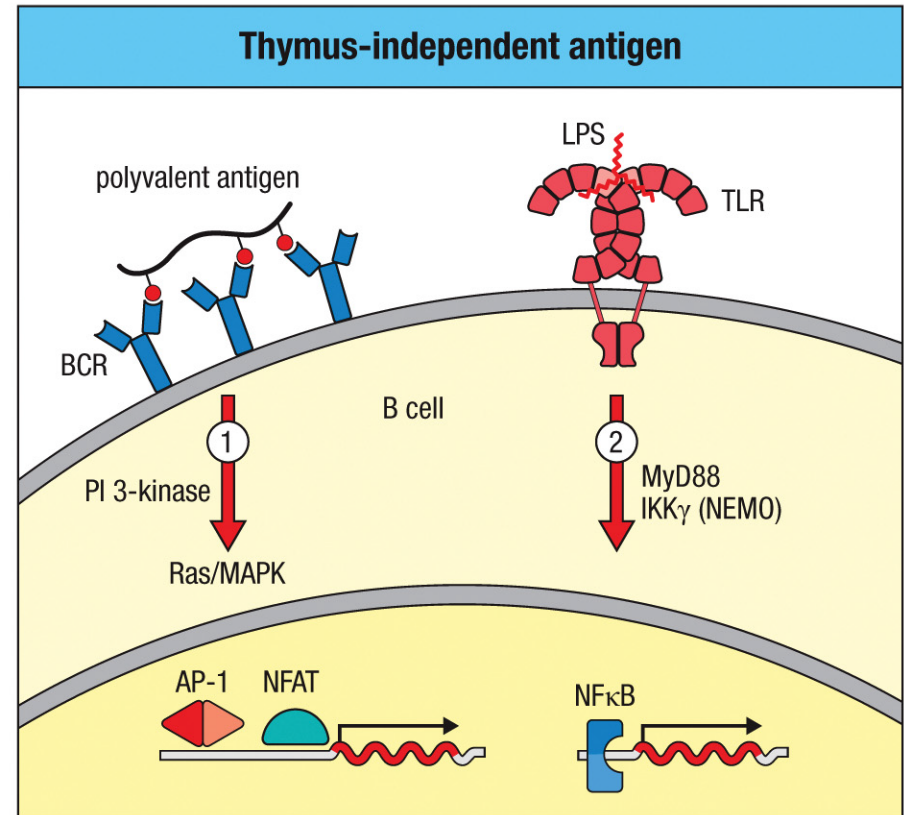
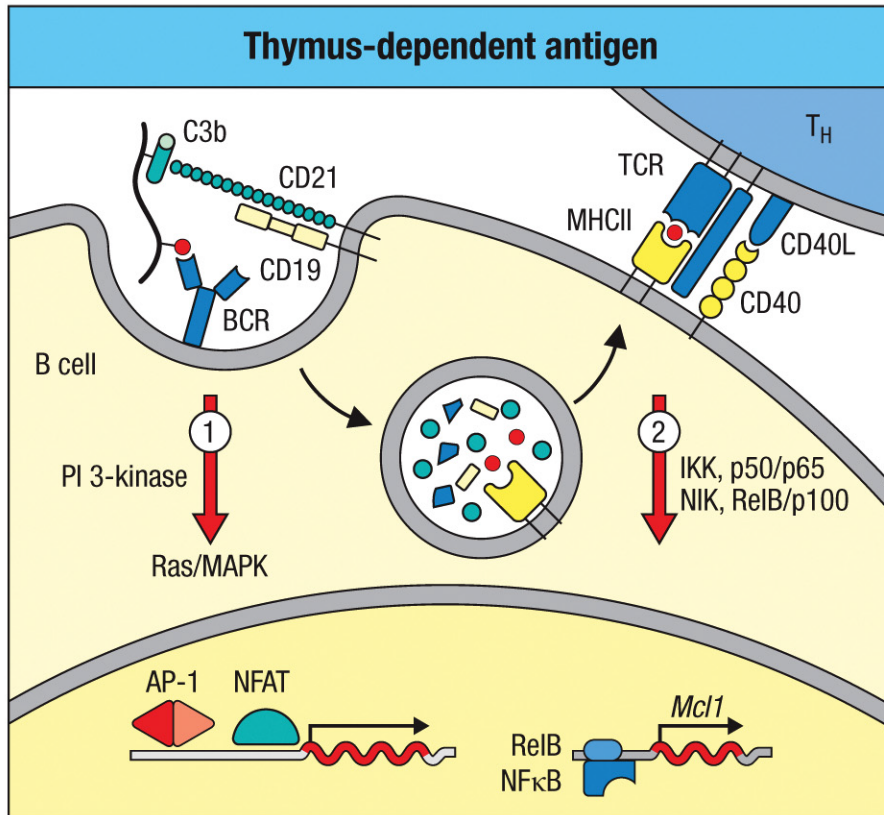


Two Signals Are Required for B Cell Activation



1. Crosslinking of the BCR
2. Signal from T helper Cells

1. Extensive cross linking of BCR (IgM)
2. Activation of TLR

Immunoglobulin Diversification

<i>Diversification mechanism</i>	<i>Effect</i>
Somatic hypermutation	Antigen specificity
Class switching	Effector activity

Irreversible changes at the DNA level

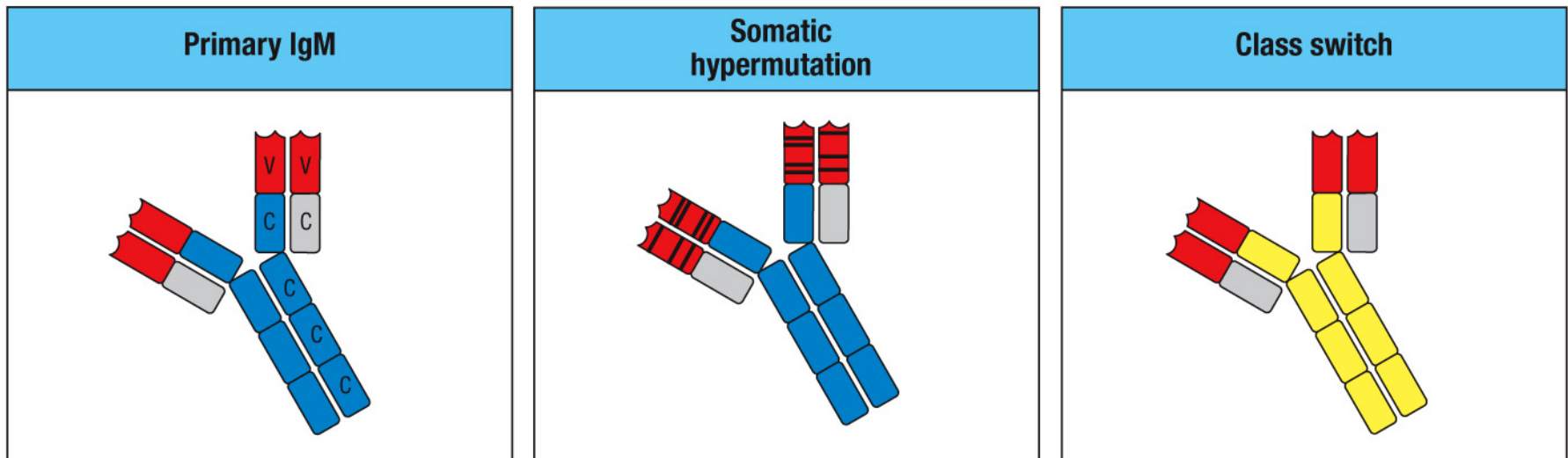
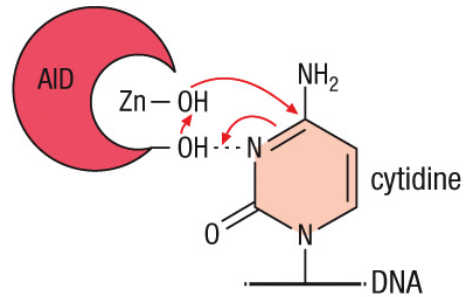


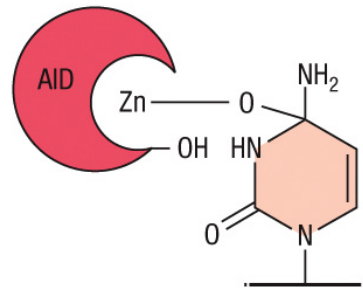
Figure 10.13 Janeway's Immunobiology, 9th ed. (© Garland Science 2017)

C-U Switch at Single-Stranded DNA

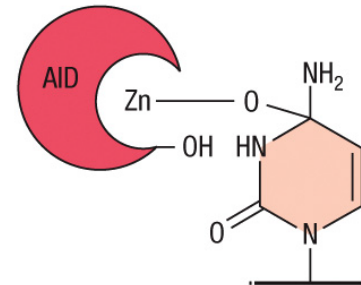
Single-stranded DNA attacked by AID



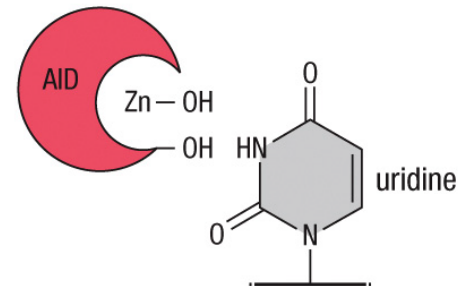
AID transition state



AID transition state



Regeneration of AID and formation of uridine



Outline

- Biological Functions of antibodies
 - Neutralization
 - Opsonization
 - Complement activation
 - Mast cell activation
 - Antibody dependent toxicity
- Application of antibodies
 - Biomedical
 - Technical

Functions of Ig Classes

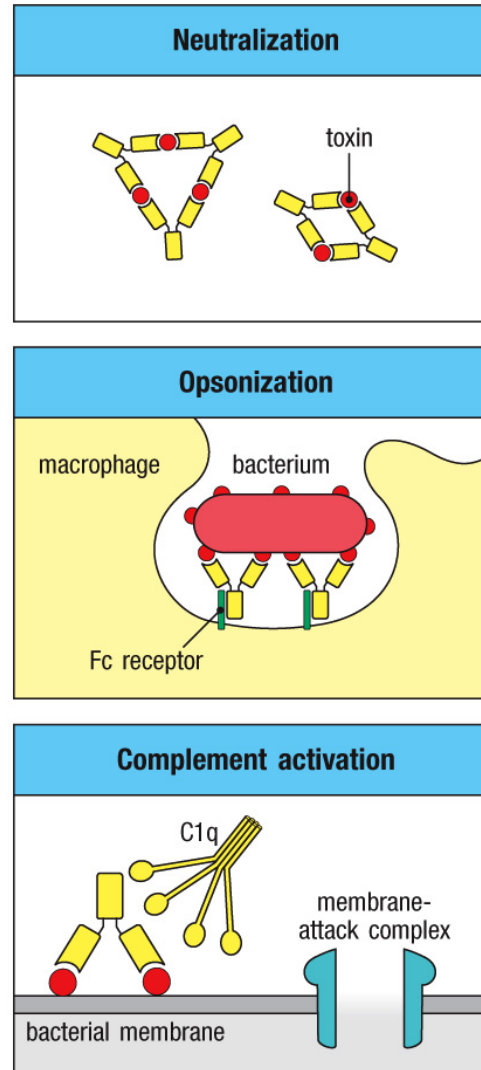
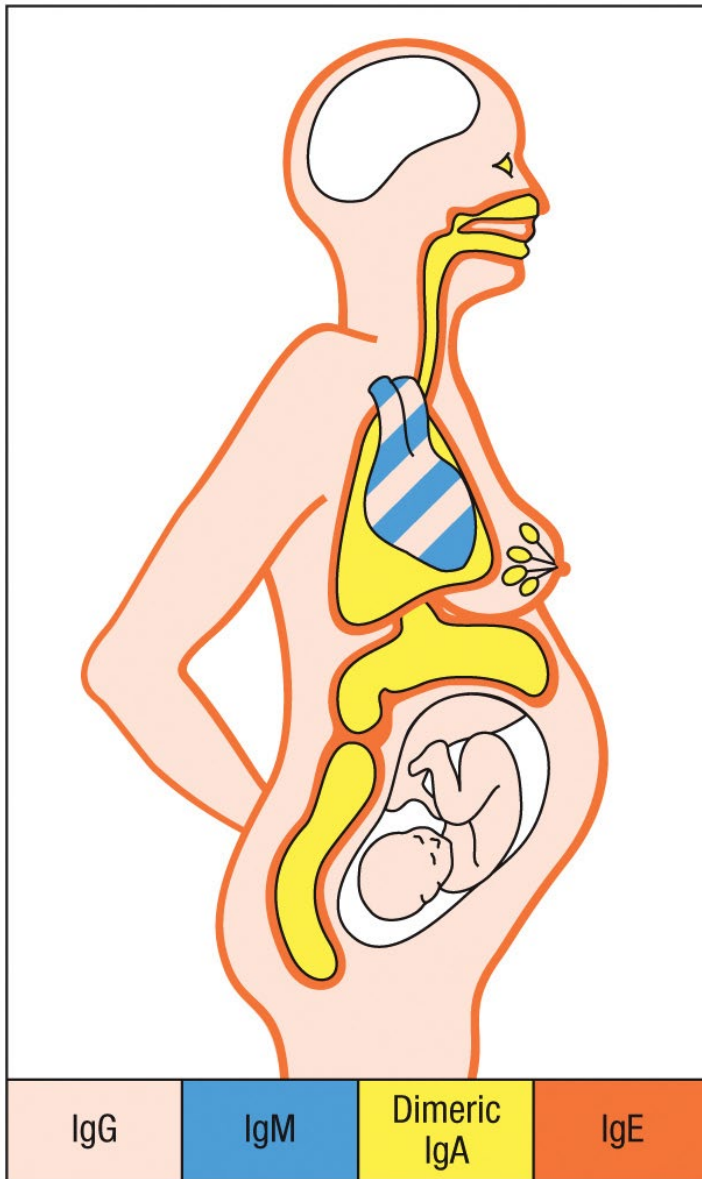


Figure 10.1 Janeway's Immunobiology, 9th ed. (© Garland Science 2017)

Distribution of Ig Classes



IgM:

Pentamer, circulation

IgG:

Monomer, Circulation, extravascular Spaces
Penetrates the placenta

IgA:

Dimer, gut lumen, exocrine organ
Monomer, extravascular Spaces

IgE:

Fixed on cell surface, underneath epithelium

Distribution of Ig Classes

Distribution	IgM	IgD	IgG1	IgG2	IgG3	IgG4	IgA	IgE
Transport across epithelium	+	—	—	—	—	—	+++ (dimer)	—
Transport across placenta	—	—	+++	+	++	+/-	—	—
Diffusion into extravascular sites	+/-	—	+++	+++	+++	+++	++ (monomer)	+
Mean serum level (mg ml ⁻¹)	1.5	0.04	9	3	1	0.5	2.1	3×10 ⁻⁵

IgM and IgA Form Multimers

	Immunoglobulin								
	IgG1	IgG2	IgG3	IgG4	IgM	IgA1	IgA2	IgD	IgE
Heavy chain	γ_1	γ_2	γ_3	γ_4	μ	α_1	α_2	δ	ϵ
Molecular mass (kDa)	146	146	165	146	970	160	160	184	188
Serum level (mean adult mg/ml)	9	3	1	0.5	1.5	3.0	0.5	0.03	5×10^{-5}
Half-life in serum (days)	21	20	7	21	10	6	6	3	2

Pentamer in plasma

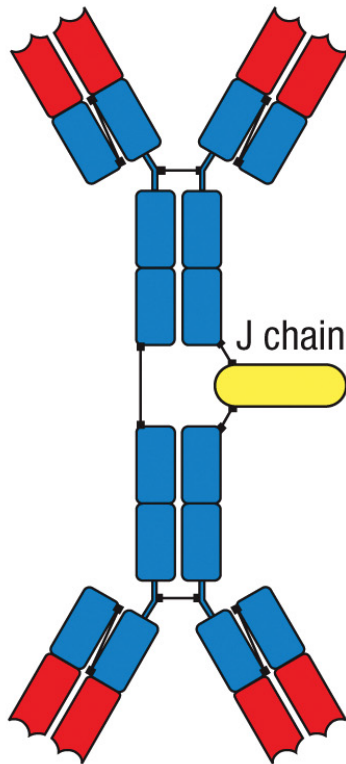
Dimers

Higher levels in mucous membranes (gut, etc.)

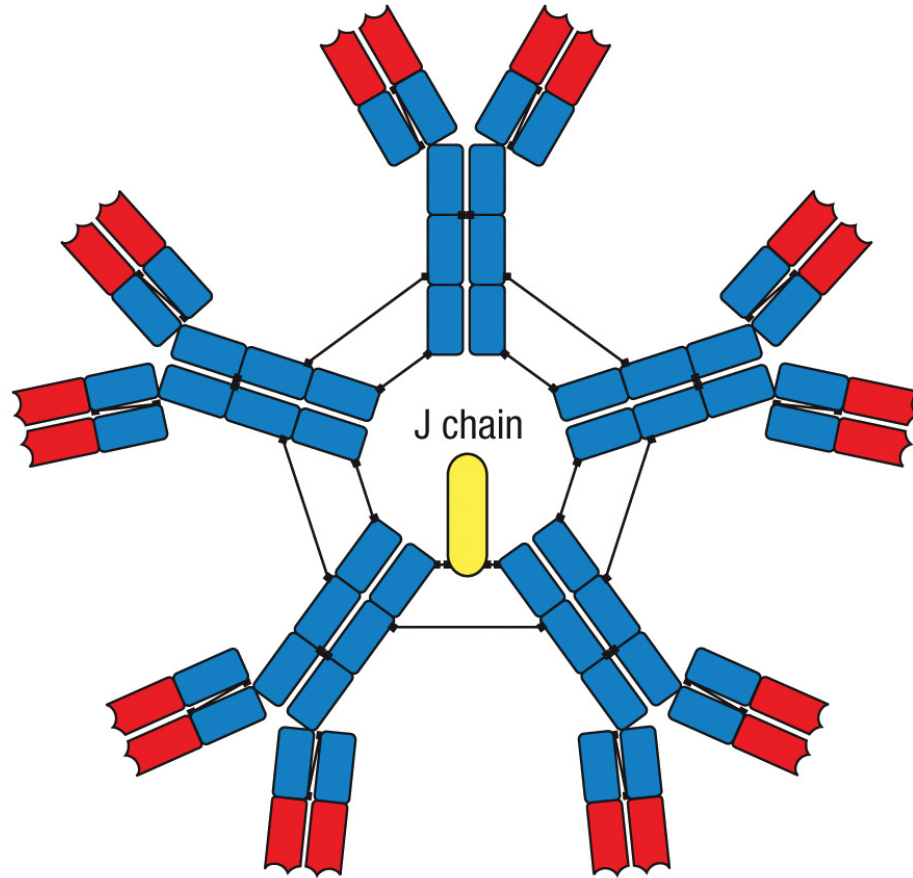
Longer $t_{1/2}$ when fixed to mast cells

IgM and IgA Form Multimers

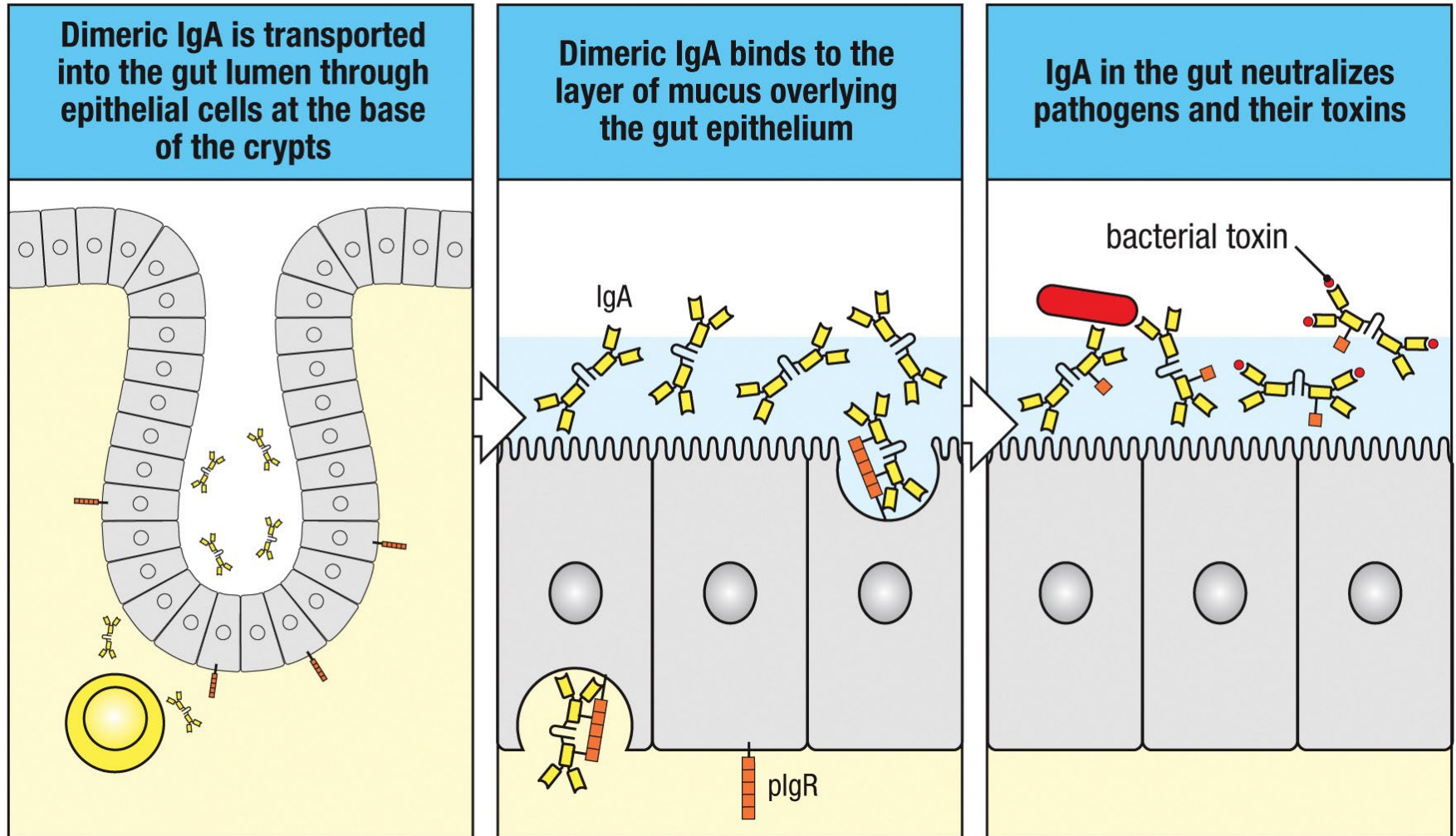
Dimeric IgA



Pentameric IgM



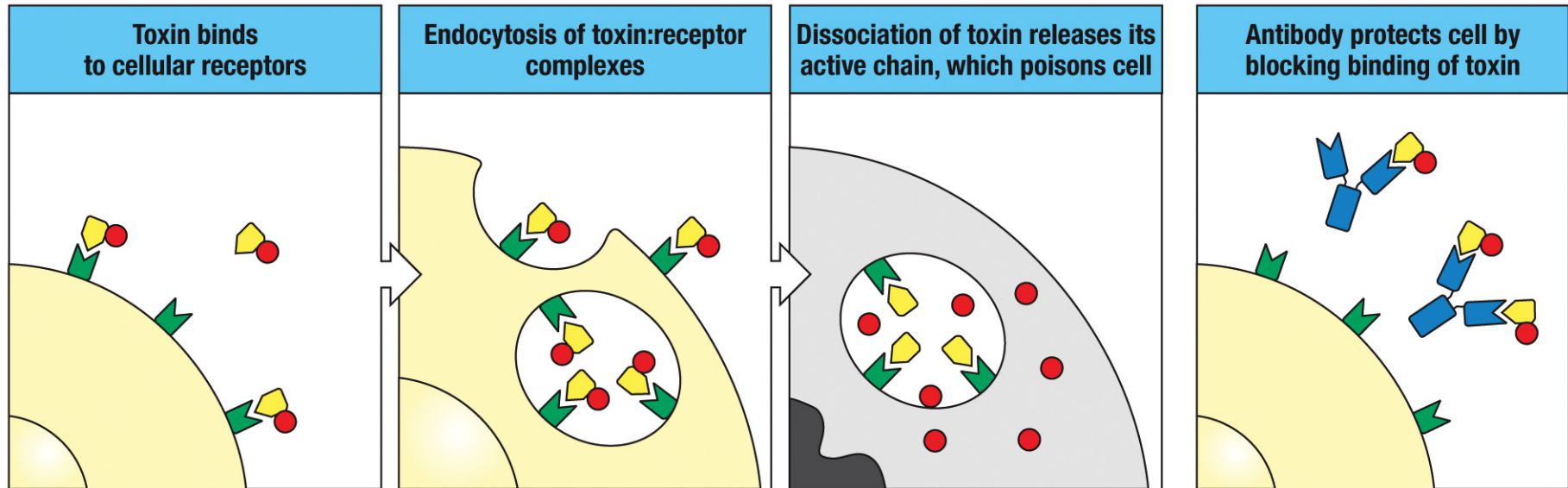
Dimeric IgA is transported into the gut Lumen



Polymeric immunoglobulin receptor and J chain

Neutralization - Prevent Binding

IgA=IgG>IgM



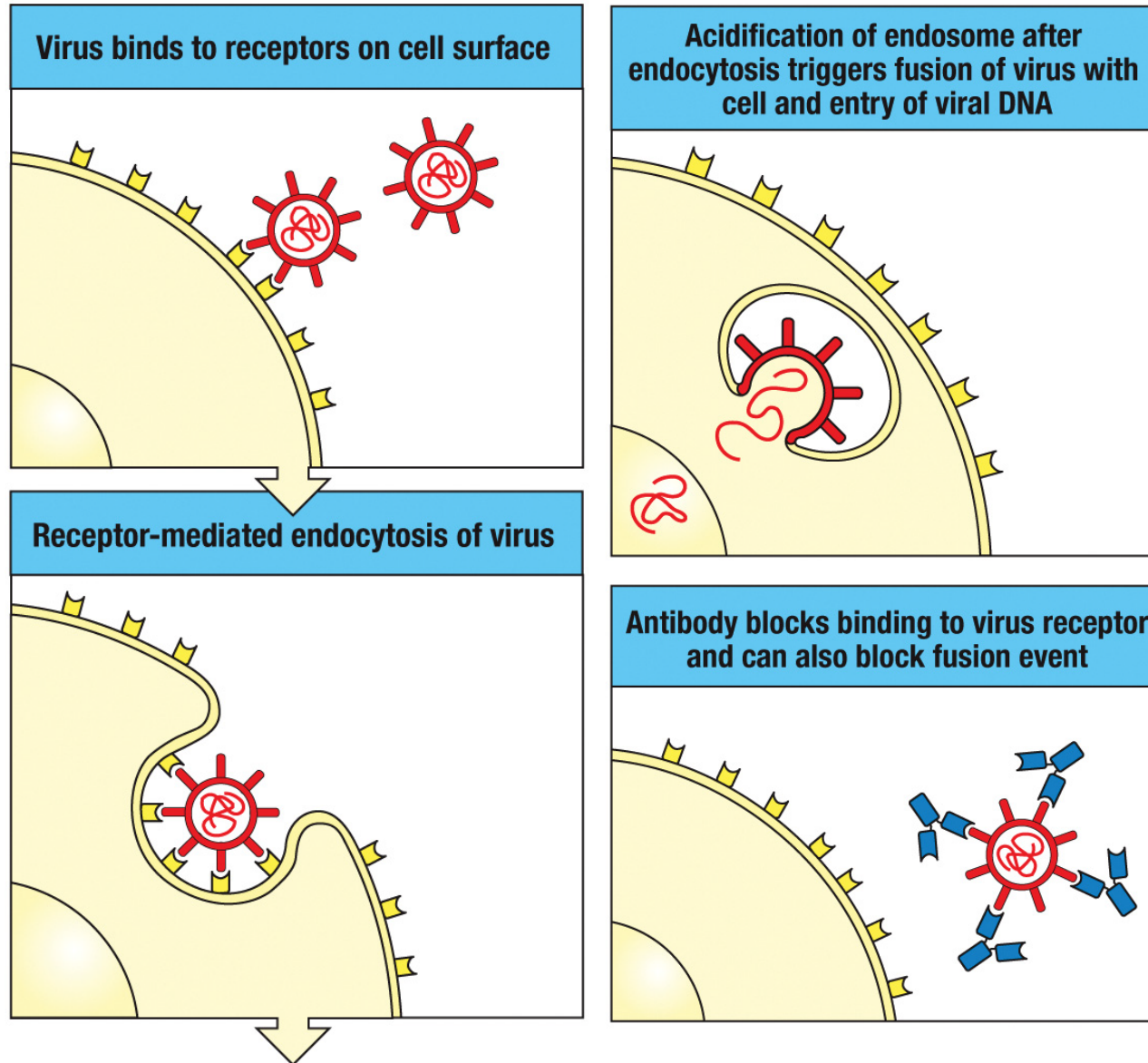
Neutralization - Prevent Binding

Disease	Organism	Toxin	Effects <i>in vivo</i>
Tetanus	<i>Clostridium tetani</i>	Tetanus toxin	Blocks inhibitory neuron action, leading to chronic muscle contraction
Diphtheria	<i>Corynebacterium diphtheriae</i>	Diphtheria toxin	Inhibits protein synthesis, leading to epithelial cell damage and myocarditis
Gas gangrene	<i>Clostridium perfringens</i>	Clostridial toxin	Phospholipase activation, leading to cell death
Cholera	<i>Vibrio cholerae</i>	Cholera toxin	Activates adenylate cyclase, elevates cAMP in cells, leading to changes in intestinal epithelial cells that result in loss of water and electrolytes
Anthrax	<i>Bacillus anthracis</i>	Anthrax toxic complex	Increases vascular permeability, leading to edema, hemorrhage, and circulatory collapse
Botulism	<i>Clostridium botulinum</i>	Botulinum toxin	Blocks release of acetylcholine, leading to paralysis

Neutralization - Prevent Binding

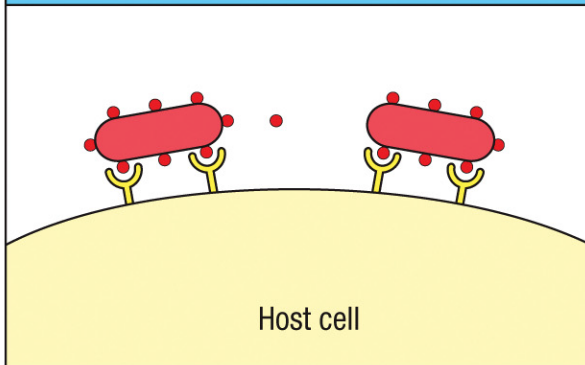
Disease	Organism	Toxin	Effects <i>in vivo</i>
Whooping cough	<i>Bordetella pertussis</i>	Pertussis toxin Tracheal cytotoxin	ADP-ribosylation of G proteins, leading to lymphoproliferation Inhibits cilia and causes epithelial cell loss
Scarlet fever	<i>Streptococcus pyogenes</i>	Erythrogenic toxin Leukocidin Streptolysins	Vasodilation, leading to scarlet fever rash Kill phagocytes, allowing bacterial survival
Food poisoning	<i>Staphylococcus aureus</i>	Staphylococcal enterotoxin	Acts on intestinal neurons to induce vomiting. Also a potent T-cell mitogen (SE superantigen)
Toxic-shock syndrome	<i>Staphylococcus aureus</i>	Toxic-shock syndrome toxin	Causes hypotension and skin loss. Also a potent T-cell mitogen (TSST-1 superantigen)

Neutralization - Prevent Binding

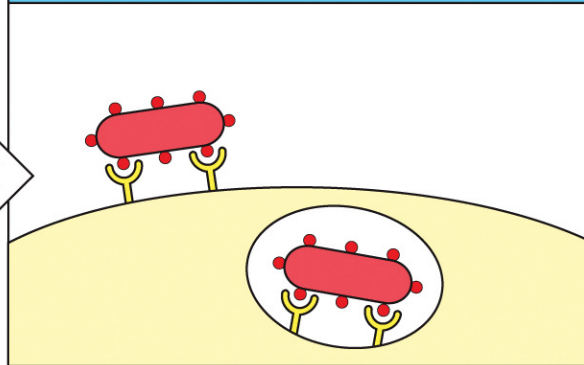


Neutralization - Prevent Binding

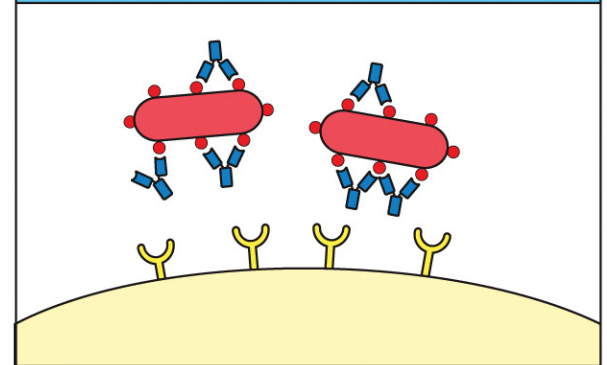
Colonization of cell surface by bacteria that bind to the surface via bacterial adhesins



Some bacteria become internalized and propagate in internal vesicles

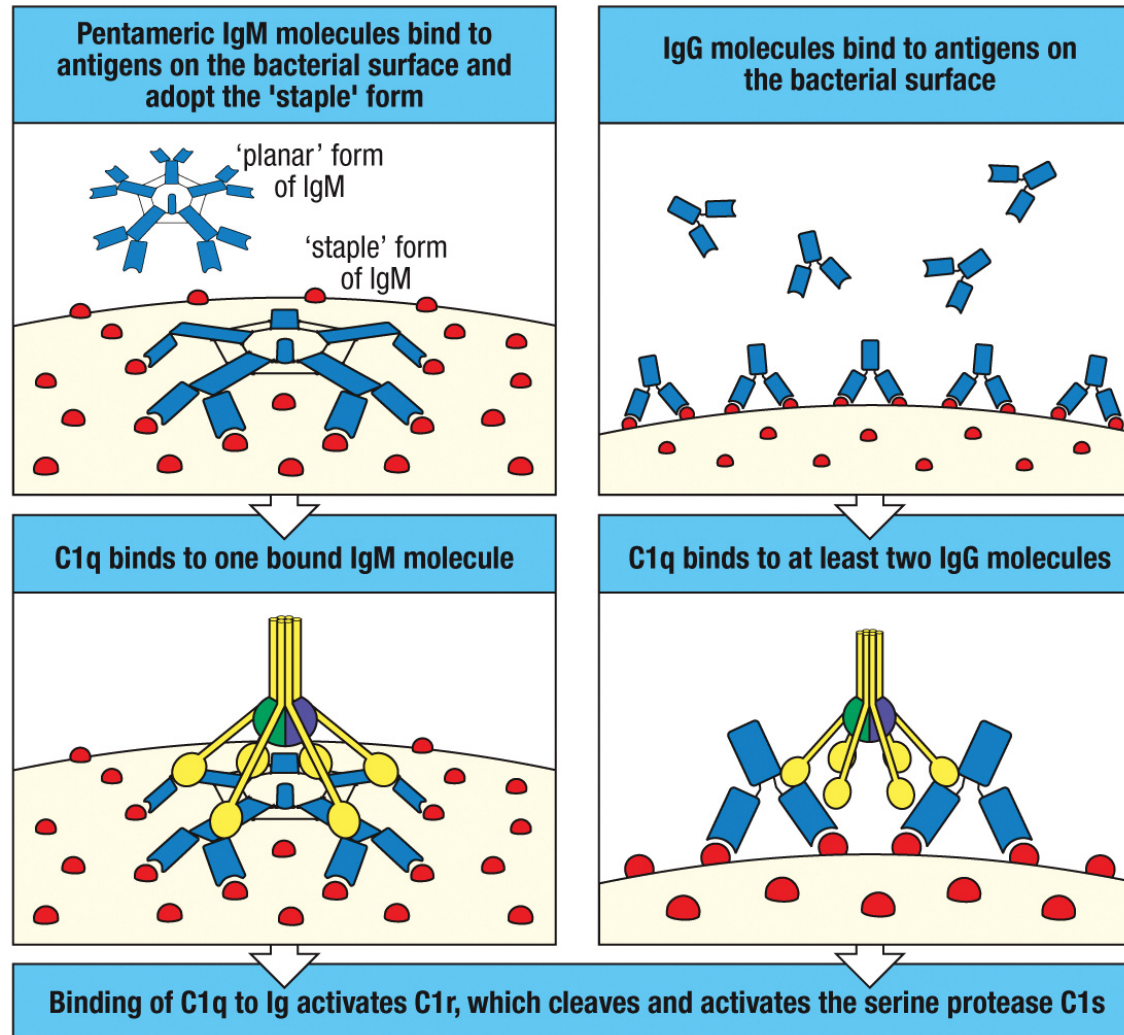


Antibodies against adhesins block colonization and uptake



Complement Activation

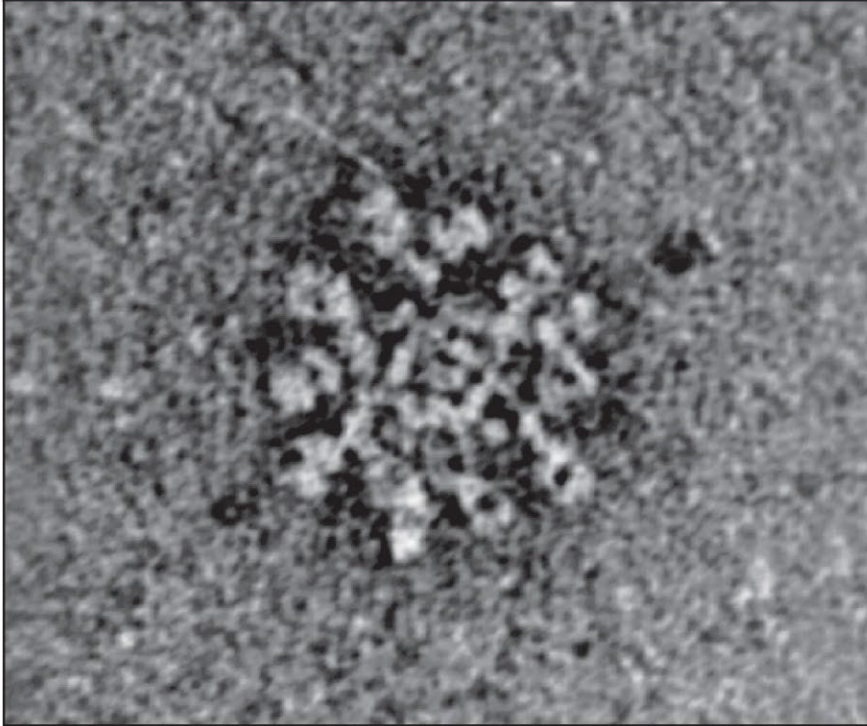
$\text{IgM} = \text{IgG} > \text{IgA}$,



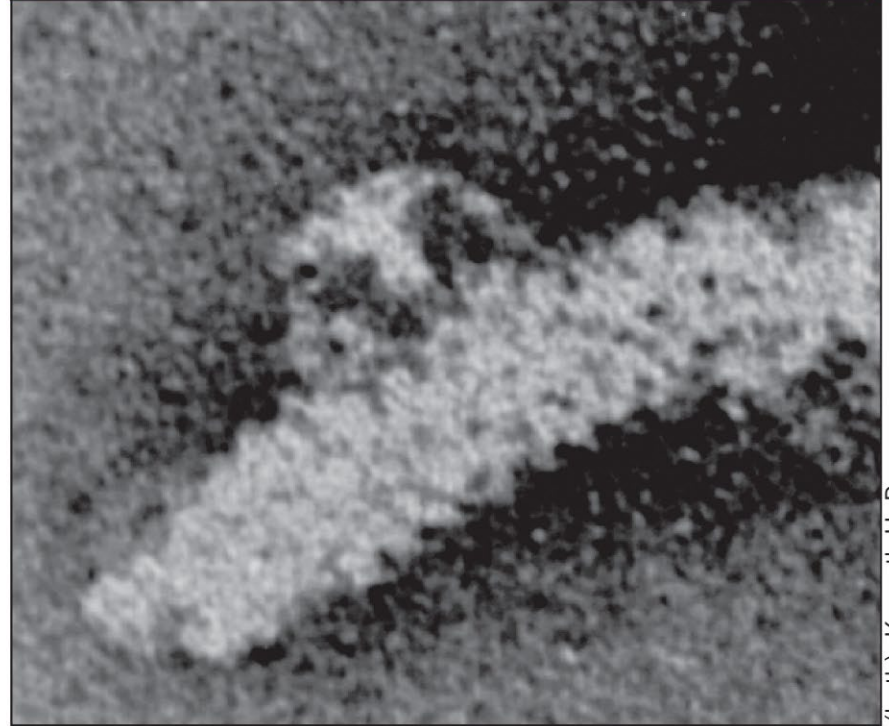
Complement Activation

Complement is activated when antibodies bind to the pathogen surface

IgM 'planar' conformation



IgM 'staple' conformation



(both): Kenneth H. Roux

Complement Activation

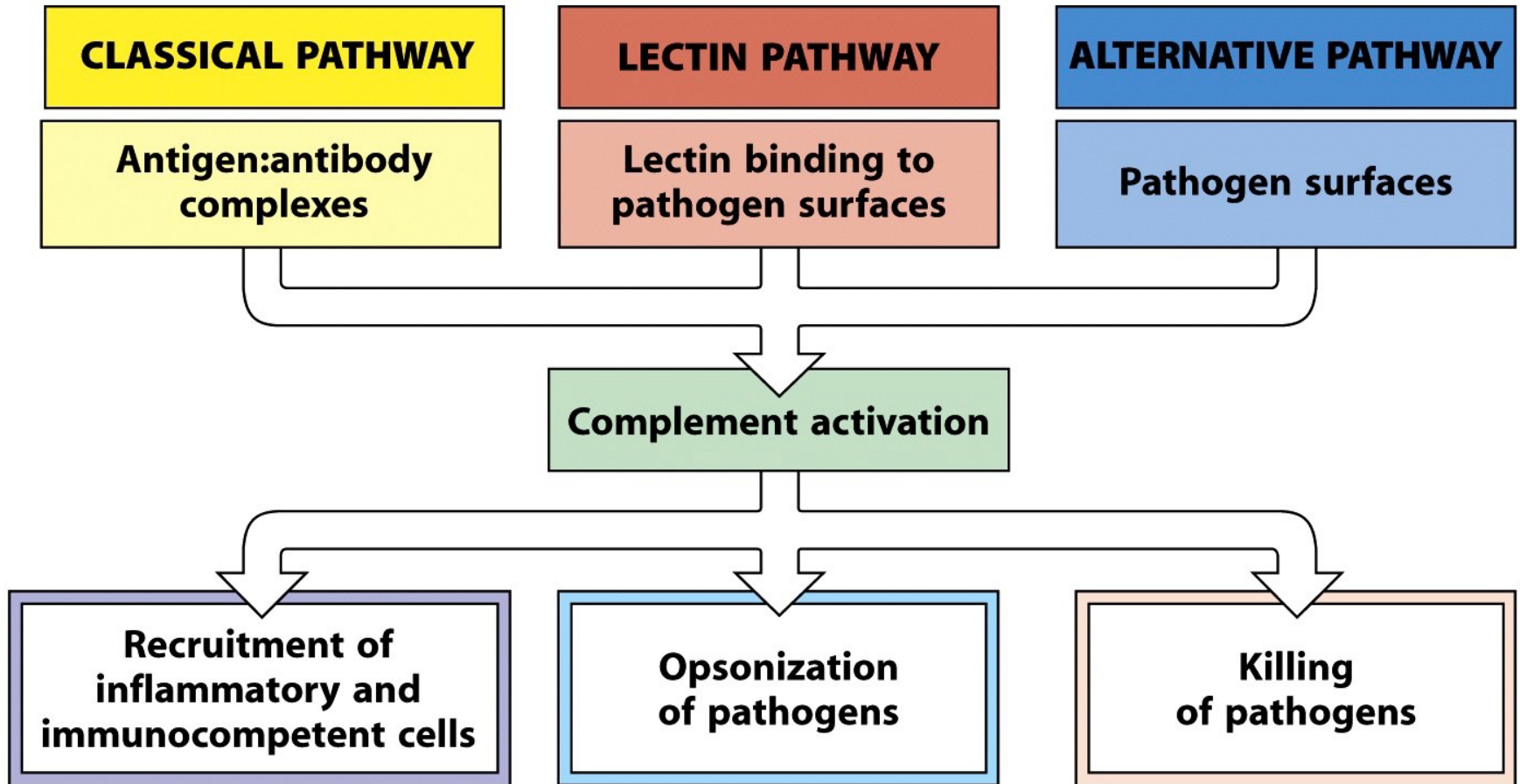
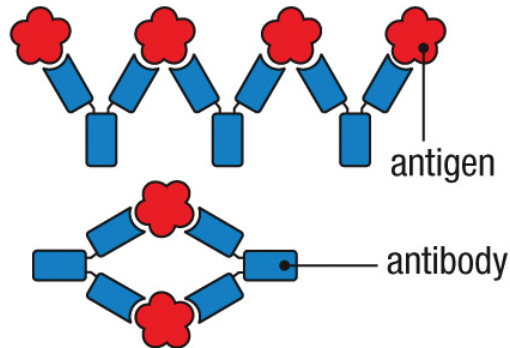


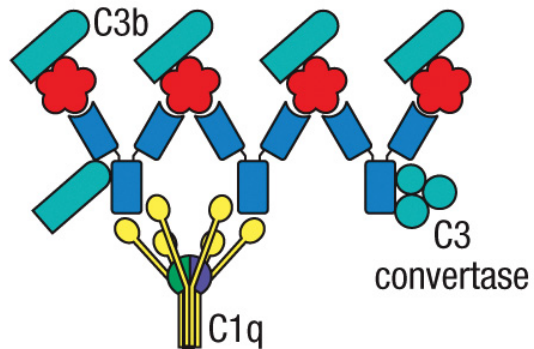
Figure 2-24 Immunobiology, 7ed. (© Garland Science 2008)

Immune Complex is Removed in the Spleen

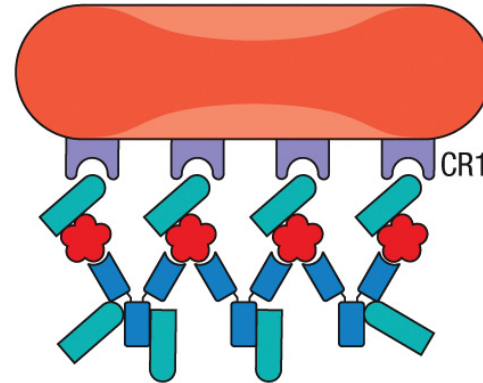
Small antigen:antibody complexes form in the circulation



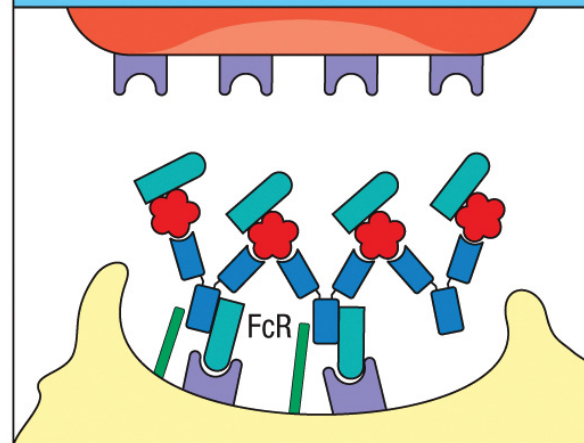
Activation of complement leads to the deposition of many molecules of C3b on the immune complex



Complement receptor CR1 on erythrocytes binds the immune complexes via bound C3b

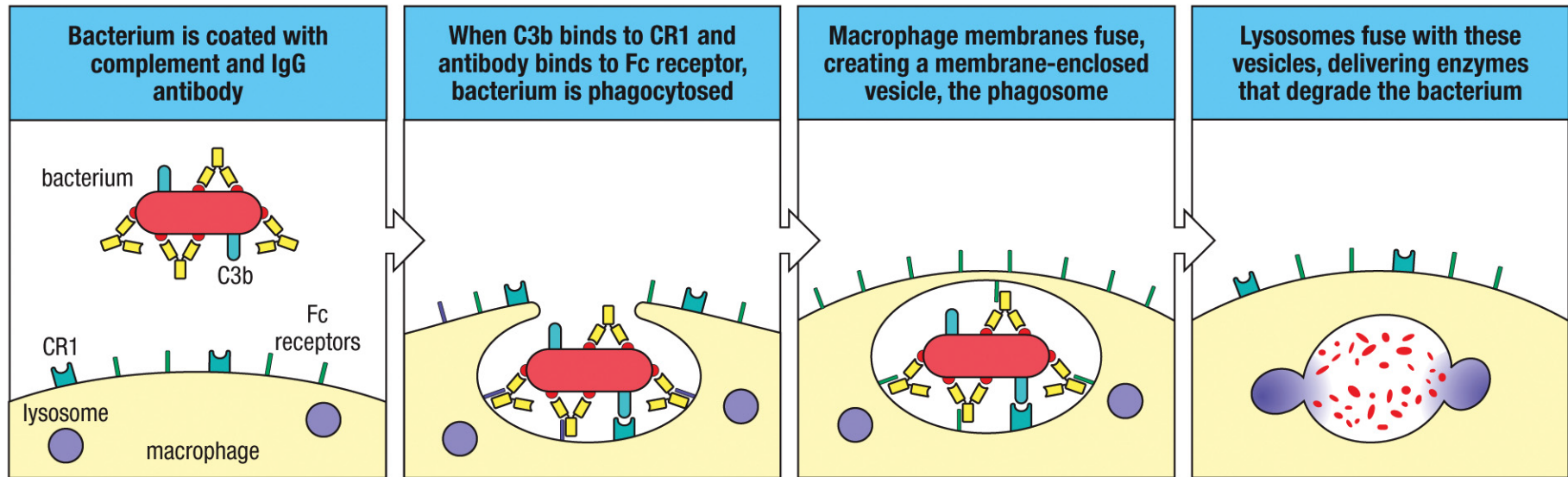


In the spleen and liver, phagocytic cells remove the immune complexes from the erythrocyte surface

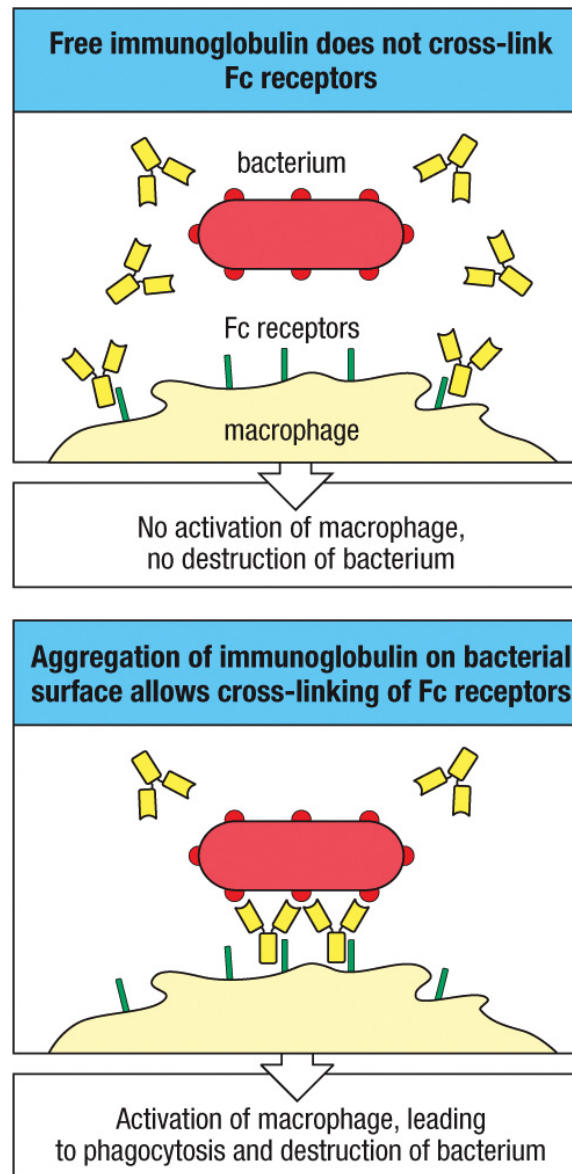


Opsonization- Enhance Phagocytosis

IgG>IgM=IgA

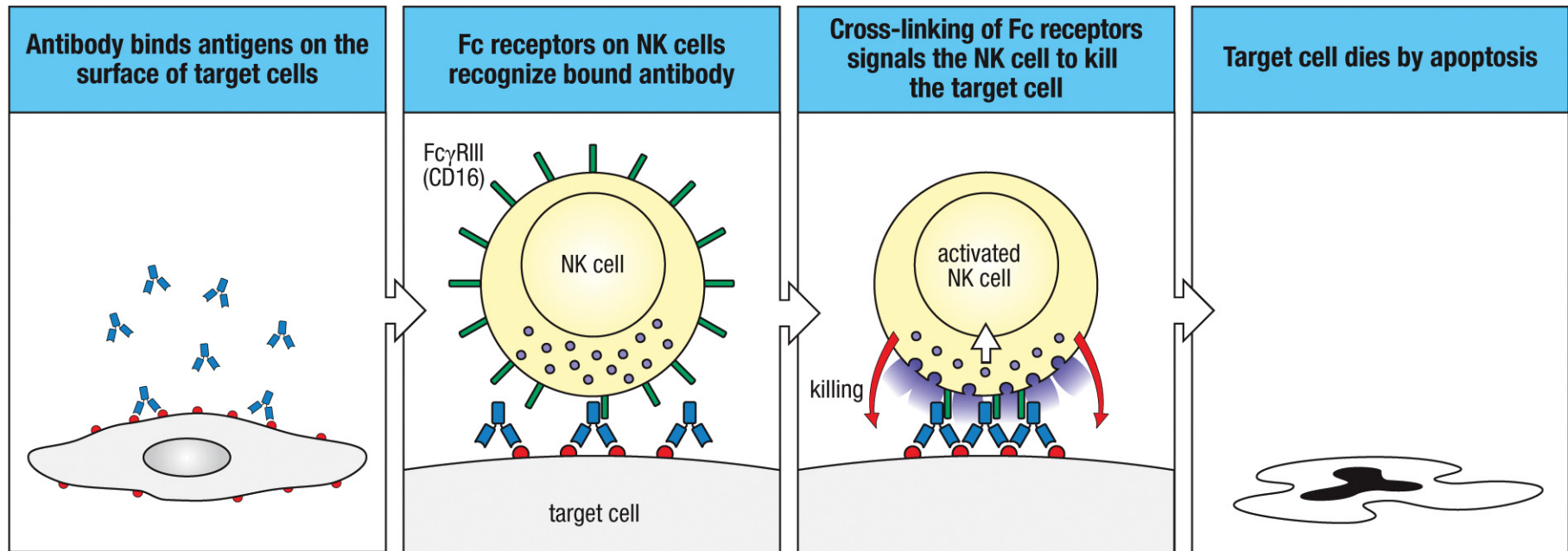


Opsonization Requires Aggregation of Ig on Bacterial Surface



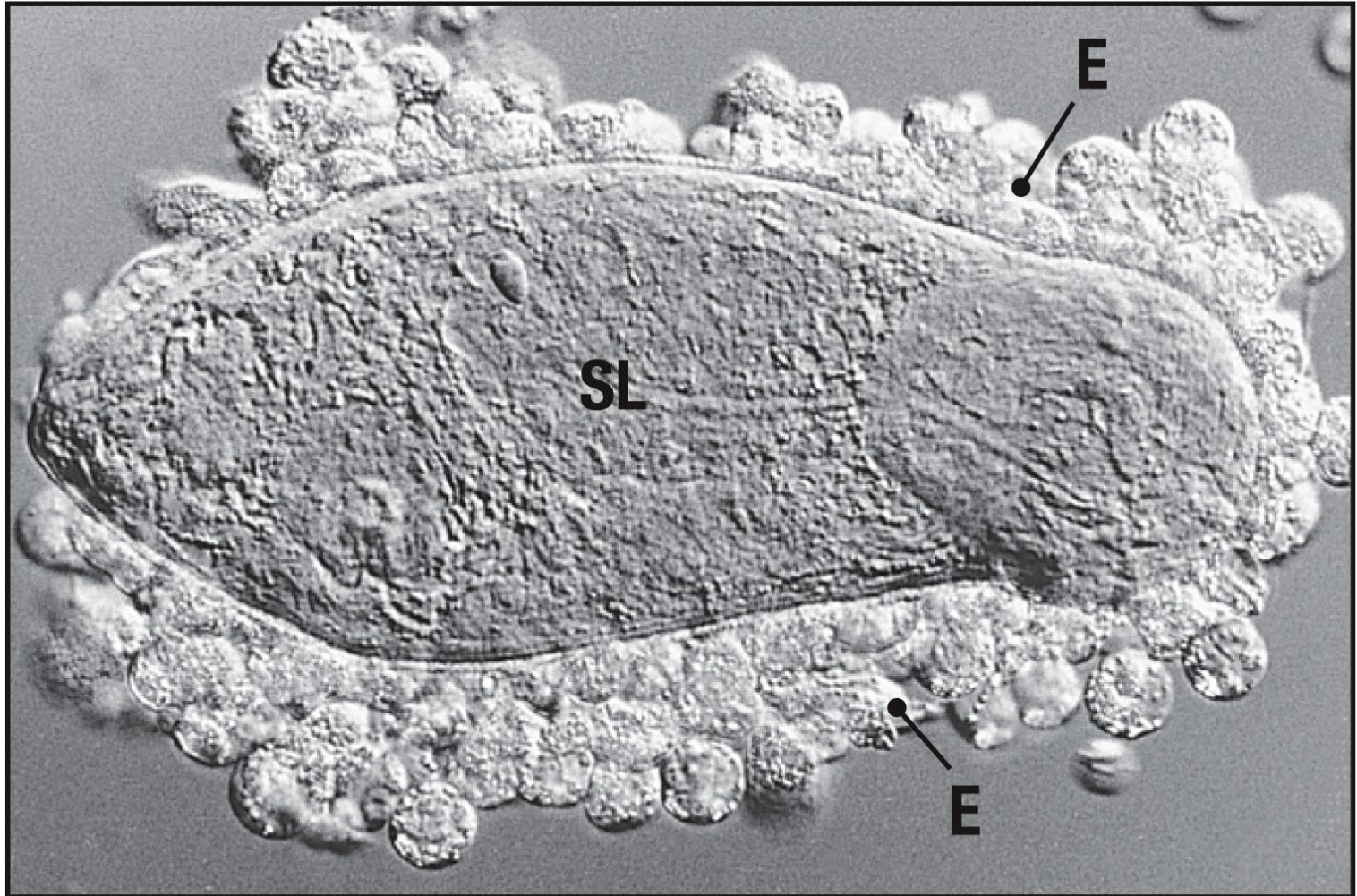
Antibody Dependent Cytotoxicity

IgG, killing of antibody coated host cells



Eosinophils Attacking a Schistosome Larva

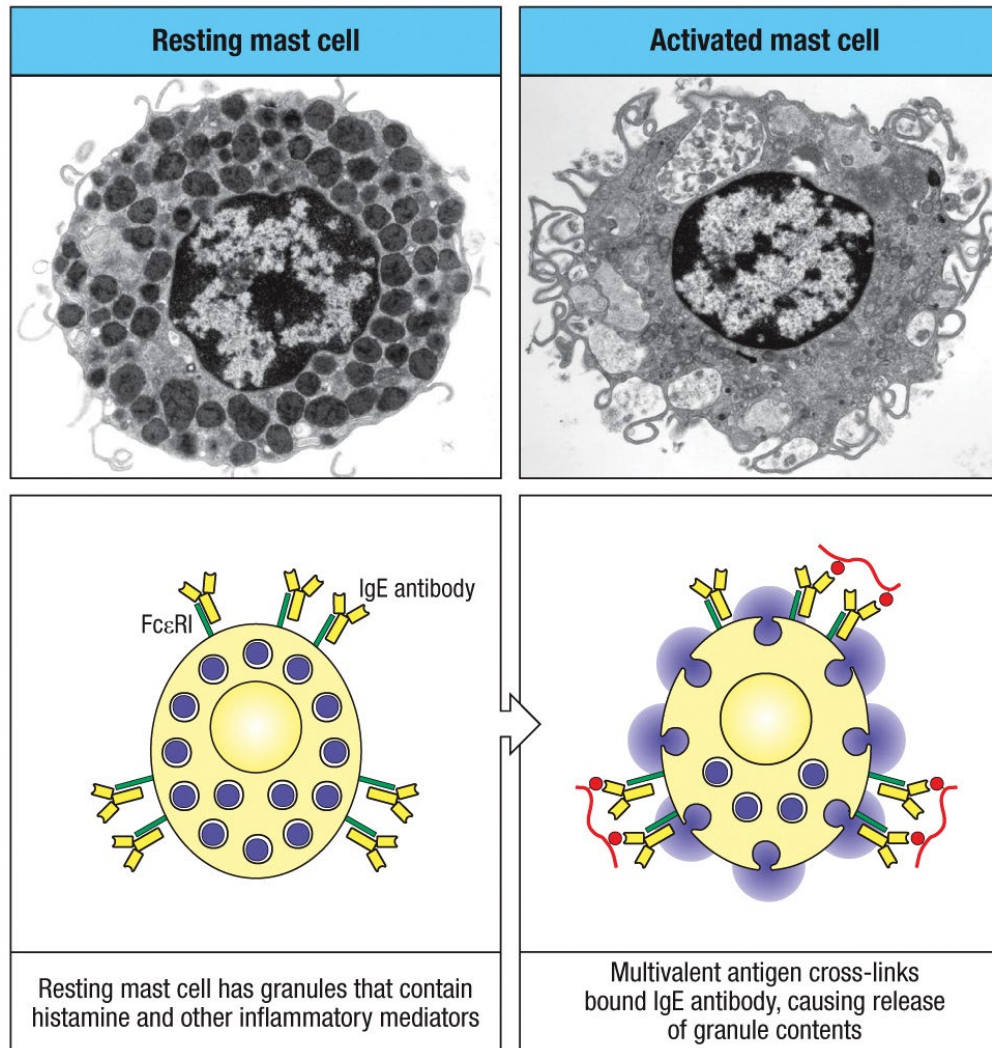
IgE, IgA and IgG



Photograph courtesy of Anthony Butterworth

Mast Cell Activation by IgE

Activates Blood Vessel; Defend against parasites; Allergy



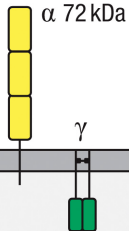
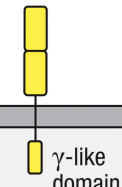
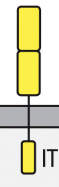
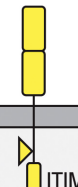
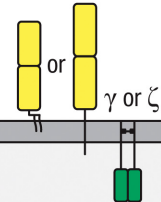
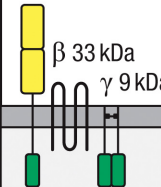
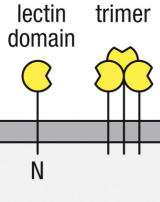
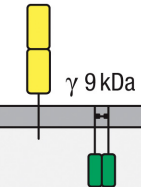
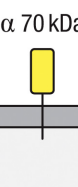
Photographs courtesy of Dr. Ann M. Dvorak

Distribution and Function of Ig Classes

Functional activity	IgM	IgD	IgG1	IgG2	IgG3	IgG4	IgA	IgE
Neutralization	+	—	++	++	++	++	++	—
Opsonization	+	—	++	*	++	+	+	—
Sensitization for killing by NK cells	—	—	++	—	++	—	—	—
Sensitization of mast cells	—	—	+	—	+	—	—	+++
Activates complement system	+++	—	++	+	+++	—	+	—

Distribution	IgM	IgD	IgG1	IgG2	IgG3	IgG4	IgA	IgE
Transport across epithelium	+	—	—	—	—	—	+++ (dimer)	—
Transport across placenta	—	—	+++	+	++	+/-	—	—
Diffusion into extravascular sites	+/-	—	+++	+++	+++	+++	++ (monomer)	+
Mean serum level (mg ml ⁻¹)	1.5	0.04	9	3	1	0.5	2.1	3×10 ⁻⁵

Fc Receptors

Receptor	FcγRI (CD64)	FcγRII-A (CD32)	FcγRII-B2 (CD32)	FcγRII-B1 (CD32)	FcγRIII (CD16)	FcεRI	FcεRII (CD23)	FcαRI (CD89)	Fcα/μR
Structure									
Binding	IgG1	IgG1	IgG1	IgG1	IgG1	IgE	IgE	IgA1, IgA2	IgA, IgM
Order of affinity	10^8 M^{-1} 1) IgG1=IgG3 2) IgG4 3) IgG2	$2 \times 10^6 \text{ M}^{-1}$ 1) IgG1 2) IgG3=IgG2* 3) IgG4	$2 \times 10^6 \text{ M}^{-1}$ 1) IgG1=IgG3 2) IgG4 3) IgG2	$2 \times 10^6 \text{ M}^{-1}$ 1) IgG1=IgG3 2) IgG4 3) IgG2	$5 \times 10^5 \text{ M}^{-1}$ IgG1=IgG3	10^{10} M^{-1}	$2-7 \times 10^7 \text{ M}^{-1}$ (trimer) $2-7 \times 10^6 \text{ M}^{-1}$ (monomer)	10^7 M^{-1} IgA1=IgA2	$3 \times 10^9 \text{ M}^{-1}$ 1) IgM 2) IgA
Cell type	Macrophages Neutrophils Eosinophils	Macrophages Neutrophils Eosinophils Platelets Langerhans cells	Macrophages Neutrophils Eosinophils	B cells Mast cells	NK cells Eosinophils Macrophages Neutrophils Mast cells	Mast cells Basophils	Eosinophils B cells	Macrophages Eosinophils [†] Neutrophils	Macrophages B cells
Effect of ligation	Uptake Stimulation Activation of respiratory burst Induction of killing	Uptake Granule release (eosinophils)	Uptake Inhibition of stimulation	No uptake Inhibition of stimulation	Induction of killing (NK cells)	Secretion of granules	Degranulation	Uptake Induction of killing	Uptake

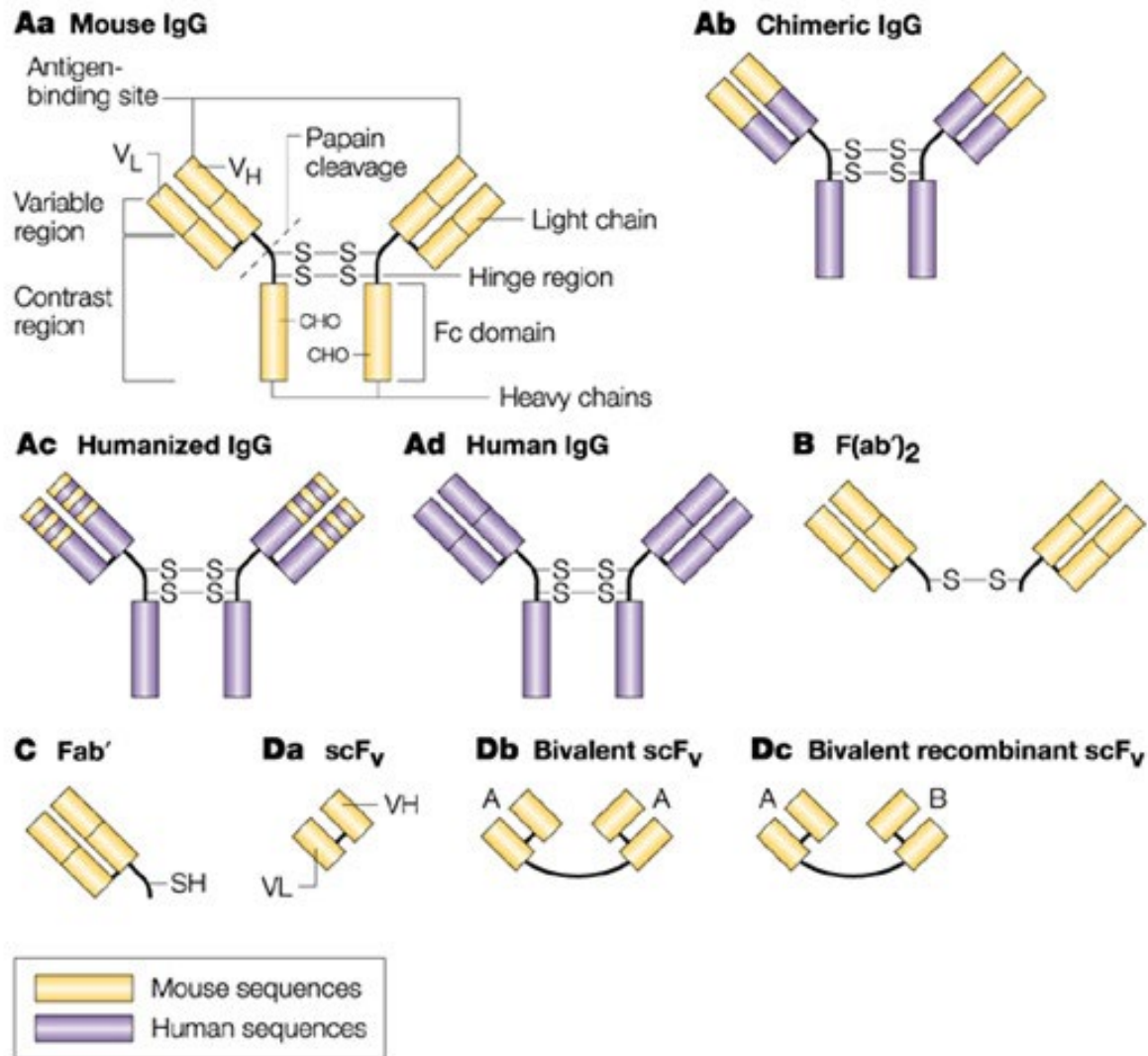
Question

- What are the three major biological functions of antibodies?
- Which type of antibody performs the most functions?

Outline

- Biological Function of antibodies
 - Neutralization
 - Opsonization
 - Complement activation
 - Mast cell activation
 - Antibody dependent toxicity
- Application of antibodies
 - Biomedical
 - Technical

Antibody in Therapy



Monoclonal and Polyclonal Antibodies

Monoclonal Abs (Ig, Gama Globulin)

- Antibodies that are identical because they were produced by one type of B cell.
- Detect only one epitope on the antigen.

Monoclonal antibody



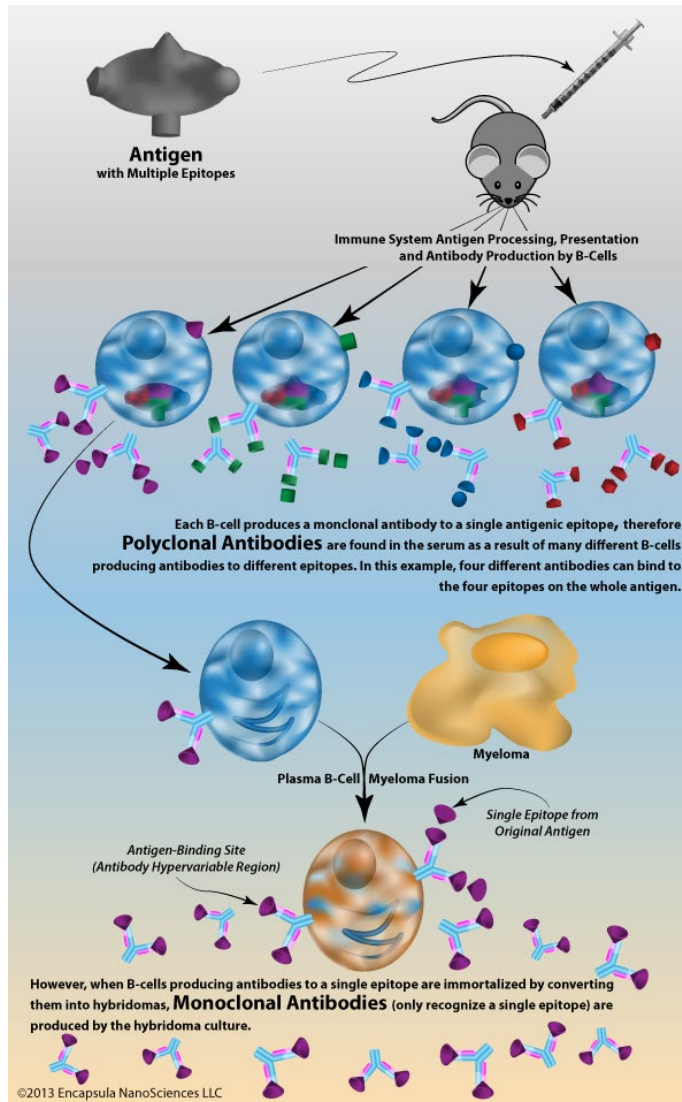
Polyclonal Abs (Ig, Gama Globulin)

- Antibodies that are non-identical because they were produced by different B cell resources.
- Detect multiple epitopes on any one antigen.

Polyclonal antibody



Production of Monoclonal Antibodies



Therapeutic Applications of Antibodies

Table 1

Antibody classification according to structure, with examples of products that are licensed or under development

mAb category	Suffix	Examples	Specificity	Reference
Chimeric	-ximab	Infliximab (Remicade®)	TNF- α	[59]
		Rituximab (Rituxan®, Mabthera®)	CD20	[60]
Humanised	-zumab	Alemtuzumab (MabCampath®)	CD52	[18]
		Tocilizumab (RoActemra®)	IL-6R	[61]
		Ocrelizumab	CD20	[62]
		Epratuzumab	CD22	[63]
		Certolizumab pegol (PEGylated Fab fragment) (Cimzia®)	TNF- α	[64]
		Otelixizumab (Aglycosyl)	CD3	[42]
		Teplizumab (Fc-mutated)	CD3	[65]
		Visilizumab (Fc-mutated)	CD3	[44]
'Fully human'	-mumab	Adalimumab (Humira®)	TNF- α	[66]
		Ofatumumab (Humax-CD20®)	CD20	[67]
		Belimumab (LymphoStat-B®)	BLyS	[68]
		Golimumab	TNF- α	[69]
Fusion proteins	-cept	Etanercept (Enbrel®)	TNF- α	[70]
		Abatacept (Orencia®)	CD80/CD86	[71]
		Atacicept	BLyS/BAFF	[72]

BAFF, B-cell activating factor; BLyS, B-lymphocyte stimulator; Fab, fragment antigen-binding; Fc, fragment crystallisable; mAb, monoclonal antibody; TNF- α , tumour necrosis factor-alpha.

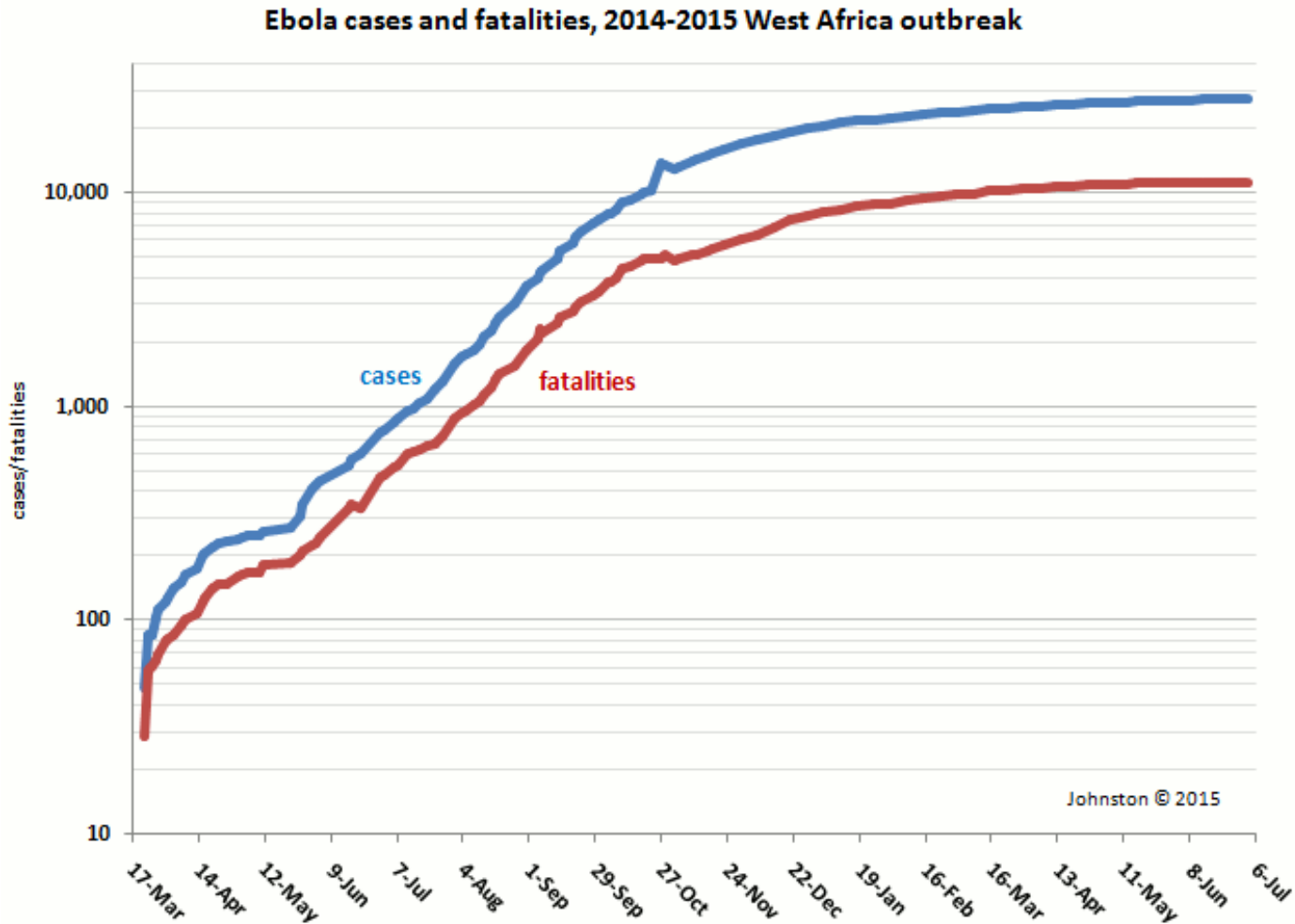
IgG Infusion

Intravenous Immunoglobulin (IVIG):

IgG antibodies extracted from the plasma of over one thousand blood donors.

- Primary Immune deficiencies
- Acquired compromised immunity conditions
- Autoimmune diseases
- Acute infections

2014 The return of Ebola



Ebola

The return of Ebola

An American doctor stricken with the deadly Ebola virus while in Liberia and brought to the United States for treatment in a special isolation ward is improving, a top U.S. health official said. Dr. Kent Brantly was able to walk, with help, from an ambulance after he was flown on Saturday to Atlanta.

Disease

Ebola is a virus that is found naturally in certain species of bats inhabiting wooded areas of Africa. Since their emergence in 1976 there have been 18 outbreaks in countries like the Democratic Republic of Congo, Gabon, Uganda and Sudan.



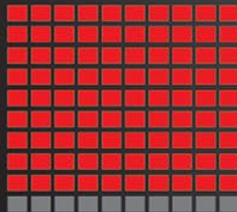
The virus.

Five species of Ebola

All of them are named after a river near the epicenter of the first outbreak in the Democratic Republic of Congo. By the place and year of discovery:

Sudan and Zaïre	(1976)
Reston	(1989)
Ivory Coast	(1994)
Bundibugyo	(2007)

The Ebola virus can have a fatality rate of 90 percent.



How is it transmitted?

Through direct blood contact or other body fluids, or through indirect contact with an environment containing contaminated fluids. In Africa, there have been documented infection cases that are associated with the handling of infected chimpanzees, gorillas, fruit bats, monkeys and antelopes.



Symptoms

Muscle weakness and intense headaches and throat pain

There is no known cure for Ebola. Instead, doctors take on this virus by tackling early symptoms, by strengthening patients' immune system.

When an infected person dies, the virus in their body does not perish immediately. The virus can live in the bodily fluids of dead organisms for a certain period.

Internal and external bleeding

Renal and hepatic dysfunction

Vomiting, diarrhea and rashes

Sudden onset of fever

Laboratory results show a reduction in the number of leukocytes and platelets, and elevated liver enzymes.

metro

Can the virus spread beyond Africa?

According to experts, the risk of contagion in Europe is low. Doctors in Guinea say that most Ebola patients are confined to remote villages and are unlikely to travel overseas.

Diagnosis

First we have to rule out other conditions such as malaria, typhoid fever, shigellosis, cholera, leptospirosis, plague, rickettsial, relapsing fever, meningitis, hepatitis and other viral haemorrhagic fevers.

Infections by the Ebola virus can only be diagnosed by various laboratory tests:



Enzyme-linked immunosorbent assay (ELISA)

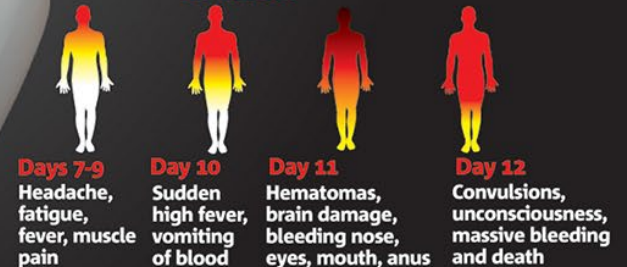
Antigen detection tests

Serum neutralization test

Virus isolation by cell culture

The patient samples represent a huge danger and must be performed under conditions of maximum biological containment.

Evolution



SOURCES: OMS / AGENCIES

Passive Transfer of Ebola Antibody

Volume 21, Number 3—March 2015

Dispatch

Treatment of Ebola Virus Infection with Antibodies from Reconvalescent Donors

Thomas R. Kreil✉

Author affiliation: Global Pathogen Safety, Baxter BioScience, Vienna, Austria

[Suggested citation for this article](#)

Abstract

Clinical evidence suggests that antibodies from reconvalescent donors (persons who have recovered from infection) may be effective in the treatment of Ebola virus infection. Administration of this treatment to Ebola virus-infected patients while preventing the transmission of other pathogenic viruses may be best accomplished by use of virus-inactivated reconvalescent plasma.

On This Page

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NEWS | IN DEPTH

but “we are not yet where we need to be.”

Ovabrite, a U.S. company in Austin, is chasing a technique that would leave the eggshell intact and sort eggs before incubation. Mass spectrometers would capture and analyze sex-specific volatile molecules that leak through the eggshell. Scientists suspect the molecules, first discovered in quail eggs, may allow parent birds to smell clues about an embryo's development and sex. But it is still a challenge to reliably detect such a faint signal from preincubation eggs, which must be refrigerated, says Ovabrite President Jonathan Hoopes.

Some predict that genetic engineering could help do away with complicated robots. Groups in Australia and Israel have used the CRISPR gene-editing technique to modify hens' sex chromosomes so that their sons carry a marker gene that makes male eggs glow under fluorescent light. That would allow hatcheries to sort out the fluorescent male eggs with a simple detector. Finding a marker that produces a strong enough signal in early embryos is a challenge, says Yehuda Elram, CEO of eggXYt (pronounced “exit”) in Jerusalem. He says eggXYt has found a solution, but declined to say whether it is close to hatchery tests.

Public opposition to genetic modification in Europe means the approach is unlikely to catch on there. But Mark Tizard, a geneticist at the Australian Animal Health



A health worker puts on protective gear at an Ebola treatment center in Beni, Democratic Republic of the Congo.

INFECTIOUS DISEASES

Successful Ebola treatments promise to tame outbreak

Antibody preparations that cut the death rate dramatically will become available to all patients in Congo

MONOCLONAL ANTIBODIES (MAB) TREATMENT FOR COVID-19 PATIENTS

HOSPITALIZATION
RATES FOR PATIENTS
OVER 60 YEARS OLD

18.7%

Without
MAB treatment

8.9%

With
MAB treatment

More than
50%
reduction

- Infusions are available for eligible patients close to home.
- It is important to consider this treatment as soon as symptoms begin.

*over 4,200 given

As of Sept. 14, 2021

SANFORD
HEALTH

Antiserum-passive Immunity

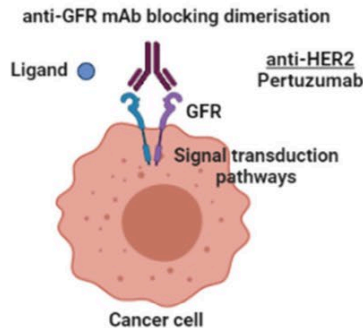


Diluted venom is used to produce antibodies in animals

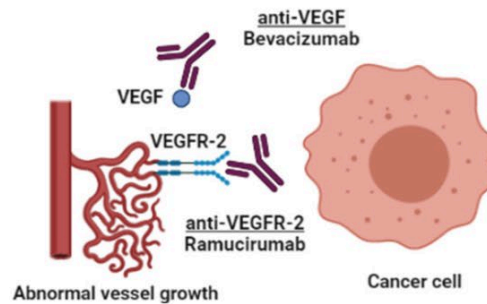
http://en.wikipedia.org/wiki/Antivenom#/media/File:Snake_Milking.jpg

Therapeutic Application of Monoclonal Antibodies

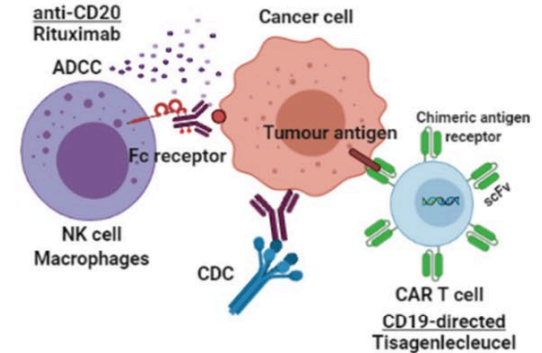
A. Targeting growth factor receptors (GFR)



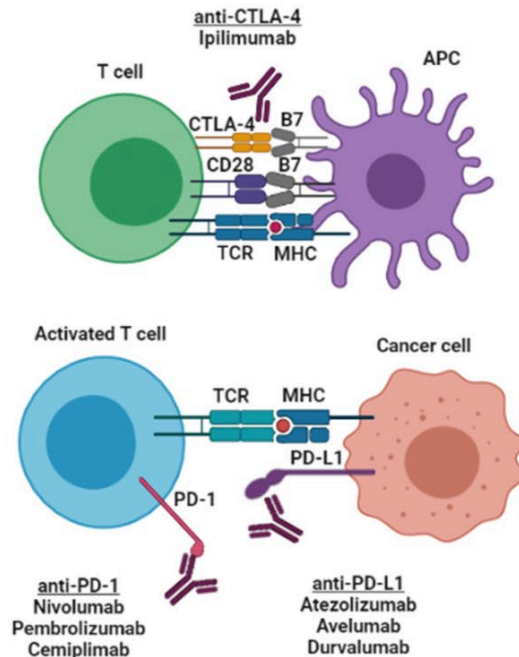
B. Targeting tumour vasculature



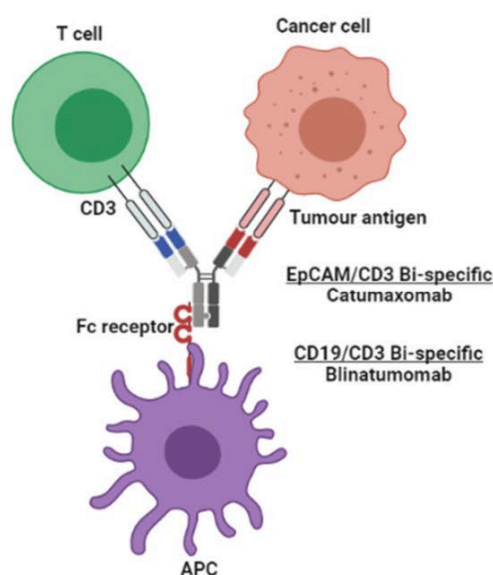
C. Immune mediated ADCC/CDC/CAR-T



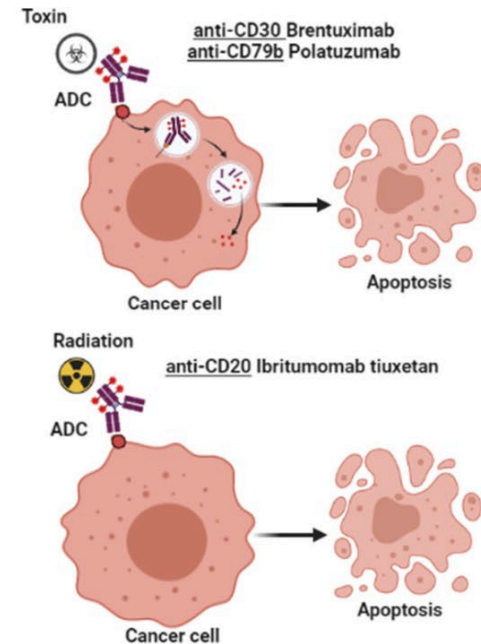
D. Immune checkpoint inhibition



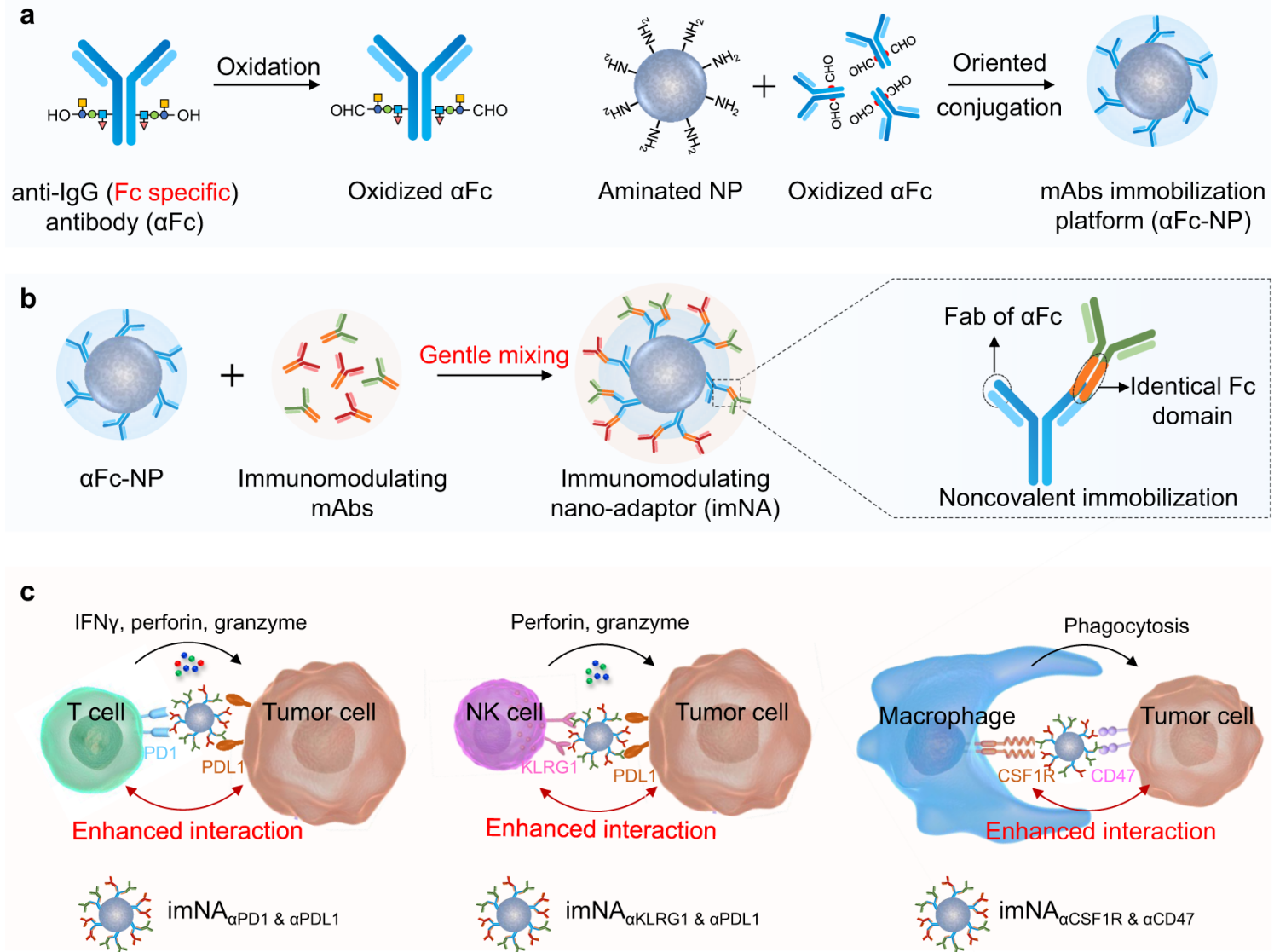
E. Bispecific antibodies



F. Delivery of payloads (ADCs)



Antibody-Based Cancer Immunotherapy



Other Applications: Flow Cytometry

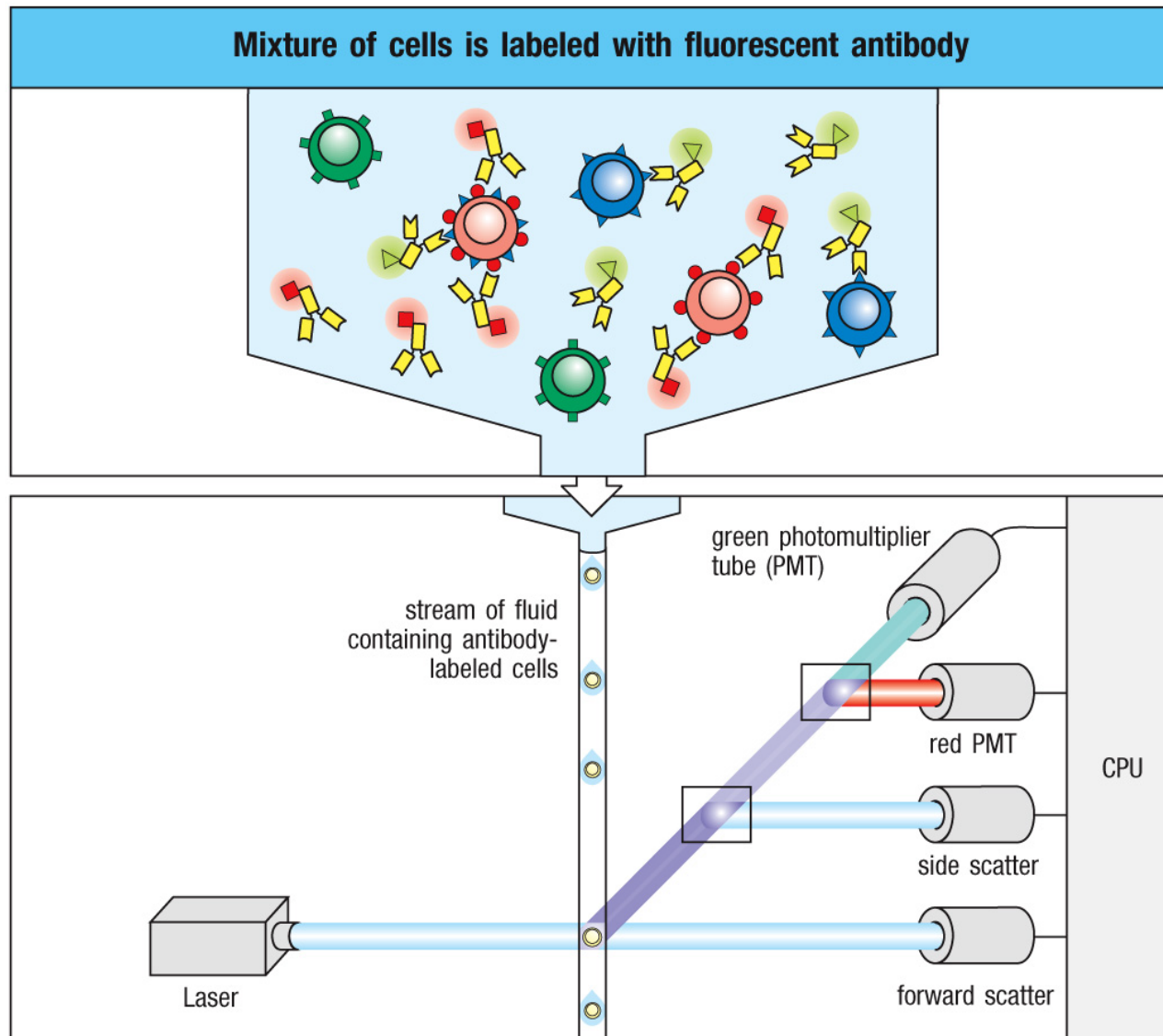


Figure A.21 (part 1 of 2) Janeway's Immunobiology, 9th ed. (© Garland Science 2017)

Surface IgM and IgD

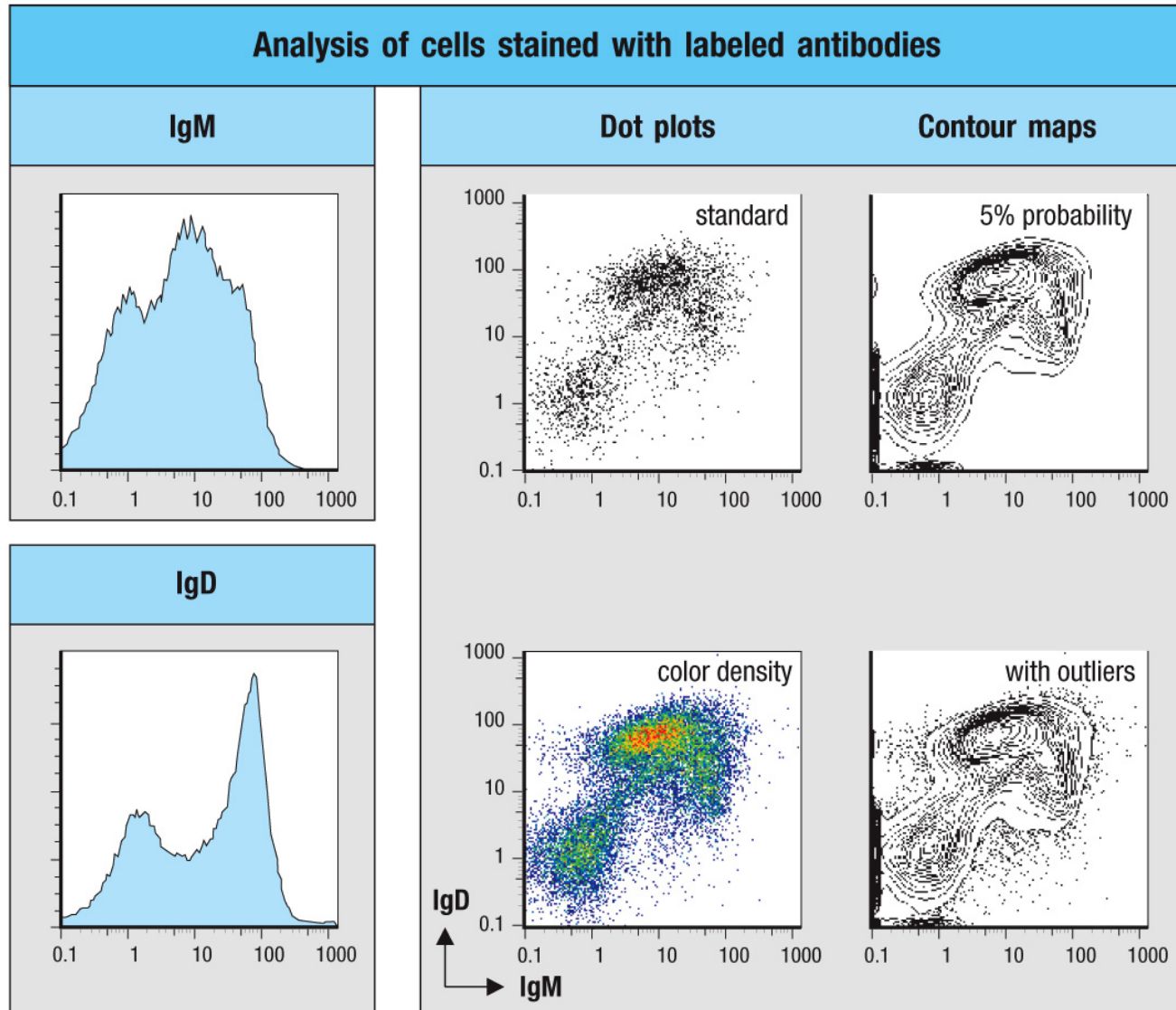
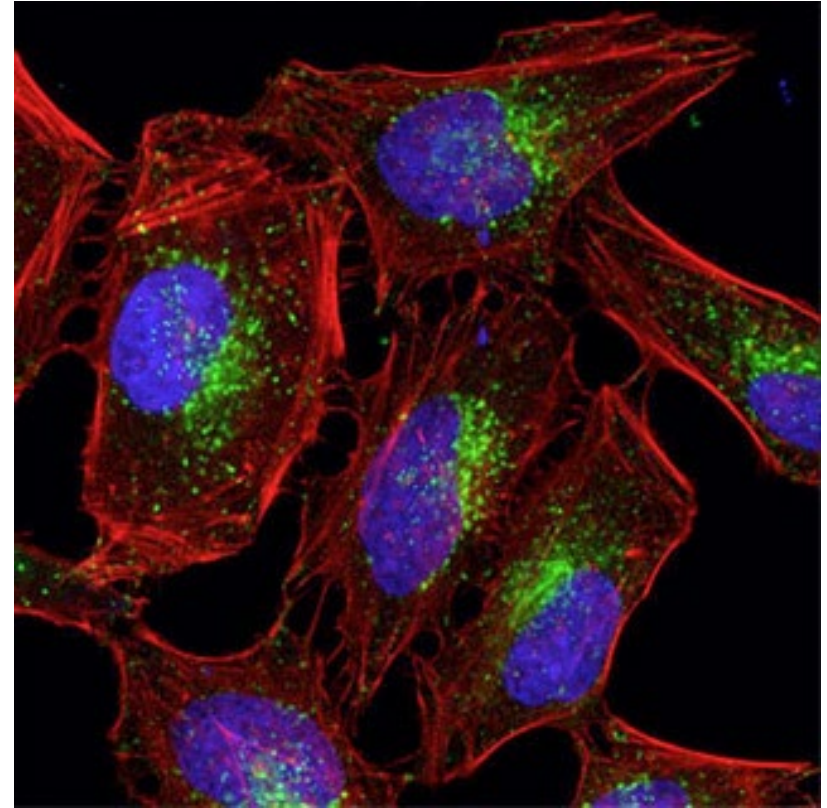
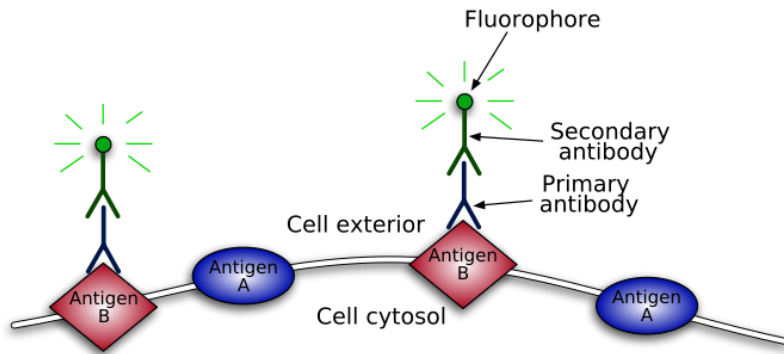


Figure A.21 (part 2 of 2) Janeway's Immunobiology, 9th ed. (© Garland Science 2017)

ImmunoFluorescence



http://php.med.unsw.edu.au/cellbiology/index.php?title=File:Primary-secondary_antibody.png

<http://www.cellsignal.com/common/content/content.jsp?id=apps-immunofluorescence>

Question

- Is it a good idea to isolate breast cancer cells from a patient and develop that into a vaccine to protect a general population?
- A) Yes
- B) No