites

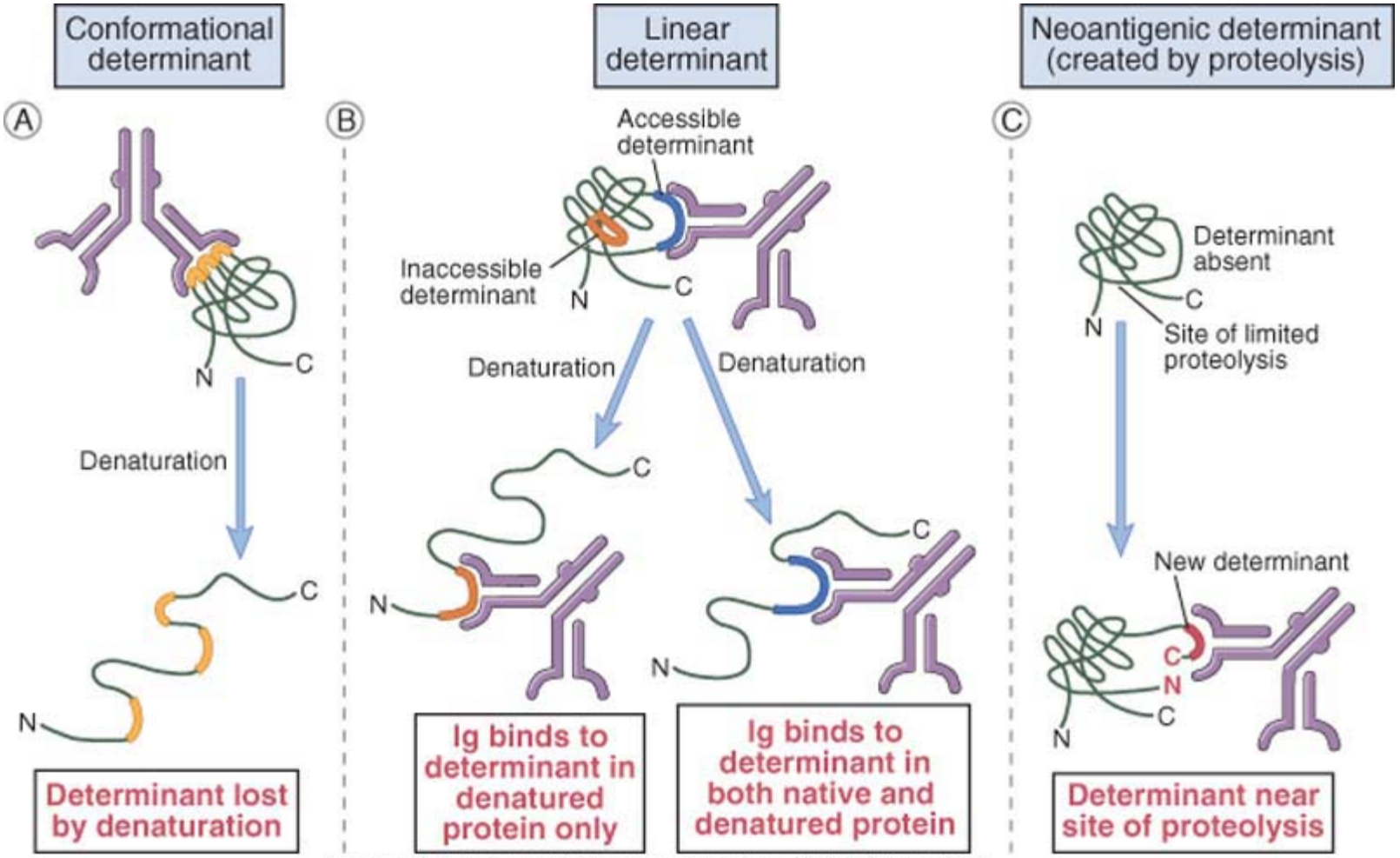
Aminoacid variability in antibody sequence



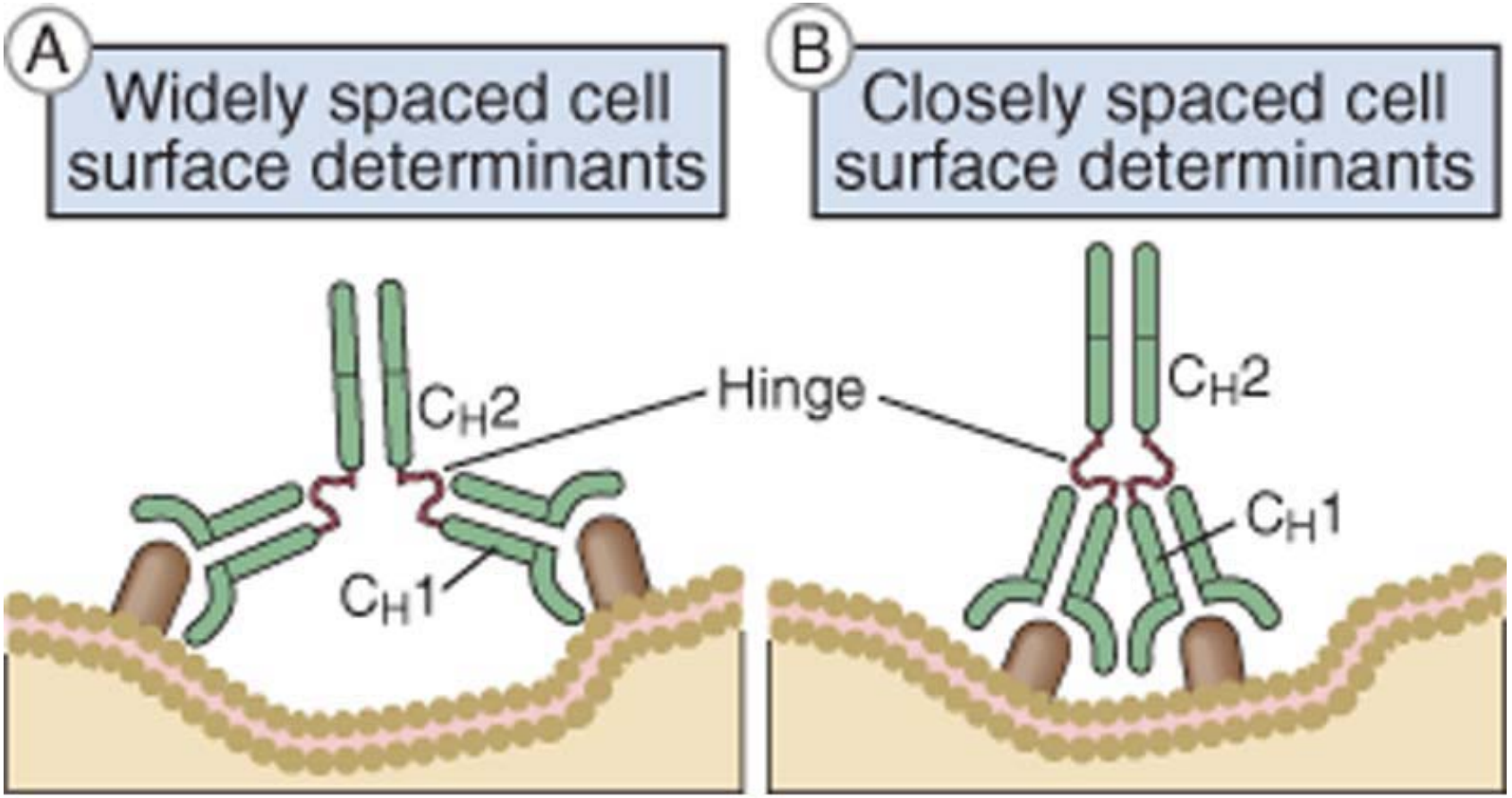
Antigen/antibody complex



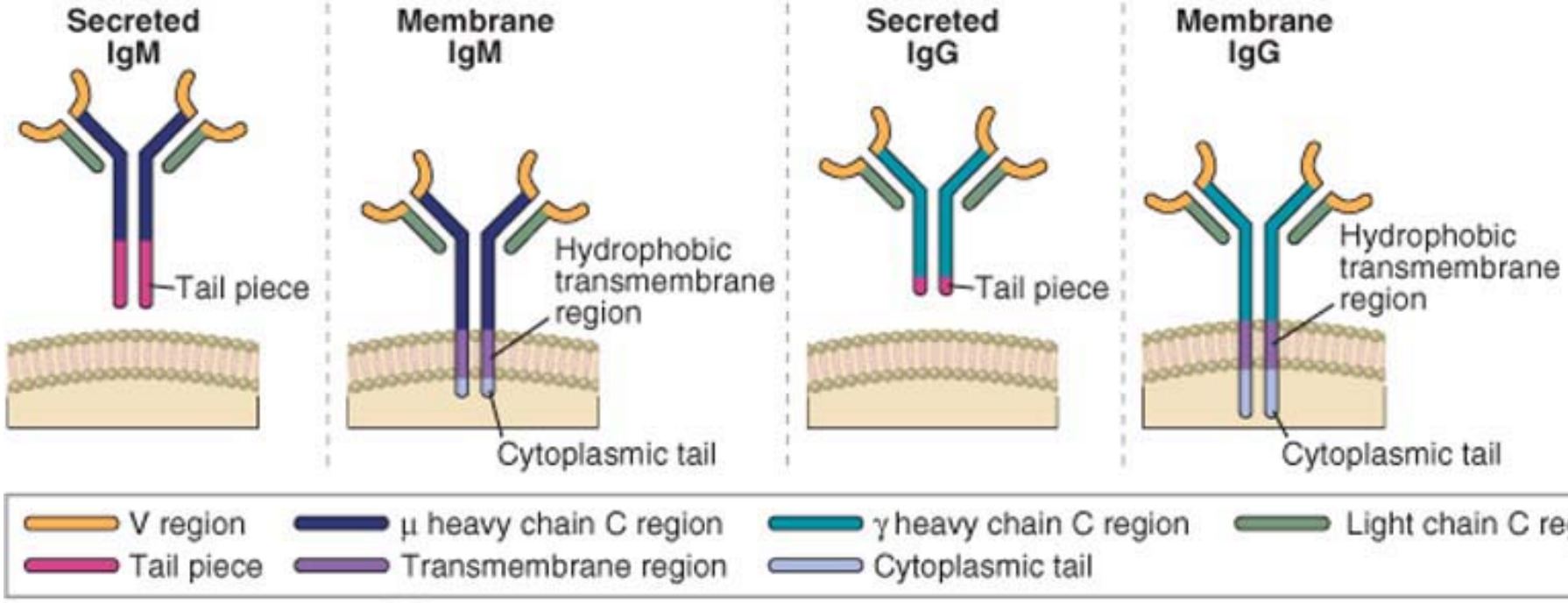
Antigenic determinant (epitope)

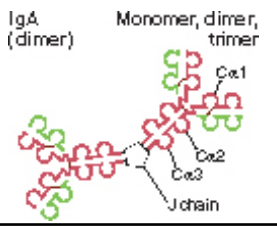
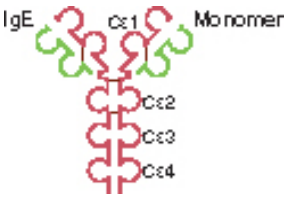
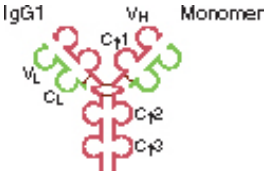
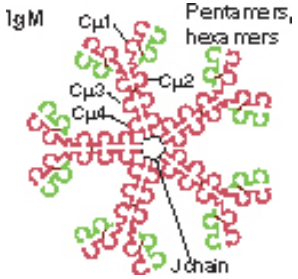


Antigen/antibody complex on cell membrane



secreted and membrane antibodies



class	subclass	plasma concentration (mg/ml)	halflife in plasma (days)	Secreted form
IgA	1,2	3,5	6	
IgD	-	-	3	-
IgE	-	0,05	2	
IgG	1-4	13,5	23	
IgM	-	1,5	5	

Antibody Isotope	Isotype-specific effector functions
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IgA	- Mucosal immunity: secretion of IgA into the lumens of the gastrointestinal and respiratory tracts - Activation of complement by the lectin pathway or by the alternative pathway
IgE	Mast cell degranulation (immediate hypersensitivity reactions)
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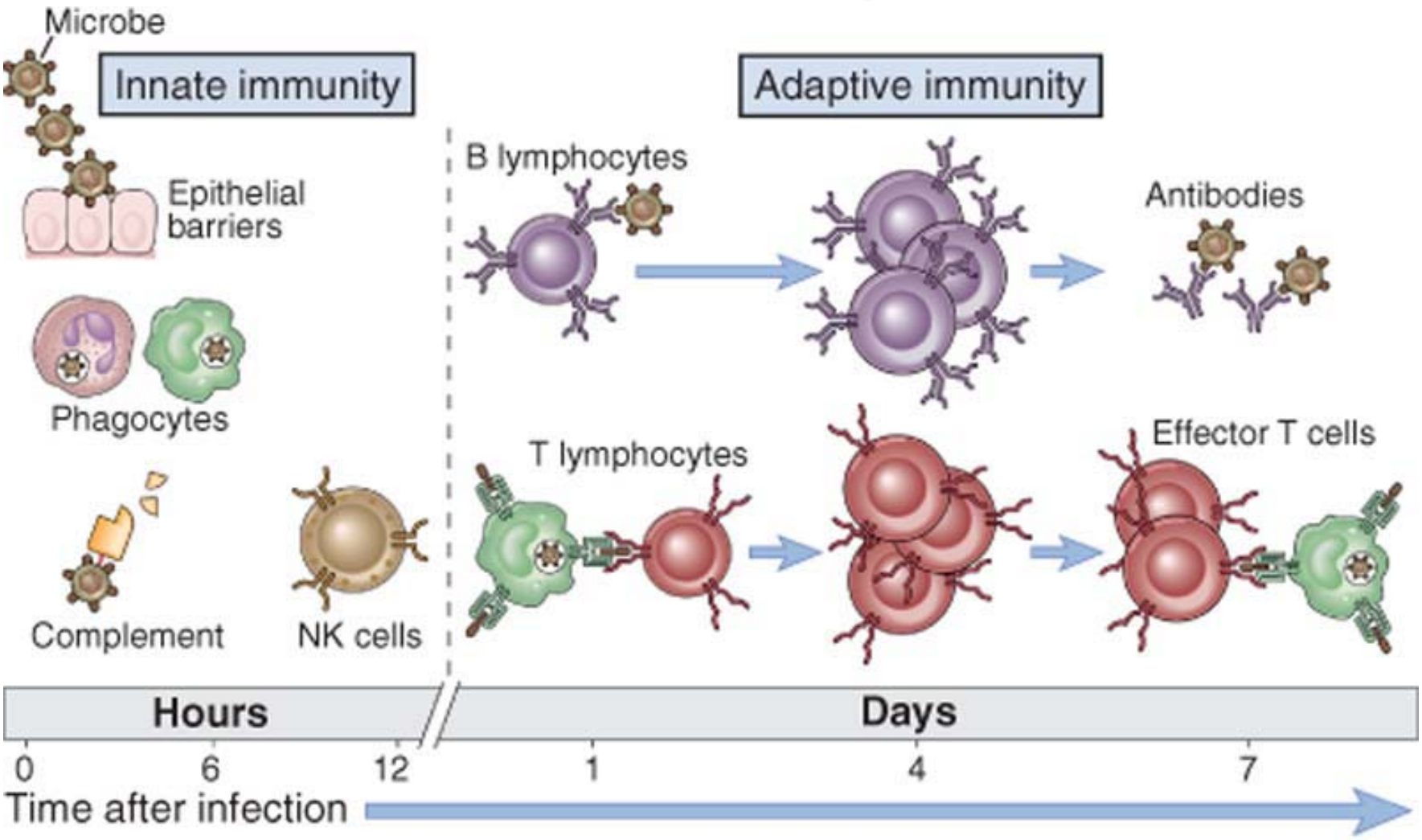
Fc ε RI	High (Kd > 10^{-10} M) binds monomeric IgE	Mast cells, basophils, eosinophils	Cell activation (degranulation)
Fc ε RII (CD23)	Low (Kd > 10^{-7} M)	B lymphocytes, eosinophils, Langerhans cells	Unknown
Fc αR (CD89)	Low (Kd > 10^{-6}M)	Neutrophils, eosinophils, monocytes	Cell activation?

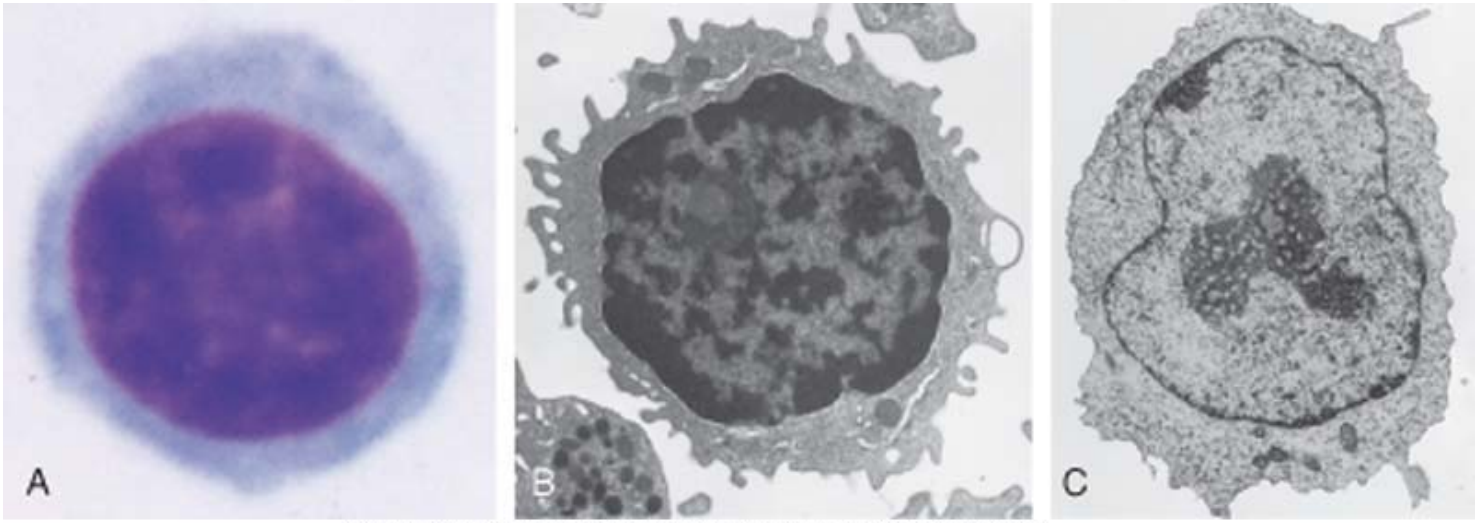
Characteristics of an antibody

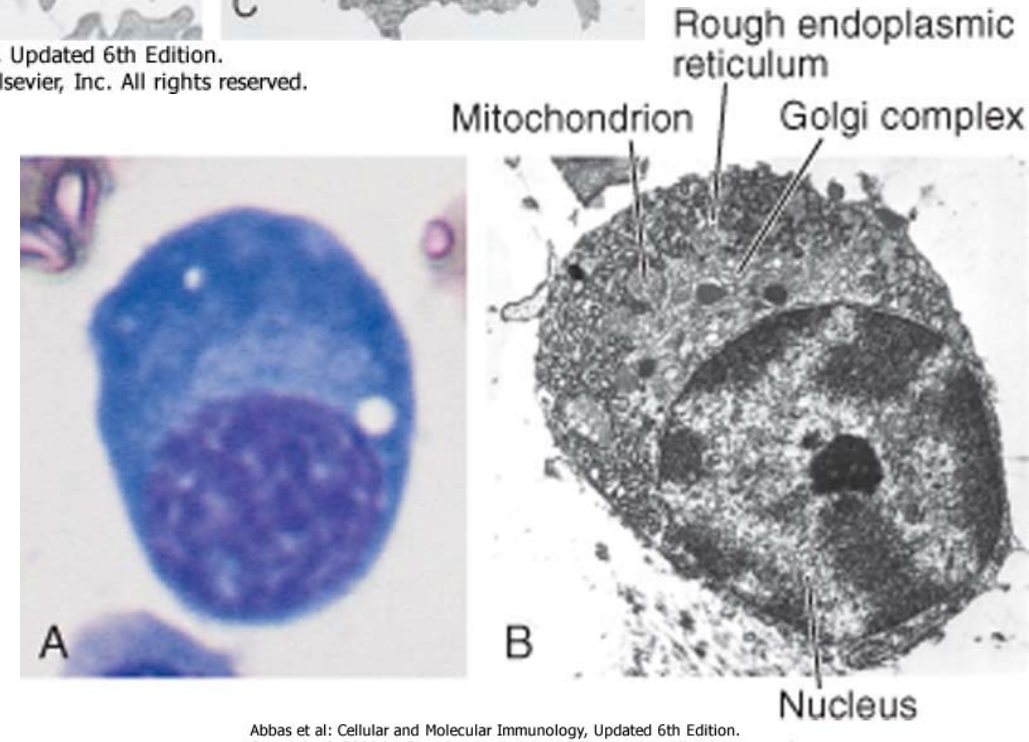
- **Specificity in its action**
- Biodistribution/halflife
- Activation of immune system

Immune response

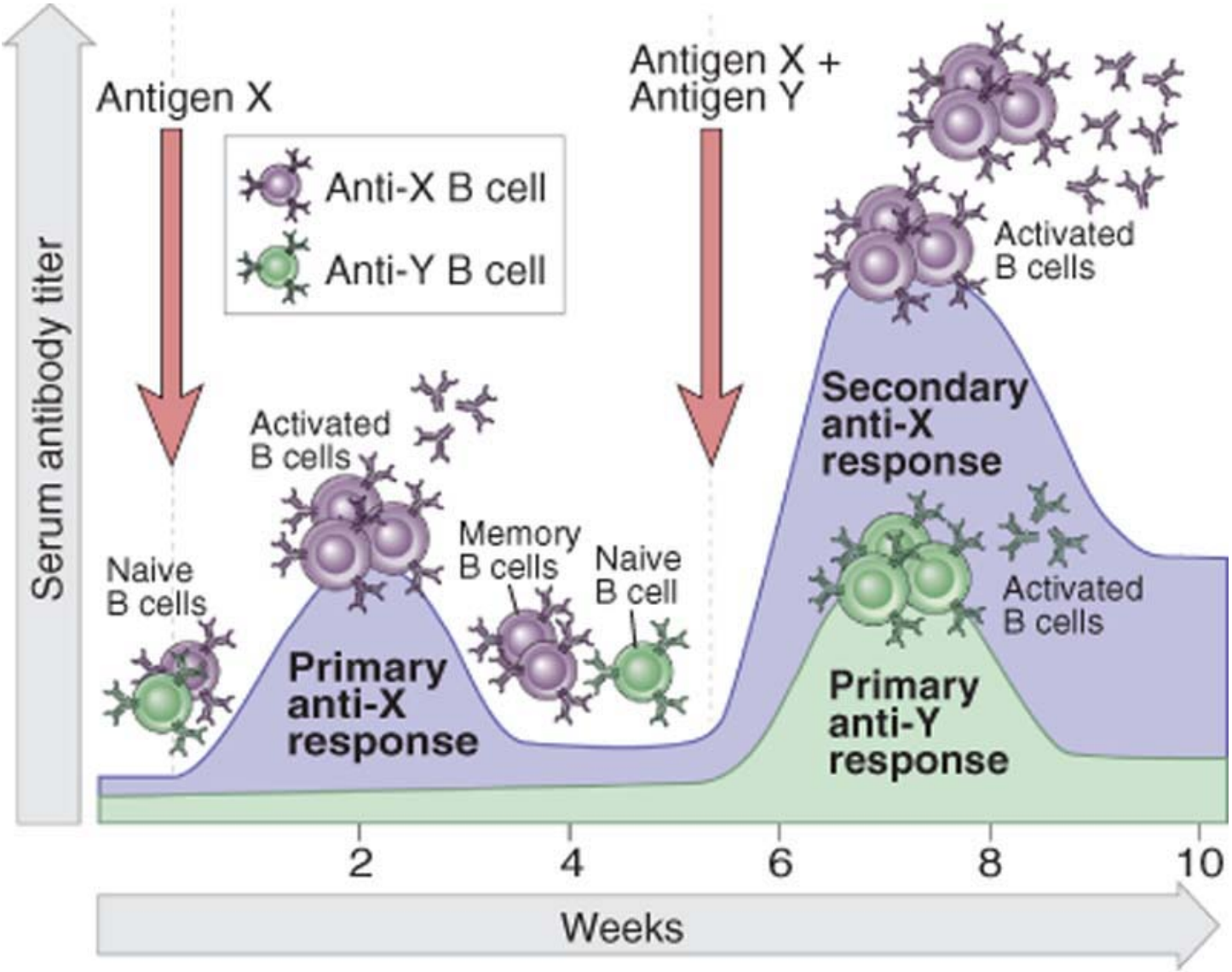


B lymphocytes and plasma cells



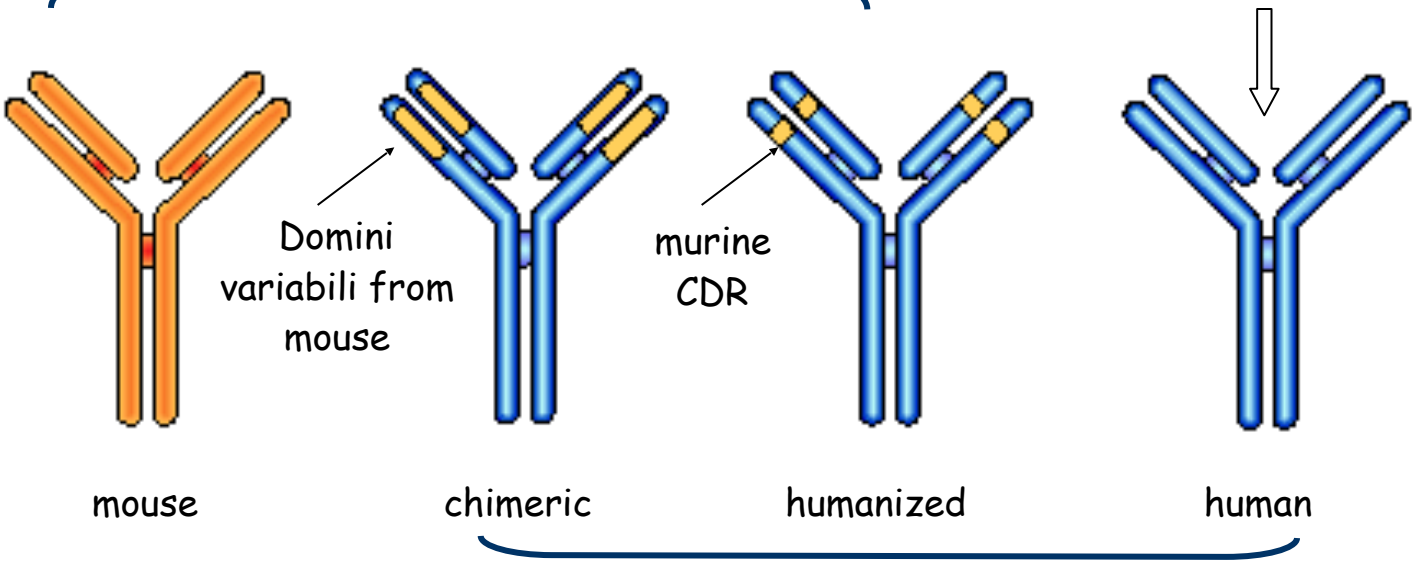


Timing for antibody production



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

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-TNFalfa (Rheumatoid arthritis, crohn disease and other inflammatory diseases)
6. Adalimumab: human anti-TNFalfa (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 indice 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 (creast 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 (IgG3) 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)

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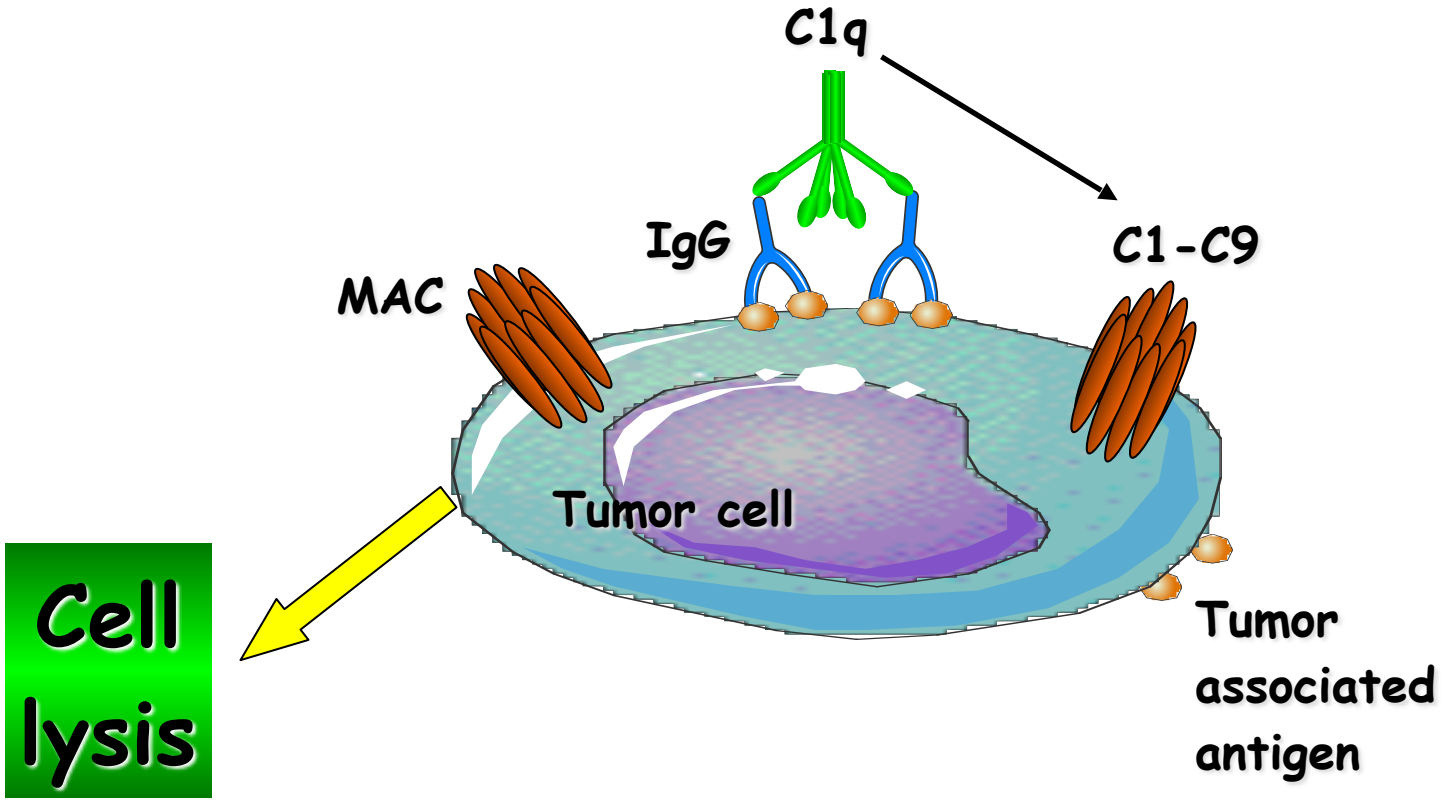
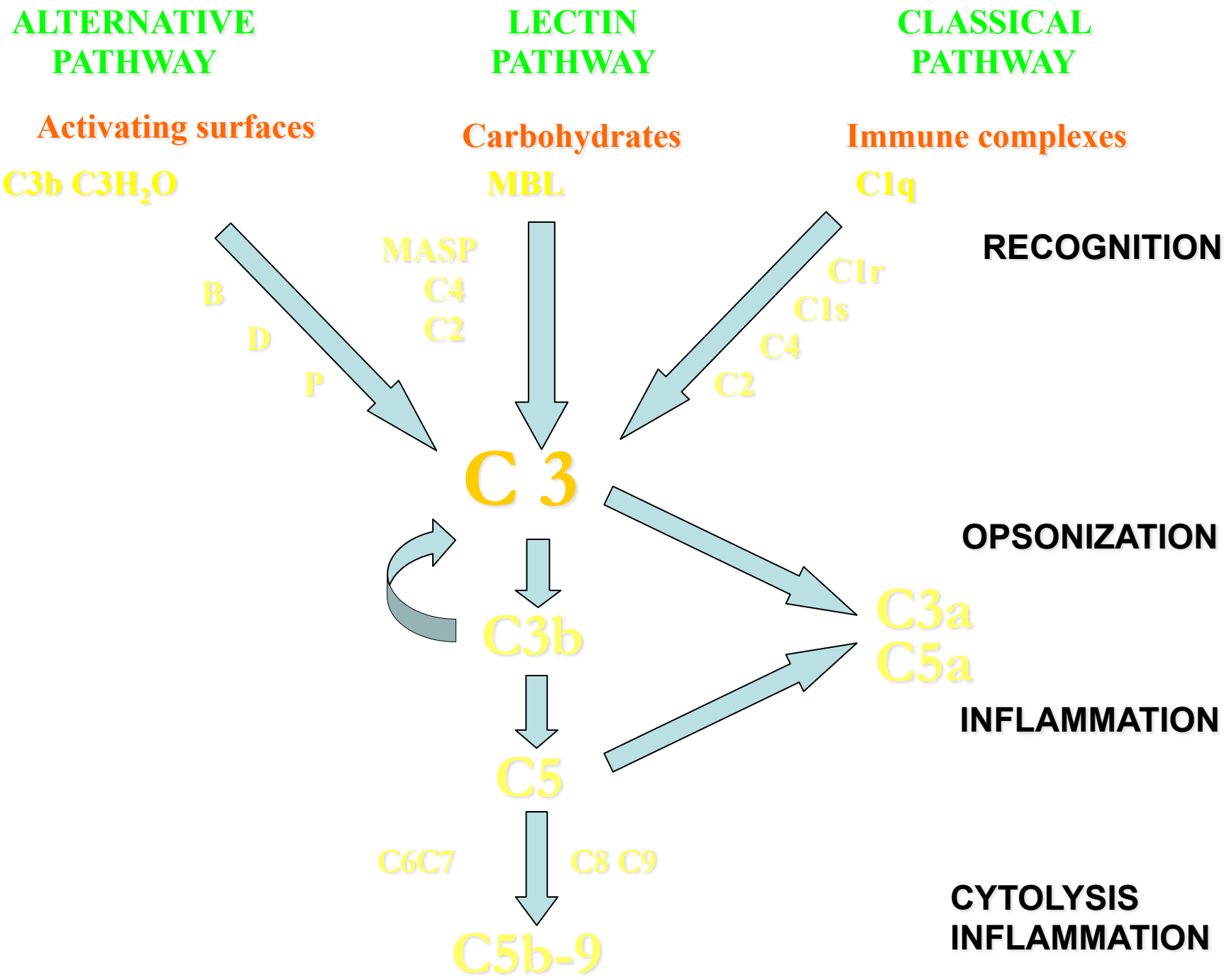
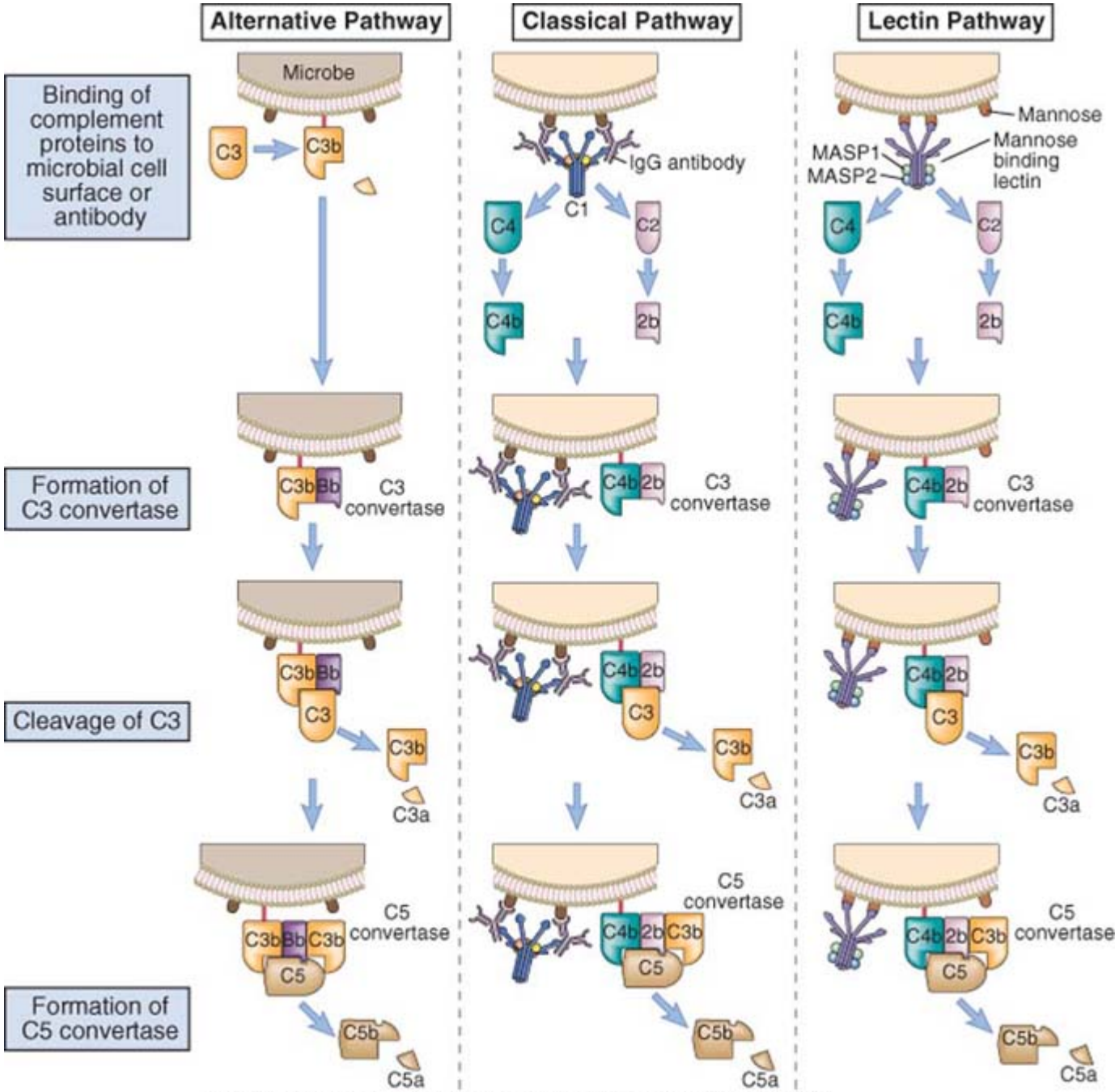
IgD	Antigen receptor of naive B lymphocytes

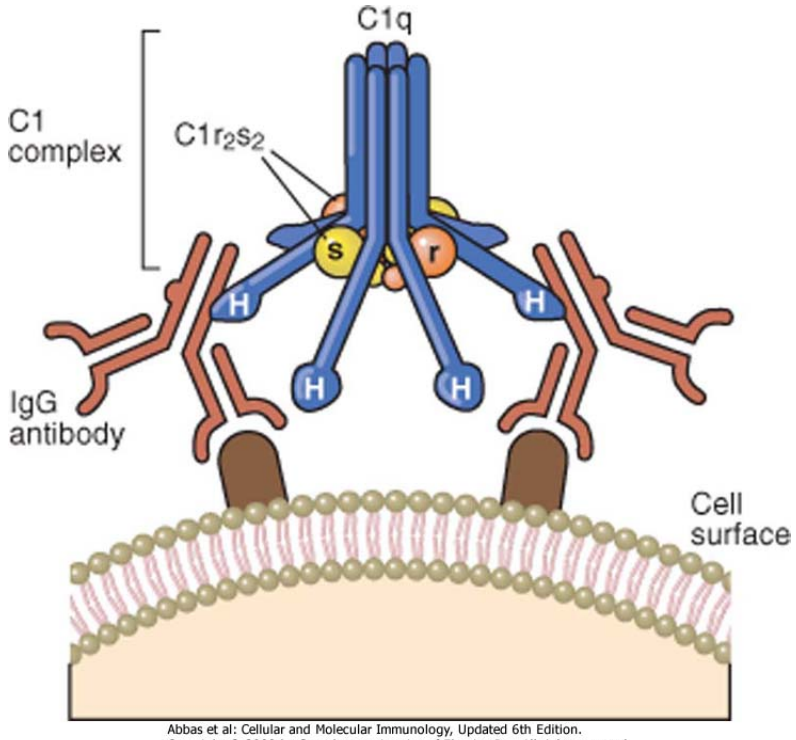
Immune-activator monoclonal antibodies:

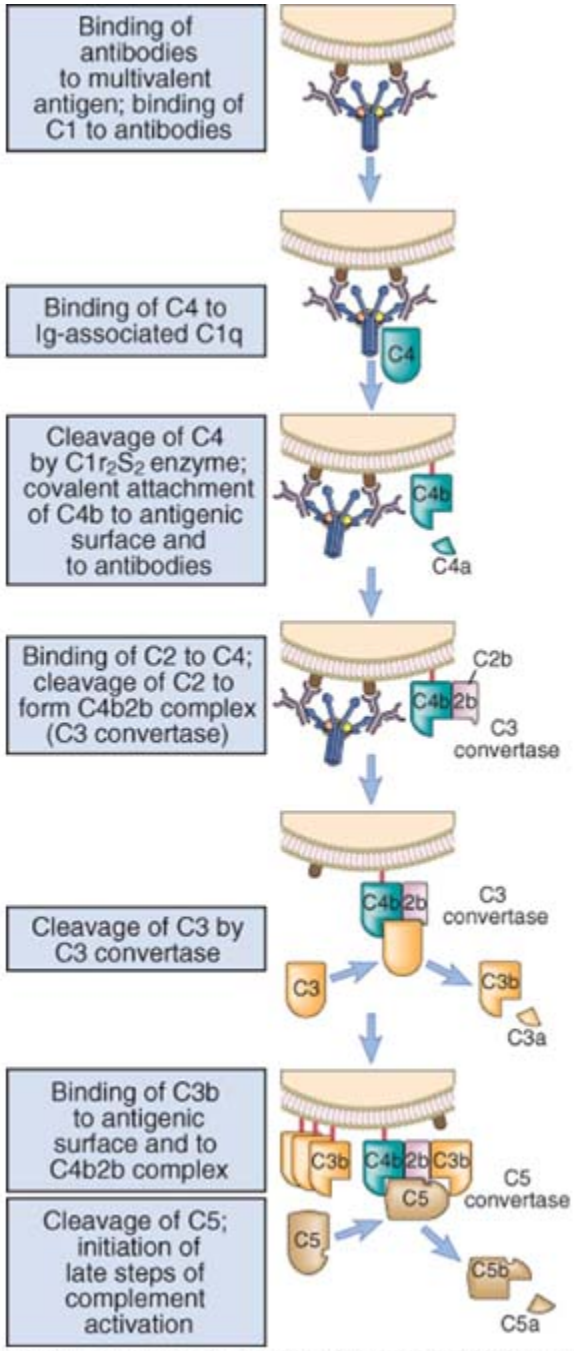
CDC (Complement-Dependent Cytotoxicity)

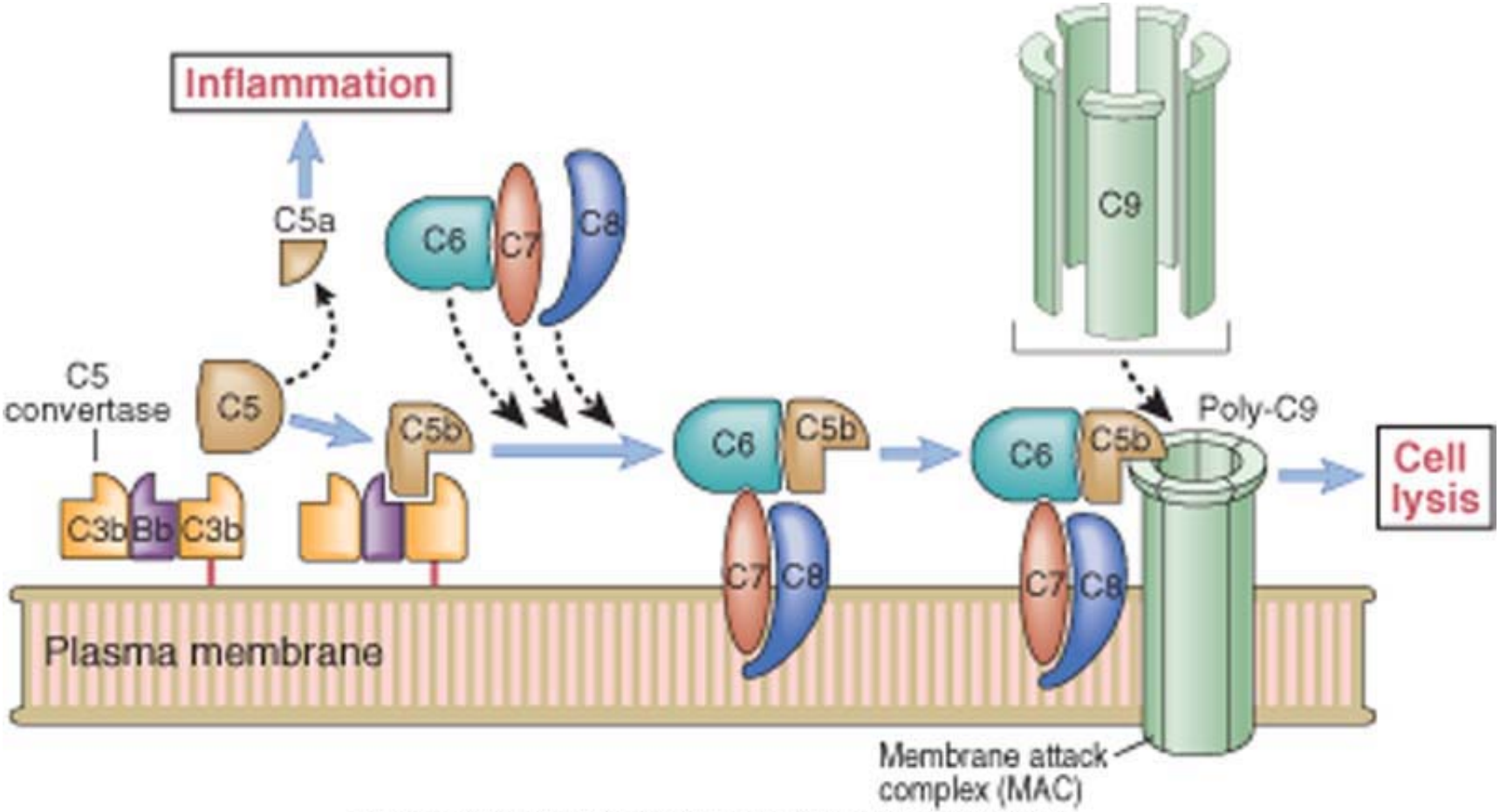


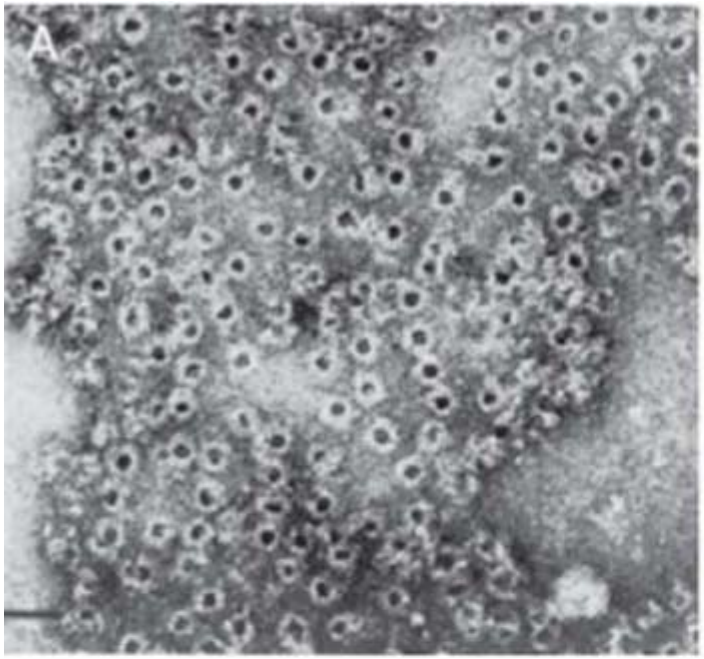

ALTERNATIVE PATHWAY
LECTIN PATHWAY
CLASSICAL PATHWAY
Activating surfaces
Carbohydrates
Immune complexes
C3b C3H2O
MBL
C1q
RECOGNITION
MASP
C4
C2
B
D
P
C1r
C1s
C4
C2
C 3
OPSONIZATION
C3b
C3a
C5a
INFLAMMATION
C5
C6C7
C8 C9
C5b-9
CYTOLYSIS
INFLAMMATION

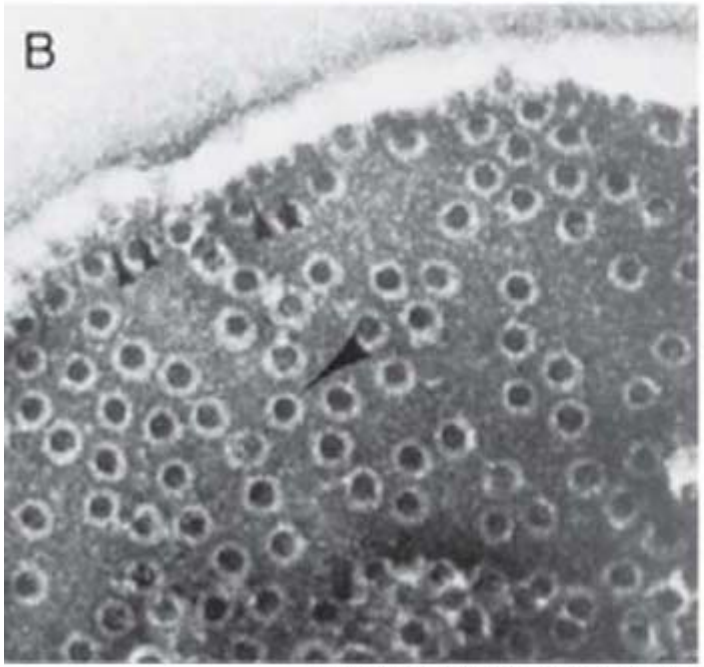


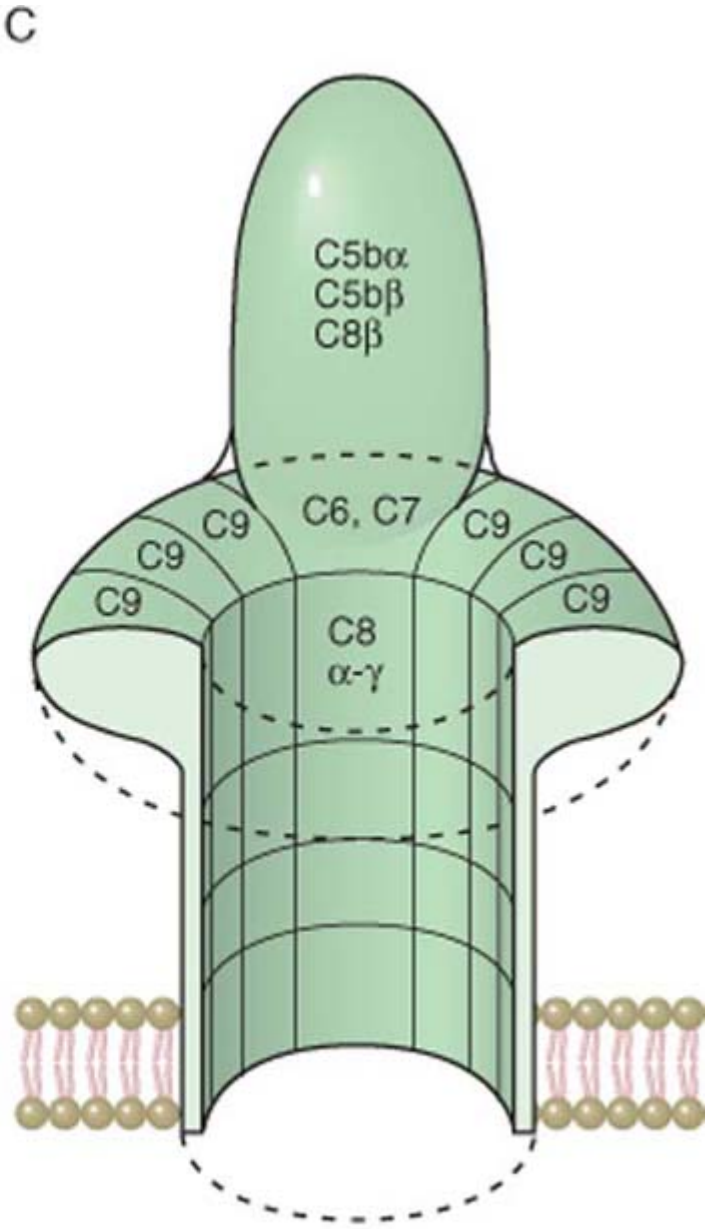


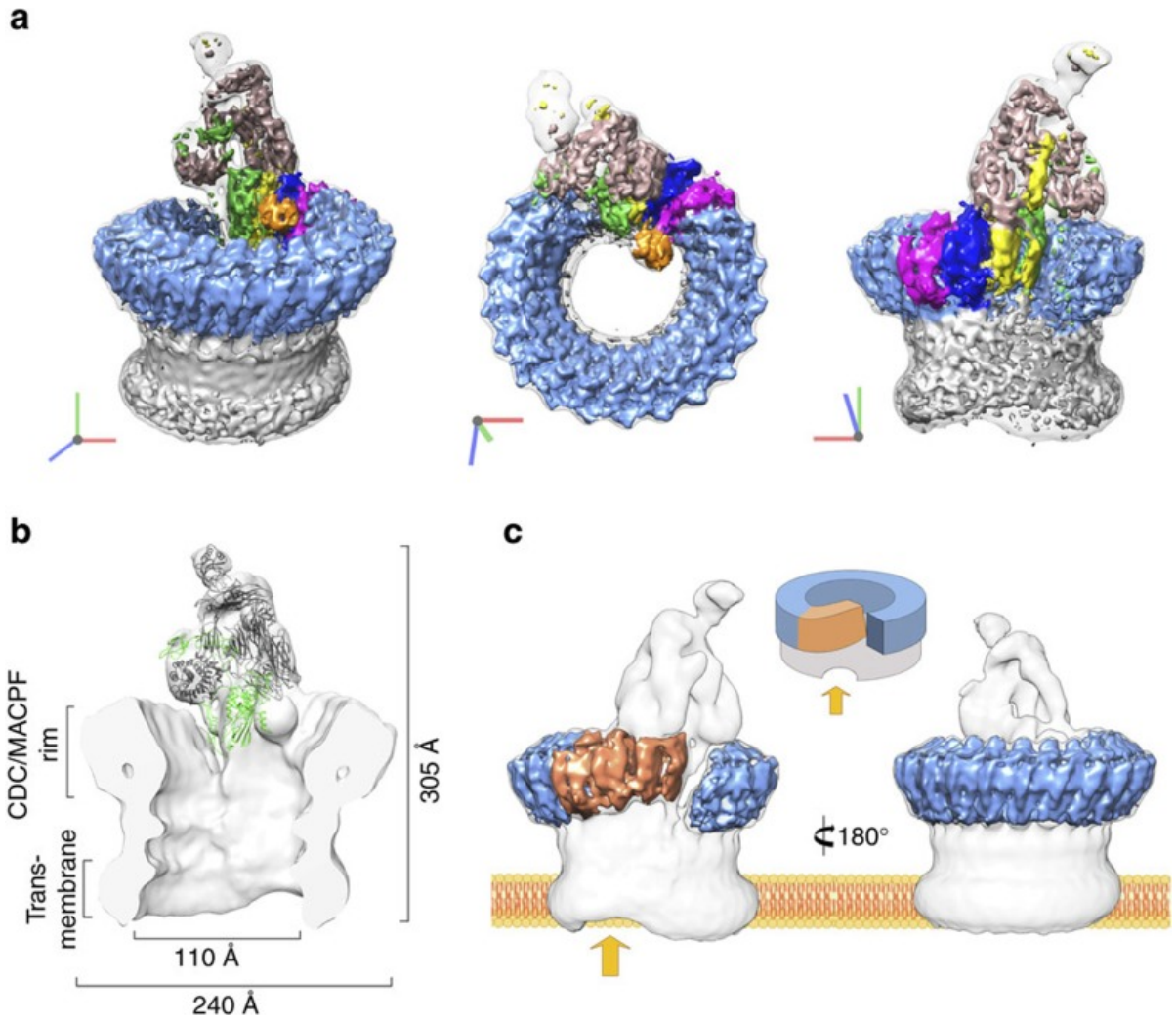




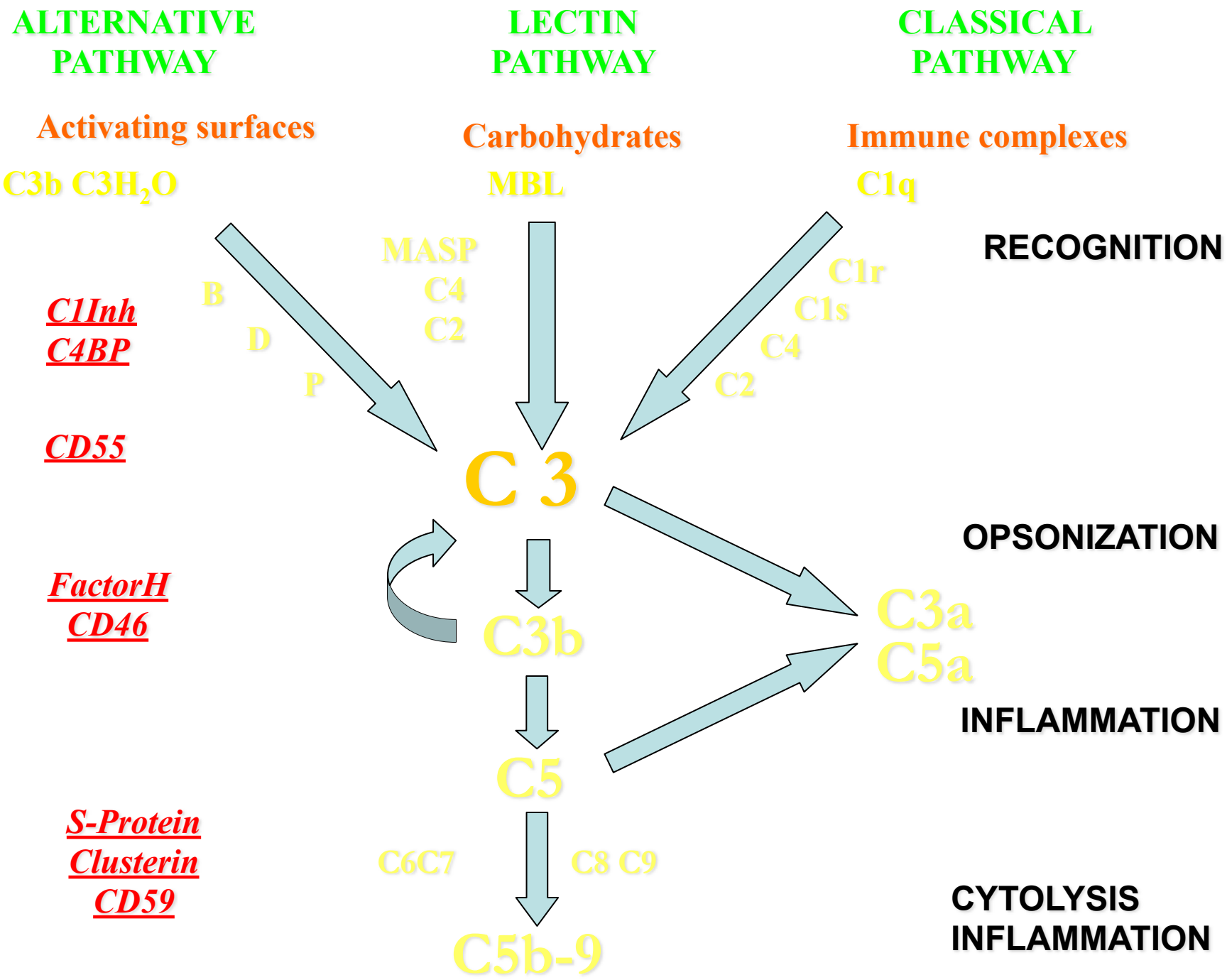








Seva et al,
Nat Comm, 2016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C4
C2
C1Inh
C4BP
CD55
C 3
OPSONIZATION
FactorH
CD46
C3b
C3a
C5a
INFLAMMATION
C5
S-Protein
Clusterin
CD59
C6C7
C8 C9
CYTOLYSIS
INFLAMMATION
C5b-9

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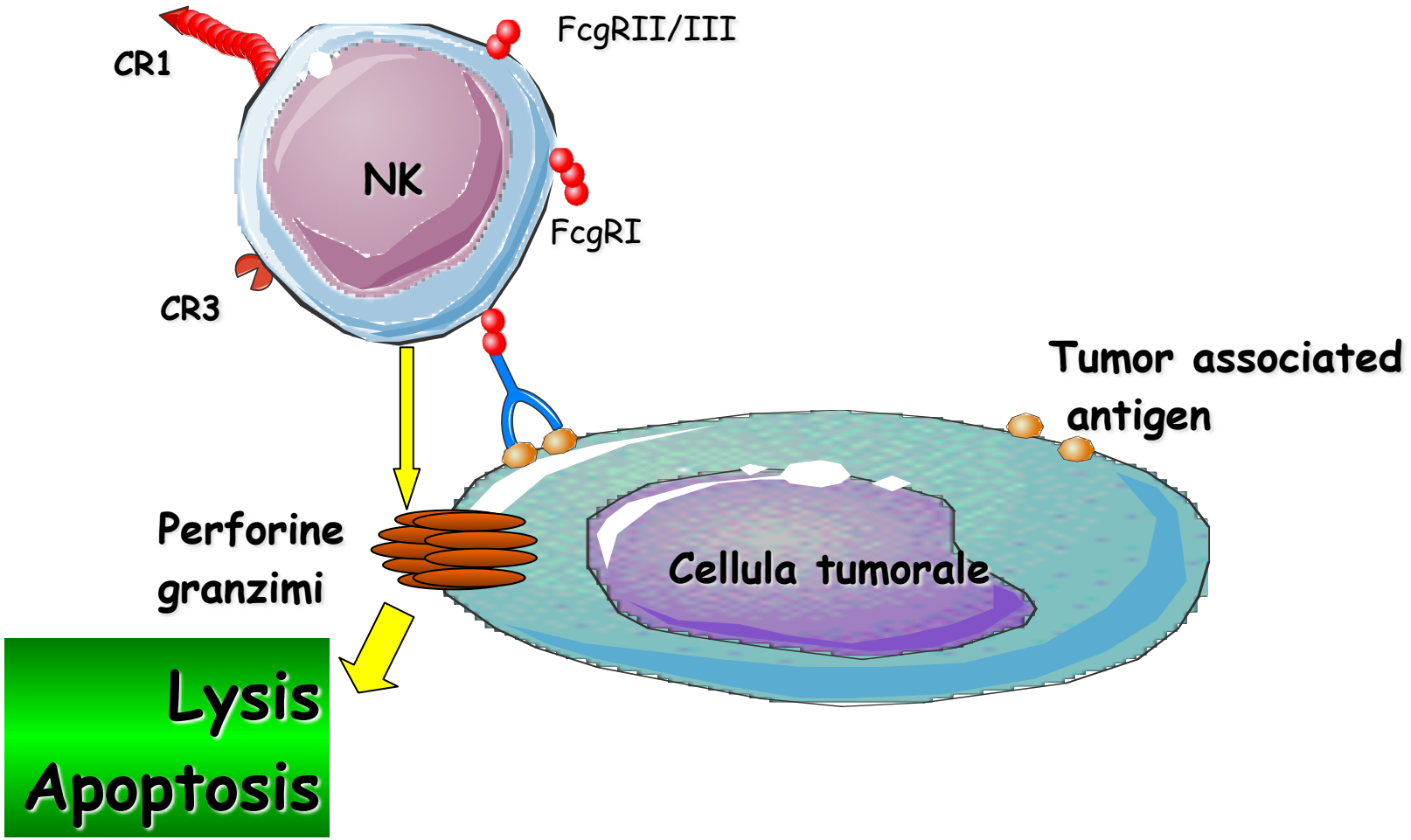
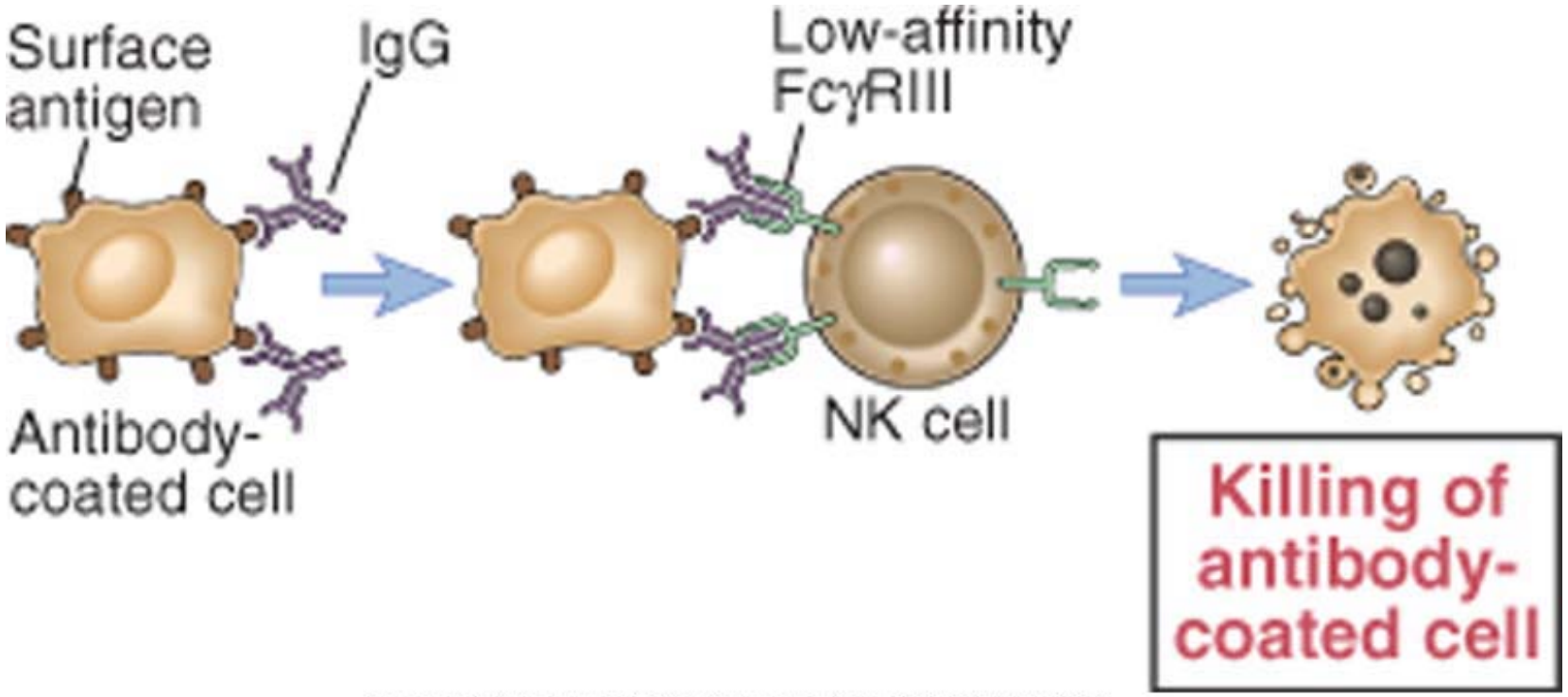
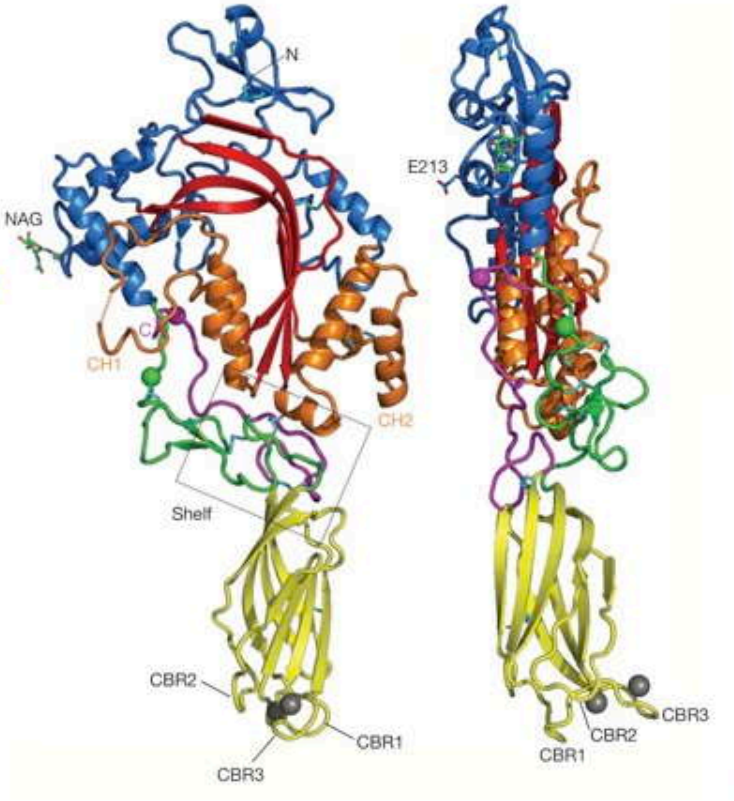
Immune-activator monoclonal antibodies:

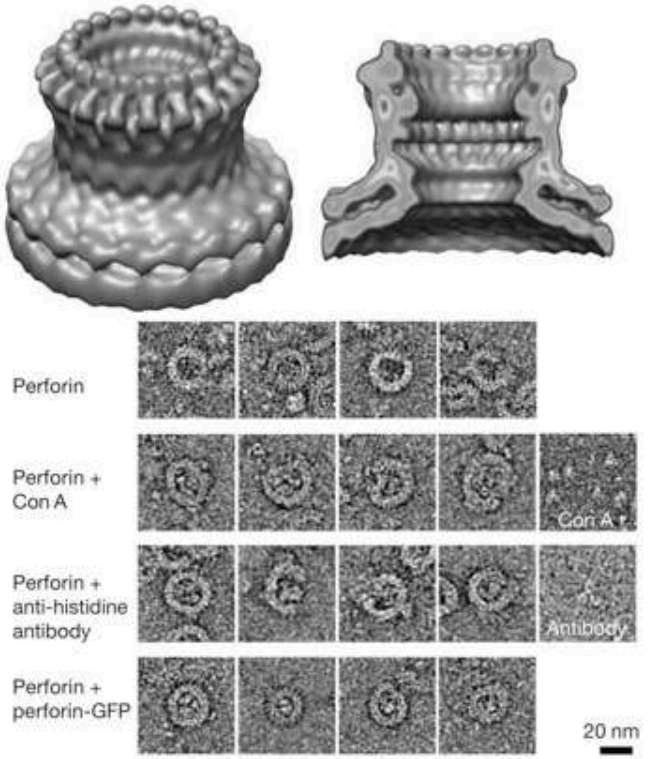
ADCC (Antibody-Dependent Cellular Cytotoxicity)



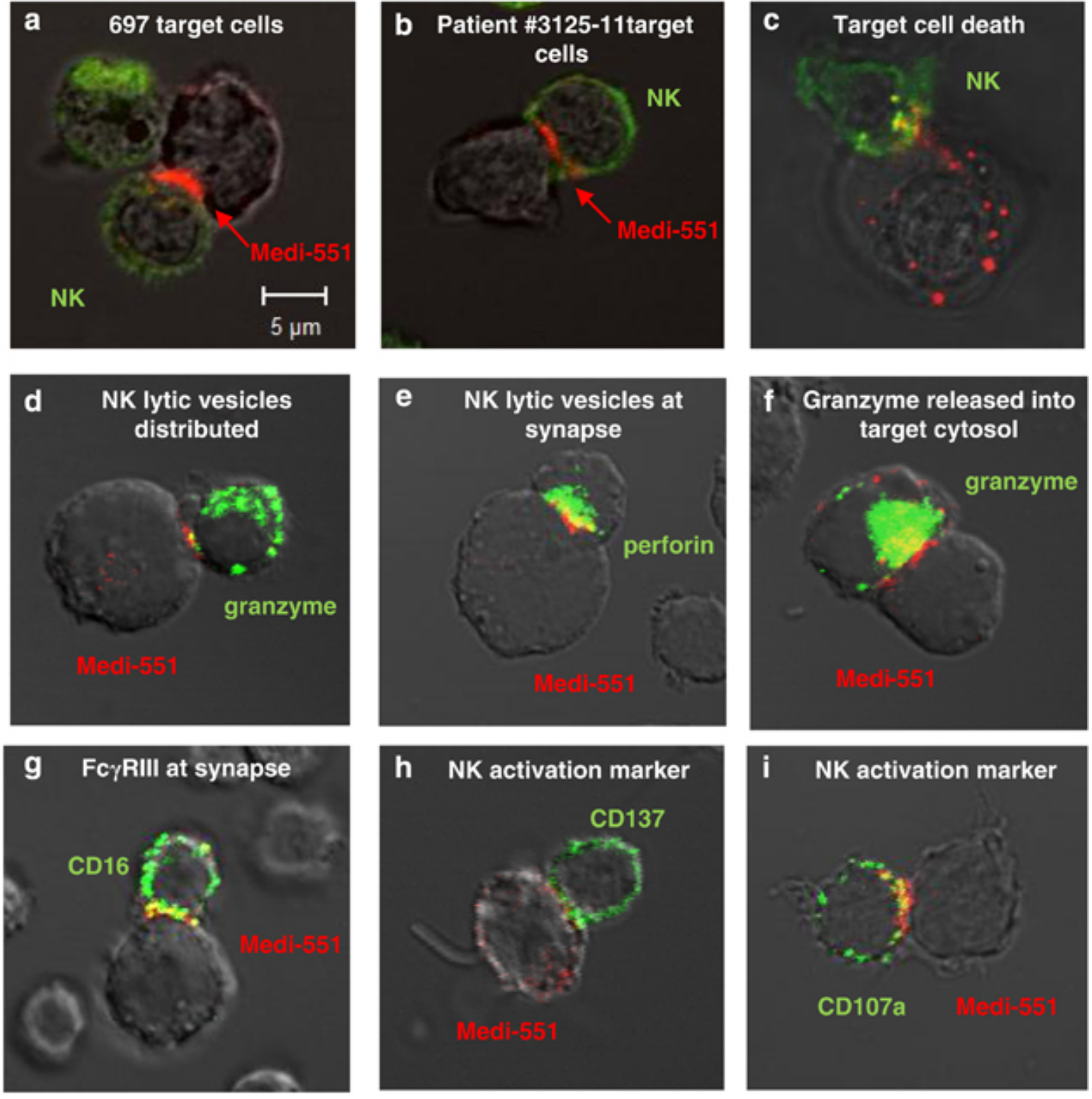


Structures of the lymphocyte perforin monomer and pore network





Law RHP et al Nature 468: 447 (2010)


a
697 target cells
Medi-551
NK
5 μm
b
Patient #3125-11target cells
NK
Medi-551
c
Target cell death
NK
d
NK lytic vesicles distributed
granzyme
Medi-551
e
NK lytic vesicles at synapse
perforin
Medi-551
f
Granzyme released into target cytosol
granzyme
Medi-551
g
FcγRIII at synapse
CD16
Medi-551
h
NK activation marker
CD137
Medi-551
i
NK activation marker
CD107a
Medi-551

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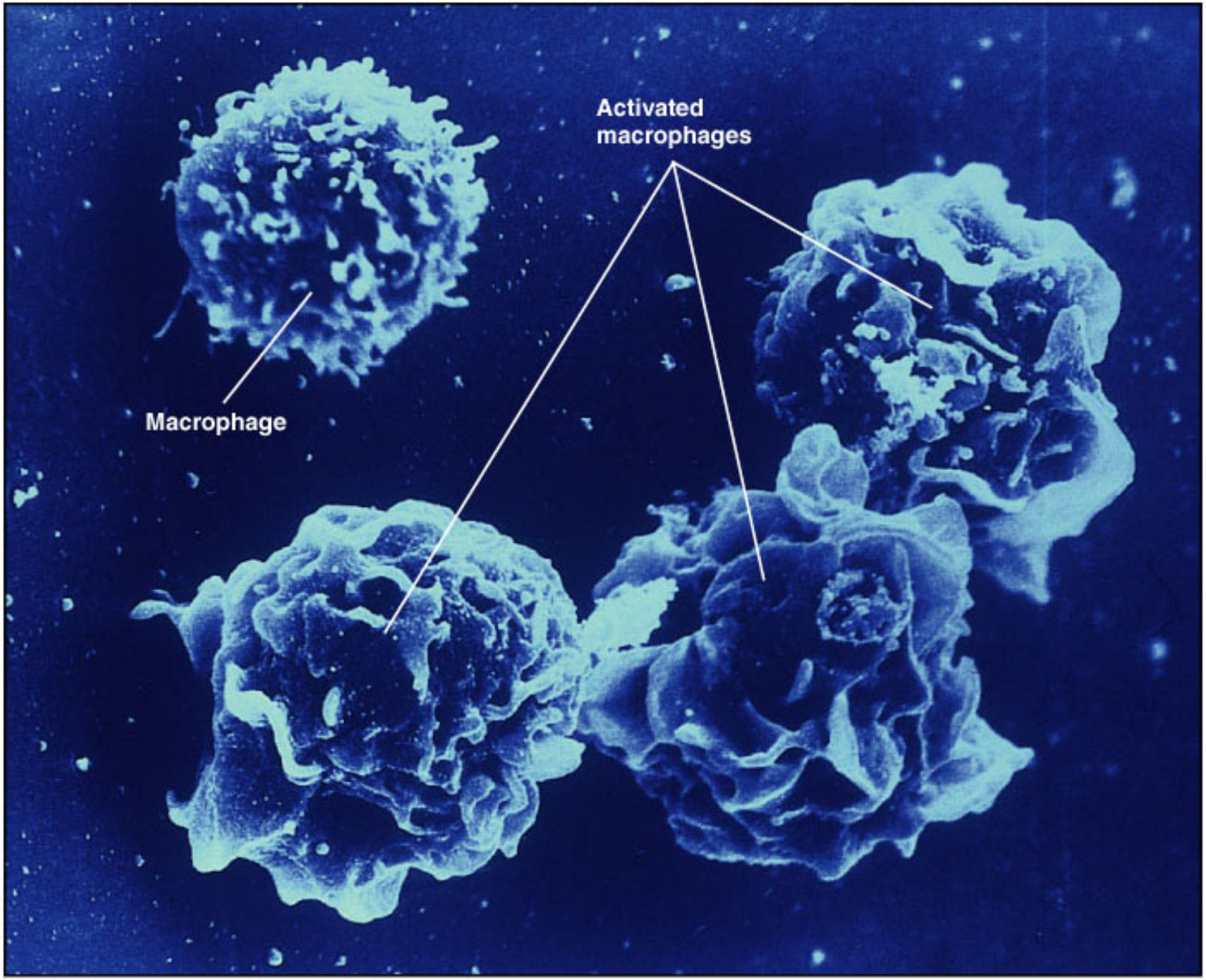
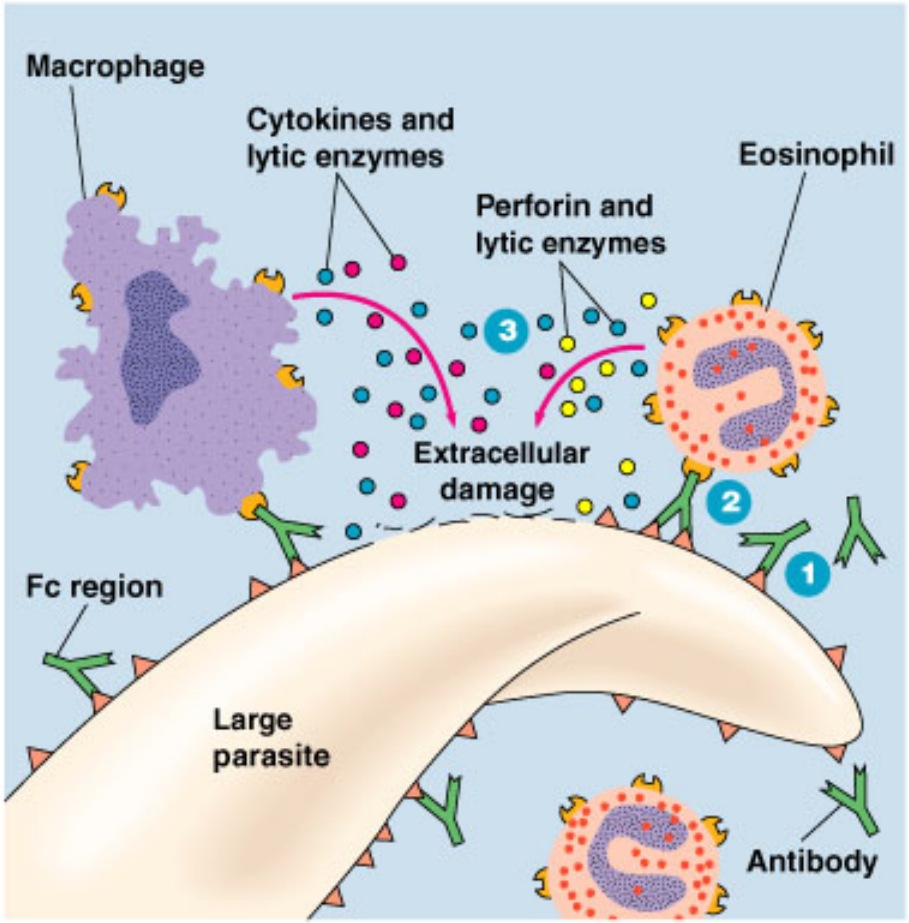
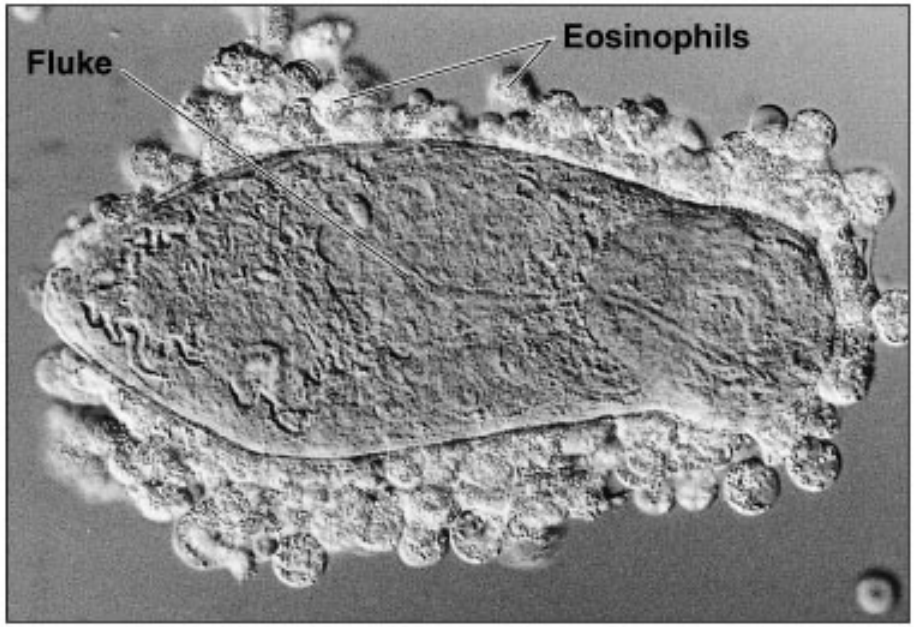


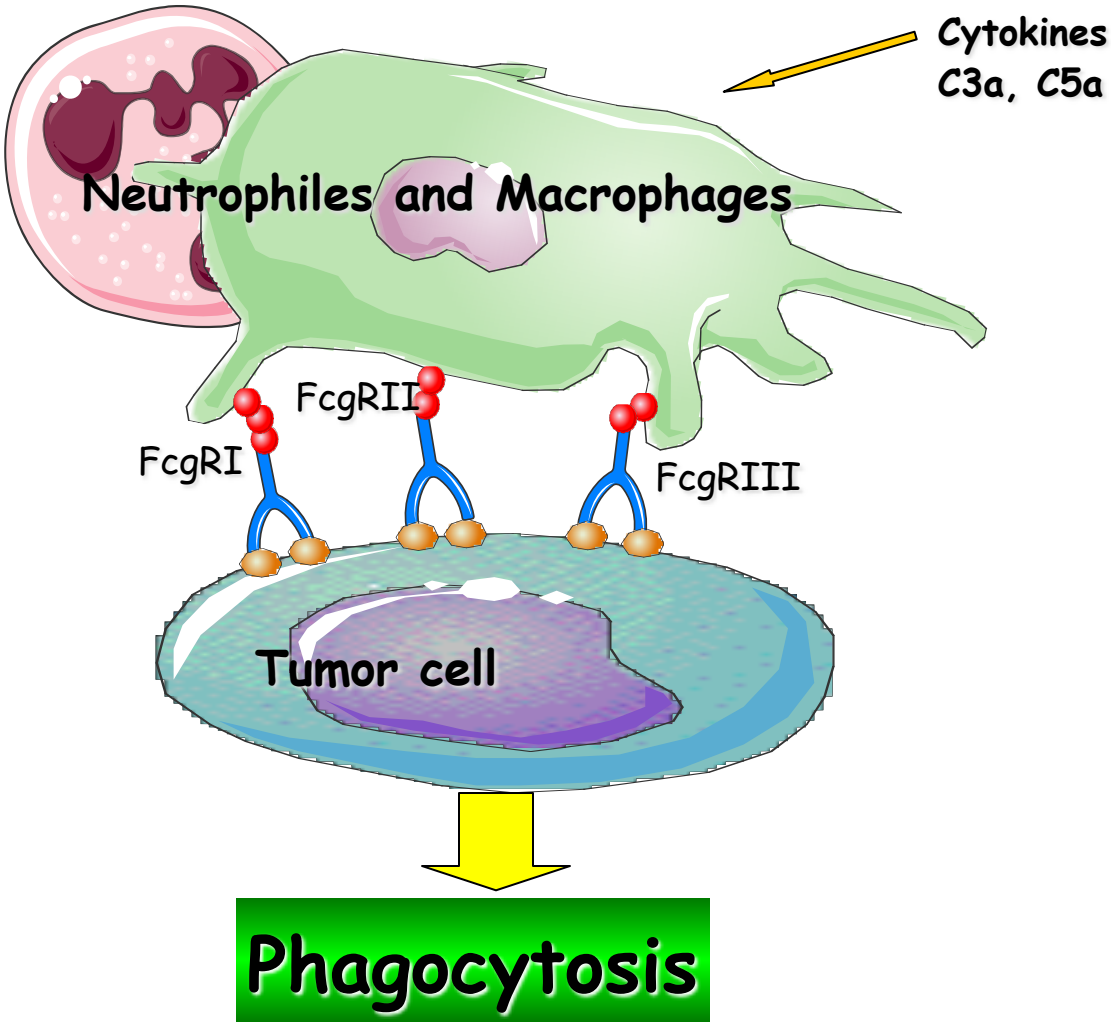


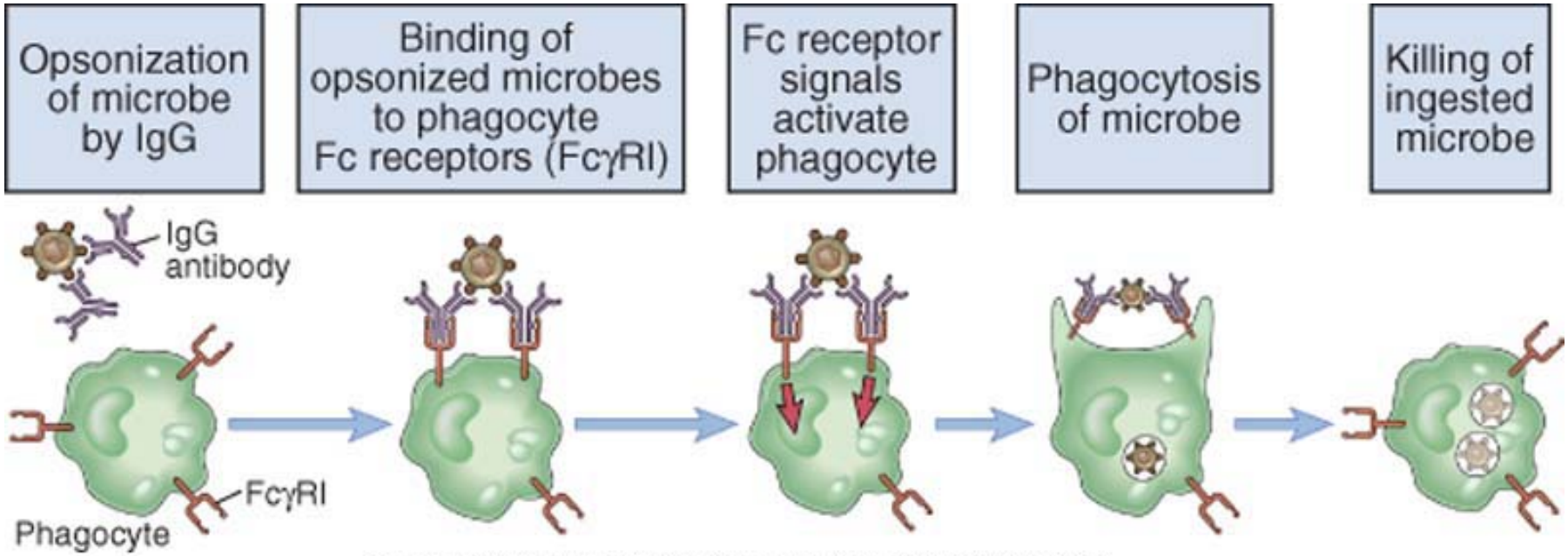
(a)

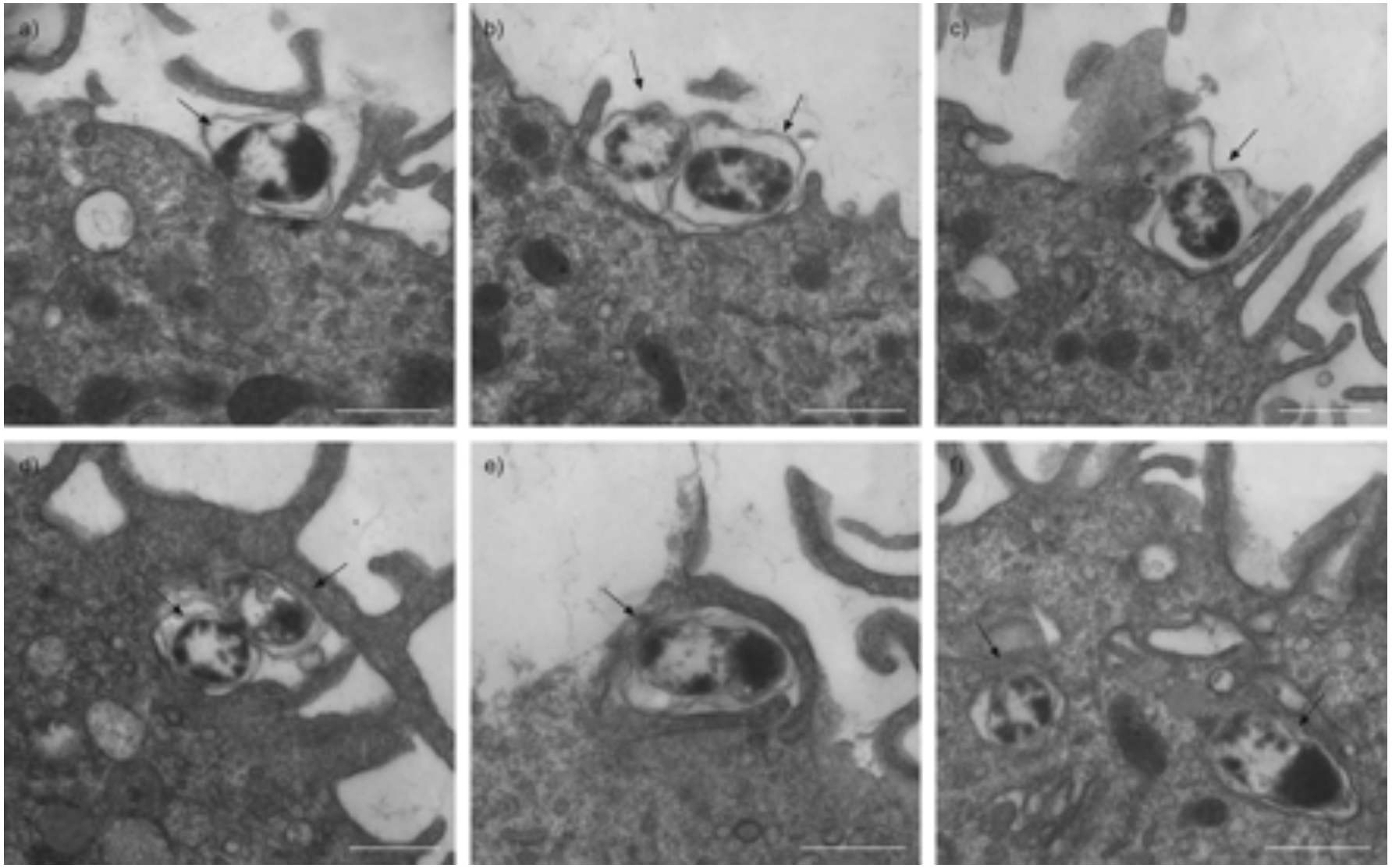


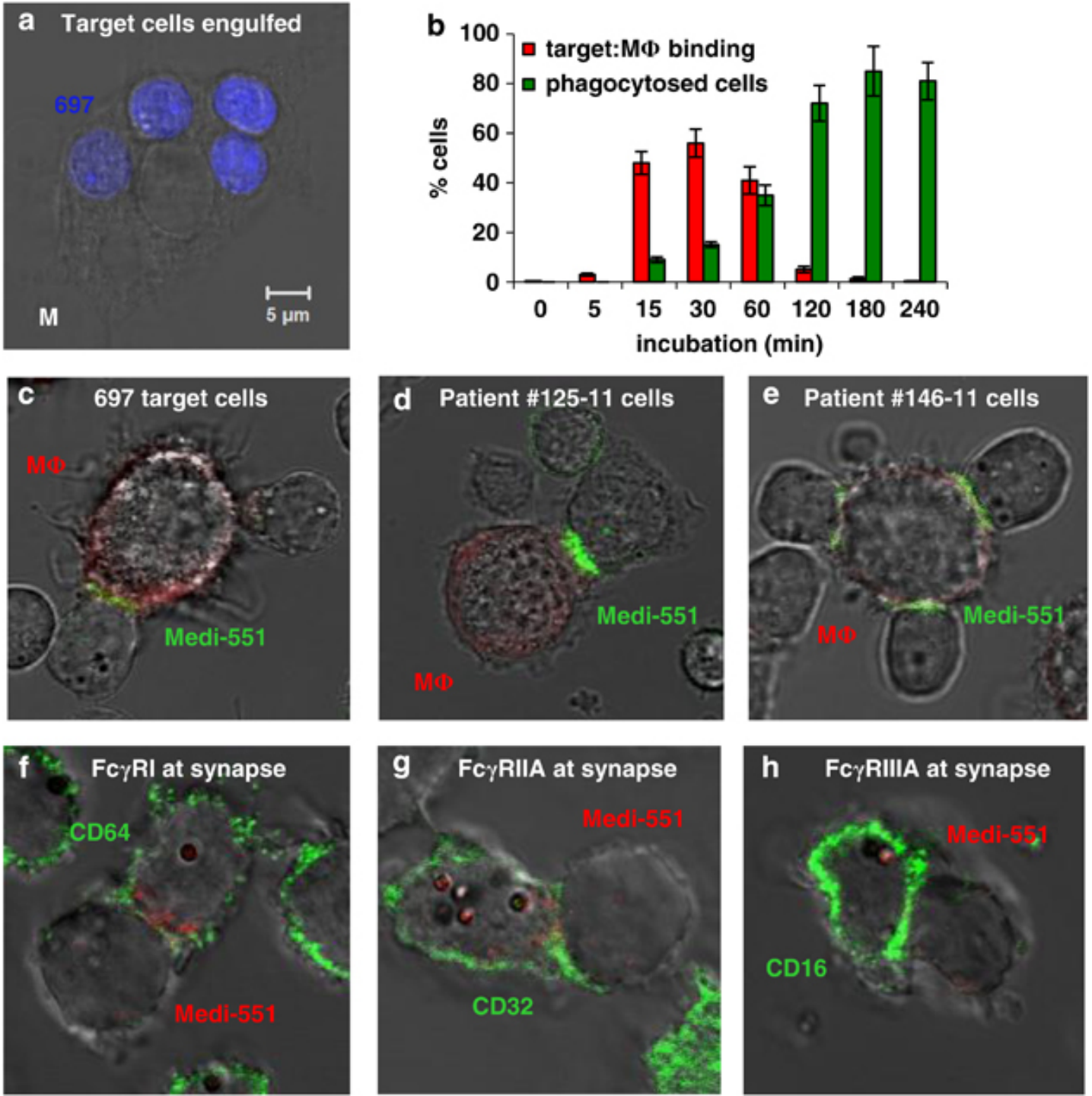
(b)

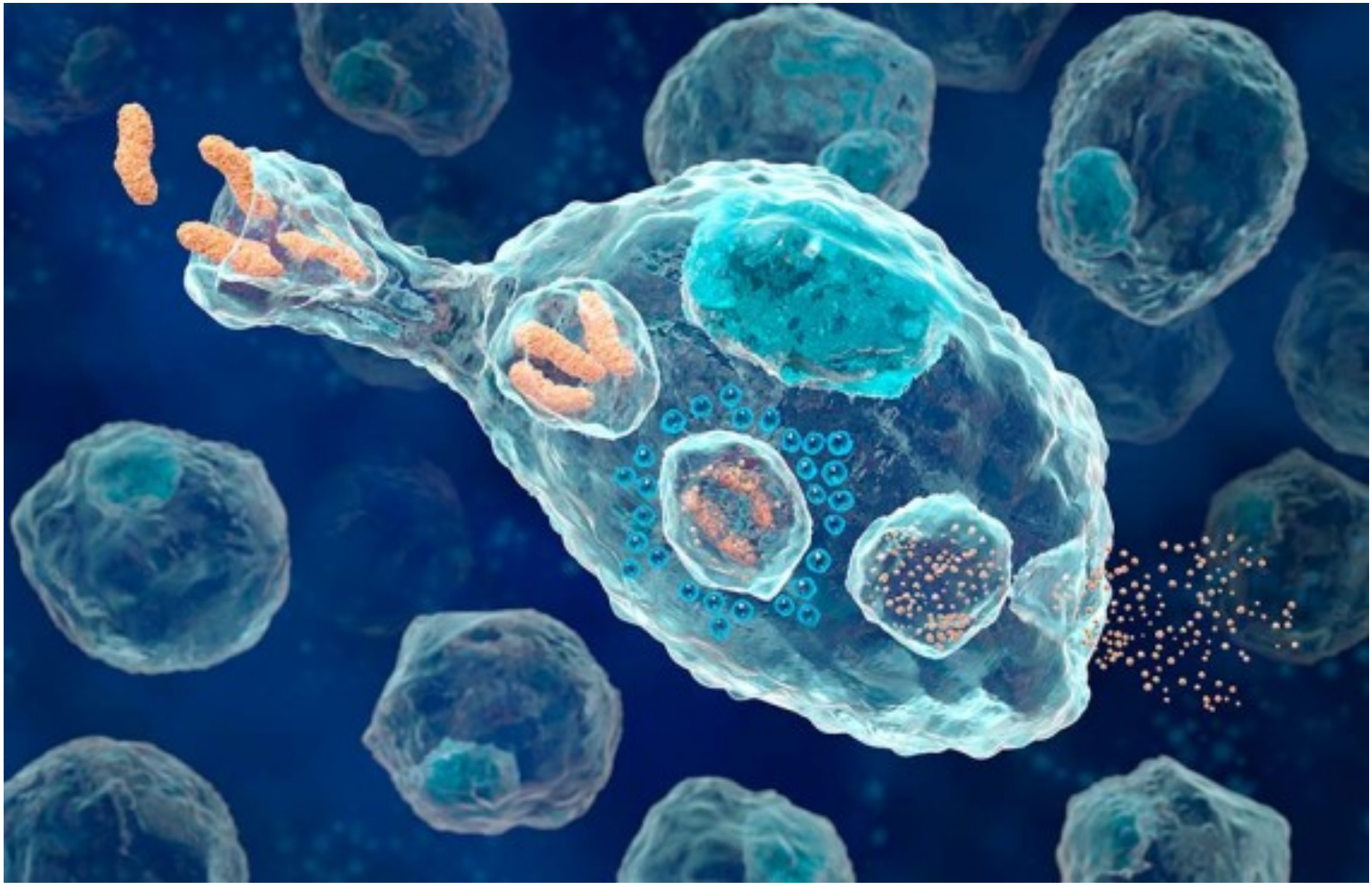
Immune-activator monoclonal antibodies: Phagocytosis





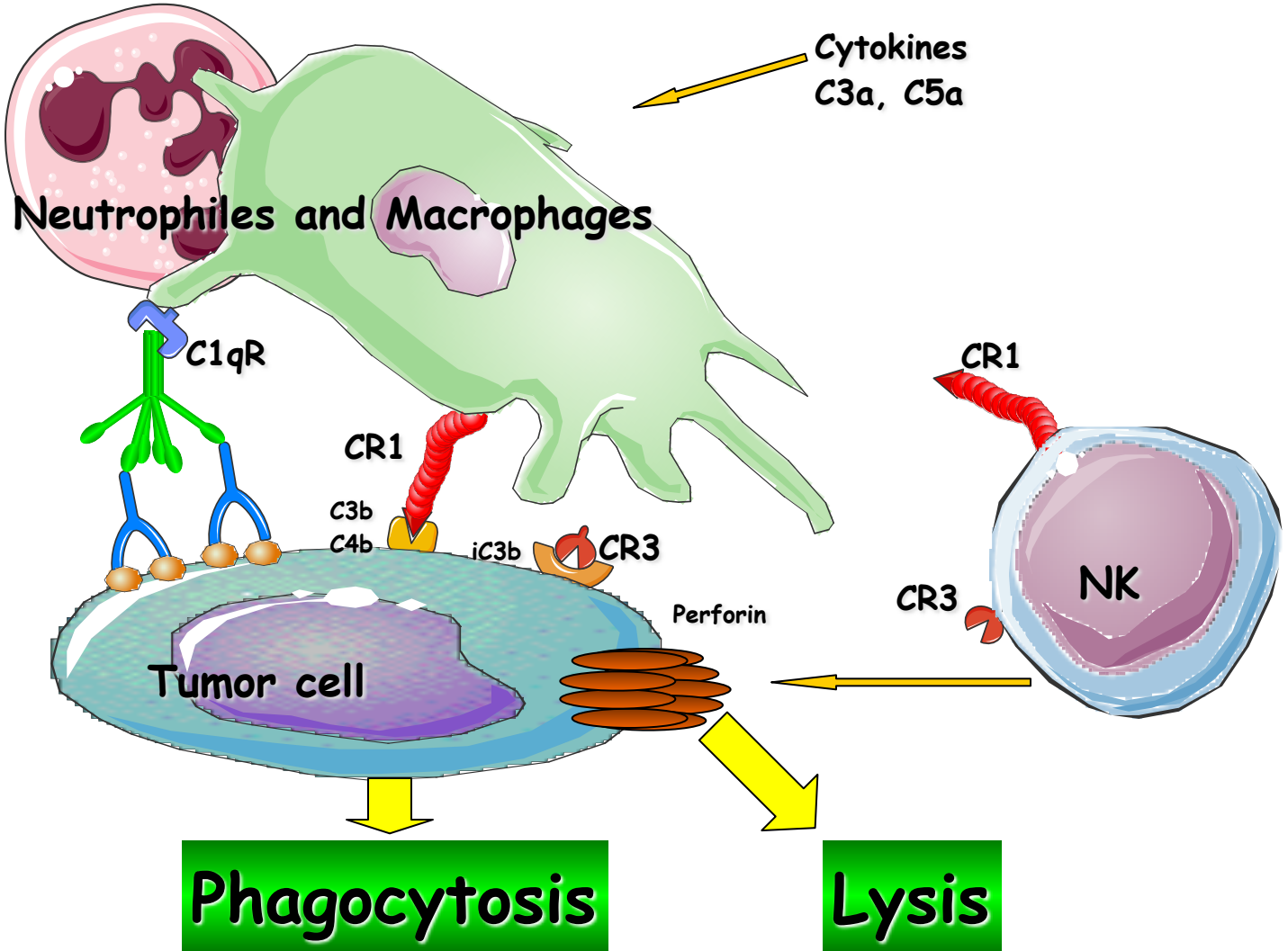

a)
b)
c)
d)
e)
f)

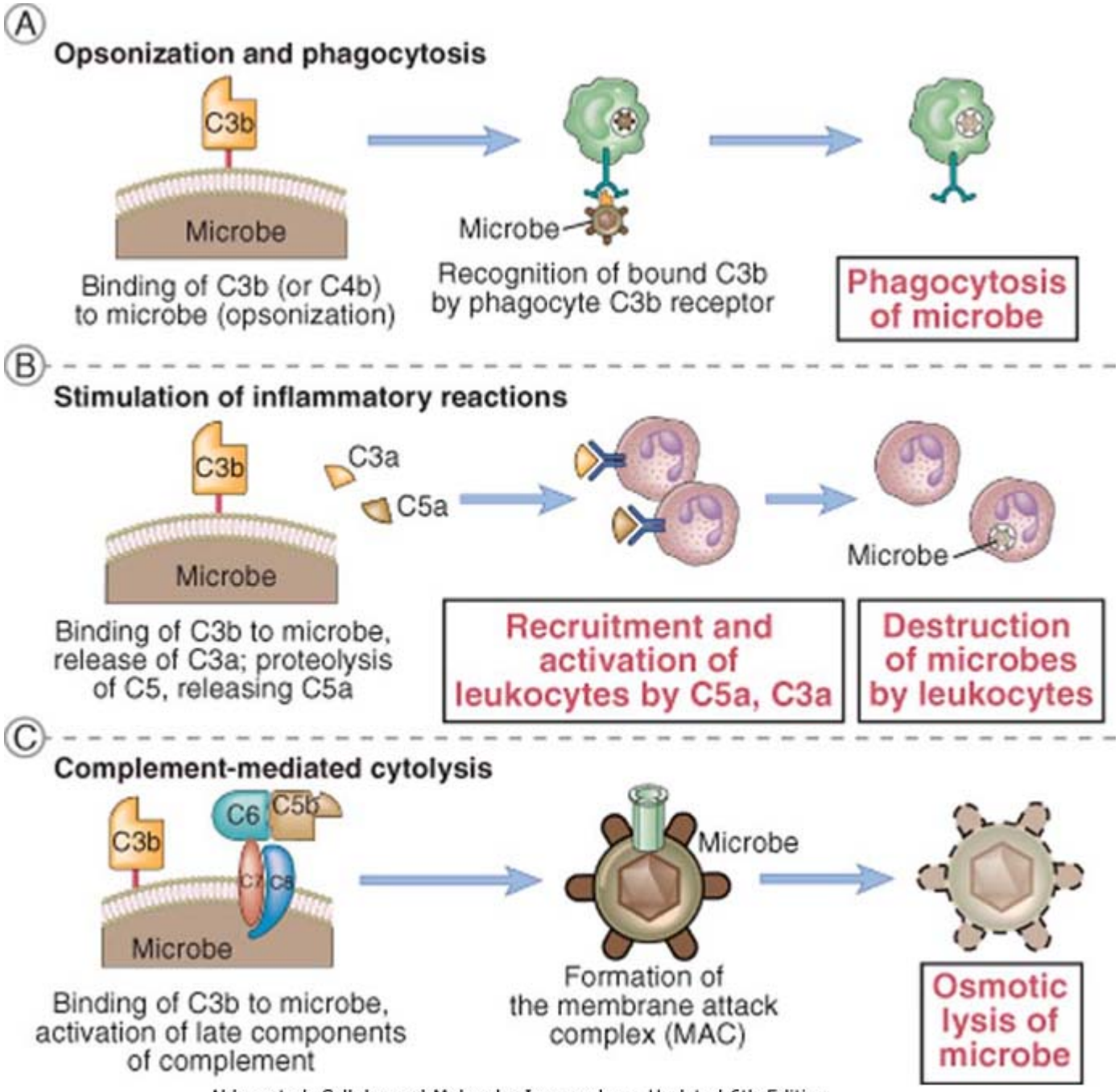

a
Target cells engulfed
697
M
5 μm
b
target:MΦ binding
phagocytosed cells
% cells
100
80
60
40
20
0
0
5
15
30
60
120
180
240
incubation (min)
c
697 target cells
MΦ
Medi-551
d
Patient #125-11 cells
Medi-551
MΦ
e
Patient #146-11 cells
MΦ
Medi-551
f
FcγRI at synapse
CD64
Medi-551
g
FcγRIIA at synapse
Medi-551
CD32
h
FcγRIIIA at synapse
Medi-551
CD16



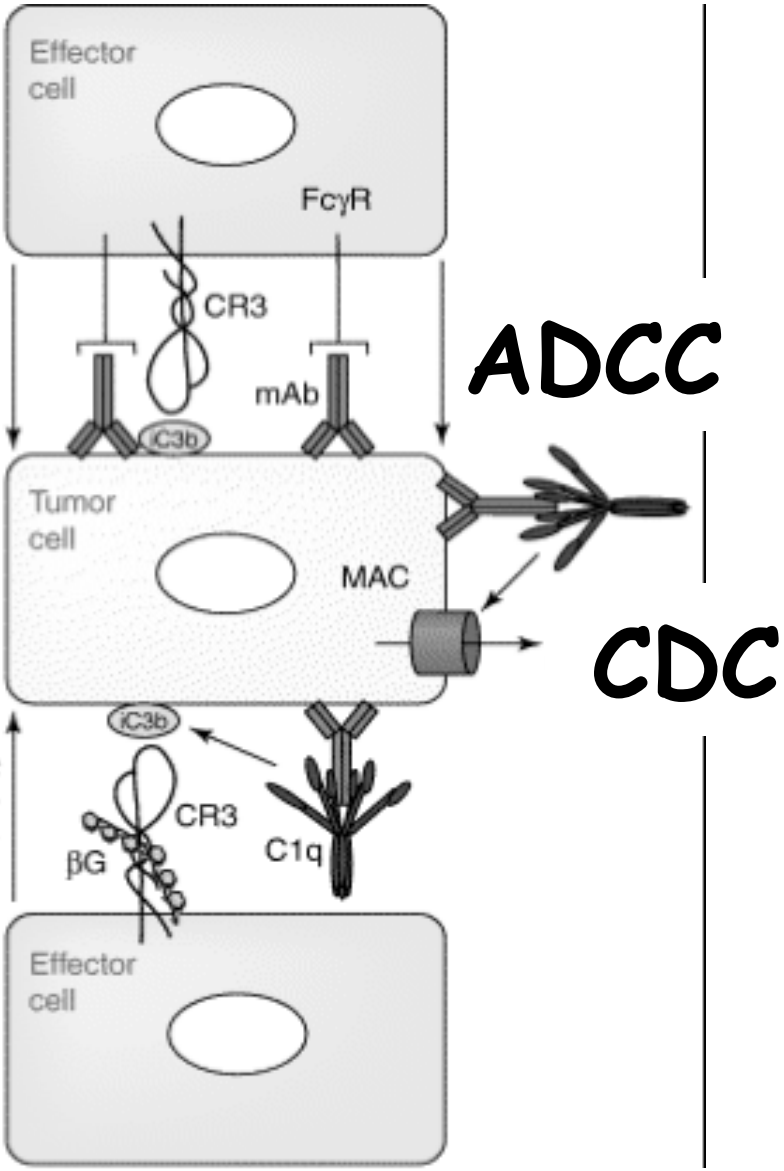
Immune-activator monoclonal antibodies:

CDCC (Complement-Dependent Cellular 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 FcgRIII 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.