Functions of antibodies

Main functions of antibodies

Neutralization	The pathogen is not destroyed, but its capability to infect cells is impaired
Elimination	The pathogen itself, or cells infected with it, are destroyed

Main functions of antibodies

Neutralization	The pathogen is not destroyed, but its capability to infect cells is impaired
	Antibodies — **Antigen binding site**
Elimination	The pathogen itself, or cells infected with it, are destroyed
	Antibodies + other components (macrophages, NKs, complement) — **Antigen binding site + Fc portion**

How many Fc Receptors?

	FcR	Affinity for Immunoglobulin	Cell Distribution	Function
FcγR	FcγRI (CD64)	High (K_d ~10^{-9} M); binds IgG1 and IgG3, can bind monomeric IgG	Macrophages, neutrophils; also eosinophils	Phagocytosis; activation of phagocytes
	FcγRIIA (CD32)	Low (K_d ~10^{-7} M)	Macrophages, neutrophils, dendritic cells, eosinophils, platelets	Phagocytosis; cell activation
	FcγRIIB (CD32)	Low (K_d ~10^{-7} M)	B lymphocytes, macrophages, dendritic cells, other cells	Feedback inhibition of various cellular responses
	FcγRIIC (CD32)	Low (K_d ~10^{-7} M)	Macrophages, neutrophils, NK cells	Phagocytosis, cell activation
	FcγRIIIA (CD16)	Low (K_d ~10^{-6} M)	NK cells, macrophages, dendritic cells	Antibody-dependent cell-mediated cytotoxicity
	FcγRIIIB (CD16)	Low (K_d ~10^{-6} M); GPI-linked protein	Neutrophils	Phagocytosis (inefficient)
FcεR	FcεRI	High (K_d ~10^{-10} M); binds monomeric IgE	Mast cells, basophils, eosinophils	Cell activation (degranulation)
	FcεRII (CD23)	Low (K_d ~10^{-7} M)	B lymphocytes, eosinophils, Langerhans cells	Unknown
FcαR	FcαR (CD89)	Low (K_d ~10^{-6} M)	Neutrophils, eosinophils, monocytes	Cell activation?

Neutralization

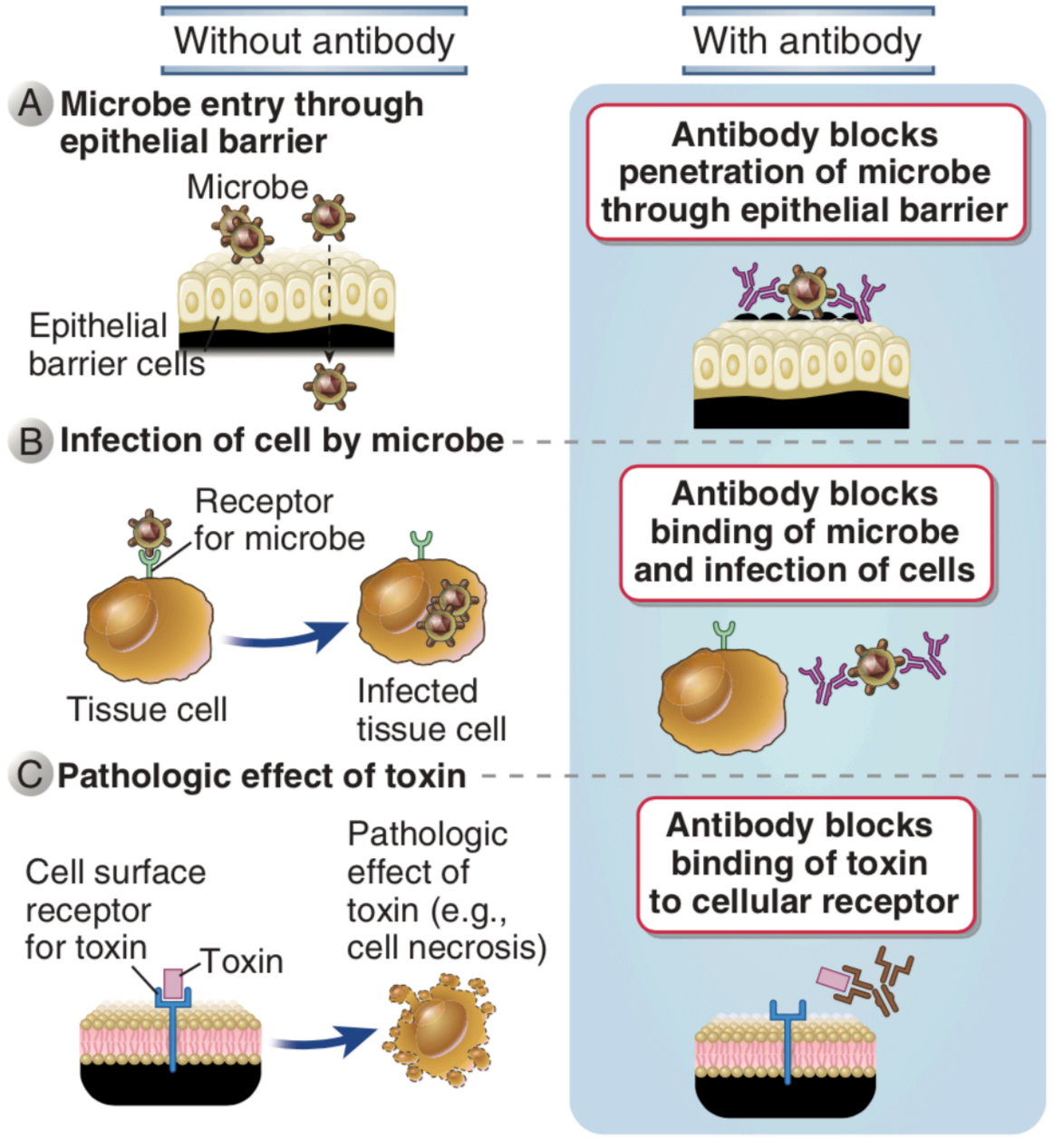
Antibodies can inhibit the infectivity of microbes as well as the potential injurious effects of microbial toxins.

Neutralization mostly occurs by **steric hindrance**

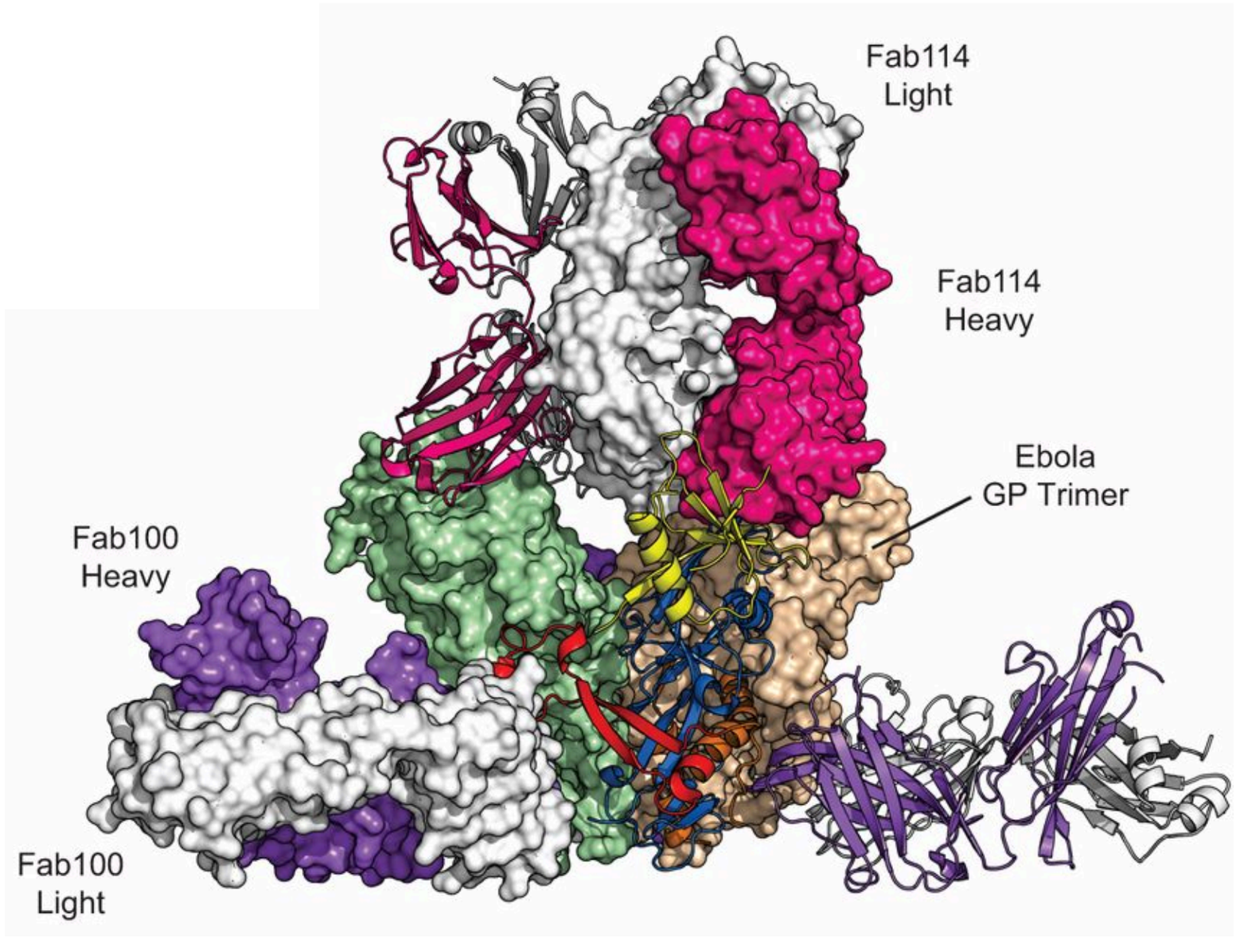
All isotypes! Actually the Fc portion is not even needed

Neutralization is the basis of all vaccines

Neutralization



Neutralization by steric hindrance



What makes a good NAb?

High affinity

Usually the antibody to be efficacious needs to bind the target (e.g. viral glycoprotein) with higher affinity than the endogenous ligand (e.g. cellular receptor)

Right epitope

The right epitope is the one that, if bound, prevent pathogen infection

Easy, right? Then why don’t we have an HIV vaccine?

HIV has a very high genetic variability. An effective vaccine would protect from multiple variants.

Conserved residues required for receptor binding in HIV glycoprotein are shielded with glycans. Our immune system finds other epitopes more attractive.

In HIV, individuals with antibodies (sieropositive) are often not “immune” since they don’t have broadly neutralizing Abs

IgG: antibody mediated opsonization and phagocytosis

IgG antibodies coat (opsonize) microbes and promote their phagocytosis by binding to Fc receptors on phagocytes.

High affinity: IgG1 and IgG3 Low affinity: IgG2 Very low affinity: IgG4

FcR	Affinity for Immunoglobulin	Cell Distribution	Function
FcγRI (CD64)	High (K_d ~10^{-9} M); binds IgG1 and IgG3, can bind monomeric IgG	Macrophages, neutrophils; also eosinophils	Phagocytosis; activation of phagocytes
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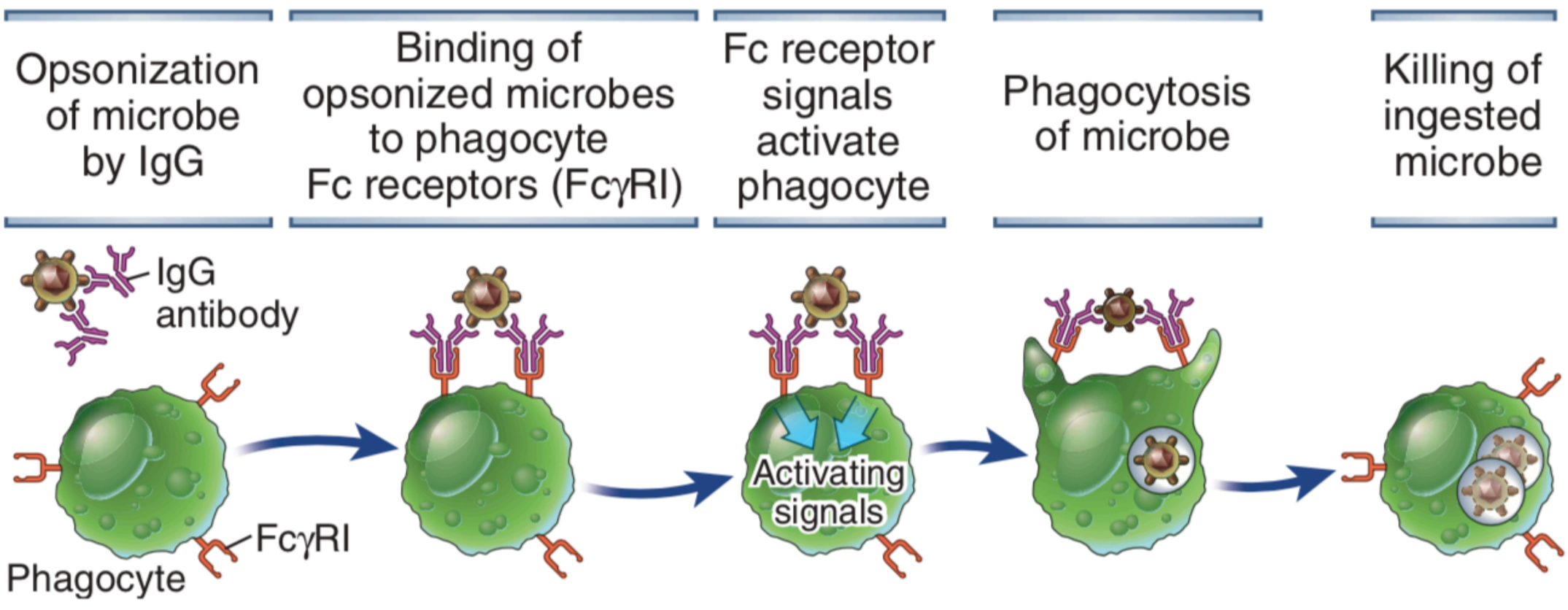
FcγRIIIB (CD16)	Low (K_d ~10^{-6} M); GPI-linked protein	Neutrophils	Phagocytosis (inefficient)

Major targets: macrophages and neutrophils

IgG: antibody mediated opsonization and phagocytosis



IgG: Antibody-Dependent Cell-Mediated Cytotoxicity (ADCC)

Natural killer (NK) cells (and macrophages?) bind to antibody-coated cells by Fc receptors and destroy these cells.

FcR	Affinity for Immunoglobulin	Cell Distribution	Function
FcγRI (CD64)	High (K_d ~10^{-9} M); binds IgG1 and IgG3, can bind monomeric IgG	Macrophages, neutrophils; also eosinophils	Phagocytosis; activation of phagocytes
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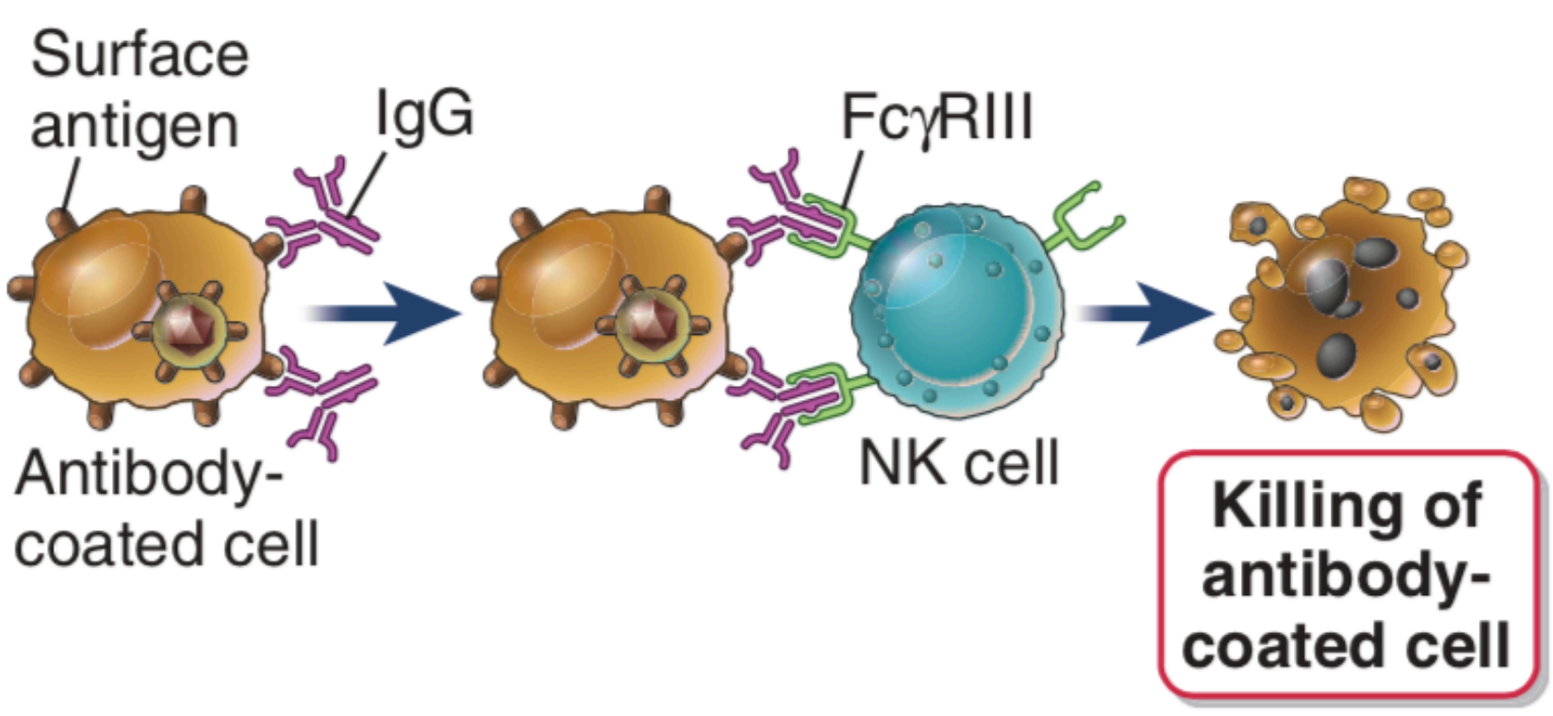
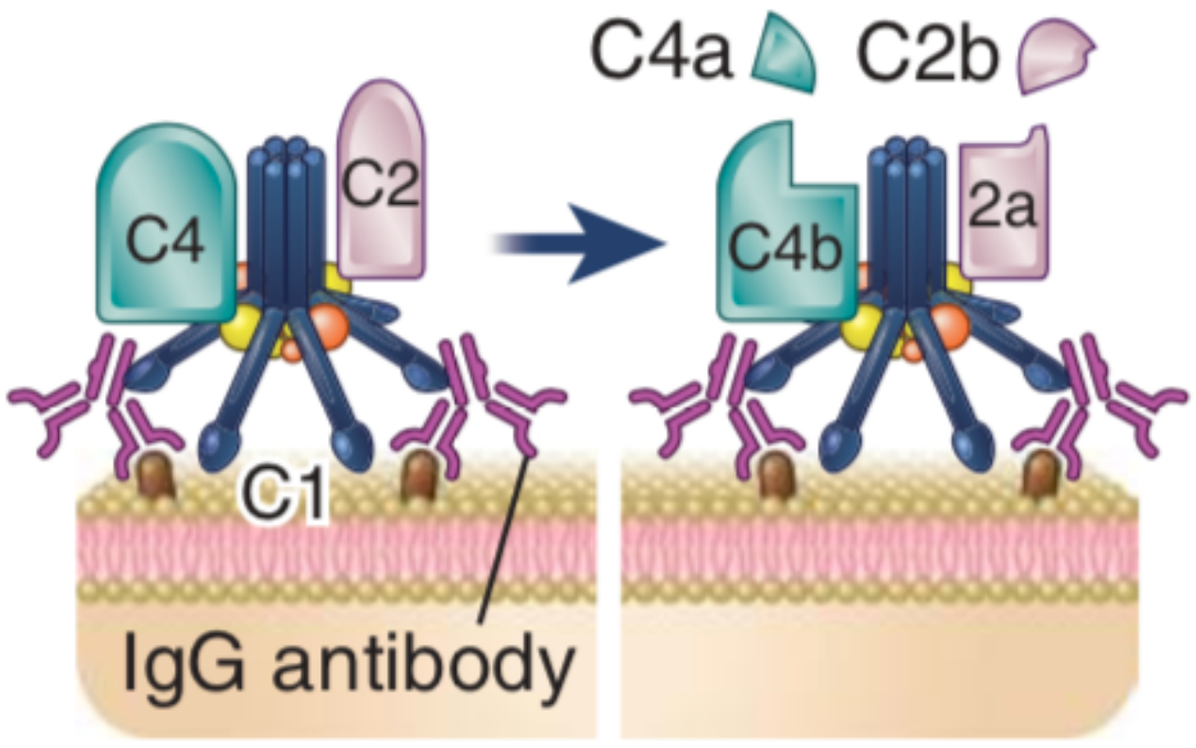
IgG: Antibody-Dependent Cell-Mediated Cytotoxicity (ADCC)



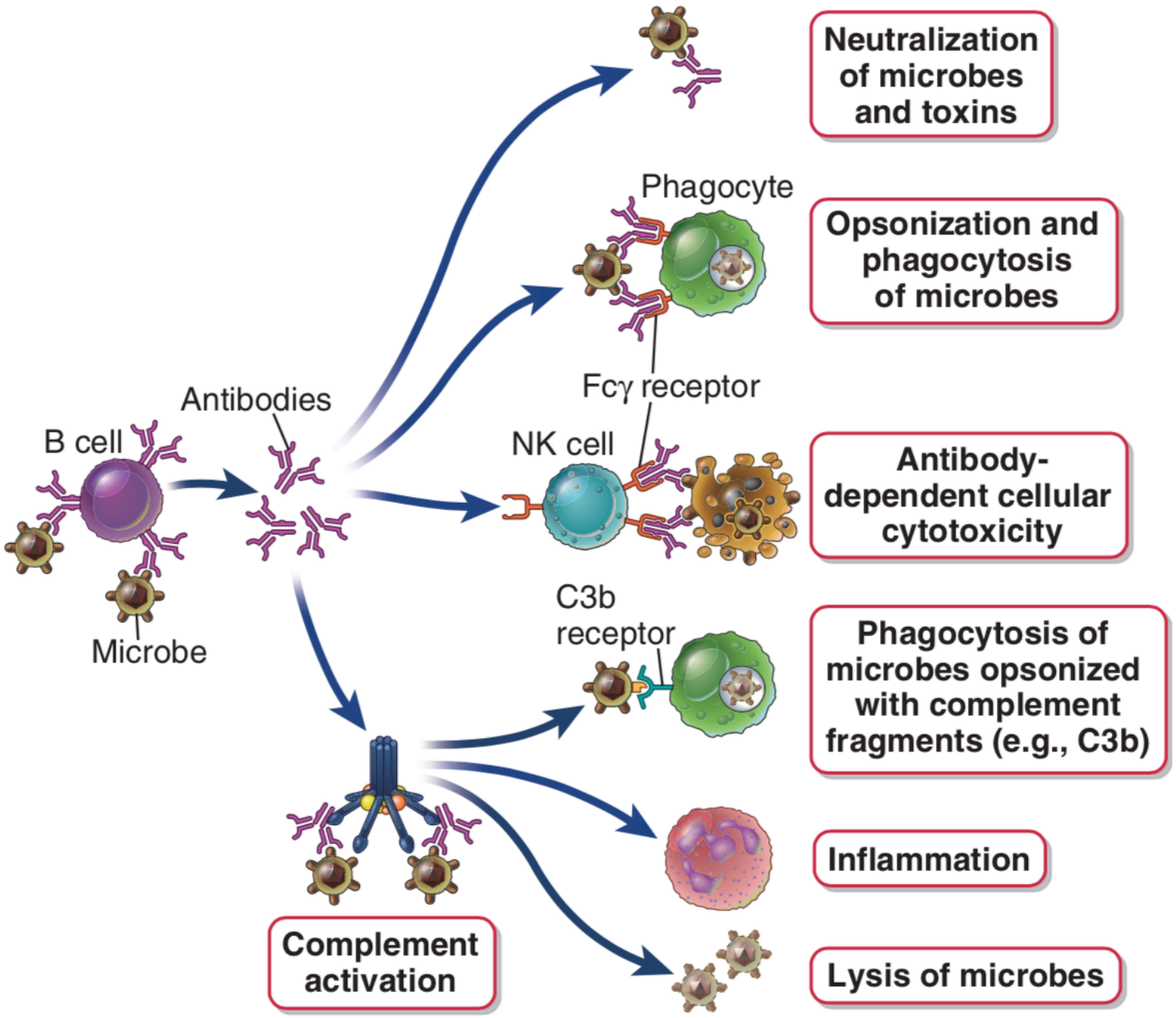
IgG and IgM: Complement activation (classical pathway)

The classical pathway is initiated by binding of the complement protein C1 to the CH2 domains of IgG or the CH3 domains of IgM molecules that have bound antigen



Because of its pentameric structure, a single molecule of IgM can bind two C1q molecules, and this is one reason that IgM is a more efficient complement-binding (also called complement-fixing) antibody than is IgG.

IgG effector functions



IgE: Antibody-Mediated Clearance of Helminths

Major targets: mast cells, basophils and eosinophils

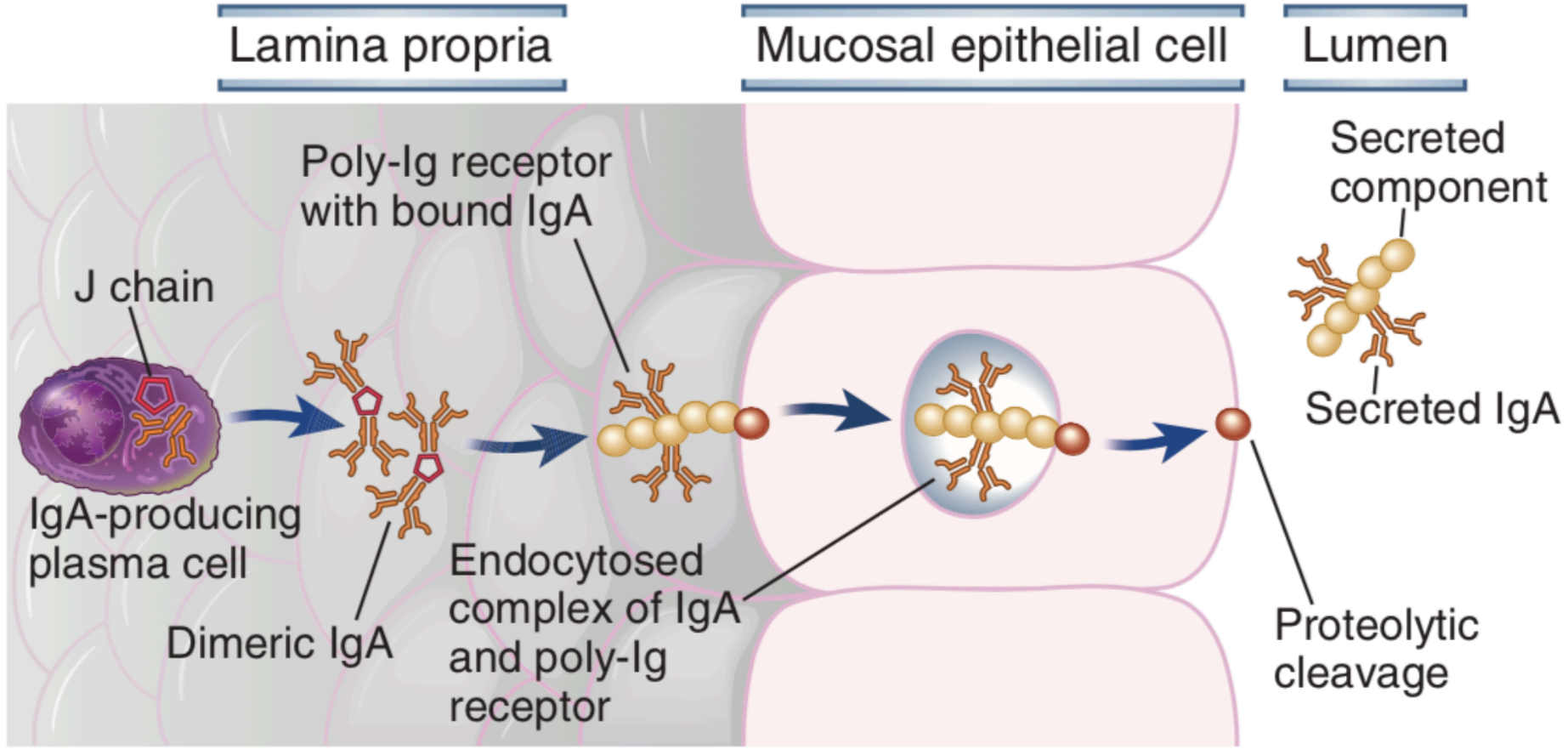
FcεRI	High (K_d ~10^{-10} M); binds monomeric IgE	Mast cells, basophils, eosinophils	Cell activation (degranulation)
FcεRII (CD23)	Low (K_d ~10^{-7} M)	B lymphocytes, eosinophils, Langerhans cells	Unknown

Eosinophils ⟶ **Major basic protein**, present in the granules of eosinophils, is responsible for the killing of helminths

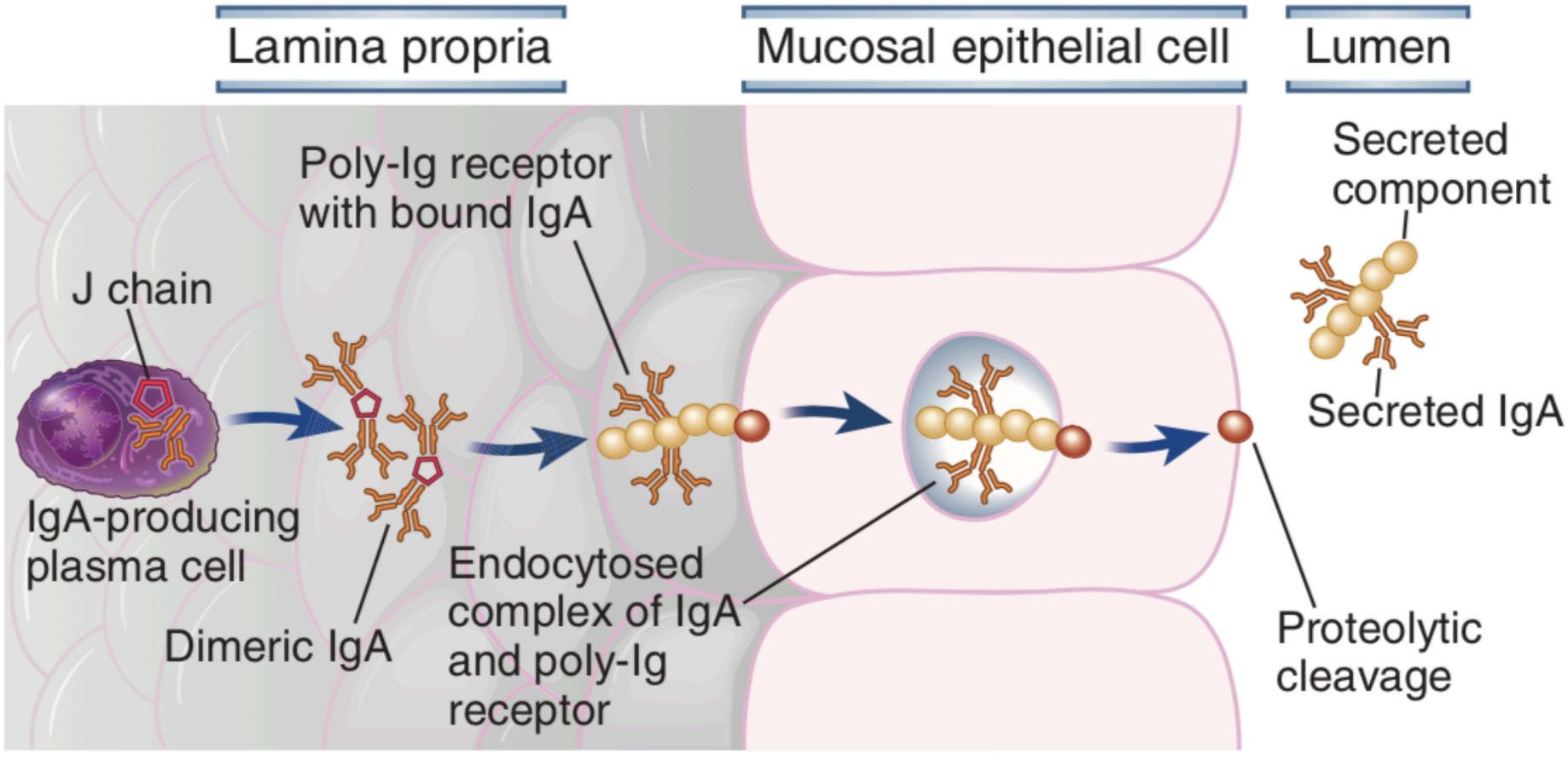
Mast cell degranulation through the high-affinity IgE receptor produce mediators may induce broncho-constriction and increased intestinal motility, contributing to the expulsion of worms from sites

IgA1 is dimeric and abundant at mucosal surfaces

Poly-Ig receptor mediate the translocation of IgA across the mucosa in the lumen (transcytosis)



Poly-Ig Receptor



The ectodomain of the poly-Ig receptor recognize the J chain, present on IgA and IgM. Upon transcytosis, the ectodomain is cleaved and remains associated to the IgA (secretory component).

IgA1 represent the first line of defense at mucosal surfaces

The main function of IgA1 is to neutralize microbes present at mucosal surfaces

IgA2 is monomeric and abundant in the serum

FcαR (CD89)	Low (K_d ~10^{-6} M)	Neutrophils, eosinophils, monocytes	Cell activation?

Seems to have different properties than IgA1 (pro-inflammatory)

Other concepts about antibodies:

Immunodominance

Isotypes, idiotypes, and allotypes

Cross-reactivity

Agglutination and precipitation

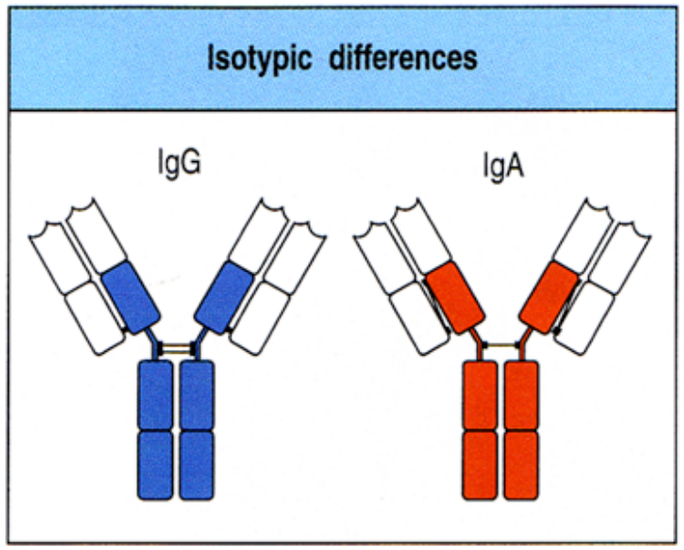
Immunodominance

(B and T cells)

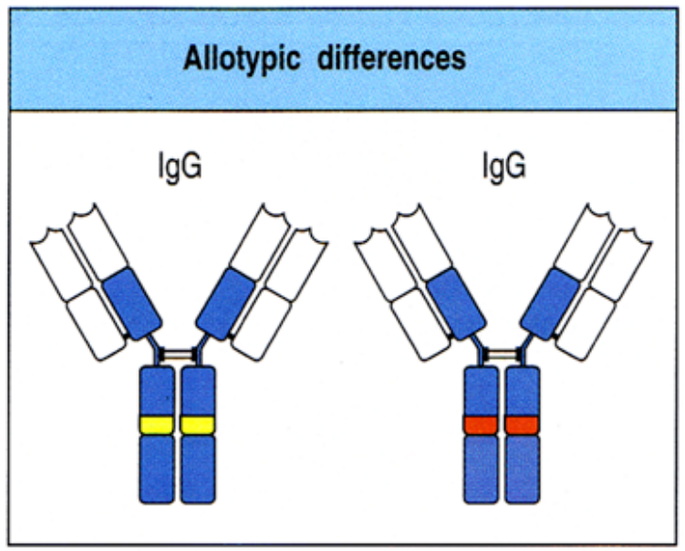
Immunodominance is the immunological phenomenon in which immune responses are mounted against only a few of the antigenic epitopes out of the many possible

Several mechanism involved: antigen processing, MHC presentation (T cells); immunological history

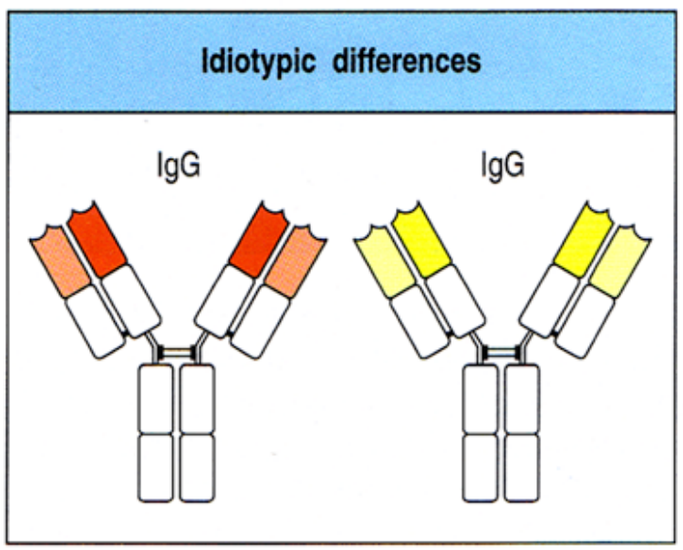
Isotypes, idiotypes, and allotypes



Different **isotypes** are immunoglobulins which differ in their constant region (e.g. IgM, IgGs, IgAs etc.)



Different **allotypes** are immunoglobulins of the same class that differs in small portions (1-4 amino acids) in their constant region (e.g. due to polymorphisms between individuals)

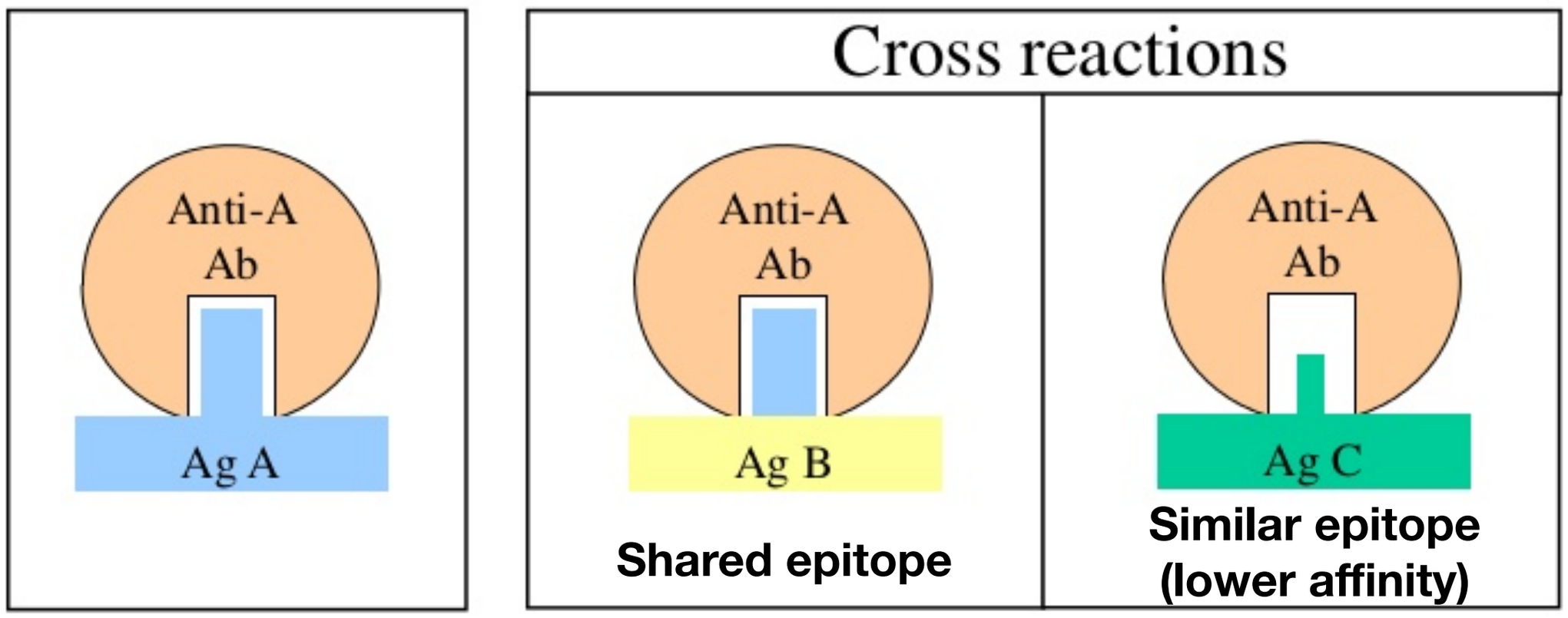


Different **idiotypes** are immunoglobulins that differs in their variable region (e.g. antibodies that recognize different antigens)

Cross-reactivity

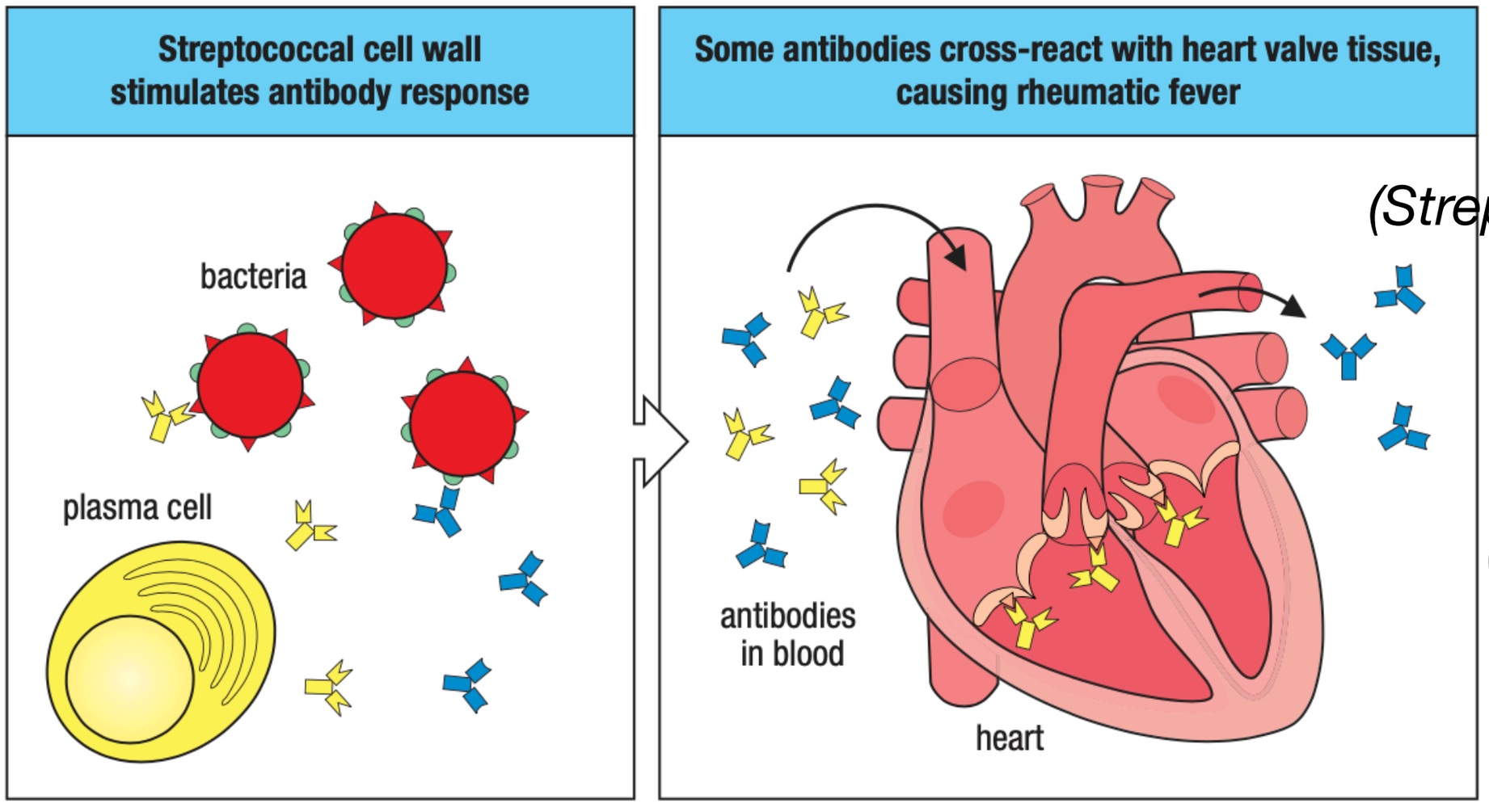
The ability of an antibody to bind to multiple antigens

Two possibilities:



Molecular mimicry and autoimmune diseases

Sequence similarities between foreign and self-peptides can result in the cross-activation of autoreactive T or B cells by pathogen-derived peptides.



Rheumatic fever (*Streptococcus pyogenes*)

Lyme arthritis (*Borrelia burgdorferi* - Lyme disease)

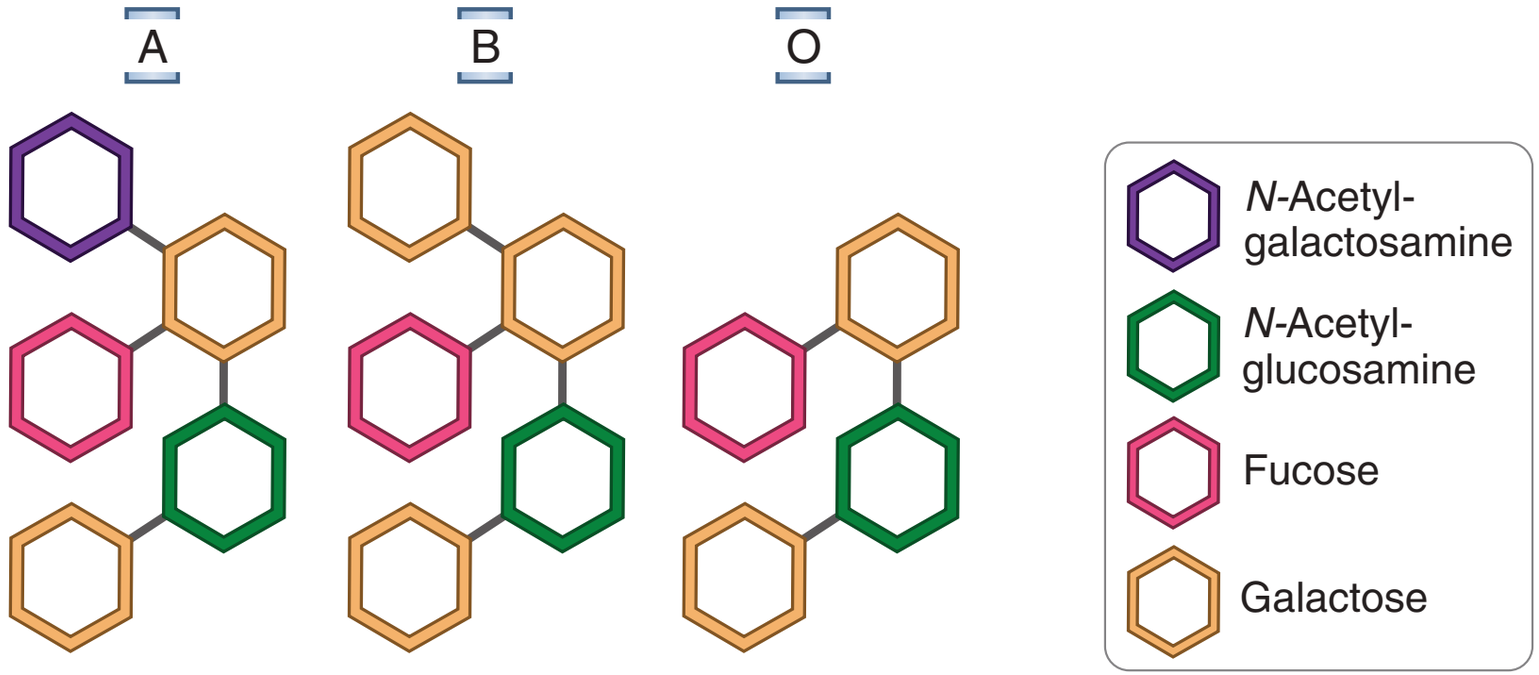
An example of cross-reactivity: ABO antibodies

ABO blood types classification is based on carbohydrates expressed on the surface of red blood cells by glycosyltransferase

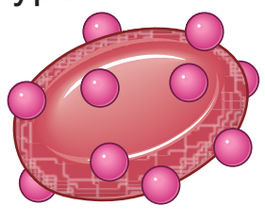
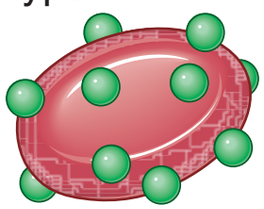
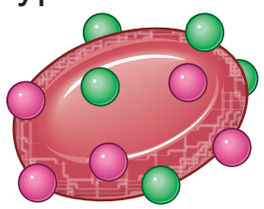
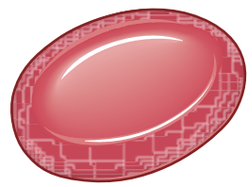
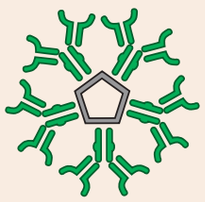
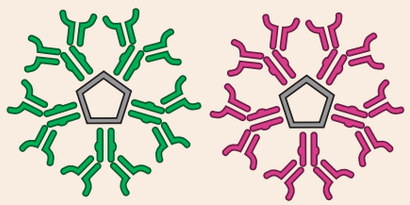
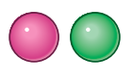
IgM antibodies directed against the ABO structures (not expressed by an individual) develops in the first months of life, and are generated by cross-reactivity with carbohydrates expressed on the surface of enterobacteria

ABO blood group antigens

A



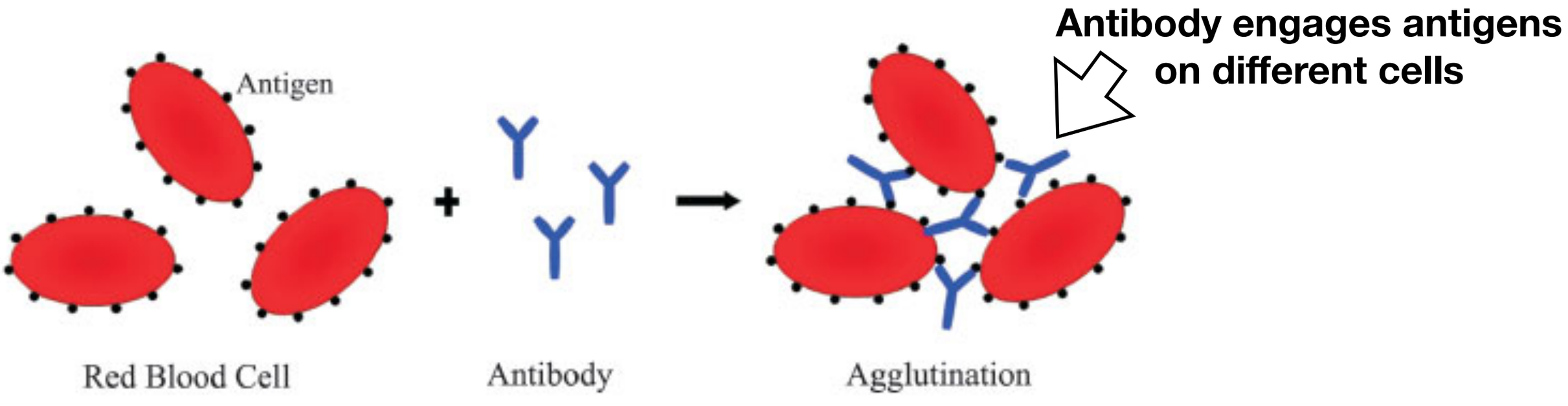
B

	Group A	Group B	Group AB	Group O
Red blood cell type	Type A 	Type B 	Type AB 	Type O 
Antibodies present	Anti-B 	Anti-A 	None	Anti-A and Anti-B 
Antigens present	A antigen 	B antigen 	A and B antigen 	None

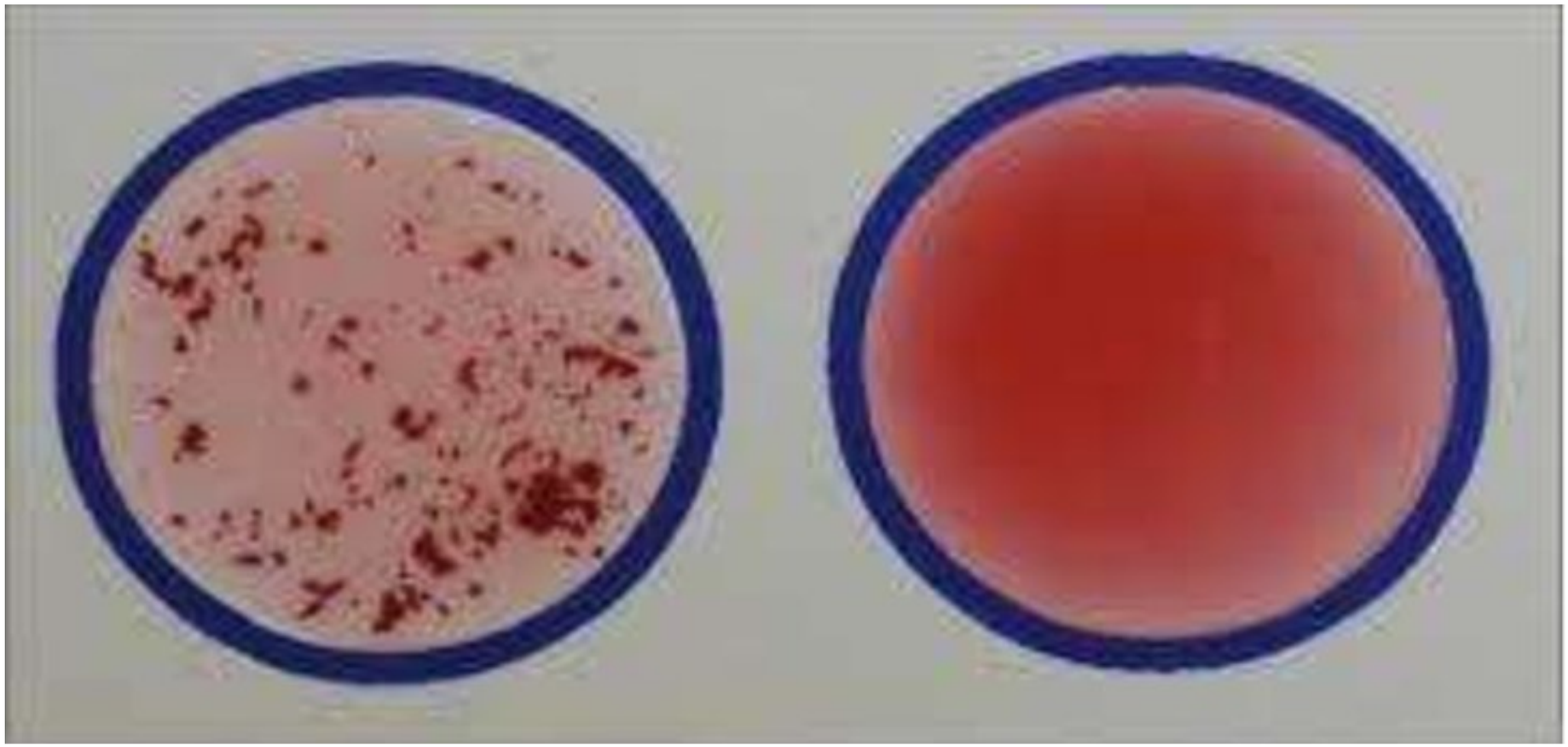
Agglutination and precipitation

Agglutination — **Bivalent antibody + particulate antigen (cells or bacteria)**

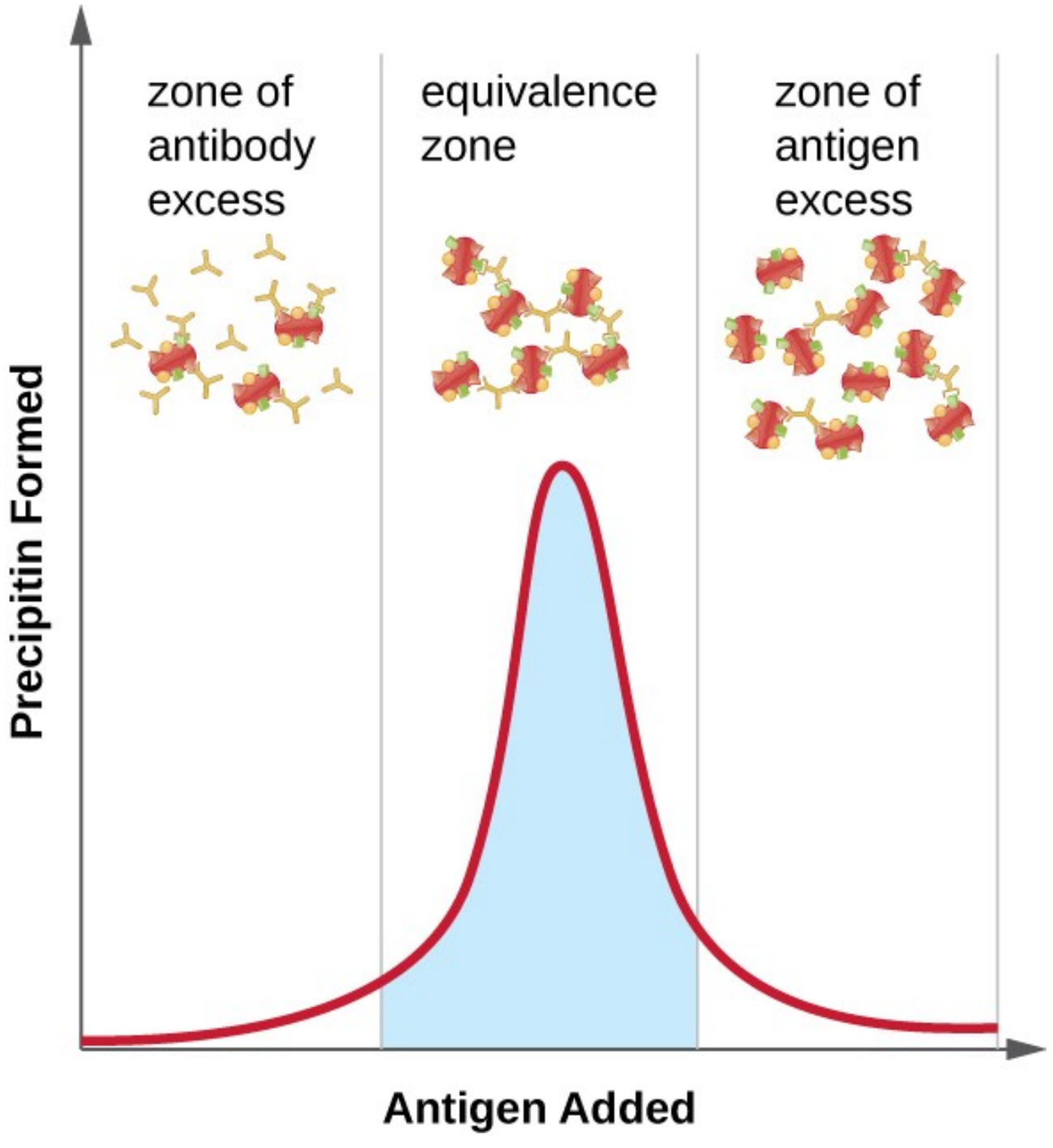
Precipitation — **Bivalent antibody + soluble antigen (proteins)**



Agglutination of blood



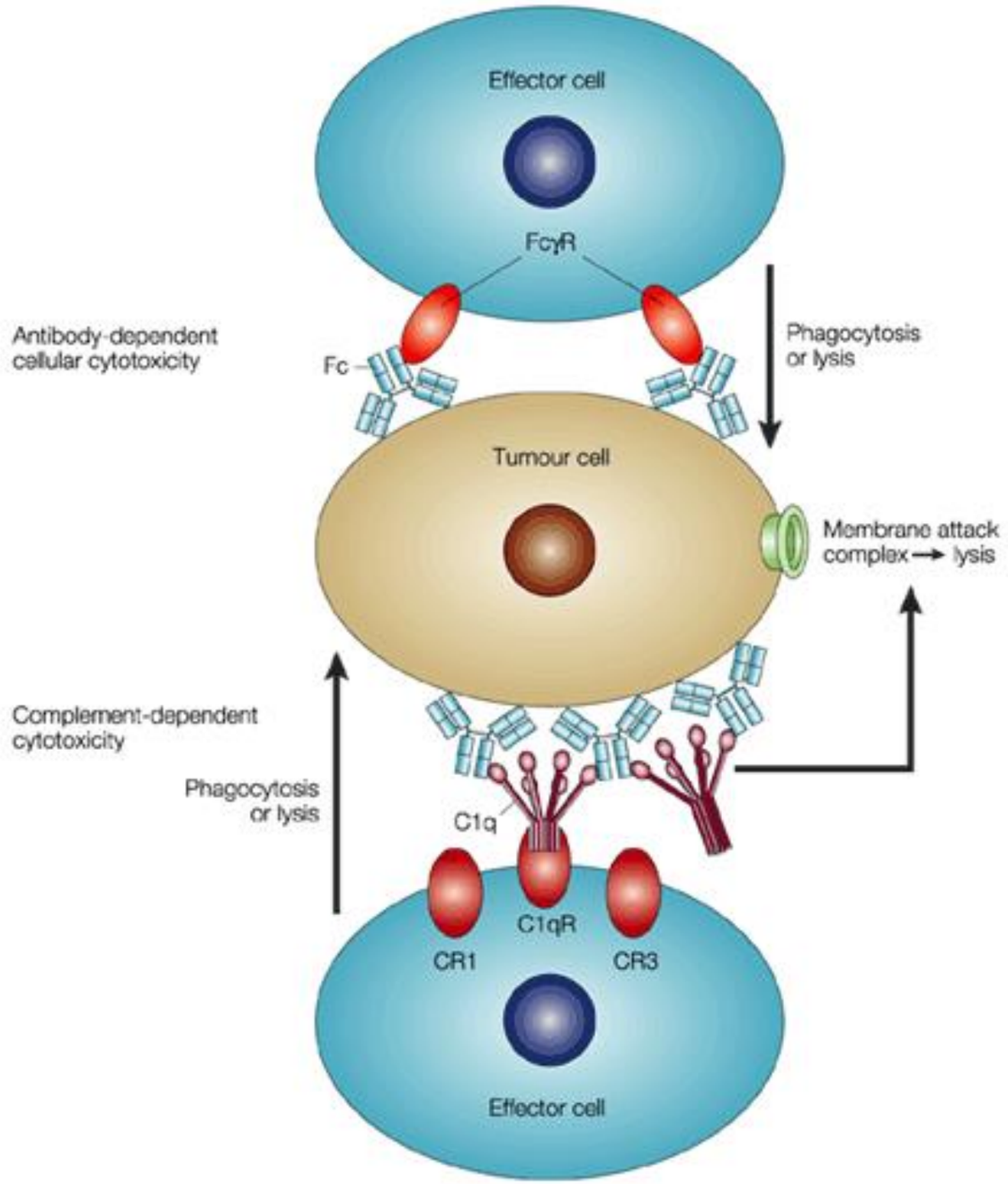
Precipitation



Same antibody bound to two Ag molecules

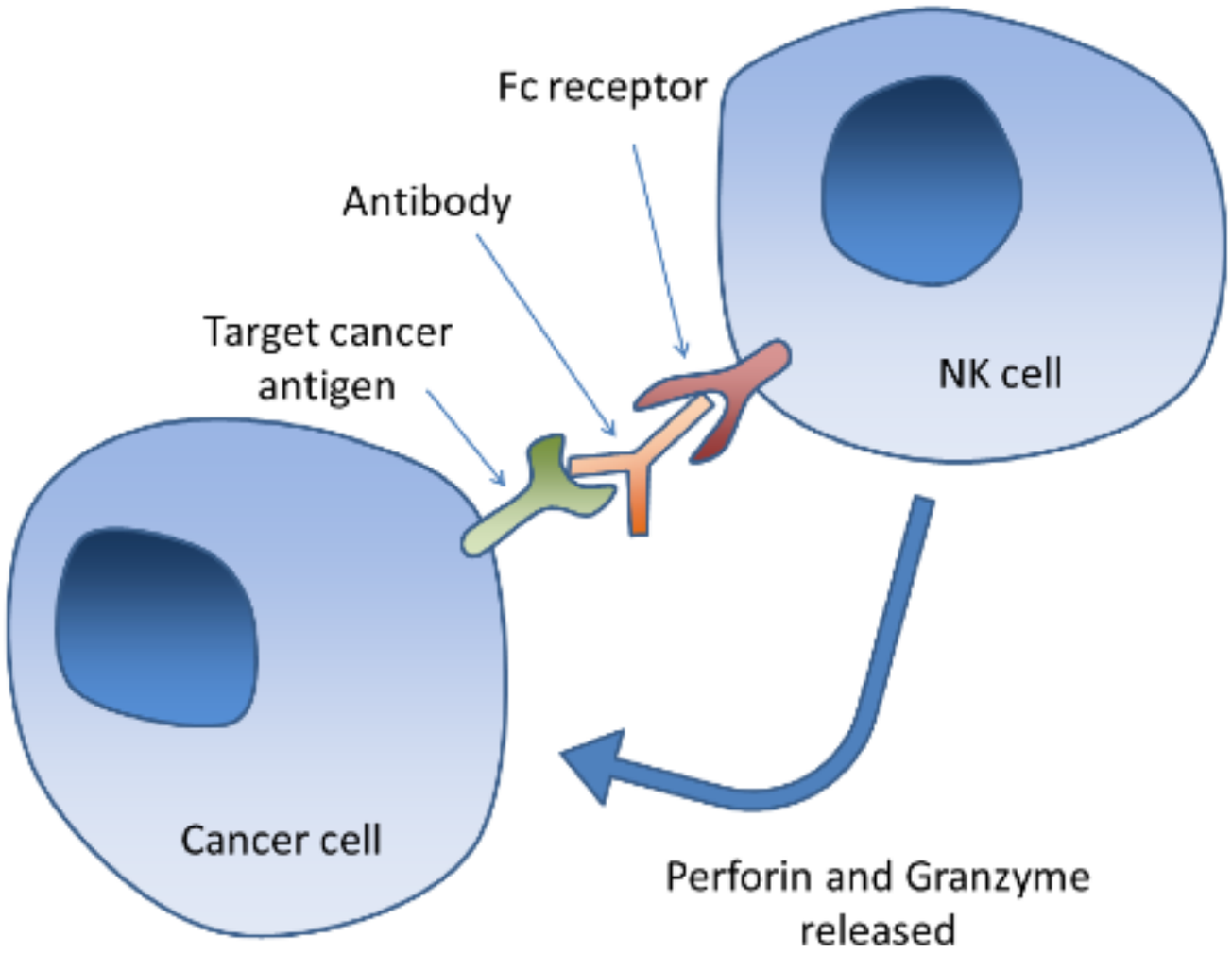
Polyclonal serum

Antibody effector functions against tumors

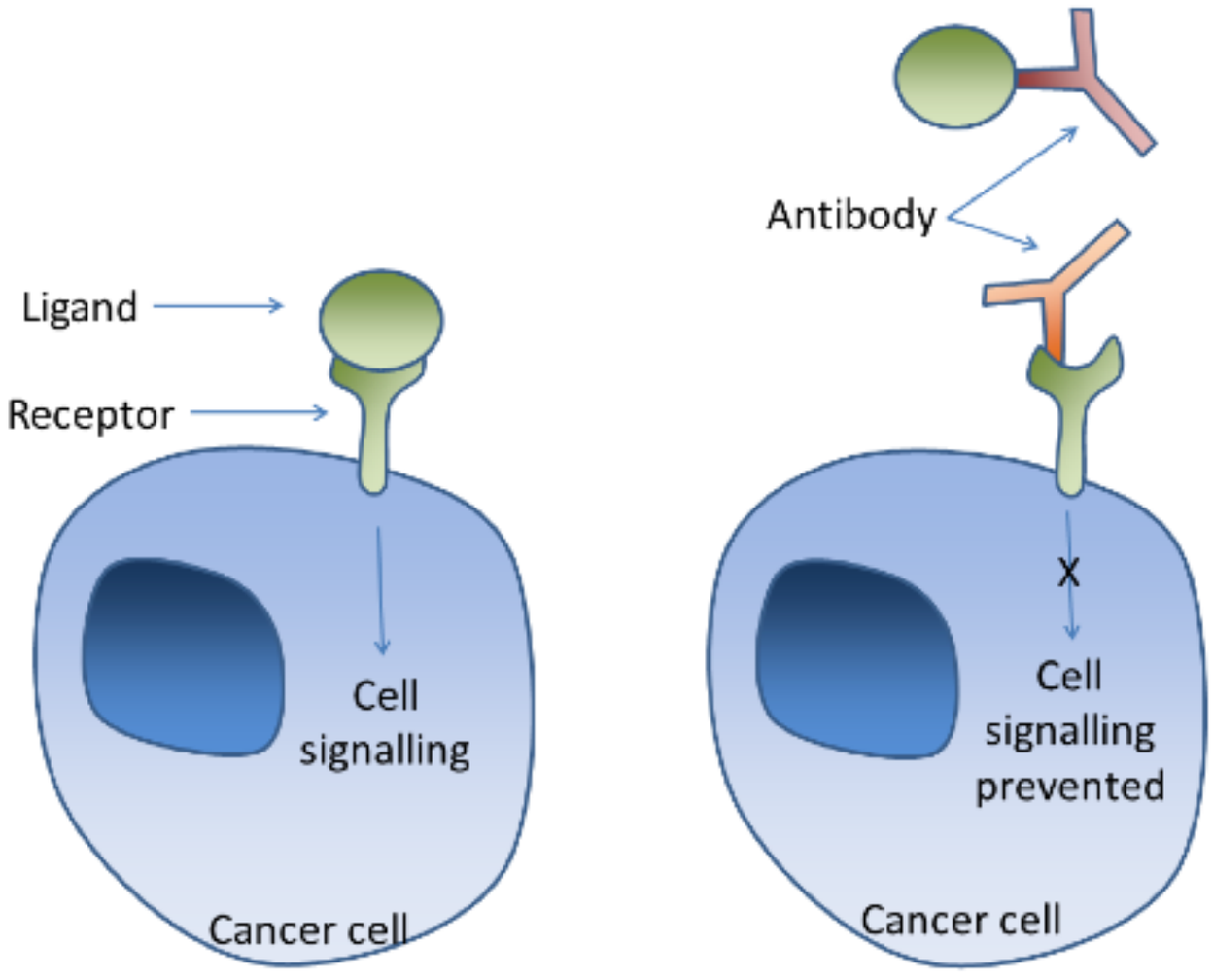


Nature Reviews | Cancer

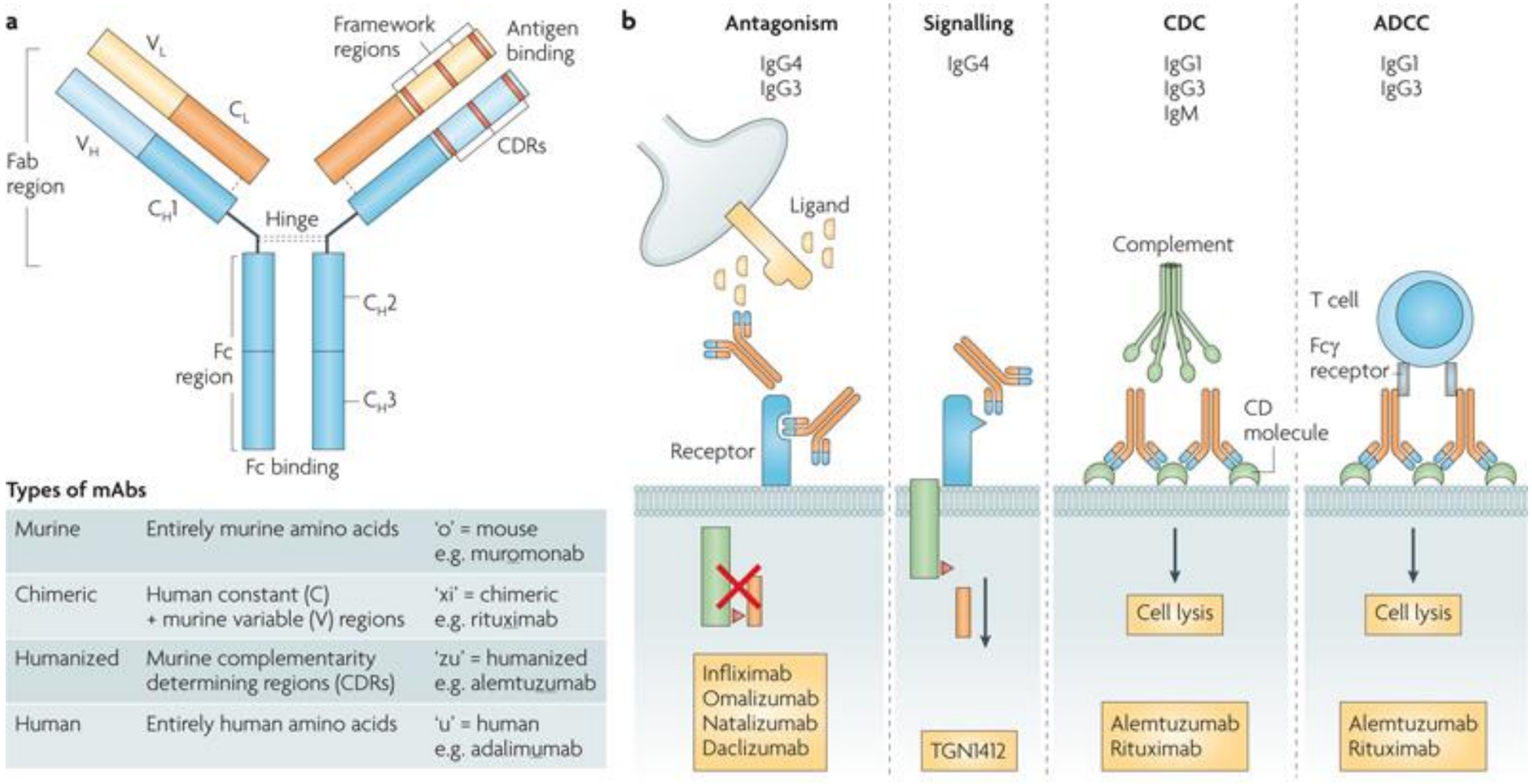
ADCC


Fc receptor
Antibody
Target cancer antigen
NK cell
Cancer cell
Perforin and Granzyme released

Antagonism/neutralization



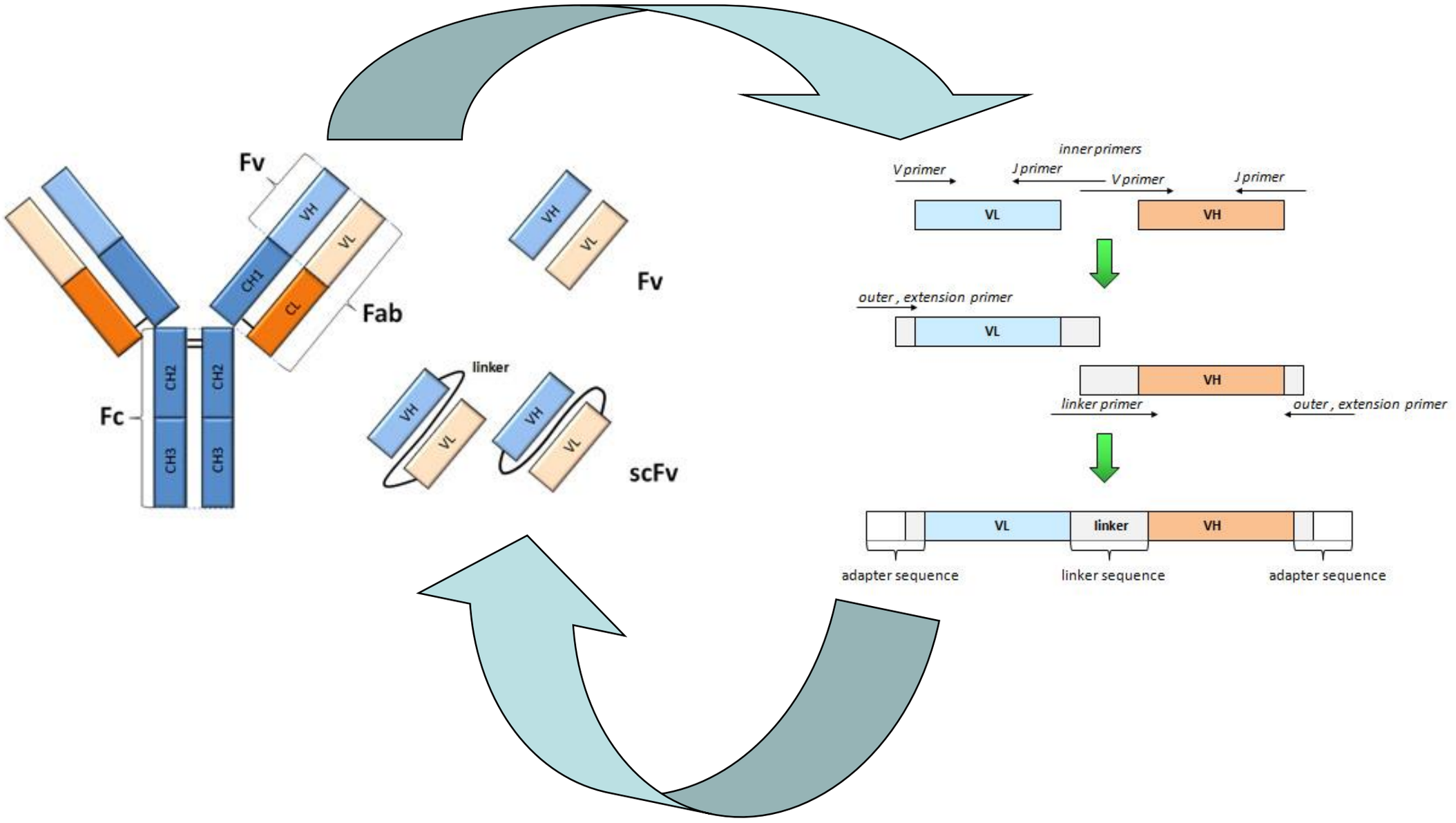
Antibody effector functions against tumors



Types of mAbs

Murine	Entirely murine amino acids	'o' = mouse e.g. muromonab
Chimeric	Human constant (C) + murine variable (V) regions	'xi' = chimeric e.g. rituximab
Humanized	Murine complementarity determining regions (CDRs)	'zu' = humanized e.g. alemtuzumab
Human	Entirely human amino acids	'u' = human e.g. adalimumab

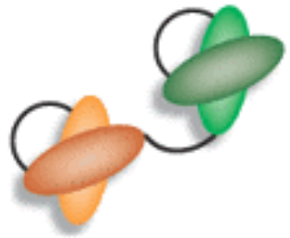
mAb derivatives



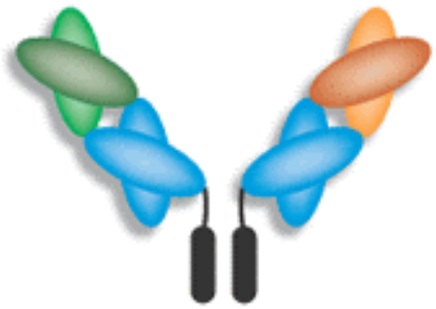
mAb derivatives

Fab
(~55 kDa)

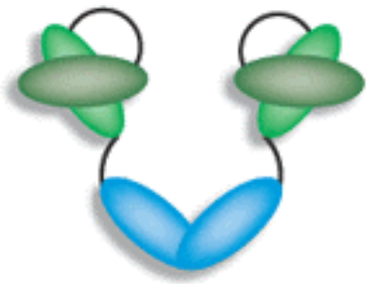
scFv
(~28 kDa)



Bis-scFv
(bispecific)
(~55 kDa)



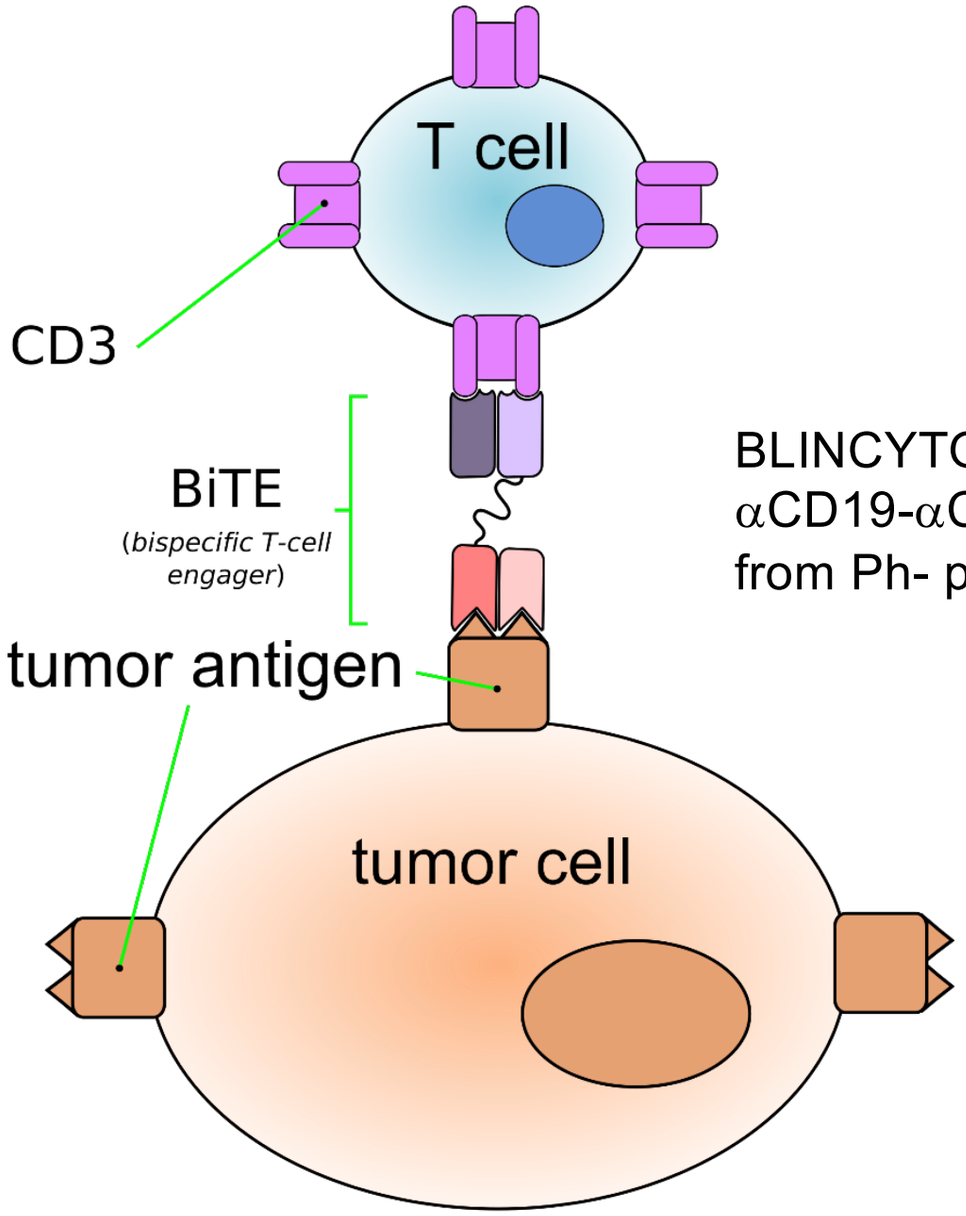
Fab_2
(bispecific)
(~110 kDa)



Minibody
(bivalent)
(~75 kDa)

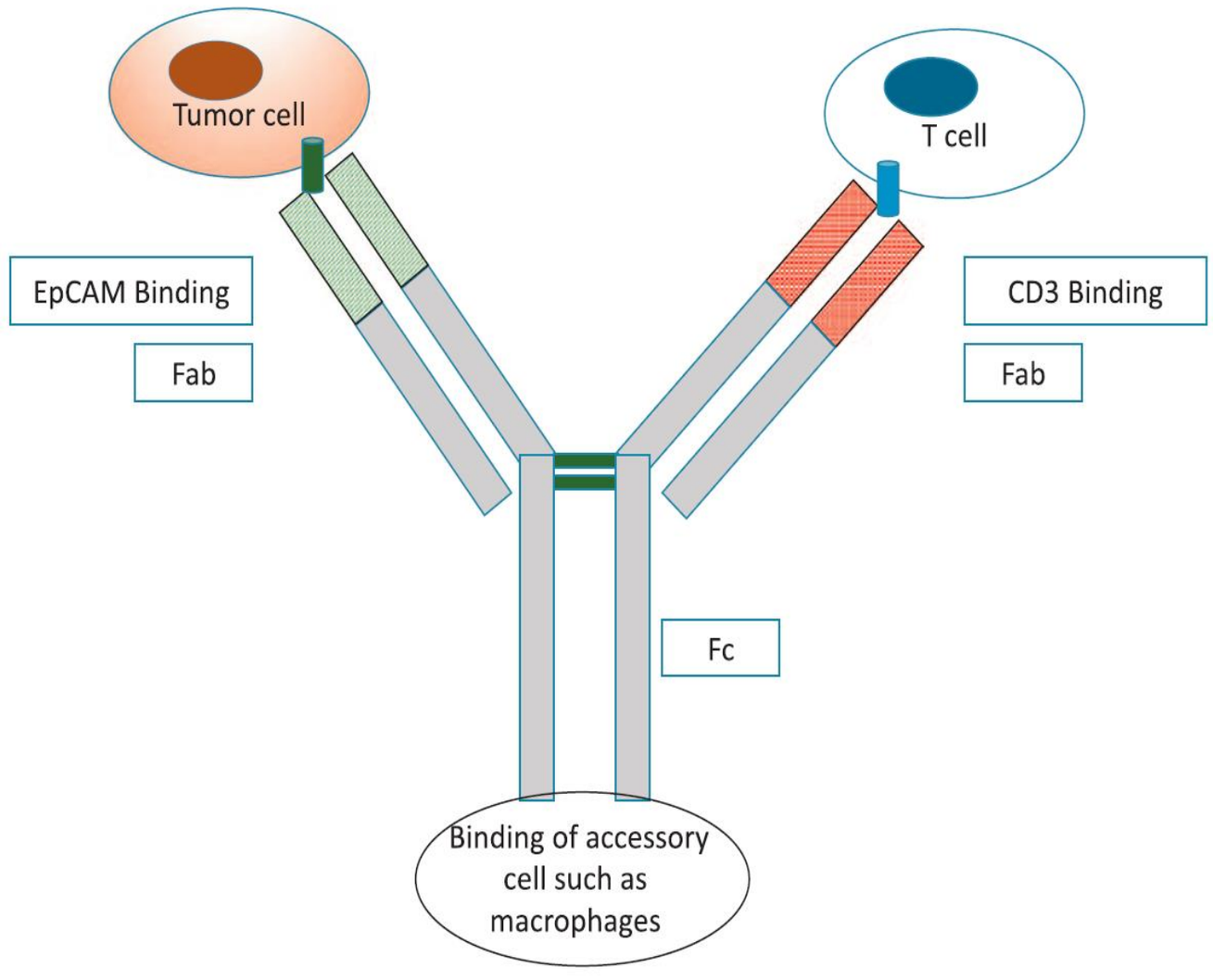
Diabody
(bispecific)
(~50 kDa)

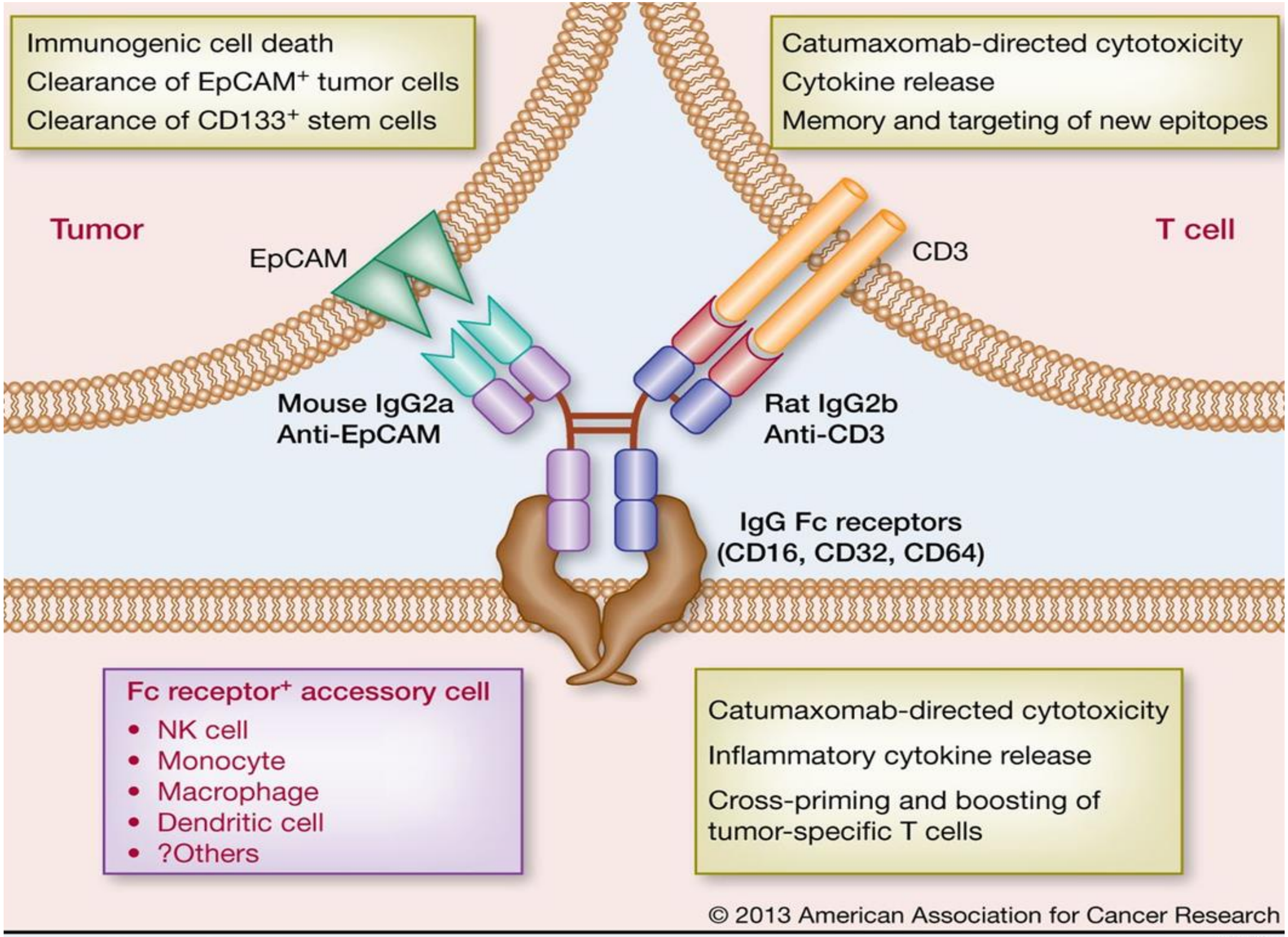
BiTE (Bispecific T cell Engager)



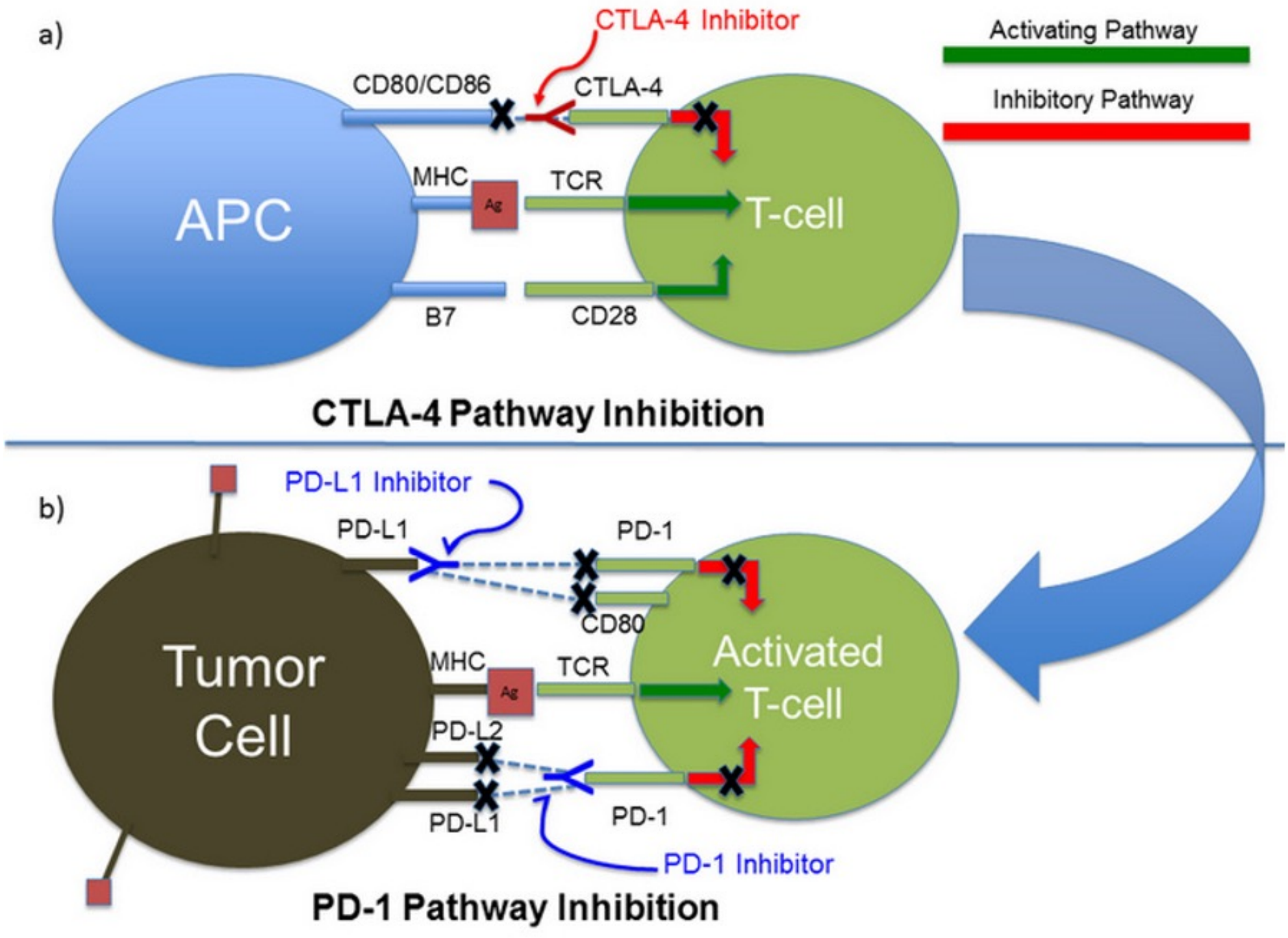
BLINCYTO® (Blinatumomab)
αCD19-αCD3 for a rare form of ALL from Ph- precursors

Catumaxomab: trifunctional antibody structure



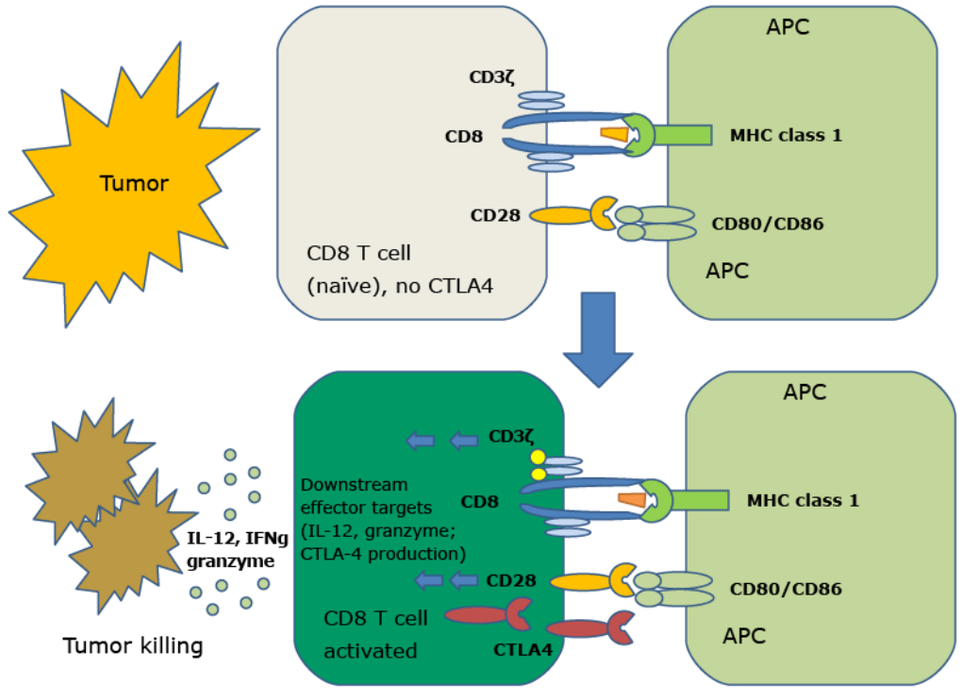


Immune checkpoint inhibitors



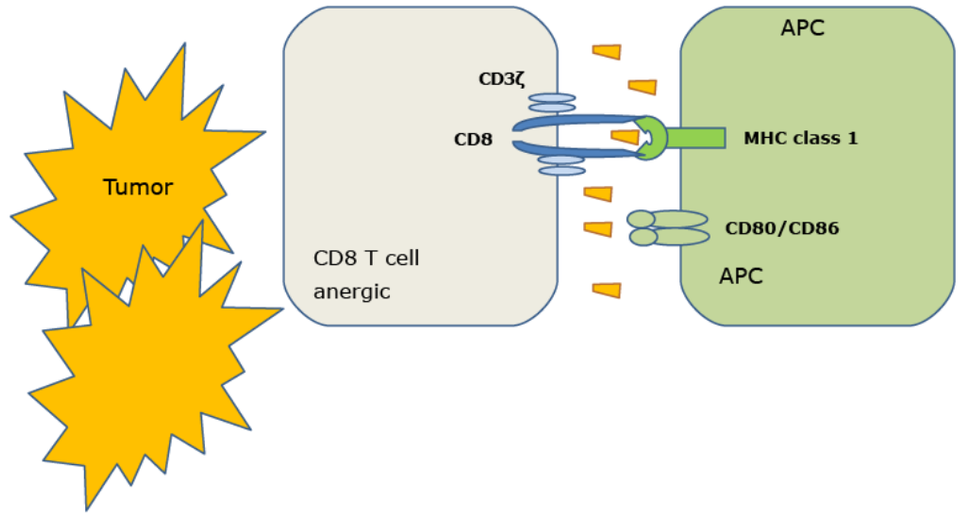
The dual signal concept in T cell activation

+ costimulation



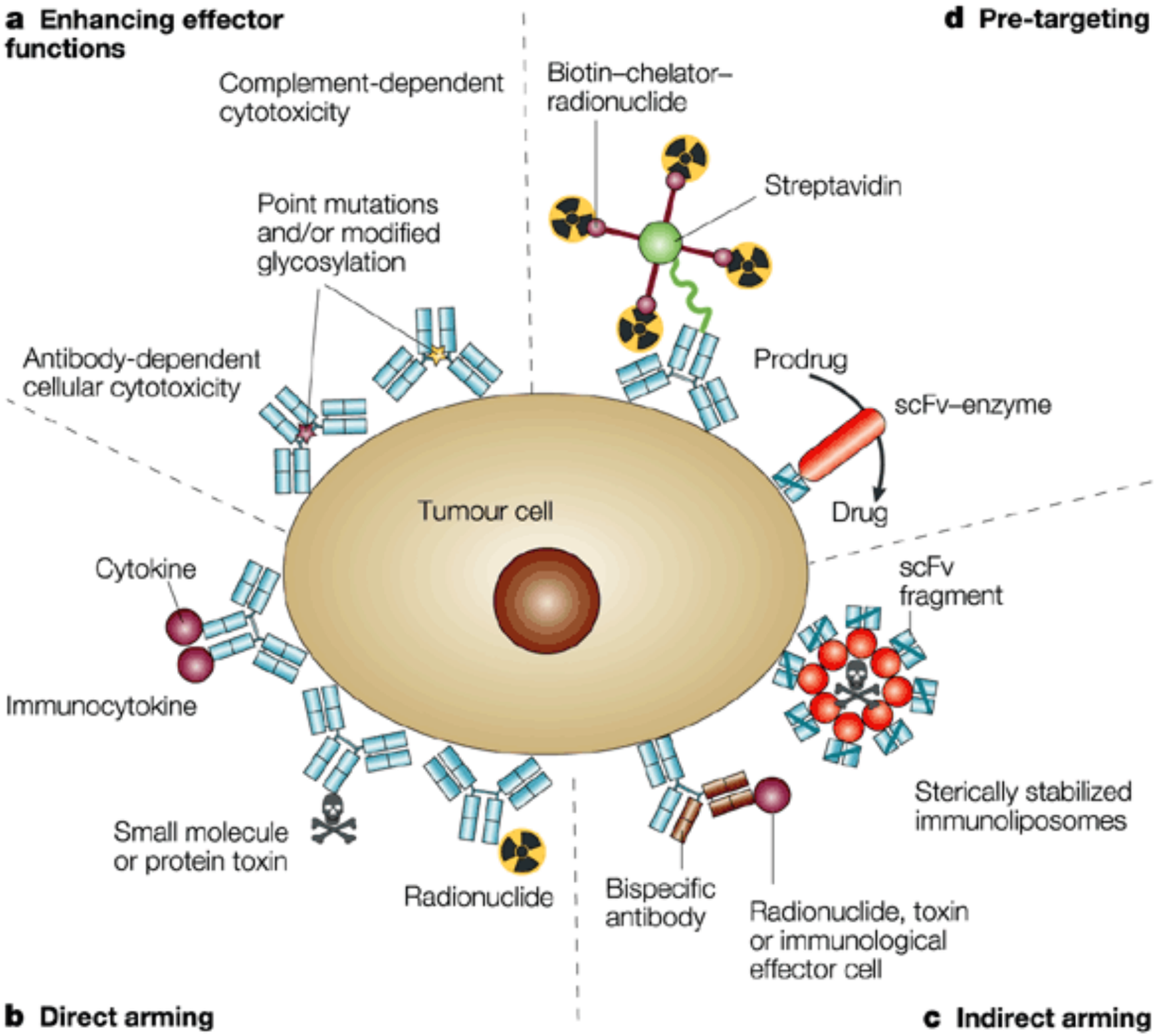
Activation

- costimulation

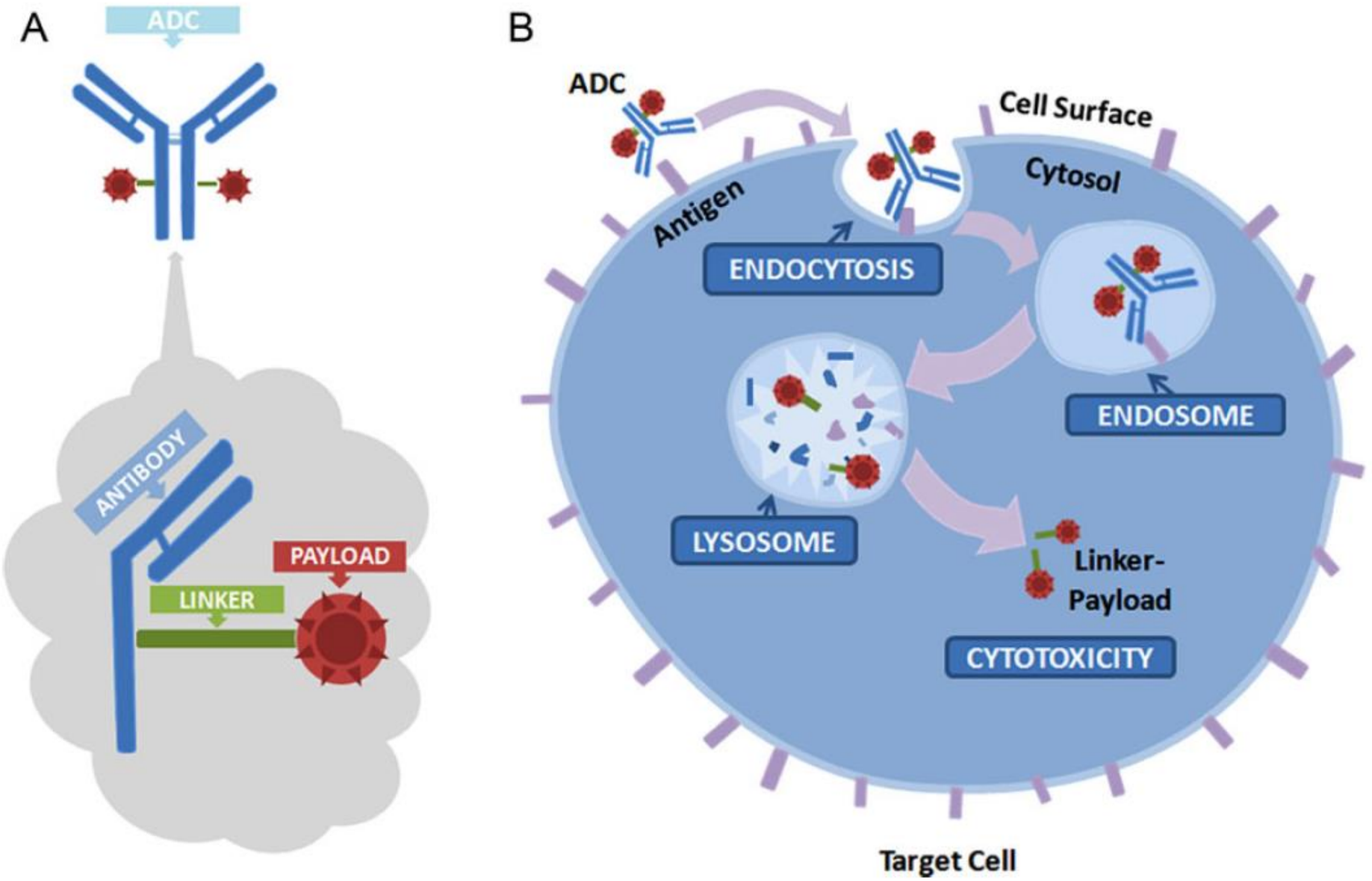


Anergy

mAb as carriers



Antibody-drug conjugates (ADC)



FDA currently approved mAb for therapy in oncology

Table 1 | **Therapeutic monoclonal antibodies approved for use in oncology**

Generic name (trade name; sponsoring companies)	Target	Antibody Format	Cancer Indication	Refs
Unconjugated antibodies				
Rituximab (Rituxan/Mabthera; Genentech/Roche/Biogen Idec)	CD20	Chimeric IgG1	Non-Hodgkin lymphoma	74,105
Trastuzumab (Herceptin; Genentech/ Roche)	HER2	Humanized IgG1	Breast cancer	19,72
Alemtuzumab (Campath/ MabCampath; Genzyme/Bayer)	CD52	Humanized IgG1	Chronic lymphocytic leukaemia	58
Cetuximab (Erbitux; ImClone Systems/Bristol–Myers Squibb)	EGFR	Chimeric IgG1	Colorectal cancer	13,106
Bevacizumab (Avastin; Genentech)	VEGFA	Humanized IgG1	Colorectal, breast and lung cancer	71, 107,108
Panitumumab (Vectibix; Amgen)	EGFR	Human IgG2	Colorectal cancer	109
Ofatumumab (Arzerra; Genmab/ GlaxoSmithKline)	CD20	Human IgG1	Chronic lymphocytic leuakemia	110
Immunoconjugates				
Gemtuzumab ozogamicin (Mylotarg; Pfizer)	CD33	Humanized IgG4	Acute myelogenous leukaemia	111
^{90}Y-Ibritumomab tiuxetan (Zevalin; Biogen Idec)	CD20	Mouse	Lymphoma	112
Tositumomab and ^{131}I-tositumomab (Bexxar; GlaxoSmithKline)	CD20	Mouse	Lymphoma	113

EGFR, epidermal growth factor receptor; HER2, human epidermal growth factor receptor 2; VEGF, vascular endothelial growth factor.